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March 2019

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On the Cover

Artist uncertain, *Georg Philipp Friedrich von Hardenberg (Novalis)*, c. 1772–1801. Oil on canvas, 22.5 in x 20.5 in/57 cm x 52.1 cm. Collection of the Forschungsstätte für Frühromantik und Novalis-Museum (Research Center for Early Romanticism and Novalis Museum), Schloss Oberwiederstedt (Oberwiederstedt Castle), Wiederstedt, Germany. With publication permission of those institutions, the Ostdeutsche Sparkassenstiftung (East German Savings Bank Foundation), and Sparkasse Mansfeld Südharz (Mansfield Southern Harz Savings Bank).

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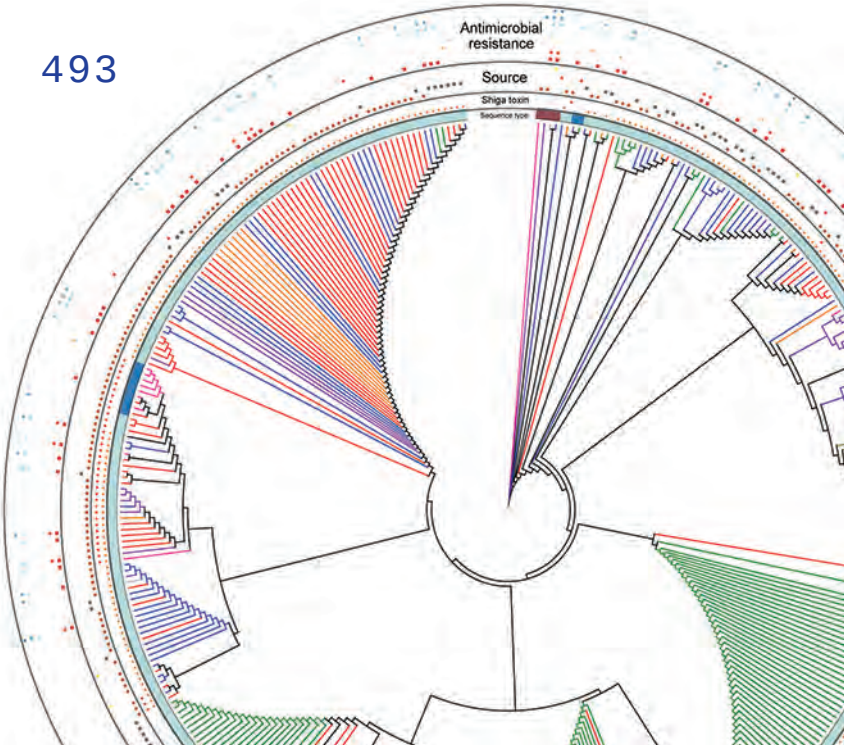

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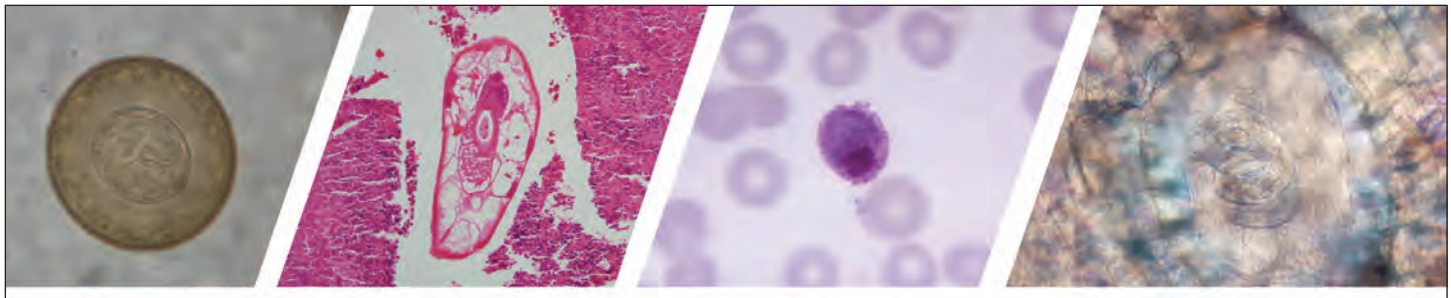

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Case Investigations of Infectious Diseases Occurring in Workplaces, United States, 2006–2015

Chia-ping Su,[1] Marie A. de Perio, Kristin J. Cummings, Anna-Binney McCague,[2] Sara E. Luckhaupt, Marie Haring Sweeney

Workers in specific settings and activities are at increased risk for certain infectious diseases. When an infectious disease case occurs in a worker, investigators need to understand the mechanisms of disease propagation in the workplace. Few publications have explored these factors in the United States; a literature search yielded 66 investigations of infectious disease occurring in US workplaces during 2006–2015. Reported cases appear to be concentrated in specific industries and occupations, especially the healthcare industry, laboratory workers, animal workers, and public service workers. A hierarchy-of-controls approach can help determine how to implement effective preventive measures in workplaces. Consideration of occupational risk factors and control of occupational exposures will help prevent disease transmission in the workplace and protect workers' health.

Despite ongoing efforts to reduce, eliminate, and eradicate infectious diseases, infections continue to pose a global threat to human health. More than 2,000 years ago, Hippocrates noted that "there are many handicrafts and arts which cause those who exercise them certain… plagues" (*1*). Recent experiences with anthrax, severe acute respiratory syndrome (SARS), influenza A(H1N1), and Ebola virus disease have highlighted the importance of focusing on workplaces not only to identify at-risk populations but also to understand mechanisms of disease propagation and to implement successful control and prevention measures (*2–5*).

In the United States, work-related infectious diseases are identified in multiple ways. Workers, employers, or workplace health and safety offices may note an unusual case or cluster of disease. Local and state public health departments receive case reports of infectious disease from clinicians and laboratories, and they may conduct investigations, sometimes with assistance from the Centers for Disease Control and Prevention (CDC). CDC's National Institute for Occupational Safety and Health (NIOSH) is the federal public health agency responsible for conducting research and making recommendations to prevent occupational safety and health risks, including work-related infectious diseases. The NIOSH Health Hazard Evaluation (HHE) program responds to requests from workers, employers, and public health agencies and conducts investigations of hazards including infectious diseases that occur in workplaces. Nongovernmental researchers also carry out investigations.

Investigators of work-related infectious disease must consider multiple factors related to the disease, workplace, and workers. Few publications have explored these factors. To illustrate the range of work-related infectious diseases that have been identified in the United States during 2006–2015 and to benefit future investigations, we examined the peer-reviewed literature and HHE reports on infectious diseases occurring in US workplaces. We describe occupational factors to consider and a systematic approach to control and prevent infectious disease in the workplace. We also note ways that specialized resources may be useful during the course of an investigation.

Literature Review

Methods

As defined by the World Health Organization, work-related diseases may have multiple causes, in which the work environment and other risk factors can play a role (https://www.who.int/occupational_health/activities/

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occupational_work_diseases/en). Work-related infectious diseases can be defined as those primarily caused by occupational exposure to biologic agents. These biologic agents, such as bacteria, fungi, viruses, and parasites, can be transmitted from human to human, from animal to human, or through environmental contact (*6*). We focused on studies of infectious disease occurring among workers in specific occupational groups or workplaces.

For the literature review, we followed the methods of Haagsma et al. (*6*). In March 2016, we searched PubMed for articles published since 2006. Our search strategy combined 3 groups of relevant keywords, including case investigation, workers/workplace/occupational, and infectious diseases. We also searched the NIOSH HHE reports database (https://www2a.cdc.gov/hhe/search.asp), using a similar strategy. We classified infectious diseases occurring in workplaces by industry category (work setting), using 2-digit codes of the North American Industry Classification System (NAICS) (https://www.census.gov/eos/www/naics), and by occupation (type of job). We describe detailed methods of our search of the literature and HHE reports in the Appendix (https://wwwnc.cdc.gov/EID/article/25/3/18-0708-App1.pdf).

Results

The literature search yielded 67 articles and 7 HHE reports from 66 investigations of infectious diseases occurring in US workplaces during 2006–2015 (Table 1; Appendix). Cases were concentrated in specific industries and occupations, especially healthcare and work involving contact with animals, reflecting the potential for disease transmission from patients and animals. Complementing previous findings from a systematic review by Haagsma et al. (*6*), our review of worksite case investigations found that several work-related infectious disease outbreaks or individual cases have been reported among laboratory and public service workers.

Work-related cases were associated with a variety of infectious pathogens. Bacteria were responsible for most reported cases, followed by viruses, fungi, and parasites or protozoa. As noted previously (*6*), respiratory viruses and zoonotic pathogens still threaten workers' health, especially for healthcare personnel and animal-contact workers. However, we also found reports of some emerging or reemerging pathogens, such as Ebola virus, lymphocytic choriomeningitis virus, norovirus, *Bacillus anthracis*, and *Yersinia pestis*, that caused several workplace disease clusters.

Specific Considerations

Many factors may combine to increase the risk for infection among workers during pathogen transmission. Categories of risk factors for work-related infections include disease factors (such as transmission mode), workplace factors, and worker factors. Considering each of these categories during case investigations is useful in planning and implementing prevention and control strategies.

Disease Factors

Infectious disease can be transmitted via direct contact (including percutaneous), droplet, airborne (aerosol), vehicles (such as food, water, and fomites), and vectors (Table 2). The studies included in our review suggest that occupations involving interaction with the general population, particularly ill persons, pose an increased risk for infection.

As one example, teachers and public service workers may acquire respiratory virus infections, including influenza and measles, because their work may bring them in contact with persons who are ill (*7–10*). Workers in the healthcare industry are also at risk for influenza as well as airborne (such as tuberculosis [TB]) and percutaneously transmitted (such as HIV) infection from patients (*11–13*). In 2014, work-related Ebola virus infections among healthcare personnel were a substantial component of the Ebola epidemic worldwide; 2 healthcare personnel acquired Ebola virus disease within the United States (*14,15*). Occupational contact with human corpses can also result in infectious disease. In 2007, an embalmer in New York, New York, USA, contracted *Mycobacterium tuberculosis* from a cadaver (*16*).

Disease transmission patterns are also relevant to those whose work brings them in contact with animals, putting them at risk for zoonotic infections. Occupational exposure to livestock and poultry contributed substantially to work-related infectious diseases. Twenty-nine cases of *Campylobacter* infection occurred over a period of several years among workers at a poultry processing plant (*17*), and sealpox virus infections were reported among animal rescue workers in a marine mammal rehabilitation center (*18*).

Transmission of pathogens in the workplace may occur in 2 directions: workers can acquire infections in the workplace and then also may serve as vectors that spread the disease to others, such as clients and co-workers. We found that workers in food preparation and serving-related occupations have been identified as sources of transmission in foodborne outbreaks. Two delicatessen workers infected with *Salmonella* from occupational contact with chicken became the source of disease transmission in a 2007 salmonellosis cluster in Minnesota (*19*). Transmission of norovirus gastroenteritis among workers and customers at a restaurant has also been reported (*20*). These examples show that preventing workers from acquiring infections in workplaces may also prevent disease transmission among the general public.

Pathogens in the environment can also serve as a source of worker infections through the respiratory route.

Table 1. Reported case investigations of infectious disease occurring in workplaces, by industry categories, occupations, and diseases, United States, 2006–2015*

Industry category (NAICS code)	Occupations	Infectious diseases	References†
Agriculture, forestry, fishing, and hunting (11)	Hunter	Brucellosis	(*61*)
	Farmer	Variant influenza A(H3N2); *Escherichia coli* infection	(*83*); (*71*)
	Rodent breeder	LCMV infection	(*82*)
Construction (23)	Laborer	Coccidioidomycosis	(*23,25*)
Manufacturing (31–33)	Drum maker	Anthrax	(*2*)
	Poultry vaccine production worker	Salmonellosis	(*29*)
	Poultry-processing worker	Campylobacteriosis	(*17*)
	Furniture company worker	Tuberculosis	(*54*)
	Slaughterhouse inspector	Q fever	(*65*)
	Automobile manufacturing worker	Legionnaires' disease	(*81*)
Transportation (48)	Truck driver	*Streptococcus suis* infection; cryptosporidiosis	(*79*); (*88*)
	Pilot, flight attendant	Malaria	(*37*)
Professional, scientific, and technical services (54)	Laboratory worker	Vaccinia virus infection, HIV infection, plague, cowpox, meningococcal disease, brucellosis	(*13,30–35,86*)
Administrative support and waste management and remediation services (56)	Landscaper	Tularemia	(*21*)
Education services (61)	School employee, teacher	Influenza	(*8*)
Healthcare and social assistance (62)	Healthcare worker (security guard, nurse, nursing aide, physician, volunteer, environmental services)	Mumps; MRSA skin infection; norovirus gastroenteritis; adenovirus 14 infection; RSV infection; *Trichophyton tonsurans* skin infection; meningococcal disease; influenza; salmonellosis; Ebola virus disease; measles; TB;	(*51*); (*52*); (*56*); (*57*); (*62*); (*64*); (*66*); (*11,68*); (*87*); (*14*); (*92*); (*12,77*)
	Childcare worker	*E. coli* infection	(*72*)
Arts, entertainment, and recreation (71)	Wildlife biologist	Plague	(*59*)
	Animal caretaker	MRSA skin infection	(*63*)
	Adult film performer	HIV infection	(*36*)
	Spa maintenance worker	MAC infection	(*22*)
	Filmmaker	Coccidioidomycosis	(*24*)
	Day camp counselor	Histoplasmosis	(*26*)
Food services (72)	Cook, food server	Norovirus gastroenteritis; salmonellosis; *E. coli* infection	(*20*); (*19*); (*89*)
Other services except public administration (81)	Embalmer	TB	(*16*)
	Animal refugee worker	Tuberculosis; sealpox virus infection	(*28*); (*18*)
	Pet store worker	Salmonellosis	(*74*)
	Missionary worker	Melioidosis; dengue fever	(*75*); (*70*)
Public administration (92)	US Customs officer	Measles	(*9,10*)
	Police officer	Meningococcal disease	(*66*)
	Firefighter	Cryptosporidiosis	(*88*)
	Correctional officer	Cryptosporidiosis; Shiga toxin–producing *E. coli* infection; TB; coccidioidomycosis;	(*78*); (*71*); (*12*); (*90*)
	Military	Legionellosis; TB	(*73*); (*53*)

*An expanded version of this table showing complete details on all cases is available online (https://wwwnc.cdc.gov/EID/article/25/3/18-0708-T1.htm). HIV, human immunodeficiency virus; LCMV, lymphocytic choriomeningitis virus; MAC, *Mycobacterium avium* complex; MRSA, methicillin-resistant *Staphylococcus aureus*; RSV, respiratory syncytial virus; TB, tuberculosis. NAICS, 2012 North American Industry Classification System (https://www.census.gov/eos/www/naics/).
†Reference numbers >50 and additional details on the literature search are available in the Appendix (https://wwwnc.cdc.gov/EID/article/25/3/18-0708-App1.pdf).

A 2006 report described a 21-year-old healthy landscaper diagnosed with tularemia. Traditionally thought of as a zoonotic pathogen, *Francisella tularensis* can also be acquired via aerosolized bacteria during occupational activities such as lawn mowing and leaf blowing (*21*). Another report described 2 spa maintenance workers infected with *Mycobacterium avium* complex organisms, which live in water and are highly resistant to disinfectants, such as chlorine. Occupational exposure to aerosolized bacteria during routine cleaning and maintenance of spa filters and tubs was the likely cause of this outbreak (*22*).

Workplace Factors

Regardless of transmission mode, workplace factors can contribute to the propagation of infection. It is crucial to identify aspects of the workplace that pose biologic haz-

Table 2. Reported case investigations of infectious disease pathogens occurring in workplaces, by mode of transmission and source of disease, United States, 2006–2015*

Mode of transmission	Disease (reference no.)†		
	Human	Animal	Environment
Direct contact (including percutaneous)	MRSA (*52,63*) *Trichophyton tonsurans* (*64*) Ebola virus (*14,15*) HIV (*13,36*)	MRSA (*63*) *Bacillus anthracis* (*2*) *Brucella* spp. (*61*) *Coxiella burnetii* (*65*) *Yersinia pestis* (*59*) Sealpox virus (*18*) *Streptococcus suis* (*79*) LCMV (*82*) *Burkholderia pseudomallei* (*75*)	
Droplet	Adenovirus 14 (*57*) influenza virus (*7,8,11,68*) *Neisseria meningitides* (*66*) RSV (*62*) Mumps virus (*51*)	Influenza virus (*83*)	
Airborne	*Mycobacterium tuberculosis* (*12,40,53,54,77,85*) Measles virus (*9,10,92*)	*Mycobacterium tuberculosis* (*28*)	*Coccidioides* spp. (*23–25,90*) MAC (*22*) *Legionella pneumophila* (*73,81*) *Francisella tularensis* (*21*) *Histoplasma capsulatum* (*26*)
Vehicles (fecal–oral)	Norovirus (*20,56*) *E. coli* (*72*)	*Salmonella* spp. (*19,74*) *E. coli* (*71,89*) *Cryptosporidium* (*78,88*) *Campylobacter jejuni (17)*	
Vectors		*Plasmodium* spp. (*37*) Dengue virus (*70*)	

*LCMV, lymphocytic choriomeningitis virus; MAC, *Mycobacterium avium* complex; MRSA, methicillin-resistant *Staphylococcus aureus*; RSV, respiratory syncytial virus.
†Reference numbers >50 and additional details on the literature search are available in the Appendix (https://wwwnc.cdc.gov/EID/article/25/3/18-0708-App1.pdf).

ards, because those aspects may be amenable to controls. Such factors can include workplace characteristics, work practices and processes, and engineering and administrative issues. For example, work-related fungal respiratory infection is a concern in some areas. Outbreaks of coccidioidomycosis have occurred among construction workers and outdoor film production workers in California (*23*,*24*); during 2011–2014, a total of 44 cases of coccidioidomycosis were identified among workers constructing solar power farms (*25*). Outdoor workplaces with hot, dry conditions in areas of endemicity pose a risk for coccidioidomycosis because soil-disruptive activities and high winds expose workers to dust harboring *Coccidioides* spores. Other factors may also contribute. In 2012, Nebraska officials reported an investigation of a cluster of histoplasmosis among 32 day camp counselors (*26*). The probable infection source was campsite contamination of soil and picnic tables by bat guano, which probably became aerosolized during camp activities or cleanup.

Engineering factors can also promote disease transmission in the workplace. Previous studies have shown that workplace environmental characteristics contributed to *M. tuberculosis* transmission among workers (*27*). During the outbreak investigation of TB among workers at an elephant refuge in 2009, investigators found that shared air between the administrative area and the barn, along with pressure-washing of the barn by workers, contributed to transmission (*28*). Laboratories are another example of unique workplaces where engineering controls (such as biosafety cabinets and local exhaust ventilation) are essential for reducing or eliminating potential biologic hazards to workers. Laboratory workers have acquired *Salmonella* infection, vaccinia virus infection, plague, cowpox, brucellosis, meningococcal disease, and HIV infection through accidental direct contact with pathogens in the workplace over the past decade (*13*,*29–35*).

Administrative issues, including workplace policies and practices, can also play an important role in disease transmission in the workplace. In 2014, the California Department of Public Health reported an occupational HIV outbreak among adult film performers (*36*). Some adult film production companies rely on HIV testing results as a control and require performers to engage in penetrative sex without a condom. This approach is problematic, because during acute infection, a performer can transmit the infection even when HIV test results are negative. This example highlights that the lack of a protective administrative policy in the workplace could lead to infectious disease transmission among workers.

For workers in high-risk environments, employers have a responsibility to provide adequate prophylaxis for prevention of infectious disease. However, sometimes

employers or workers are unfamiliar with or unable to comply with the relevant recommendations. For example, CDC staff found 4 malaria cases among employees of a commercial airline who had all traveled to Ghana and stayed at the same hotel before disease onset. None had used antimalarial chemoprophylaxis provided by the company (*37*). It is unclear why they did not use the chemoprophylaxis, but this cluster underscores the importance of a comprehensive malaria prevention program that includes education and counseling.

Worker Factors

Individual characteristics, such as impaired immunity, inadequate prophylaxis, and socioeconomic and language factors, may increase the risk for acquisition and transmission of infectious diseases. For example, a case of fatal laboratory-acquired infection caused by *Y. pestis* occurred in a laboratorian in 2009 (*32*). No additional cases or major deficiencies in engineering controls were identified in this laboratory. A postmortem examination revealed that the affected worker had hereditary hemochromatosis, a condition that increases susceptibility to infection with certain bacterial pathogens. In another situation, *Campylobacter* infection among poultry-processing workers was found to occur most frequently during the first weeks of work, after which the workers develop immunity that may be protective against future infection (*17*). Therefore, investigators should consider that individual host susceptibility to certain diseases may play a role in disease transmission in workplaces.

Documented nosocomial transmission puts healthcare personnel at substantial risk for acquiring or transmitting several vaccine-preventable diseases, including hepatitis B, influenza, measles, mumps, rubella, pertussis, and varicella. The Advisory Committee on Immunization Practices and the Hospital Infection Control Practices Advisory Committee recommend that healthcare personnel be vaccinated or have documentation of immunity for all of these diseases; employers must formulate a comprehensive vaccination policy for all healthcare personnel (*38*,*39*). Workers in other settings, such as public services, may also be at risk for exposure to vaccine-preventable diseases, such as measles. In 2007, an airport officer contracted measles from an international traveler and may have transmitted it to a second airport worker (*9*). In 2011, a US Customs and Border Protection officer contracted measles after processing an arriving refugee with measles (*10*). All of these situations underscore that immunizations should be kept current for workers whose jobs involve frequent contact with the public.

In addition to individual susceptibility and immunity, a worker's socioeconomic status could contribute to risk. Texas officials reported a TB outbreak investigation among workers in a meatpacking plant in 2011. The index case was in a foreign-born patient who had cavitary TB with acid-fast–positive smears. Most of the patient's work contacts were foreign-born. Investigators found that low economic status, limited access to healthcare, and communication and language barriers caused delays in diagnosis and played significant roles in TB transmission among immigrant workers (*40*).

Approach to Controlling Exposure to Protect Workers

Investigators of an infectious disease outbreak that is potentially related to a workplace must consider the "who, what, where, when, and why" questions of field epidemiology in the context of a unique and sometimes contentious environment. Stakeholders, including workers, employers, labor unions, trade associations, regulatory agencies, and members of the public, may have distinct and, at times, competing priorities. Barriers to implementing solutions may include those common to all epidemics, such as expense and time constraints, and others more specific to the occupational setting, such as a poorly developed safety culture.

To reduce worker exposure to potential occupational hazards, including biologic agents, we need feasible, effective measures that can be implemented in the workplace. Occupational health and safety specialists have long used the hierarchy of controls (Figure 1) to eliminate or minimize exposure to any occupational hazard. The control methods at the top of the graphic are potentially more effective and protective than those at the bottom. Here are examples of control recommendations made for identified occupational hazards using the hierarchy.

Elimination and Substitution

Elimination and substitution are the most effective ways to reduce occupational hazards but sometimes can be difficult to implement in an existing work process. For infectious diseases, one should first assess the feasibility of not working in an area in which biohazards are present. If avoidance is not feasible, then decontaminating surfaces, items, and areas in a hazardous workplace can eliminate the possibility of transmission of infectious agents to workers. For environmentally associated laboratory infections, CDC has published guidance on methods for sterilization and disinfection in laboratories and on the levels of antimicrobial activity associated with liquid chemical germicides (*41*). However, employers and workers should also be aware of potential health risks when using such disinfectants (*42*).

Training on early recognition of the key signs and symptoms of common communicable diseases and the

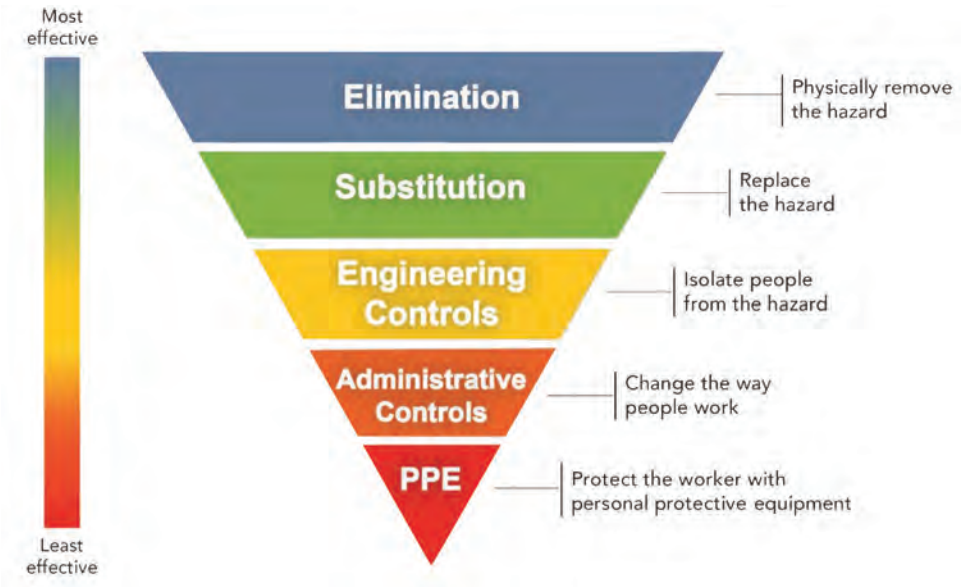

Figure. The hierarchy of controls for controlling exposures to occupational hazards. Source: NIOSH, https://www.cdc.gov/niosh/topics/hierarchy/default.html.

use of sick leave by workers may help to reduce disease transmission in the workplace. For example, CDC guidance for responding to influenza in schools during the 2009 influenza A(H1N1) pandemic included directing students and staff with influenza-like illness to stay home when ill (*43*). A recent study showed that offering paid sick leave to workers is likely to reduce the spread of disease in workplaces by increasing the rate at which sick workers stay home (*44*). However, the effectiveness of those measures still needs to be closely evaluated.

Engineering Controls

Engineering controls in the field of occupational health and safety are physical changes to work processes or equipment to remove hazardous conditions or to place a barrier between workers and hazards. Engineering controls effectively protect workers without placing the primary responsibility of implementation on the worker. Ventilation is by far the most common engineering control, especially for airborne pathogens. Previous modeling data showed that the risk for TB infection in healthcare settings decreases exponentially as room ventilation rates increase (*45*). Thus, guidelines for preventing transmission of TB in healthcare settings recommend engineering controls such as local exhaust and general ventilation to prevent the spread and reduce the concentration of infectious droplet nuclei in the air. Supplementing ventilation systems with high-efficiency particulate air filtration, ultraviolet germicidal irradiation, or both can further enhance system performance and reduce the spread of airborne disease (*46*).

Nonventilation engineering controls also can reduce or eliminate pathogen exposure in the workplace. For example, dust suppression during construction, including continuous soil-wetting and proper covering, could decrease the risk for coccidioidomycosis among outdoor workers (*23*). In addition, evidence shows that engineering controls can reduce healthcare personnel's exposure to bloodborne pathogens. Safety needle devices with built-in engineering controls reduce the risk for needlestick injury among healthcare personnel (*47*).

Administrative Controls

Administrative controls are methods such as standard operating procedures that change the way work is performed. Their effectiveness depends on the availability of the control, employer commitment, and worker acceptance. Regular monitoring and reinforcement are necessary to ensure that workers follow policies and procedures consistently. The CDC publication Biosafety in Microbiological and Biomedical Laboratories addresses recommended practices for working safely from a biosafety perspective (*41*).

Developing and implementing a mandated workplace health regulation can be an effective administrative control. In December 1991, mandatory hepatitis B vaccination of all healthcare personnel (at employers' expense) became a federal standard under the Occupational Safety and Health Act (*48*). The regulation accelerated the use of hepatitis B vaccine for healthcare personnel. As the result of routine vaccination and improved infection control precautions, the number of hepatitis B virus infections among healthcare personnel decreased from ≈10,000 in 1982 to ≈304 in 2004 (*39*). In addition, administrative measures of TB control, such as developing and implementing a written TB infection–control plan, prevent disease transmission in healthcare settings (*46*).

Personal Protective Equipment

Various types of personal protective equipment (PPE) are available to minimize exposure to hazards in workplaces. PPE, such as respirators, gloves, goggles, and coveralls, provides a physical barrier between the worker and the infectious agent. Compared with other methods of controlling exposure, PPE is the least effective but probably best-known method used for infectious disease prevention. Proper use of PPE requires a comprehensive program and a high level of worker involvement and commitment. When engineering, work practice, and administrative controls are not feasible or do not provide sufficient protection, PPE may be the only reliable method of disease prevention for workers. Therefore, employers must provide their workers with PPE that is appropriate to the task and the correct size for the user, along with proper training on use and on donning and doffing methods.

In the early stages of epidemics of emerging infectious diseases, such as Ebola virus disease, lack or misuse of PPE can lead to infections in healthcare personnel. In 2014, the transmission of Ebola virus to 2 nurses who provided care to an Ebola-infected patient at a US hospital revealed the importance of directive PPE recommendations and standardized training efforts for healthcare personnel. Interventions included a system of trained observers supervising the donning and doffing of PPE (*5*,*49*). Even though the quality of evidence is low, the risk for contamination may be reduced by double-gloving, following directives for donning and doffing procedures, and instituting more active training (*50*).

Additional Resources

The NIOSH Surveillance Program works with partners at CDC and the Council of State and Territorial Epidemiologists to promote inclusion of standard occupational information in the National Notifiable Disease Surveillance system (in which most conditions included are infectious) and in electronic medical records, to facilitate detection of possible disease transmission in workplaces (https://www.cdc.gov/niosh/topics/ehr/default.html). Once a disease cluster or outbreak is suspected, the NIOSH HHE program can be a resource for technical assistance and consultation during the case investigation in a workplace. Workers, employers, or public health professionals can request an evaluation of health hazards in the workplace (https://www.cdc.gov/niosh/hhe). NIOSH makes recommendations aimed at controlling the hazard; these are voluntary but can reduce risk and improve the health and safety of the workforce.

Conclusions

We found that cases of work-related infectious diseases in the United States during 2006–2015 appeared to be concentrated in specific industries and occupations, especially in healthcare and among laboratory, animal, and public service workers. The biosafety programs in these industries could be strengthened. Case investigations of infectious disease occurring in a workplace can be challenging with regard to linking the symptoms of a disease to a specific pathogen or exposure source and identifying effective preventive strategies. A multidisciplinary approach that includes epidemiologists, physicians, industrial hygienists, and engineers may be beneficial.

Emerging and reemerging work-related infectious diseases will continue to threaten workers' health. Previously published literature has identified several high-risk occupations, but other occupations may also be at risk. Indeed, we cannot know with certainty which industry or which workers will be at risk in the future. Considering occupational risk factors and controlling exposures among workers when investigating infectious disease may help prevent disease transmission in the workplace. In addition, because person-to-person transmission between workers and members of the public can propagate some disease outbreaks, assessment of worker infection may contribute to control of disease outbreaks in communities.

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Cross-Border Movement of Highly Drug-Resistant *Mycobacterium tuberculosis* from Papua New Guinea to Australia through Torres Strait Protected Zone, 2010–2015

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In this retrospective study, we used whole-genome sequencing (WGS) to delineate transmission dynamics, characterize drug-resistance markers, and identify risk factors of transmission among Papua New Guinea residents of the Torres Strait Protected Zone (TSPZ) who had tuberculosis diagnoses during 2010–2015. Of 117 isolates collected, we could acquire WGS data for 100; 79 were Beijing sublineage 2.2.1.1, which was associated with active transmission (odds ratio 6.190, 95% CI 2.221–18.077). Strains were distributed widely throughout the TSPZ. Clustering occurred more often within than between villages (p = 0.0013). Including 4 multidrug-resistant tuberculosis isolates from Australia citizens epidemiologically linked to the TSPZ into the transmission network analysis revealed 2 probable cross-border transmission events. All multidrug-resistant isolates (33/104) belonged to Beijing sublineage 2.2.1.1 and had high-level isoniazid and ethionamide co-resistance; 2 isolates were extensively drug resistant. Including WGS in regional surveillance could improve tuberculosis transmission tracking and control strategies within the TSPZ.

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Tuberculosis (TB) is the leading infectious cause of death globally (*1*). To reduce the burden of TB, many countries committed to achieving a 90% reduction in TB incidence by 2035 as part of the End TB Strategy (*2*). Australia has already achieved preelimination targets (<10 cases/1 million population) in the nonindigenous, Australia-born population (*3*). However, achieving TB elimination in Australia remains a daunting challenge, given high population mobility, increased importation of TB cases from high-incidence settings, and cross-border spread from neighboring countries, such as Papua New Guinea (*4*). In 2013, ≈90% of the TB cases reported in Australia were in persons born overseas (*3*). The state of Queensland has one of the lowest TB notification rates in Australia (4.0 cases/100,000 population) (*5*); the Western Province of neighboring country Papua New Guinea has a significantly higher incidence, estimated at 2,901 cases/100,000 population at the provincial capital, Daru (*6*,*7*).

Daru General Hospital (Port Moresby, Papua New Guinea) is the main health center that offers TB health services to residents of Western Province, including those in the surrounding areas, such as the Torres Strait Protected Zone (TSPZ). The TSPZ is an area where free bidirectional cross-border movement (without passports or visas) is permitted for purposes of traditional customs and economic activities (Figure 1); the zone was created with the signing of the 1978 Torres Strait Treaty by Papua New Guinea and Australia. This area contains a number of Papua New Guinea villages and 14 Australia island communities (estimated population 1,526) that are part of Queensland (*9*). Cross-border movement of populations within the TSPZ provides a potential route of entry of *Mycobacterium tuberculosis* into northern Queensland and its spread elsewhere in Australia.

In 2012, an Australia resident of the TSPZ was reported to have pulmonary TB resistant to streptomycin only. After initial improvement with standard therapy, the patient deteriorated clinically. Rifampin resistance was detected by Xpert MTB/RIF assay (Cepheid, http://www.cepheid.com), and multidrug-resistant (MDR) TB was confirmed after positive culture and drug susceptibility testing. The mycobacterial interspersed repetitive unit 24 (MIRU-24) profile of the new isolate differed from the initial isolate and was characteristic of TB strains in Western Province, indicating a possible reinfection with an MDR TB isolate (*10*). This incident was the first notified case of MDR TB in a citizen of Australia in the Torres Strait Islands. During outbreak epidemiologic investigations, 3 additional MDR TB diagnoses were made in citizens of Australia who were current or previous residents of the TSPZ. These 4 cases were epidemiologically linked through a network of close contacts. At the time of diagnosis, 2 of the patients were living in different Queensland cities distant from the TSPZ.

We sought to use whole-genome sequencing (WGS) to determine the genomic relationship between these isolates and the MDR TB isolates from Papua New Guinea as proof of principle for transmission of MDR TB through the TSPZ. The discriminatory power of WGS enables the delineation of TB transmission with a higher resolution than conventional genotyping (*11*,*12*). The aim of this study was to use WGS and epidemiologic data to determine strain diversity within the TSPZ, characterize geno-resistance markers, and identify potential risk factors for transmission.

Methods

Strain Selection

All patients receiving TB diagnoses in Queensland (including Papua New Guinea citizens receiving TB

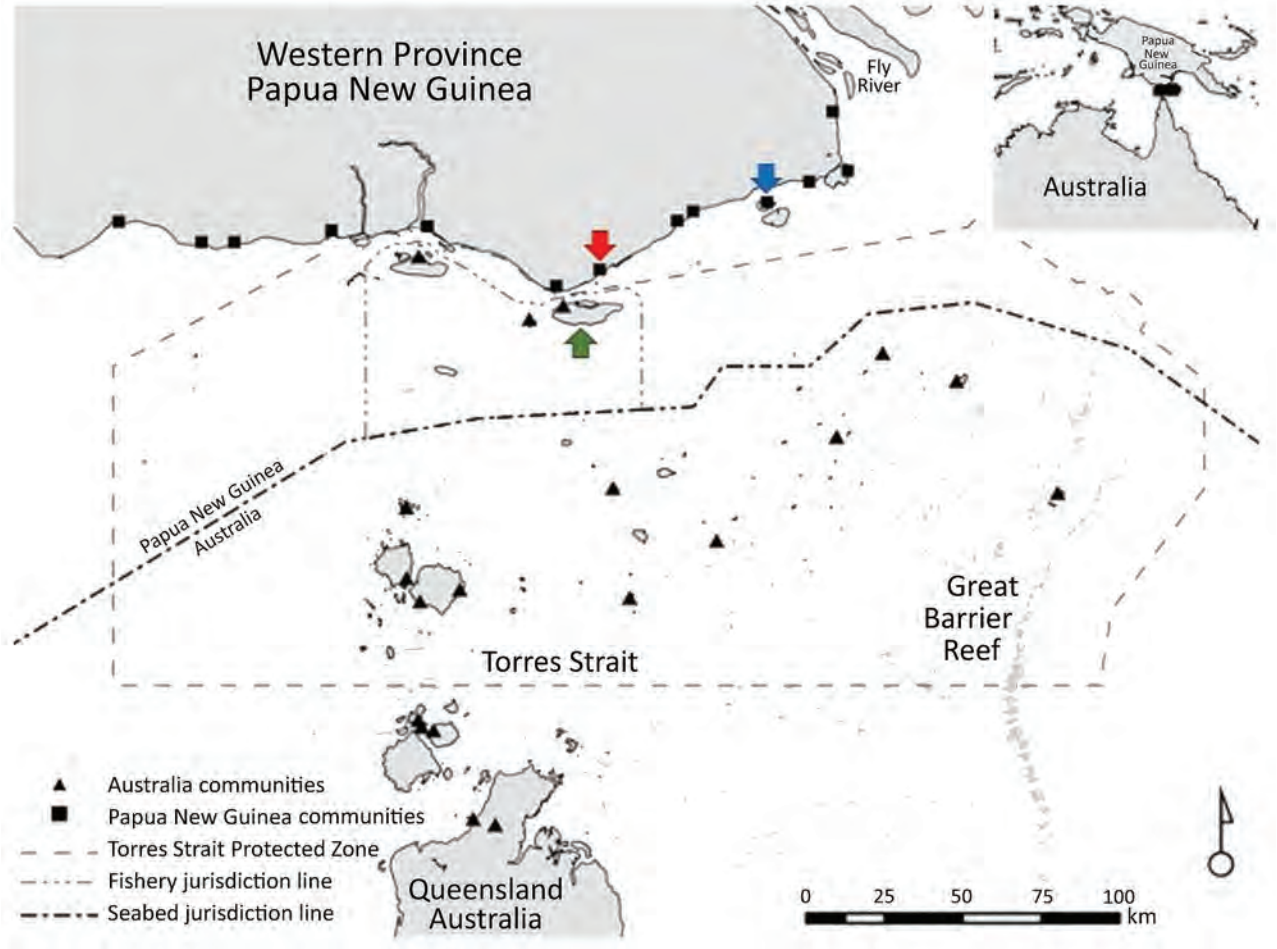

Figure 1. Communities within and boundaries of Torres Strait Protected Zone. Green arrow indicates Saibai Island, Australia; red arrow Mabaduan village, Papua New Guinea; and blue arrow Daru Island, Western province provincial Capital, Papua New Guinea. Inset depicts location of Torres Strait Protected Zone between Australia and Papua New Guinea. Operational details on how the 1978 Torres Strait Treaty functions can be accessed online (https://dfat.gov.au/geo/torres-strait/Documents/torres-strait-guidelines.pdf). Map obtained with permission from Butler JRA, Tawake A, Skewes T, McGrath V. Integrating traditional ecological knowledge and fisheries management in the Torres Strait, Australia: the catalytic role of turtles and dugong as cultural keystone species. Ecology and Society. 2012;17:34 (*8*).

diagnoses in Queensland clinics in the TSPZ) must be reported to the Queensland Department of Health in Brisbane. In this study, we included isolates from Papua New Guinea citizens residing in the TSPZ who received TB diagnoses in the TSPZ during January 1, 2010–December 31, 2015, which we refer to as cross-border TB isolates. We extracted demographic and clinical data from the Queensland Notifiable Conditions System on April 6, 2016. We included 1 patient given a TB diagnosis previously in Papua New Guinea who subsequently sought treatment in the TSPZ for extensively drug-resistant (XDR) TB; we also included the 4 previously mentioned MDR TB cases in residents of Australia because these infections were linked to the TSPZ. During the study period, a small number of drug-susceptible TB cases (n = 14) were reported among Australia citizens residing in the TSPZ, but we did not include these cases in this study.

Drug Susceptibility Testing and Genotyping

We performed *M. tuberculosis* culture, species identification, genotyping, and drug susceptibility testing at Queensland Mycobacterium Reference Laboratory (Pathology Queensland, Brisbane). MIRU-24 or MIRU-15 results obtained as previously described (*13*) were available for most isolates. We determined phenotypic susceptibility to first- and second-line drugs as previously described (*7*). On a case-by-case basis, we used SENSITITRE Microbroth Dilution Method (Trek Diagnostic Systems, https://www.thermofisher.com) to resolve differences between resistance mutations detected and phenotypic drug susceptibility test results.

WGS Analysis

We retrieved *M. tuberculosis* isolates from –80°C storage, cultured them on Lowenstein–Jensen medium, and extracted isolate DNA using an organic enzymatic method (*7*). We performed WGS at the Australia Genome Research Facility (Brisbane) using Illumina HiSeq 2000 (https://www.illumina.com) for paired-end reads, checked the quality of reads using FastQC version 0.11.2 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc), and trimmed using trimmomatic version 0.27 (*14*). We mapped reads to reference genome *M. tuberculosis* H37Rv (GenBank accession no. NC_000962.3) using BWA-MEM (https://arxiv.org/abs/1303.3997) and used GATK UnifiedGenotyper (*15*) to call single-nucleotide polymorphisms (SNPs) and small insertion/deletions (indels). We selected the SNPs and small indels with ≥10 times read depth, 80% allele frequency, and ≥10-bp difference between neighboring SNPs and indels. Using SnpEff version 4.1 (*16*), we annotated high-quality SNPs and indels. We included for analysis mutations in all known genes (including regulatory genes) that conferred resistance to TB drugs (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1003-App1.pdf) and excluded mutations in repetitive regions, such as the *PE* and *PPE* gene family regions. We submitted raw reads in the form of FASTQ files to the Sequence Read Archive (project file no. PRJNA401368). Scripts of the raw sequence data are available (https://github.com/arnoldbaino/Daru_scripts).

Phylogenetics and Bayesian Coalescent Analysis

We used data sets from 2 previous independent WGS studies, PRJEB7281 and PRJEB2358 (*17*), as TB global representatives in phylogenetic analysis. Using concatenated SNP alignment, we constructed a maximum-likelihood phylogenetic tree with RAxML version 7.4.2 (*18*). We used a general time-reversible model, with rate heterogeneity accommodated by using discrete rate categories (i.e., GTRCAT algorithm), with 1,000 bootstraps and visualized using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree). We performed molecular dating of the Beijing lineage isolates using BEAST version 1.8.2 (*19*), as previously described (*7*).

Transmission Assessment

Using ape library in R statistical package (http://cran.r-project.org), we calculated pairwise SNP differences (excluding SNPs in known drug-resistance genes) between isolates. We assessed SNP differences by lineage and compared these differences between different localities to set a threshold as a measure of putative transmission. We constructed genomic clusters using a median-joining network (Network version 5, http://www.fluxus-engineering.com/sharenet.htm). We structured links within genomic clusters assuming the existence of linear transmission among isolates from the same locality and putative transmission among isolates from different localities (if the SNP difference was within the limit of the threshold).

Statistical Analysis and Ethics

We compared patient characteristics using Fisher exact test. We performed univariate and multivariable logistic regression analyses to evaluate associations between patient characteristics and genomic clusters as outcome variables to infer transmission. We used R statistical package for all statistical analyses. This study was approved by the institutional review board of the University of Queensland (Brisbane, Queensland, Australia; approval no. HREC2015000572) and, as required by the Public Health Act of 2005, by Queensland Health (approval no. RD006697).

Results

In total, 134 Papua New Guinea citizens were reported to have TB in the TSPZ during the study period (Figure 2), and 117 (87.3%) had culture-confirmed disease. We

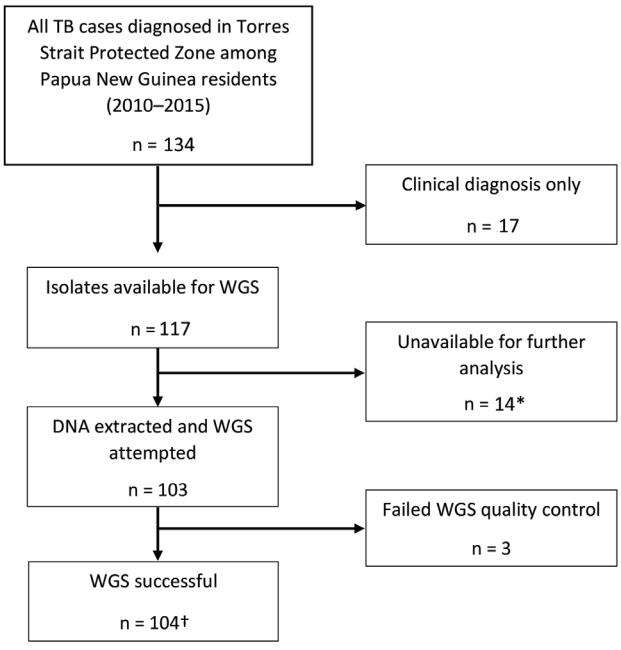

Figure 2. Flow diagram of included *Mycobacterium tuberculosis* isolates from Papua New Guinea citizens residing in Torres Strait Protected Zone, 2010–2015. *Isolates unable to grow or were contaminated. †Included were 4 additional isolates among Queensland residents that were a part of an epidemiologic cluster linked to the Torres Strait Protected Zone. TB, tuberculosis; WGS, whole-genome sequencing.

acquired and successfully sequenced 100 (85.5%) of 117 isolates from these citizens plus 4 isolates from the Australia citizens who resided or previously resided in the TSPZ. Isolates were sequenced with a mean coverage depth of 74 times (range 42–110 times) and mean coverage breadth of 98.5% (range 96.3%–99.8%). MIRU profiles were available for 98 of 104 patients (Appendix Table 2). Sequence comparisons revealed a median SNP difference of 31 (interquartile range [IQR] 24–38) for isolates with the same MIRU profile and 1,101 (IQR 1,066–1,119) for isolates with different MIRU profiles (Appendix Figure 1). SNP differences between all possible pairs within the data set were bimodal; 2 large peaks represent 2 different lineages. The possibility of cross-contamination among isolates with no SNP differences was negligible because specimens were processed and their DNA sequenced on different days. Of the 104 isolates sequenced, phylogenetic analysis revealed that 83, including the 4 MDR TB isolates from Australia citizens, were part of the modern Beijing sublineage 2.2.1.1 and 21 the Euro-American lineage, which consisted of 6 different sublineages (Appendix Table 3, Figure 2). Although the isolates were widely distributed throughout the TSPZ, 50% originated from a single Papua New Guinea village, Mabadauan (Appendix Figure 3).

Evolutionary analysis of the Beijing sublineage 2.2.1.1 isolates revealed that 59% (49/83) were related to clades previously identified in nearby Daru Island (Appendix Figure 4) (*7*) and 41% were a part of unique clades. The isolates from the Australia residents were clade C, which we inferred to have emerged in the 1980s.

To determine links between isolates from the same and different localities, especially isolates from villages with high numbers of isolates from different lineages, we deduced a threshold of 8 SNPs to distinguish transmission links (Appendix Figure 5). We found 17 genomic clusters (14 Beijing lineage, 3 Euro-American lineage) constituting 74 isolates (65 Beijing lineage, 9 Euro-American lineage) among PNG citizens (Figure 3). In total, 89% (34/38) of isolates from Mabadauan (identified as Beijing lineage) and 100% (4/4) of isolates from Sigabadaru (characterized as Euro-American lineage) clustered. More cases (36/41) formed genomic clusters in 2011 than in any other year, indicative of enhanced transmission leading up to this time point (Appendix Figure 6). The number of isolates from the same locality that formed genomic clusters (41 Beijing, 5 Euro-American) was significantly higher than the number that formed among isolates from different localities (24 Beijing, 4 Euro-American; $p = 0.0013$). The median SNP difference was 2 (IQR 1–3) among genomic clusters from the same locality and 4 (IQR 2–6) among genomic clusters from different localities. Of the 4 Australia cases, 3 formed 1 MDR TB cross-border genomic cluster having 1 SNP difference and a 15-month difference in sample collection dates. The fourth isolate was linked to another MDR TB cross-border cluster with no SNP differences and a 5-month difference in sample collection dates, suggesting 2 independent episodes of cross-border transmission into Australia citizens.

Most cross-border TB isolates in Papua New Guinea residents (74%, 74/100) were found in young persons (<35 years of age) (Table 1), and male and female sexes were equally represented. Only 1 (1.4%) of 74 patients tested had HIV co-infection. No major differences in characteristics were detectable between patients infected with the Beijing and Euro-American lineage, except for drug resistance, which was strongly associated with the Beijing lineage ($p<0.001$).

Univariate analysis revealed that the Beijing lineage (odds ratio [OR] 6.190, 95% CI 2.221–18.077; $p<0.0005$) and resistance to first- and second-line TB drugs (OR 4.677, 95% CI 1.452–20.943; $p = 0.019$) were strongly associated with transmission (Table 2). In multivariate logistic analysis, we adjusted for these 2 characteristics, and this analysis showed the Beijing lineage was associated with transmission (adjusted OR 4.484, 95% CI 1.526–13.891). The HIV-infected patient was not part of a cluster.

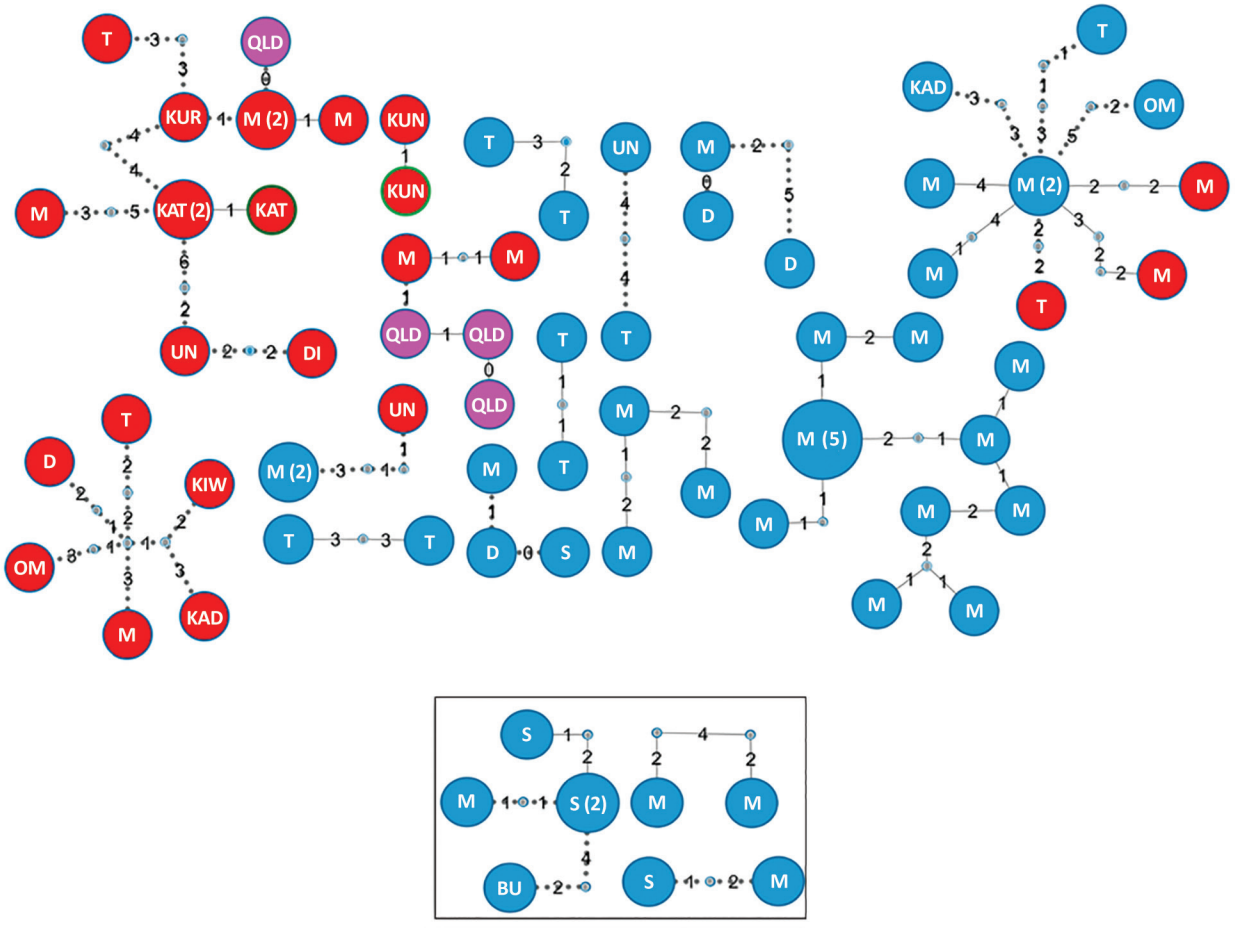

Figure 3. Genomic clusters of highly drug-resistant tuberculosis cases among Papua New Guinea and Australia citizens residing or previously residing in the Torres Strait Protected Zone, 2010–2015, by lineage. The top shows the Beijing lineage, and the box at bottom shows the Euro-American lineage. Each circle represents ≥1 isolate. Larger circles represent ≥2 isolates with identical sequences; the number of specimens with identical sequences are indicated in parentheses. Details indicated with each circle are locality (initials; QLD residents shaded in pink), drug susceptibility (blue shading with blue outline), and drug resistance (multidrug resistance is red shading with blue outline, extreme drug resistance is red shading with green outline). Solid lines illustrate plausible transmission links among isolates from the same locality, and broken lines represent plausible transmission links among strains from different localities. Small nodes between lines represent unidentified ancestral isolates. Number on lines represents single-nucleotide polymorphism differences between isolates. BU, Buji; D, Daru; DI, Dimiri; KAD, Kadawa; KAT, Katatai; KIW, Kiwia; KUN, Kunini; KUR, Kurunti; M, Mabadauan; OM, Old Mawata; QLD, Queensland; S, Sigabadaru; T, Ture Ture; UN, unknown.

Phenotypic drug susceptibility testing of all isolates showed that 53 (51.0%) were susceptible to all oral first-line drugs tested (Appendix Table 4); 1 showed streptomycin monoresistance. Drug-resistance patterns observed included 13.5% (14/104) with isoniazid and streptomycin co-resistance, 31.7% (33/104) with MDR (1 pre-XDR with fluoroquinolone resistance), and 1.9% (2/104) with XDR. In 2010 and 2011, eleven TB cases were MDR or XDR (Appendix Figure 6). All MDR and XDR isolates, including the isolates from the 4 Australia citizens, belonged to clades C and D of Beijing sublineage 2.2.1.1.

Phenotypic and genotypic resistance profiles correlated, except for ethambutol (Figure 4). Of the 35 MDR and XDR isolates, which were also tested for second-line drug susceptibility, all carried the *fabG1-inhA* mutation (C15T), and 28 (80%) had high-level (0.4 μg/mL) isoniazid resistance. In total, 27 of these 28 had both *ndh* (ΔG304) and *inhA* (p.Ile21Val) mutations, and 1 had a *katG* (p.Trp191Arg) mutation. All the rifampin-resistant isolates had the same *rpoB* mutation (p.Ser450Leu), and 29 (82.9%) of 35 had compensatory mutations in the *rpoC* gene (27 p.Val483Gly and 2 p.Trp484Gly). Of note, nearly all isolates with compensatory *rpoC* mutations (28/29) and isolates with the *fabG1-inhA* mutation (48/49) were part of a genomic cluster. Of the 2 isolates with the p.Asp94Ala *gyrA* mutation conferring fluoroquinolone resistance, 1 was XDR; this isolate had pan–second-line injectable

resistance associated with an *rrs* (A1401G) mutation. The second XDR isolate (from a patient previously treated in Papua New Guinea) had a p.Asp89Asn *gyrA* mutation and phenotypic kanamycin resistance without a detected mutation conferring aminoglycoside resistance. Mutations in genes potentially conferring resistance to newly available agents were not identified.

Discussion

We characterized *M. tuberculosis* strain diversity, transmission dynamics, and drug-resistance profiles among cross-border isolates from TB patients residing in the TSPZ. This region is known for its sparse population, unique geography, and ethnic and social diversity, all of which can make disease surveillance difficult. The identification of drug-resistant isolates in every studied year and evidence of cross-border links underscore the risk for cross-border importation of drug-resistant TB with the potential to spread. Studies conducted in other parts of Australia suggest limited local TB transmission, despite a high proportion of imported TB cases (*20*,*21*), but those findings mainly reflected occurrences in urban areas. Residents of and visitors to the TSPZ might be vulnerable to community outbreaks; cultural and family relationships were associated with TB transmission in studies of community outbreaks within Australia among indigenous populations (*22*). We show that most transmission in the TSPZ occurred during local community outbreaks, as demonstrated by the high genomic clustering among patients from the same locality. With the economic growth anticipated in this region (*23*), interactions between Australia and Papua New Guinea populations could increase and affect disease notification trends.

The Beijing sublineage 2.2.1.1 dominated among isolates, and the Euro-American sublineage isolates were diverse. In a previous report, we attributed isolates from the same Beijing sublineage responsible for a large MDR TB outbreak on Daru Island, which is ≈50 km east of the outer islands of the TSPZ (*7*). The MDR TB outbreak on Daru Island was unprecedented in scale (*24*), but no published information described the extent of this outbreak beyond the shores of Daru Island.

In our study, most cases were identified to have come from Mabadauan, indicating that this setting could have a higher migratory rate than other TSPZ villages. The high number of drug-resistant TB cases from Mabadauan suggests this setting could be another hotspot for MDR TB transmission. Control of MDR TB transmission with effective clinical and public health–related interventions in this remote setting is urgently needed. Only 59% of Beijing lineage isolates from the TSPZ were part of clades previously identified on Daru Island (*7*), illustrating a greater genomic diversity for this lineage. This finding might be an indication of ongoing microevolution, which could further influence transmissibility, acquisition of drug resistance, or severity of disease (*25*).

Our study identified 2 plausible independent episodes of drug-resistant TB transmission to Australia residents in the TSPZ, a finding that would not have been identified by conventional genotyping techniques. Others

Table 1. Characteristics of Papua New Guinea residents infected with cross-border TB isolates, Torres Strait Protected Zone, 2010–2015, by lineage*

Characteristic	Total no. (%)	Lineage		p value†
		Beijing	Euro-American	
Age, y				0.739
<15	19 (19.0)	15	4	
15–24	31 (31.0)	26	5	
25–34	24 (24.0)	19	5	
35–49	18 (18.0)	13	5	
≥50	8 (8.0)	6	2	
Year				0.324
2010	29 (29.0)	22	7	
2011	42 (42.0)	33	9	
2012	13 (13.0)	12	1	
2013	2 (2.0)	1	1	
2014	8 (8.0)	5	3	
2015	6 (6.0)	6	0	
Sex				0.876
F	45 (45.0)	35	10	
M	55 (55.0)	44	11	
Sputum smear				0.599
Negative	46 (46.0)	34	12	
Positive	54 (54.0)	45	9	
HIV status				0.234
Unknown	26 (26.0)	20	6	
Negative	73 (73.0)	59	14	
Positive	1 (1.0)	0	1	
Previous TB episode				0.653
No	87 (87.0)	68	19	
Yes	13 (13.0)	11	2	
Diagnostic delay, d				0.484
0–30	53 (53.0)	44	9	
31–60	18 (18.0)	14	4	
≥61	29 (29.0)	21	8	
MDR or XDR				<0.001
No	69 (69.0)	48	21	
Yes	31 (31.0)	31	0	
Type of TB				0.741
Pulmonary	53 (53.0)	43	10	
Extrapulmonary	7 (7.0)	5	2	
Both	40 (40.0)	31	9	
Started TB treatment				0.472
No	22 (22.0)	15	7	
Yes	78 (78.0)	64	14	
TB treatment outcome				0.216
Transferred or lost to follow-up	66 (66.0)	52	14	
Cure or complete treatment	22 (22.0)	15	7	
Defaulted	3 (3.0)	3	0	
Died	9 (9.0)	9	0	

*MDR, multidrug resistant; TB, tuberculosis; XDR, extensively drug resistant.
†p value calculated by using Fisher exact test.

Table 2. Characteristics associated with *Mycobacterium tuberculosis* isolate clustering among Papua New Guinea residents, Torres Strait Protected Zone, 2010–2015*

Characteristic	No. clustered/total no.	OR (95% CI)	p value	aOR (95% CI)†	p value
Beijing lineage	74/100				
No	9/21	Referent		Referent	
Yes	65/79	6.190 (2.221–18.077)	0.0005	4.484 (1.526–13.891)	0.007
Age, y					
<15	14/19	Referent		Referent	
15–24	25/31	1.488 (0.369–5.841)	0.565	1.298 (0.291–5.614)	0.725
25–34	19/24	1.357 (0.320–5.783)	0.673	1.242 (0.260–5.981)	0.782
35–49	12/18	0.714 (0.166–2.955)	0.641	0.722 (0.148–3.415)	0.679
≥50	4/8	0.357 (0.059–2.016)	0.241	0.262 (0.035–1.785)	0.173
Sex					
F	34/45	Referent		Referent	
M	40/55	0.862 (0.343–2.118)	0.748	0.856 (0.311–2.284)	0.757
Sputum smear					
Negative	32/46	Referent		Referent	
Positive	42/54	1.531 (0.624–3.808)	0.352	1.147 (0.423–3.095)	0.784
Case type					
New case	63/87	Referent		Referent	
Recurrent	11/13	2.095 (0.513–14.183)	0.358	1.447 (0.270–11.282)	0.684
Diagnostic delay, d					
0–30	40/53	Referent		Referent	
31–59	13/18	0.845 (0.260–3.033)	0.784	1.042 (0.290–4.154)	0.95
≥60	21/29	0.853 (0.308–2.455)	0.761	1.157 (0.380–3.743)	0.799
MDR or XDR					
No	46/69	Referent		Referent	
Yes	28/31	4.677 (1.452–20.943)	0.019	2.774 (0.779–13.123)	0.143
Treatment outcome					
Cure, treatment completed	13/22	Referent		Referent	
Transferred, lost to follow-up	50/66	2.163 (0.767–6.006)	0.138	1.794 (0.584–5.407)	0.298
Defaulted	2/3	NA	NA	NA	NA
Died	9/9	NA	NA	NA	NA

*aOR, adjusted OR; MDR, multidrug resistant; NA, not applicable (large value); OR, odds ratio; XDR, extensively drug resistant.
†Adjusted for Beijing lineage and drug resistance (MDR or XDR).

have shown the superiority of WGS over conventional genotyping tools for resolving transmission (*11*,*26*). Two of the Australia patients with MDR TB were residing in major urban centers in northern Queensland at the time of diagnosis; 1 was living in a residential congregate setting, demonstrating the potential for diffuse community spread. WGS confirmed that the initial isolate noted in the index Australia patient was a lineage 4 isolate with only streptomycin resistance (*gidB*, Leu79Ser) (data not shown). This isolate was unrelated to the other Euro-American isolates identified and different from the subsequent MDR TB Beijing isolate identified, consistent with exogenous reinfection or endogenous reactivation rather than persistent infection with acquired resistance (*27*).

In another study conducted in Europe, WGS was used to investigate MDR TB outbreaks among immigrants and traced transmission routes to strains circulating in northern Somalia or Djibouti (*28*). Although no evidence of transmission to citizens of Europe was noted in that study, our investigation proves the principle of MDR TB cross-border transmission. Our findings highlight the challenge faced by Australia and Papua New Guinea TB control programs to prevent TB transmission through the TSPZ. Over the study period, the number of TB cases among citizens of Australia in the TSPZ was low (18 cases). Most TB notifications on the Australia island communities of the TSPZ were of diagnoses among Papua New Guinea citizens treated in outreach clinics located on the outer Torres Strait Islands close to the Papua New Guinea border. These clinics were closed in 2012, and all Papua New Guinea patients were handed over to the Western Province TB program for treatment, and enhanced TB services were coordinated from Daru Island. This closure and change in services accounts for the sharp fall in notifications in 2012 (Appendix Figure 6).

We observed transmission of a locally evolved Beijing sublineage strain that was associated with resistance to first- and second-line TB drugs. Beijing strains are known to be associated with increased transmissibility (*29*–*31*), drug resistance, treatment failure, and rapid progression to active disease (*32*,*33*).

Although the World Health Organization estimates a high TB incidence among persons living with HIV in Papua New Guinea (44 cases/100,000 population) (*1*), HIV incidence in our study was low and did not account for the highly successful transmission of Beijing sublineage 2.2.1.1 in this region. In another study in Gulf Province, Papua New Guinea, only 2 HIV-infected persons were identified among 105 TB patients (*34*).

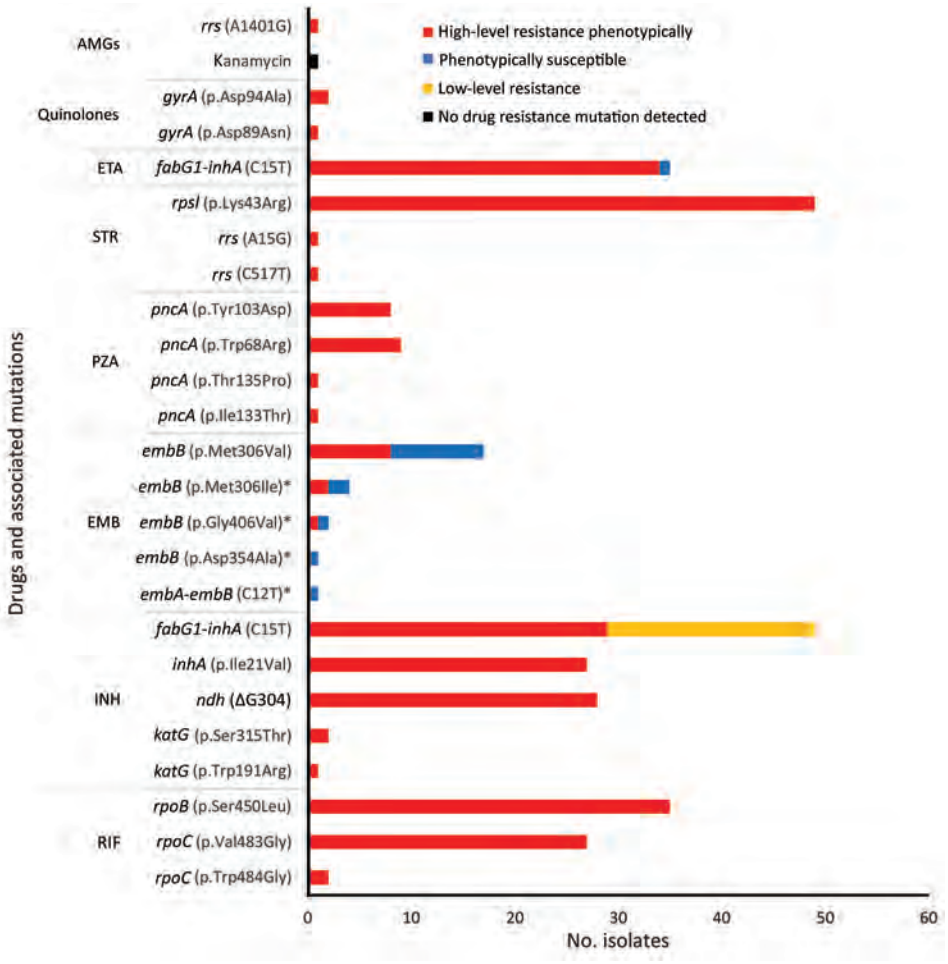

Figure 4. Correlation of phenotypic and genotypic drug resistance among modern Beijing isolates from Papua New Guinea and Australia citizens residing or previously residing in the Torres Strait Protected Zone, 2010–2015. No resistance mutation detected in 1 kanamycin-resistant strain, despite targeted sequencing of *rrs* and *eis* genes. *Pairs of co-occurring mutations observed within the same isolates were *embA-embB* (C12T) with *embB* (p.Asp354Ala) and *embB* (p.Gly406Val) with *embB* (p.Met306Ile). AMGs, aminoglycosides; EMB, ethambutol; ETA, ethionamide; INH, isoniazid; PZA, pyrazinamide; RIF, rifampin; STR, streptomycin.

The high proportion (74%) of TB cases among young adults (<35 years of age) in our study suggests ongoing TB transmission and is consistent with the finding of another study that identified 76.6% of pediatric TB notifications in Queensland were from Papua New Guinea residents in the TSPZ (*35*). Further assessment is needed of other risk factors, such as medical conditions (e.g., diabetes mellitus, high blood pressure) and socioeconomic status, that could affect TB transmission.

Excluding ethambutol, we observed a near-perfect correlation between resistance mutations and phenotypic in vitro resistance. One XDR TB isolate had resistance to kanamycin (mycobacterial growth indicator tube system critical concentration 2.5 μg/mL and a microbroth dilution MIC of 10 μg/mL), but no known resistance-conferring mutations were documented. Isolates with low-level kanamycin resistance are generally thought to display resistance to only kanamycin among the aminoglycoside drugs (*36*), but this isolate had a high level of kanamycin resistance. A previous study determined that only 80% of phenotypic kanamycin resistance could be accounted for by known mutations, indicating unexplained mechanisms of kanamycin resistance (*37*). Although the *fabG1-inhA* mutation is typically associated with low-level isoniazid resistance, we usually observed high-level isoniazid resistance with this mutation, possibly because of the accompanying *inhA* (p.Ile21Val) mutation (*38*). The *fabG1-inhA* (C15T) mutation does not seem to compromise successful spread and has previously been documented in clustered *M. tuberculosis* isolates in South Africa (*39*). One isolate had a *fabG1-inhA* (C15T) mutation with a rare *katG* mutation (p.Trp191Arg), which could affect the conformation of the catalase peroxidase protein, considering this mutation is located outside the active binding site of isoniazid (*40*).

Unexpectedly, we observed most rifampin-resistant isolates had mutations in *rpoC*. The fitness cost associated with some drug-resistant mutations can be ameliorated by compensatory mutations (*41*). Most isolates (96.5%) with *rpoC* compensatory mutations demonstrated genomic clustering, signifying their probable role in MDR or XDR TB transmission. In an assessment of MDR TB isolates in Argentina, no evidence was found to support the role of *rpoC* mutations in fitness restoration and increased transmission (*42*). Higher proportions of compensatory mutations

in MDR TB outbreaks were reported in Shanghai, China (66%) (*32*), and Samara, Russia (87%) (*11*). More studies are needed to assess the role of compensatory mutations in MDR and XDR strain transmission.

This study had a few limitations. The retrospective study design involved the selection of only 1 isolate per patient, and some of the stored isolates were unable to be studied because of failure to recover from culture. Close social contact between patients with genomically linked isolates could not be confirmed. In other studies, a refined breakdown of transmission patterns was shown with a similar approach that included data on social contacts (*28*,*32*,*43*). Although the current scope of the study comprised mostly Papua New Guinea residents, additional information is needed regarding the evolution and transmission dynamics among all residents in the TSPZ, including drug-susceptible isolates in Australia citizens residing in the TSPZ, to assess the implications of transmission to populations on the Australia mainland.

The combination of genomic and epidemiologic data in this study highlighted the wide distribution of a Beijing sublineage strain associated with MDR and XDR TB in the TSPZ that threatens regional TB control through cross-border transmission. Further investigation of the environmental, sociocultural, and clinical factors that facilitate TB transmission in this region is warranted. Supplementation of regional TB surveillance programs with WGS technology could improve control strategies.

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Emergence and Spread of Cephalosporin-Resistant *Neisseria gonorrhoeae* with Mosaic *penA* Alleles, South Korea, 2012–2017

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In South Korea, surveillance of antimicrobial drug resistance in *Neisseria gonorrhoeae* is extremely limited. We describe the emergence and subsequent national spread of *N. gonorrhoeae* strains with mosaic *penA* alleles associated with decreased susceptibility and resistance to extended-spectrum cephalosporins. From 2012 through 2017, the proportion of mosaic *penA* alleles in gonococcal-positive nucleic acid amplification test (NAAT) specimens across South Korea increased from 1.1% to 23.9%. Gonococcal strains with mosaic *penA* alleles emerged in the international hubs of Seoul in Gyeonggi Province and Busan in South Gyeongsang Province and subsequently spread across South Korea. Most common was mosaic *penA*-10.001 (n = 572 isolates; 94.7%), which is associated with cefixime resistance. We also identified mosaic *penA*-34.001 and *penA*-60.001, both of which are associated with multidrug-resistant gonococcal strains and spread of cefixime and ceftriaxone resistance. Implementation of molecular resistance prediction from *N. gonorrhoeae*–positive nucleic acid amplification test specimens is imperative in South Korea and internationally.

Neisseria gonorrhoeae remains a major public health threat globally (*1*). During the past 2 decades, *N. gonorrhoeae* strains with resistance to extended-spectrum cephalosporins (ESCs), including ceftriaxone, the last remaining option for empiric first-line gonorrhea treatment, have emerged and spread internationally, which is a serious concern worldwide (*2*–*23*). Enhanced global antimicrobial resistance (AMR) surveillance is crucial to controlling further spread of AMR strains. However, in many countries, culture-based AMR testing cannot be performed because most patients are treated empirically at primary-care facilities or hospitals where culture is unavailable and nucleic acid amplification tests (NAATs) have replaced culture for diagnosis. Consequently, molecular methods for detection of AMR determinants in *N. gonorrhoeae*–positive NAAT specimens are essential (*21*).

For the currently recommended ESCs, resistance is influenced by many different mutations in various genes that collaboratively increase the MICs of ESCs (*4*,*5*,*7*,*24*). Nevertheless, the main mechanism for ESC resistance is the acquisition of a mosaic *penA* allele encoding a mosaic version of the ESC lethal target, penicillin-binding protein 2 (*24*–*32*). Nearly all *N. gonorrhoeae* isolates with clinical or in vitro resistance to ESCs contain a mosaic *penA* allele. However, isolates with decreased susceptibility or susceptibility to ESCs also can contain a mosaic *penA* allele; that is, different mosaic *penA* alleles can affect the ESC MICs differently (*3*–*8*,*10*–*19*,*24*–*38*). Accordingly, a molecular assay detecting all types of mosaic *penA* alleles can predict ESC resistance with a high sensitivity but lower specificity (*24*–*31*). Currently, no molecular assays for detection of mosaic *penA* alleles and prediction of ESC resistance are commercially available.

In South Korea, ≈15,000 gonorrhea patients (≈29 patients/100,000 population) are reported to the Korean Health Insurance System (http://opendata.hira.or.kr) annually, but culture and AMR testing of *N. gonorrhoeae* are exceedingly limited (*2*,*3*). ESC resistance and ESC-resistant *N. gonorrhoeae* strains with the mosaic *penA*-10.001 allele were observed in 2011 in South Korea (*3*). Since the early 2000s, ESC-resistant *N. gonorrhoeae* strains containing the mosaic *penA*-10.001, associated with cefixime resistance, have been prevalent and caused cefixime treatment failures in Japan (*4*,*5*,*7*,*33*). In South

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Korea, molecular prediction of AMR directly from *N. gonorrhoeae*–positive NAAT samples is imperative for large-scale screening and prediction of the level and spread of ESC resistance in Korea.

We describe the emergence and subsequent national spread of *N. gonorrhoeae* strains with mosaic *penA* alleles in gonococcal-positive NAAT specimens across South Korea from 2012 through 2017. For molecular epidemiology, *penA* sequencing, *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), and multilocus sequence typing (MLST) were performed on all mosaic *penA*-positive specimens.

Materials and Methods

N. gonorrhoeae–Positive NAAT Specimens

We examined DNA extracts of NAAT specimens, mainly first-voided urine (55%) and vaginal swab specimens (27%), but also urethral and cervical swab specimens (18%), positive for *N. gonorrhoeae* (n = 3,884; 1 specimen per patient) by Seeplex NAATs (Seegene, http://www.seegene.com). These DNA extracts were obtained from commercial centralized accredited laboratories (2012–2017, Seegene Medical Foundation and SamKwang Medical Laboratories; 2016–2017, U2Bio; and 2017, Green Cross Medical Foundation) in Seoul, South Korea. The DNA extracts were stored at –70°C before further analysis.

All samples were collected and preserved as part of the routine diagnostics, and no patient identification data were available during the study. Therefore, ethics approval was not required.

Detection of Mosaic *penA* Alleles

We examined all DNA extracts for mosaic *penA* alleles in 2 steps: 1) screening of all types of mosaic *penA* alleles (*7*,*34*); and 2) detection of the specific mosaic *penA* alleles that caused high-level resistance to ceftriaxone in the strains H041 (*penA*-37.001; *7*) and F89 (*penA*-42.001; *6*). For screening of all types of mosaic *penA* alleles, we used a previously described TaqMan probe–based real-time PCR method on a Rotor-Gene 6000 (QIAGEN, https://www.qiagen.com) (*34*). We then tested all DNA extracts positive for a mosaic *penA* allele by using a real-time PCR specific for *penA*-37.001 (*35*) and a modified hybridization probe-based real-time PCR detecting *penA*-42.001 (*36*).

Sequencing of Mosaic *penA* Alleles

We sequenced the entire *penA* gene in all DNA specimens positive for a mosaic *penA* allele, as previously described (*3*,*37*). Among 604 mosaic *penA*-positive specimens, we were able to sequence 601 (99.5%) specimens. Three (0.5%) specimens failed to sequence due to low DNA content. The mosaic *penA* alleles were named by using the *Neisseria gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR; *38*) database (https://ngstar.canada.ca/welcome/home).

Molecular Epidemiologic Characterization

We performed NG-MAST (*39*) and MLST on all mosaic *penA*-positive specimens using the methods described on the NG-MAST (http://www.ng-mast.net) and MLST (http://pubmlst.org/neisseria) websites. We successfully typed 594 (98.3%) of the mosaic *penA*-positive specimens with NG-MAST and 593 (98.2%) specimens with MLST.

Antimicrobial Agents Used for Gonorrhea Treatment

We acquired prescription data for patients treated for gonorrhea from 2010 through 2017 in South Korea from the Korea Health Insurance Review & Assessment Service (http://opendata.hira.or.kr). In South Korea, all clinics and hospitals participate in the national health insurance system, and reporting of prescription data and diagnosis to the Korea Health Insurance Review & Assessment Service HIRA is mandatory. We analyzed the percentage of diagnosed gonococcal infections in patients who did not have additional sexually transmitted infections and who were treated with each specific antimicrobial drug.

Statistical Analysis

We used the Student *t*-test and a test of proportions for statistical analysis (Statistica 12.0 PL software, https://www.tibco.com). The level of significance was set at $\alpha = 0.05$.

Results

N. gonorrhoeae–Positive NAAT Specimens and Corresponding Patients

The collection of DNA extracts we examined consisted of 3,884 *N. gonorrhoeae*–positive NAAT specimens collected across South Korea from 2012 through 2017 (Table 1). The number of specimens per year varied from 428 in 2012 to 901 in 2017 (Table 1). Data on the sex and age of patients were available for 3,422 (88.1%) of specimens. The ratio of men to women was 1:0.37, and 71.1% of the specimens were collected from patients in their 20s (43.9%) and 30s (27.2%).

Molecular Typing of Gonococcal Mosaic *penA*-Positive Specimens

The proportion of mosaic *penA* alleles in the specimens increased annually, from 1.2% (5/428) in 2012 to 23.9% (215/901) in 2017 (Table 2). The mosaic *penA*-10.001, previously associated with resistance to ESCs, particularly cefixime and other oral ESCs (*4*,*5*,*33*), was the most common mosaic *penA* allele (n = 572; 94.7% of all mosaic

Table 1. Number of *Neisseria gonorrhoeae*–positive nucleic acid amplification test specimens examined, by year and location, South Korea, 2012–2017

Location	No. (%) tested specimens						
	2012	2013	2014	2015	2016	2017	Total
Seoul metropolitan area	192 (44.9)	171 (32.6)	185 (30.6)	141 (23.3)	338 (41.2)	252 (28.0)	1,279 (32.9)
Gyeonggi area, including Incheon	71 (16.6)	96 (18.3)	100 (16.6)	156 (25.7)	191 (23.3)	157 (17.4)	771 (19.9)
Gangwon	4 (0.9)	12 (2.3)	7 (1.2)	19 (3.1)	16 (1.9)	14 (1.6)	72 (1.9)
North Chungcheong	6 (1.4)	1 (0.2)	16 (2.6)	19 (3.1)	25 (3.0)	73 (8.1)	140 (3.6)
South Chungcheong, including Daejeon	22 (5.1)	21 (4.0)	69 (11.4)	62 (10.2)	53 (6.5)	103 (11.4)	330 (8.5)
North Jeolla	2 (0.5)	2 (0.4)	13 (2.2)	12 (2.0)	24 (2.9)	42 (4.7)	95 (2.4)
South Jeolla, including Gwanju	17 (4.0)	6 (1.1)	39 (6.5)	43 (7.1)	42 (5.1)	61 (6.8)	208 (5.4)
North Gyeongsang, including Daegu	49 (11.4)	93 (17.7)	78 (12.9)	93 (15.3)	83 (10.1)	103 (11.4)	499 (12.8)
South Gyeongsang, including Busan and Ulsan	65 (15.2)	121 (23.1)	97 (16.1)	61 (10.1)	46 (5.6)	93 (10.3)	483 (12.4)
Jeju	0	1 (0.2)	0	0	3 (0.4)	3 (0.3)	7 (0.2)
Total	428 (100)	524 (100)	604 (100)	606 (100)	821 (100)	901 (100)	3,884 (100)

penA-positive specimens). The annual proportion of mosaic *penA*-10.001 among the mosaic *penA*-positive specimens varied from 60% to 100% (Table 2). Most (76.9%) mosaic *penA*-10.001–positive samples belonged to MLST ST1901, but the proportion of sequence type (ST) 1901 among the mosaic *penA*-10.001 specimens decreased from 100% (5/5) in 2012 to 68% (143/210) in 2017. In contrast, the proportion of other MLST STs, including ST1588 and ST7363, increased. In 2012 and 2013, NG-MAST ST2958 was the most prevalent ST, but the genetically closely related NG-MAST ST10668 was most common in 2014 and 2015. In 2016 and 2017, NG-MAST STs diversified, and ST15014 became the most common.

The mosaic *penA*-34.001 was found in 1–3 samples annually between 2013 and 2017, and the mosaic *penA*-72.001 (*penA*-34.001+P551S) was identified in 10 samples between 2013 and 2016. These mosaic *penA* alleles also have been previously associated with ESC resistance internationally (*4*,*5*,*31*,*40*). Of particular concern, mosaic *penA*-60.001, which has been found in internationally spreading ceftriaxone-resistant gonococcal strains since 2015 (*12*,*14*–*16*,*18*,*19*), was identified in a sample in 2017. This sample belonged to MLST ST1903 and NG-MAST ST16327 and was collected from a man in Seoul (Table 2).

Distribution of Mosaic *penA* Alleles in Different Provinces

We summarized the annual proportions of mosaic *penA* alleles in the 10 different provinces of South Korea from 2012 through 2017 (Figure 1). In 2012, only 5 mosaic *penA*-positive specimens were identified: 1 each in the capital city Seoul (0.5% of the total number of isolates collected in this location), Gyeonggi Province surrounding Seoul (1.4%), and North Gyeongsang Province (2.0%) and 2 (3.1%) in South Gyeongsang Province, which includes Busan, the second largest city in South Korea. In 2013, the proportions of mosaic *penA*-positive specimens in these 4 provinces increased to 4.1%–7.4%, but no mosaic *penA*-positive specimens were detected in any of the other provinces. However, in 2014, while the proportion of mosaic *penA*-positive specimens further increased in these 4 provinces (to 6.4%–16.5%), mosaic *penA*-positive specimens also emerged in 4 of the 6 additional provinces (proportions of 7.2%–15.4%). Mosaic *penA*-positive specimens were widely spread in all provinces of South Korea, except Jeju Island, in 2015 (9.7%–34.5%) and 2016 (3.8%–32.0%). In 2017, the proportion of mosaic *penA*-positive specimens was >20% in all provinces, except South Jeolla Province (13.1%), and mosaic *penA*-positive specimens also were found on Jeju Island (33.3%) (Figure 1).

Antimicrobial Drugs Used for Gonorrhea Treatment

In 2010, 13.1% of gonorrhea patients were treated with ceftriaxone, and 17.4% were treated with any ESC, but these proportions increased to 30.5% treated with ceftriaxone and 38.8% treated with ESCs in 2017 (Figure 2). With the exception of ceftriaxone, cefixime is the most widely used ESC; the proportion of patients treated with cefixime increased from 2.9% in 2010 to 6.6% in 2017. In 2010, 32.8% of patients were treated with spectinomycin and 49.6% with fluoroquinolone, but these proportions decreased in 2017 to 16.0% treated with spectinomycin and 26.7% treated with fluoroquinolone (Table 3).

Discussion

The emergence of multidrug-resistant *N. gonorrhoeae* is compromising the treatment of gonorrhea globally. In most countries, only ESCs, azithromycin combined with ESCs, and spectinomycin remain recommended for the empiric treatment of gonorrhea (*3*–*5*,*8*–*10*,*20*–*23*,*31*,*32*,*41*–*44*).

Among the ESCs, oral cefixime and the more potent injectable ceftriaxone have been recommended for the treatment of gonorrhea internationally (*4*,*5*,*10*,*41*–*44*). However, resistance to cefixime emerged in Japan in the early 2000s, and cefixime resistance subsequently has been reported in many countries globally. Since 2010, resistance

Table 2. Molecular typing of specimens positive for a *Neisseria gonorrhoeae* mosaic *penA* allele by year, South Korea, 2012–2017*

Year	No. (%) specimens with mosaic *penA*	Mosaic *penA* allele (no. specimens)	MLST ST (no. specimens)	NG-MAST ST (no. specimens)
2012	5 (1.2)	10.001 (5)	1901 (5)	2958 (5)
2013	27 (5.2)	10.001 (17)	1901 (21)	2958 (8), 10668 (6), 5682 (1), 6783 (1), 10669 (1)
		72.001 (4)		1407 (3), 436 (1)
		10.001 (1)	7363 (1)	5308 (1)
		10.001 (3)	ND (5)	ND (3)
		34.001 (2)		10670 (1), ND (1)
2014	60 (9.9)	10.001 (1)	1588 (1)	6696 (1)
		10.001 (54)	1901 (56)	10668 (33), 2958 (15), 11495 (2), 11493 (1), 11494 (1), 11496 (1), 11497 (1)
		34.001 (1)		5622 (1)
		72.001 (1)		1407 (1)
		10.001 (3)	ND (3)	ND (3)
2015	109 (18.0)	10.001 (2)	1579 (2)	13376 (2)
		10.001 (7)	1588 (7)	12399 (3), 3611 (2), 6696 (1), 10679 (1)
		10.001 (82)	1901 (82)	10668 (38), 2958 (20), 6734 (7), 11495 (4), 1407 (3), 6481 (3), 4502 (2), 6373 (2), 11497 (1), 13374 (1), 13388 (1), 13391 (1)
		10.001 (1)	1922 (1)	13393 (1)
		10.001 (10)	7363 (10)	5308 (8), 11562 (1), 13389 (1)
		10.001 (2)	7371 (2)	13390 (1), 13394 (1)
		34.001 (2)	7827 (2)	3702 (2)
		10.001 (2)	8137 (2)	13373 (1), 13392 (1)
		10.001 (1)	10931 (1)	12402 (1)
2016	185 (22.5)	10.001 (11)	1588 (11)	15024 (7), 15026 (3), 15086 (1)
		10.001 (133)	1901 (148)	15014 (39), 15016 (31), 15015 (28), 7307 (8), 15025 (3), 15041 (3), 15029 (2), 15045 (2), 11497 (1), 15013 (1), 15017 (1), 15018 (1), 15019 (1), 15020 (1), 15021 (1), 15022 (1), 15023 (1), 15027 (1), 15031 (1), 15033 (1), 15034 (1), 15038 (1), 15042 (1), 15044 (1), 15048 (1)
		27.001 (2)		15015 (2)
		34.001 (3)		4431 (1), 15032 (1), 15039 (1)
		72.001 (10)		15018 (7), 4431 (1), 15016 (1), 15030 (1)
		10.001 (26)	7363 (26)	6910 (13), 15028 (4), 15037 (2), 15046 (2), 15012 (1), 15040 (1), 15043 (1), 15049 (1), 15050 (1)
2017	215 (23.9)	10.001 (40)	1588 (40)	15024 (24), 5576 (4), 3611 (3), 15025 (3), 15026 (2), 6696 (1), 7684 (1), 14668 (1), 16331 (1)
		10.001 (143)	1901 (145)	15014 (33), 15016 (25), 7307 (17), 15015 (16), 5446 (10), 15032 (3), 16324 (3), 16341 (3), 6734 (2), 15045 (2), 16270 (2), 16325 (2), 16335 (2), 12402 (1), 13973 (1), 15018 (1), 15019 (1), 15028 (1), 15029 (1), 16319 (1), 16320 (1), 16321 (1), 16323 (1), 16328 (1), 16329 (1), 16332 (1), 16333 (1), 16334 (1), 16336 (1), 16338 (1), 16342 (1), 16343 (1), 16344 (1), 16346 (1)
		34.001 (2)		15032 (2)
		10.001 (1)	1902 (1)	16339 (1)
		60.001 (1)	1903 (1)	16327 (1)
		10.001 (24)	7363 (25)	6910 (13), 16322 (3), 13044 (1), 13876 (1), 15037 (1), 15046 (1), 16318 (1), 16337 (1), 16340 (1)
		34.001 (1)		11624 (1)
		10.001 (1)	7371 (1)	16330 (1)
		10.001 (1)	10899 (1)	11895 (1)
		10.001 (1)	11179 (1)	16345 (1)

*MLST, multilocus sequence typing; ND, not determined (due to insufficient amount of DNA); NG-MAST, *N. gonorrhoeae* multiantigen sequence typing; ST, sequence type.

to ceftriaxone, the last remaining option for empiric first-line monotherapy, also has been described in many countries (*3*–*19*,*21*,*32*). The mechanisms of resistance to these ESCs are complex and involve different mutations in the *penA* gene, in the promoter or coding region of *mtrR*, and in the *porB1b* gene. However, the main ESC-resistance determinant is evidently the acquisition of a mosaic *penA* allele. The mosaic *penA*-10.001 was shown to be strongly associated with cefixime resistance in gonococcal isolates in Japan in the early 2000s (*3*–*5*,*7*,*33*), which resulted in the exclusion of cefixime from the Japanese treatment guidelines in 2006 (*43*). In South Korea, 1 cefixime-resistant isolate cultured in 2004 (*2*), 1 in 2011, and 3 in 2013 previously were reported to contain mosaic *penA*-10.001

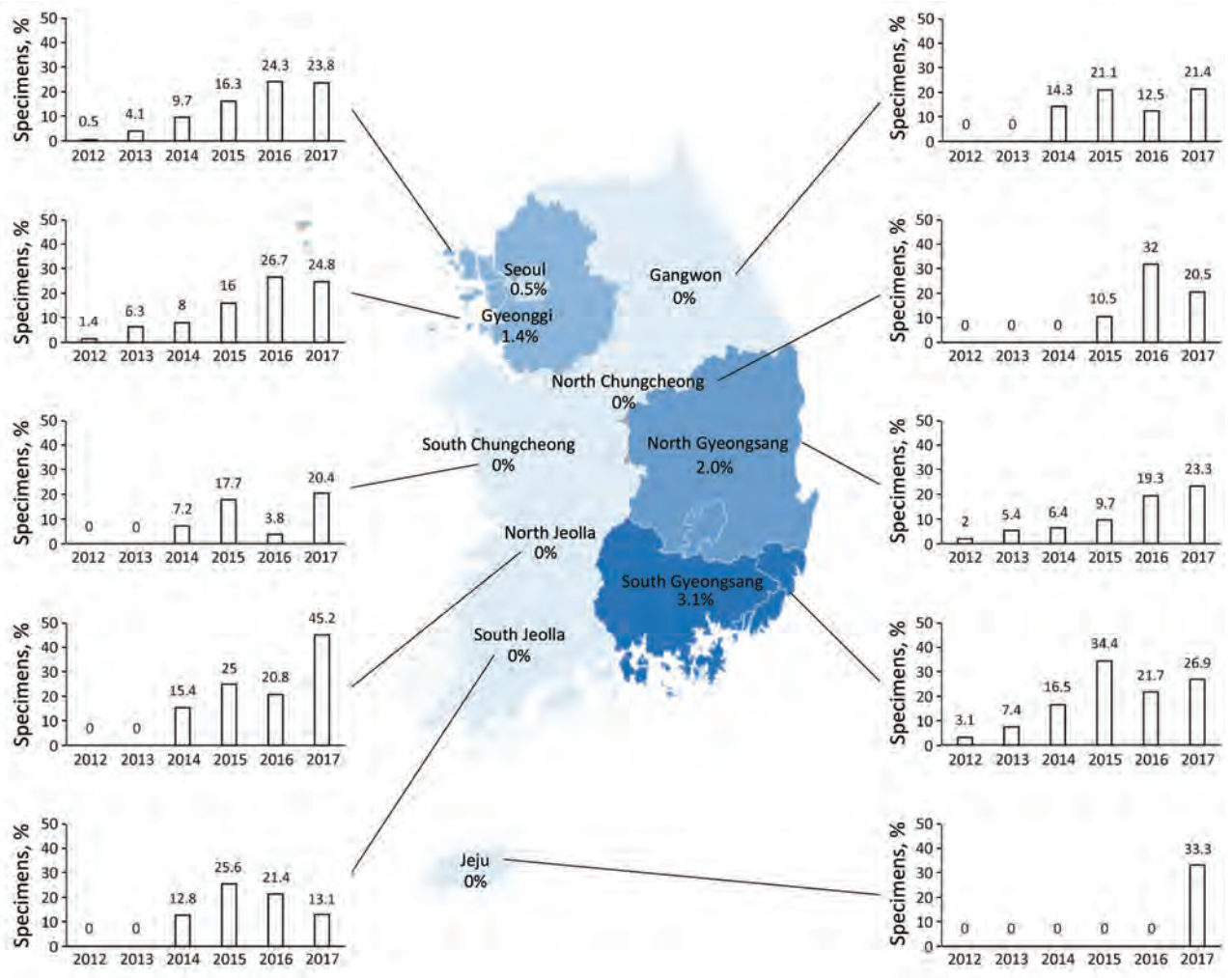

Figure 1. Distribution of specimens positive for a *Neisseria gonorrhoeae* mosaic *penA* allele, by year and province, South Korea, 2010–2017. Numbers shown in each province denote the proportion of samples positive for a mosaic *penA* allele in 2012. The bar graphs describe the percentage of specimens positive for a *N. gonorrhoeae* mosaic *penA* allele in each province and year. Seoul is the capital city of South Korea, and Gyeonggi Province contains an international airport.

(*3*). In our study, 1.1% (n = 5) of *N. gonorrhoeae*–positive NAAT specimens contained a mosaic *penA* allele in 2012. This proportion increased significantly in 2017 to 23.9% (n = 215; p<0.05). The proportion of mosaic *penA*-positive specimens (n = 209) containing mosaic *penA*-10.001 was 100% (n = 5) in 2012 and >90% during all the following years, except 2013 (77.8%; 21/27).

Twenty-five (96.2%) of the NAAT specimens containing mosaic *penA*-10.001 in 2012 and 2013 were collected from the capital region of Seoul and surrounding Gyeonggi Province, as well as the Gyeongsang area (North and South). Seoul (population 8.8 million; 19.8% of the population of South Korea) and the Gyeonggi area (29.6% of the population) share 2 large international airports and an international port, which have pivotal roles in trading with foreign countries, including China and Japan. The South Gyeongsang area (15.6% of the population) includes Ulsan, Gyeonsangnam-do, and the second largest city in South Korea, Busan, which comprises the main port in northeast Asia, with close and frequent trading routes to Japan. In 2012 and 2013, the proportions of mosaic *penA*-10.001 were 2.2% in the capital area and 4.1% in the Gyeongsang area, whereas the proportion was only 0.8% in the remaining provinces. This indicates that *N. gonorrhoeae* strains with mosaic *penA*-10.001, associated with decreased susceptibility and resistance to ESCs, initially emerged or were imported to at least 2 areas (Seoul and Gyeongsang) of South Korea, possibly from Japan, where these strains have been prevalent for many years (*4*,*5*,*7*,*33*). From 2014, the proportion of *N. gonorrhoeae* mosaic *penA*-10.001 in the other provinces significantly increased (from 9.1% in 2014 to 20.9% in 2017; p<0.05);

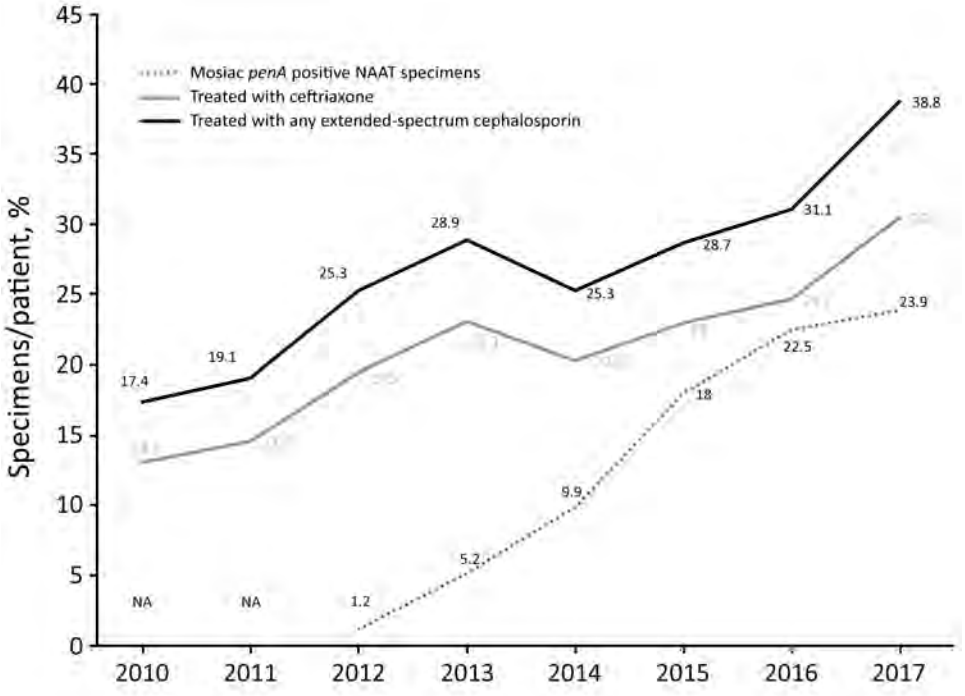

Figure 2. Percentages of *Neisseria gonorrhoeae* mosaic *penA* in NAAT specimens and gonorrhea patients treated with ceftriaxone or any extended-spectrum cephalosporin, South Korea, 2010–2017. Data for antimicrobial drug use were acquired from the Korea Health Insurance Review and Assessment Service in South Korea. In 2010 and 2011, no NAAT samples were available for screening of mosaic *penA* alleles. NA, not assessed; NAAT, nucleic acid amplification test.

accordingly, *N. gonorrhoeae* strains with mosaic *penA*-10.001 spread and diversified, based on NG-MAST STs, nationally in South Korea.

Gonococcal resistance to cefixime and other oral ESCs is common in South Korea, according to a smaller study (*3*), and the prevalent mosaic *penA*-10.001 we report causes decreased susceptibility and frequently resistance to cefixime and other oral ESCs (*4*,*5*,*7*,*33*). With these facts in mind, oral ESCs should be abandoned from the treatment of gonorrhea in South Korea. Gonococcal strains with mosaic *penA*-10.001 also can cause ceftriaxone treatment failure (*17*). However, mosaic *penA*-10.001 affects the MICs of ceftriaxone less than those of cefixime in general. Thus, ceftriaxone, combined with azithromycin, should be the first-line empiric treatment in South Korea. However, *N. gonorrhoeae* specimens containing mosaic *penA*-34.001 (n = 11) and *penA*-72.001 (n = 15) also were detected. These mosaic *penA* alleles have been associated with ESC resistance globally (*4*,*5*,*31*,*40*) and require only a single additional penicillin binding protein 2 amino acid alteration (A501P) to develop high-level ceftriaxone resistance (*6*,*45*). Of most concern, mosaic *penA*-60.001 was found in a sample collected in Seoul in 2017 and this mosaic *penA* allele has been found in internationally spreading ceftriaxone-resistant gonococcal strains in Japan, Australia, Canada, Denmark, France, the United Kingdom, and Ireland since 2015 (*12*,*14*–*16*,*18*,*19*,*46*).

The detailed reasons for the emergence and subsequent national spread of gonococcal strains with mosaic *penA*-10.001 in South Korea are not clear. One possibility is importation of cefixime-resistant gonococcal strains containing the mosaic *penA*-10.001 from Japan, as previously mentioned. However, increased therapeutic use of ESCs, particularly oral ESCs, in South Korea cannot be excluded as contributing to the increased spread of ESC-resistant gonococcal strains. In South Korea, spectinomycin, fluoroquinolones, and ESCs have been used to treat gonorrhea since the early 2000s (*3*). In 2002, most patients were treated with spectinomycin; only 8.4% of all patients were treated with ceftriaxone, and oral ESCs were rarely prescribed. However, 38.8% of patients were treated with ESCs (30.5% with ceftriaxone) in 2017. Despite the high use of spectinomycin in South Korea for decades, susceptibility to spectinomycin in *N. gonorrhoeae* has remained high. No spectinomycin-resistant gonococcal strain has been reported since 1993. Nevertheless, spectinomycin has a low eradication rate for pharyngeal gonorrhea (*47*) and, if pharyngeal infection has not been excluded, should be used only in dual antimicrobial therapy (e.g., in combination with azithromycin). In the 2016 South Korea guideline, dual antimicrobial therapy (ceftriaxone 500 mg or 1 g plus azithromycin 1 g) is the recommended first-line empiric therapy for uncomplicated gonorrhea (*48*).

The limitations of our study include the unavailability of gonococcal isolates, MIC data, rectal or pharyngeal specimens (which are not covered by the national insurance system in South Korea), and clinical or epidemiologic information regarding patients or treatment outcome. Accordingly, phenotypic ESC resistance was not measured; instead molecular detection of mosaic *penA* alleles was used to reflect the ESC resistance. Nearly all gonococcal strains with ESC

Table 3. Percentage of patients treated with each antimicrobial drug used against gonorrhea in South Korea, 2010–2017

Year	% Patients					
	Fluoroquinolones*	Spectinomycin	Ceftriaxone	Cefixime	Other cephalosporin	Penicillin G
2010	49.6	32.8	13.1	2.9	1.4	0.2
2011	51.4	29.2	14.6	3.7	0.8	0.2
2012	52.3	22.2	19.5	4.6	1.2	0.2
2013	47.0	23.9	23.1	4.6	1.3	0.2
2014	38.8	22.0	20.3	3.9	1.1	0.1
2015	39.3	17.7	23.0	4.5	1.1	0.1
2016	36.7	17.3	24.7	5.0	1.4	0.1
2017	26.7	16.0	30.5	6.6	1.7	0.1

*Mainly ciprofloxacin.

resistance contain a mosaic *penA* allele; however, isolates with decreased susceptibility or susceptibility to ESCs also can contain a mosaic *penA* allele, and different mosaic *penA* alleles affect the ESC MICs differently (*3*–*8*,*10*–*19*,*24*–*38*). Even so, the mosaic *penA*-10.001 in 94.7% of our mosaic *penA* allele-positive specimens is relatively strongly associated with resistance to cefixime particularly and other oral ESCs, although with a weaker association with ceftriaxone resistance. In addition, our molecular approach to identifying decreased susceptibility and resistance to ESCs can overestimate the ESC resistance. The mosaic *penA* allele PCR assay (*34*) did not include any internal control for gonococcal DNA. However, the assay has been previously evaluated on gonococcal strains and detects 1–10 copies of a mosaic *penA* per PCR reaction (*34*) on urogenital and extragenital gonococcal-positive and gonococcal-negative NAAT samples (*49*,*50*). Finally, the distribution of specimens could not be allocated by population in an ideal way and the suboptimal number of samples, especially from the Gyeonggi area, Jeju, Gangwon, South Gyeongsang, South Jeolla, and North Jeolla provinces in some years, might have underestimated the prevalence of *N. gonorrhoeae* specimens with mosaic *penA* allele in these provinces.

In conclusion, we describe the initial emergence and subsequent national spread of *N. gonorrhoeae* strains with mosaic *penA* alleles (*penA*-10.001 in 94.7% of specimens), associated with decreased susceptibility and resistance to ESCs, in NAAT specimens collected across South Korea from 2012 through 2017. The proportion of *penA*-10.001, frequently causing resistance to cefixime and other oral ESCs, increased during these years. Only a few additional mutations in *penA*-10.001 are required for development of high-level resistance to ceftriaxone. Furthermore, we identified mosaic *penA*-34.001 and *penA*-60.001, both associated with multidrug-resistant gonorrhea and international spread of cefixime and ceftriaxone resistance (*4*,*5*,*12*,*14*–*16*,*18*,*19*,*31*,*40*). The mosaic *penA*-60.001 has been found in internationally spreading ceftriaxone-resistant gonococcal strains in Japan, Australia, Canada, Denmark, the United Kingdom, France, and Ireland since 2015 (*12*,*14*–*16*,*18*,*19*,*46*). Nevertheless, *N. gonorrhoeae* strains with ESC resistance due to nonmosaic *penA* alleles, such as *penA*-13.001, also are spreading in South Korea (*3*). Consequently, it is essential to establish a systematic, regular, and quality-assured phenotypic AMR surveillance system for *N. gonorrhoeae* in South Korea. Implementing the use of molecular methods for prediction of AMR or antimicrobial susceptibility is also crucial. These molecular methods will effectively support phenotypic AMR surveillance and enable large-scale screening of *N. gonorrhoeae*–positive NAAT specimens, which represent most of *N. gonorrhoeae* diagnostic specimens in many countries internationally.

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H.L., M.U., and K.L. designed and initiated the study; S.L., Y.-K.K., M.-S.H. and H.G.B. provided NAAT specimens; H.L., S.L., Y.-K.K., and Y.H.S. coordinated and performed all the laboratory analyses; H.L., K.L., and M.U. analyzed and interpreted all the data and wrote a first draft of the paper; and all authors read, commented on, and approved the final manuscript.

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Donor-Derived Genotype 4 Hepatitis E Virus Infection, Hong Kong, China, 2018

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Hepatitis E virus (HEV) genotype 4 (HEV-4) is an emerging cause of acute hepatitis in China. Less is known about the clinical characteristics and natural history of HEV-4 than HEV genotype 3 infections in immunocompromised patients. We report transmission of HEV-4 from a deceased organ donor to 5 transplant recipients. The donor had been viremic but HEV IgM and IgG seronegative, and liver function test results were within reference ranges. After a mean of 52 days after transplantation, hepatitis developed in all 5 recipients; in the liver graft recipient, disease was severe and with progressive portal hypertension. Despite reduced immunosuppression, all HEV-4 infections progressed to persistent hepatitis. Four patients received ribavirin and showed evidence of response after 2 months. This study highlights the role of organ donation in HEV transmission, provides additional data on the natural history of HEV-4 infection, and points out differences between genotype 3 and 4 infections in immunocompromised patients.

Hepatitis E virus (HEV; genus *Orthohepevirus*) is a major cause of hepatitis globally (*1*). In immunocompetent persons, it typically causes self-limited acute hepatitis. In immunocompromised persons, such as transplant recipients, HEV infection can persist, causing chronic hepatitis and cirrhosis (*2*). HEV-A, the main HEV virus of relevance to human health, is classified into 8 genotypes, of which 5 can infect humans (*3*). Persistent hepatitis E is most commonly reported for patients infected with HEV genotype 3 (HEV-3), the prevalent genotype infecting humans in Europe and the Americas (*4*–*8*). In many parts of China, HEV genotype 4 (HEV-4) has rapidly emerged as the most common genotype causing acute hepatitis (*9*,*10*). We recently demonstrated that, similar to HEV-3, HEV-4 can cause persistent infections in transplant recipients (*11*). However, data on the natural history and ribavirin responsiveness of HEV-4 infections in immunocompromised patients are limited.

HEV-3 and HEV-4 are predominantly transmitted by food, specifically undercooked pork products. Transmission through blood product transfusion has also been well documented (*12*,*13*), prompting many countries to consider screening blood products for HEV (*14*). Less frequently reported is HEV transmission via transplanted organs (*15*,*16*).

In August 2018, chronic HEV-4 infection developed in 2 transplant recipients who had received organs from a common donor. In response, we conducted an investigation to 1) identify other infected recipients from the same donor, 2) confirm that the infection was indeed donor derived, 3) investigate the natural history of HEV-4 infection, and 4) study outcomes of infected patients who received ribavirin.

Materials and Methods

Study Setting, Patients, and Samples

In Hong Kong, Queen Mary Hospital is the liver and heart–lung transplantation center, Princess Margaret Hospital is a kidney transplant center, and Tuen Mun Hospital offers specialist follow-up services for kidney transplant recipients. HEV diagnostic testing for this study was performed at the University of Hong Kong Department of Microbiology, based at Queen Mary Hospital. We retrieved patient identifiers for the organ donor and recipients from the organ donor registry and retrieved clinical details for donor and recipients from the electronic patient record. Archived serum samples from the donor and all recipients were retrieved and subjected to HEV quantitative real-time reverse transcription PCR (qRT-PCR) and serologic testing. Ethics

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approval for this study was obtained from the institutional review board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

HEV Diagnostic Testing, Sequencing, and Phylogenetic Analysis

For serologic testing, we used HEV IgM and HEV IgG ELISA kits (Wantai, http://www.ystwt.cn/HEV.html). In addition, we tested donor and recipient serum samples by using IgG and IgM Western blots as previously described (*17*). HEV qRT-PCR was performed as previously described (*18*). Protocols for the Western blot, HEV qRT-PCR, sequencing, and phylogenetic analysis are described in the Appendix (https://wwwnc.cdc.gov/EID/article/25/3/18-1563-App1.pdf).

Liver Histology and Immunohistochemistry

Liver tissue sections from the organ donor and liver graft recipient were available for histologic analysis with hematoxylin and eosin staining. We performed immunohistochemical staining with monoclonal antibody against HEV open reading frame 2 (kindly provided by N.S. Xia, Xiamen University, Xiamen, China) on tissue sections, as previously described (*19*).

Case Definitions

Patients were defined as having hepatitis E if HEV qRT-PCR detected HEV RNA in their plasma. Patients were defined as having probable donor-derived hepatitis E if they had received organ transplant from the viremic donor and subsequently hepatitis E developed. To prove that the infections were definitely donor derived, we phylogenetically compared recipient HEV sequences with the original donor sequence. Persistent HEV infection was defined as detection of HEV RNA in plasma for >3 months (*20*). If archived samples were unavailable, persistent hepatitis for 3 months before the first positive HEV RNA result was also defined as persistent HEV infection.

Results

The Outbreak

On posttransplantation day 127, detection of HEV RNA in plasma from a liver transplant recipient at Queen Mary Hospital led to a diagnosis of hepatitis E, at the time attributed to autochthonous foodborne acquisition. On posttransplantation day 162, HEV RNA was detected in plasma from a lung transplant recipient at Queen Mary Hospital. Both patients had undergone transplantation at Queen Mary Hospital on February 28, 2018, and had received organs from the same deceased donor. Archived donor serum was tested by HEV qRT-PCR and found to be positive. Because both kidneys and the heart had also been harvested from this donor, samples were collected from the 3 corresponding recipients, and HEV RNA was detected in the plasma of all 3. Phylogenetic analysis confirmed that HEV partial RNA-dependent RNA polymerase sequences from the donor and 5 organ recipients were identical and belonged to genotype 4b (Figure 1). All 5 recipients fulfilled criteria for persistent HEV infection according to the case definition used in this study. Except for the lung transplant recipient, who died before hepatitis E infection could be confirmed, all patients were counseled about the diagnosis and prescribed ribavirin. When possible, their immunosuppression was reduced. For each patient, plasma and serum samples archived before and after transplantation were retrieved for HEV qRT-PCR and serology testing. The timeline of the outbreak is depicted in Figure 2, and individual case details are summarized in Table 1.

Organ Donor

The organ donor was a previously healthy 29-year-old woman admitted to Princess Margaret Hospital for acute subarachnoid hemorrhage that led to brainstem death. Alanine aminotransferase (ALT) level was 18 U/L (reference range 8–58 U/L), aspartate aminotransferase (AST) 22 U/L (reference range 15–38 U/L), and bilirubin 16 μmol/L (reference range 4–23 μmol/L). On hospitalization day 4 (February 28, 2018), her heart, liver, lungs, and kidneys were harvested and transplanted to 5 recipients. After hepatitis E in the organ recipients was recognized, HEV qRT-PCR was performed on archived donor serum collected on the day of organ harvest; viral load was 2.87×10^5 copies/mL. This serum sample was negative for HEV IgM and IgG by both ELISAs and the Western blot (Appendix Figure 1). HEV RNA was detected in liver graft tissue; viral load was 2.88×10^2 copies/reaction. Histology of liver graft tissue showed minimal inflammation at portal tracts, and immunohistochemical staining that used HEV monoclonal antibodies did not show positive signals (Appendix Figure 2, panels A, B).

Case-Patient 1 (Liver Graft Recipient)

The index case-patient was a 66-year-old man with a history of liver cirrhosis caused by hepatitis C virus infection that had been cured with direct-acting antiviral medications. During his liver transplantation, he received basiliximab and hydrocortisone for intraoperative immunosuppression. Postoperatively, immunosuppression was maintained with tacrolimus, mycophenolate, and prednisolone. After transplantation, his liver function test results initially normalized, but on posttransplantation day 40, ALT rose to 62 U/L. Liver function test results abruptly worsened, reaching a nadir on posttransplantation day 123 (bilirubin 85 μmol/L, ALT 1,478 U/L, and AST 561 U/L). Prothrombin time was elevated to 14.2 seconds. Liver histology on posttransplantation day 122 showed moderate inflammation at

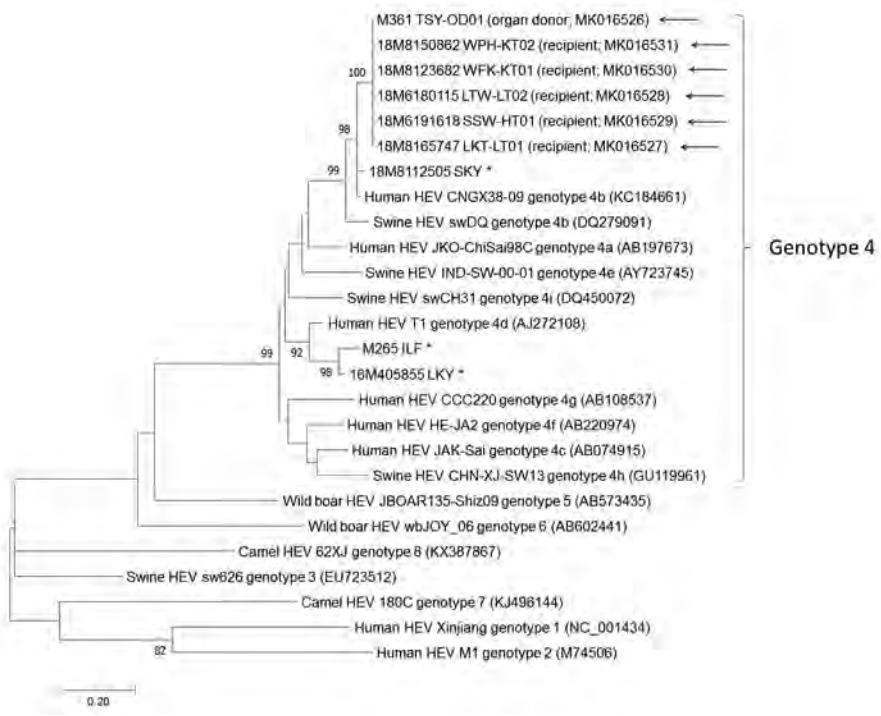

Figure 1. Phylogenetic analyses of the partial RNA-dependent RNA polymerase region of HEV strains involved in study of donor-derived genotype 4 HEV infection, Hong Kong, China, 2018, and other HEV genotypes. The bootstrap analysis was performed with 1,000 replicates. Bootstrap values <70% are not shown. The analysis included 382 nt positions. GenBank accession numbers are shown in parentheses. Asterisks (*) indicate locally identified cases of HEV infection; arrows (←) indicate HEV outbreak cases from this study. Scale bar indicates estimated number of substitutions per site. HEV, hepatitis E virus.

portal tracts (Figure 3, panel A). Acute organ rejection was treated by increasing immunosuppression. Hepatitis E was diagnosed after HEV RNA was detected in EDTA-treated blood collected on posttransplantation day 127. Immunohistochemical staining of the posttransplantation day 122 liver biopsy sample, with monoclonal antibodies against HEV, showed granular cytoplasmic staining of groups of hepatocytes (Figure 3, panel B). HEV RNA load in this biopsy sample was 1.21×10^7 copies/reaction. Because the patient's liver function test results were spontaneously improving (Figure 4, panel A), immunosuppression was reduced and HEV RNA loads monitored. However, the patient was repeatedly admitted for ascites, and HEV RNA was persistently detected in plasma with mildly elevated ALT. In view of persistent HEV infection and portal hypertension, oral ribavirin (200 mg/400 mg on alternate days, dose adjusted for renal impairment) was started on posttransplantation day 177; viremia cleared within 2 months (Figure 4, panel A).

Case-Patient 2 (Lung Transplant Recipient)

A 59-year-old man with a history of bronchiectasis and chronic obstructive pulmonary disease underwent sequential bilateral lung transplantation. The procedure was complicated by bleeding requiring exploratory thoracotomy for hemostasis. For postoperative immunosuppression, the patient was administered tacrolimus, mycophenolate, and prednisolone. The patient required prolonged hospitalization for ventilator-associated pneumonia. Starting at posttransplantation day 65, he had low-grade hepatitis. ALT was elevated to 65 U/L, AST to 45 U/L, while bilirubin remained within normal limits. Over the next 3 months, ALT elevations persisted (Figure 4, panel B). On posttransplantation day 161, the patient experienced generalized tonic–clonic convulsion and vesicular rash over the groin. Disseminated herpes zoster was confirmed by detection of varicella zoster virus DNA in cerebrospinal fluid, plasma, and swab samples from the vesicular rash. Despite intravenous acyclovir, the patient's condition progressively deteriorated to refractory shock, coagulopathy, and then death on posttransplantation day 171. Postmortem qRT-PCR testing of a plasma sample sent on posttransplantation day 162 confirmed a diagnosis of hepatitis E. An archived sample from posttransplantation day 21 that had been positive for HEV RNA confirmed persistent HEV infection (Figure 4, panel B). IgG in baseline pretransplant serum was detectable by ELISA, but this finding could not be confirmed by Western blot (Appendix Figure 1).

Case-Patient 3 (Kidney Transplant Recipient)

A 6-year-old boy with focal segmental glomerulosclerosis underwent kidney transplantation at Princess Margaret Hospital. Immunosuppression was induced with methylprednisolone and basiliximab and maintained with oral tacrolimus, mycophenolate, and prednisolone. Mycophenolate was switched to azathioprine because of BK virus reactivation. Starting at posttransplantation day 67, persistent ALT level abnormalities were noted. Because hepatitis E had been

diagnosed in 2 other recipients of organs from the same donor, HEV qRT-PCR testing of plasma collected on posttransplantation day 177 was performed, and results confirmed the diagnosis of persistent HEV infection. Administration of tacrolimus and azathioprine was stopped, and immunosuppression was continued with cyclosporin A, everolimus, and prednisolone. On posttransplantation day 180, oral ribavirin (100 mg 2×/d) was started. Liver function test results improved; HEV viral load underwent a 1-log reduction after the patient had received ribavirin for 1 month (Figure 4, panel C). However, during the second month of treatment, viral load and liver function test results plateaued.

Case-Patient 4 (Kidney Transplant Recipient)

A 54-year-old woman with chronic glomerulonephritis underwent kidney transplantation at Princess Margaret Hospital. Preoperatively, she received antithymocyte globulin and methylprednisolone. Postoperative immunosuppression included tacrolimus, mycophenolate, and prednisolone. During follow-up at Tuen Mun Hospital on posttransplantation day 54, abnormal parenchymal enzymes were first noted, coinciding with an increasing trend in tacrolimus levels detected in May (Figure 4, panel D); in addition, ALT was elevated to 59 U/L, ALP was 442 U/L, and bilirubin levels were within reference range at 8 μmol/L. The result of HEV qRT-PCR of plasma collected on posttransplantation day 176 was positive. Tacrolimus dosage was reduced, and oral ribavirin (400 mg 1×/day) was started. Within 2 months of treatment, ALT levels normalized and viremia cleared (Figure 4, panel D).

Case-Patient 5 (Heart Transplant Recipient)

A 49-year-old woman received a heart transplant for severe myocarditis at Queen Mary Hospital. Intraoperatively, she received 500 mg intravenous methylprednisolone; postoperatively, as an outpatient, she received immunosuppression with tacrolimus, mycophenolate, and prednisolone. On posttransplantation day 34, abnormal liver function

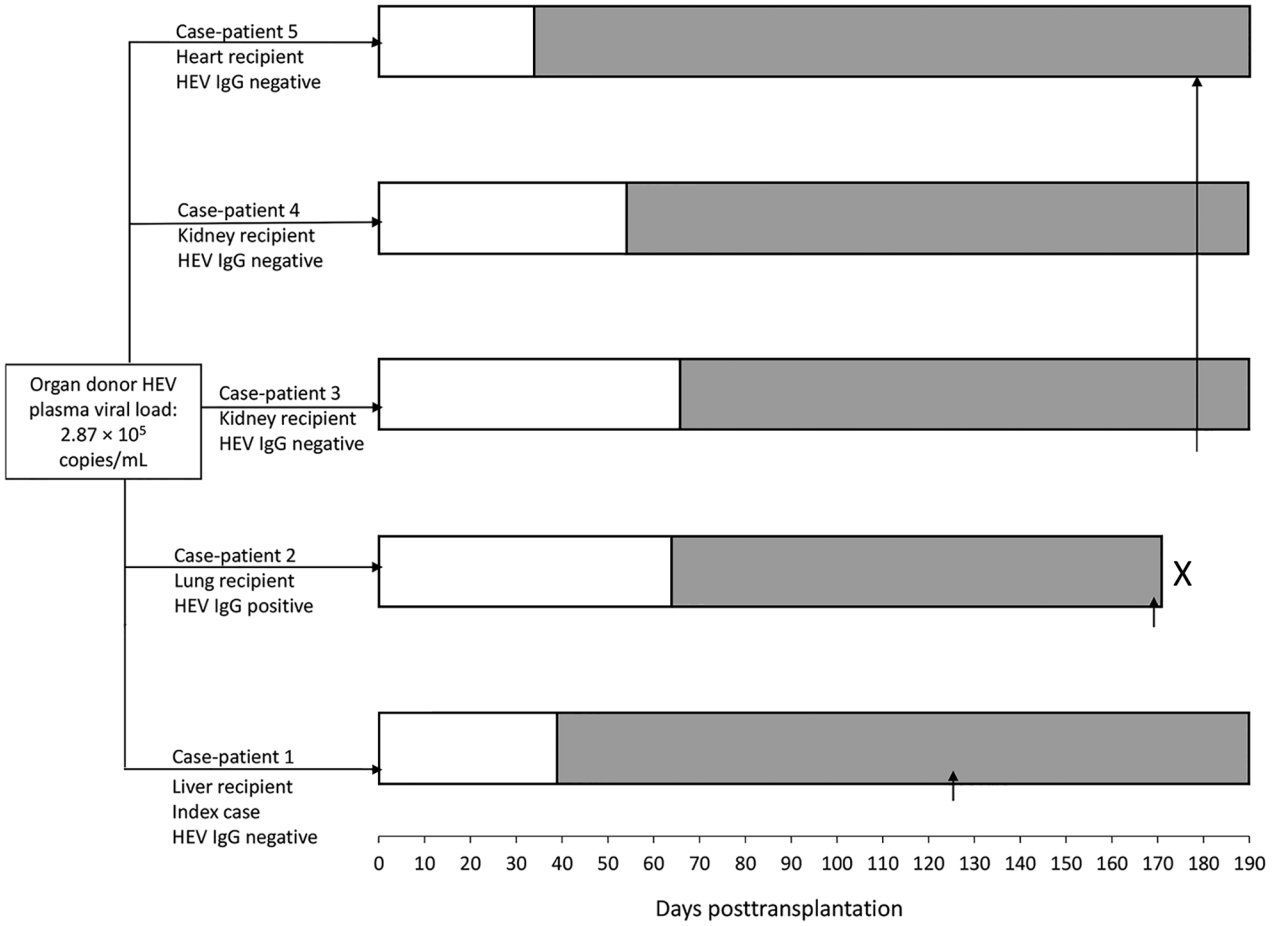

Figure 2. Timeline of outbreak in study of donor-derived genotype 4 HEV infection, Hong Kong, China, 2018, showing baseline HEV IgG status of each organ recipient. White bars indicate incubation period during which liver function test results were within reference range. Gray bars indicate timeline of alanine aminotransferase derangement after transplantation. X indicates patient death. Vertical arrows (↑) indicate time of hepatitis E diagnosis. HEV, hepatitis E virus.

Table 1. Characteristics of 5 patients who received organs from the same donor and had donor-derived genotype 4 hepatitis E virus infection, Hong Kong, China, 2018*

Case-patient	1	2	3	4	5
Organ transplanted	Liver	Lung	Kidney	Kidney	Heart
Age, y/sex	66/M	59/M	6/M	54/F	48/F
Incubation period, d†	40	65	67	54	34
Signs/symptoms	Ascites	None	None	None	None
Peak ALT level, U/L	1,385	138	490	186	130
Lymphocytes at hepatitis onset, $\times 10^9$ cells/L	0.43	0.59	1.4	0.37	1.12
Pretransplantation HEV IgG‡	–	+	–	–	–
Pretransplantation HEV-IgG§	–	–	–	–	–
Posttransplantation HEV-IgM/HEV-IgG¶	+/+	+/+	–/–	+/–	–/–
Posttransplantation HEV-IgM/HEV-IgG§	+/+	+/+	–/–	+/+	–/–

*All patients had received tacrolimus, mycophenolate, and prednisolone before onset of hepatitis E. ALT, alanine aminotransferase; HEV, hepatitis E virus.
†Time between transplantation and onset of abnormal ALT level.
‡HEV IgG ELISA (http://www.ystwt.cn/HEV.html).
§Western blot.
¶HEV IgM/HEV IgG ELISA (http://www.ystwt.cn/HEV.html).

test results were first noted; ALT was 123 U/L, AST 94 U/L, and ALP 123 U/L, but bilirubin levels were within reference range. Subsequent blood testing showed ongoing low-grade hepatitis (Figure 4, panel E). Because other transplant-transmitted hepatitis E infections from the same donor had been recognized, HEV qRT-PCR was performed on posttransplantation day 177; plasma contained an HEV RNA load of 1.51×10^8 copies/mL. Because the hepatitis had persisted for >3 months, on posttransplantation day 181, oral ribavirin (200 mg/d, adjusted for renal function) was started, and all immunosuppressant dosages were reduced. At the end of the second month of ribavirin treatment, ALT and HEV RNA loads were improving.

Discussion

This hepatitis E outbreak affected 5 transplant recipients who had received organs from a donor with HEV viremia. Two previous studies have described HEV transmission from organ donors to recipients (*15*,*16*). In both of those studies, liver function test results were abnormal for donors at the time of organ donation; 1 donor had subclinical hepatic HEV carriage (without serum HEV IgM or RNA positivity), and the other had acute hepatitis E (with serum HEV IgM and RNA positivity) (*15*,*16*). The donor in our study was a young woman whose liver function test results were within reference ranges and whose premortem serum was negative for HEV IgM but positive for HEV RNA; these values are compatible with organ donation during the window period of hepatitis E infection. However, subclinical long-term HEV carriage without seroconversion, a recently described entity in immunocompetent blood donors (*21*,*22*), cannot be excluded. Adoptive transfer of functional hepatitis B immunity via transplantation has been reported (*23*,*24*). The lack of measurable HEV humoral antibodies in the donor's serum may have facilitated HEV transmission in the absence of adoptive transfer of anti-HEV immune responses.

The risk for HEV transmission during transplantation has been recognized in the United Kingdom and Spain (*25*,*26*); guidelines from both countries recommend organ donor HEV screening. This recommendation is further supported by a recent study that reported that 1 (0.95%) of every 105 liver grafts is contaminated with HEV (*27*). The screening method of choice is nucleic acid amplification testing (NAAT), which is more sensitive than serologic testing (*13*,*28*). Undetectable HEV RNA in donor

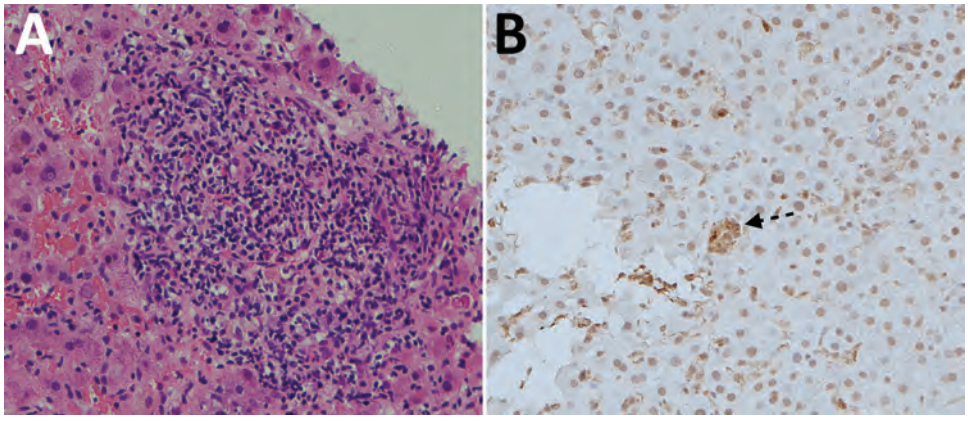

Figure 3. Histology of tissue from liver graft of hepatitis E virus case-patient 1, a 66-year-old man, on posttransplantation day 122. A) Hematoxylin and eosin staining showing moderate (grade 2) inflammation. B) Immunohistochemical staining (using hepatitis E virus monoclonal antibody); arrow indicates small groups of hepatocytes with positive cytoplasmic signals. Original magnification ×200.

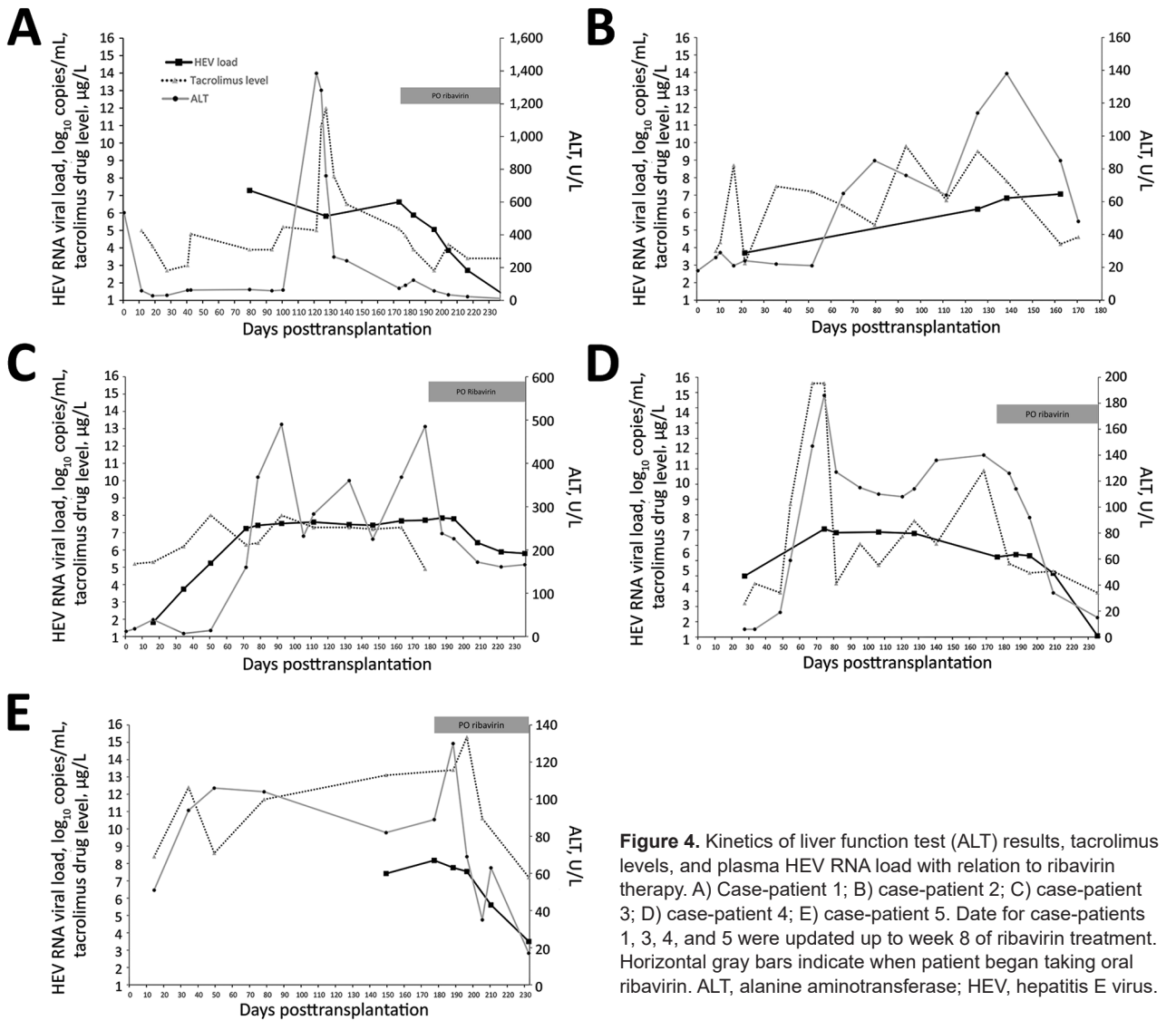

Figure 4. Kinetics of liver function test (ALT) results, tacrolimus levels, and plasma HEV RNA load with relation to ribavirin therapy. A) Case-patient 1; B) case-patient 2; C) case-patient 3; D) case-patient 4; E) case-patient 5. Date for case-patients 1, 3, 4, and 5 were updated up to week 8 of ribavirin treatment. Horizontal gray bars indicate when patient began taking oral ribavirin. ALT, alanine aminotransferase; HEV, hepatitis E virus.

serum would indicate low risk for HEV transmission, but the experience of Schlosser et al. suggests that liver grafts may harbor infectious HEV RNA even in the absence of systemic markers of infection (*15*). The most sensitive screening method may be HEV NAAT of graft tissue samples obtained at the time of transplantation. Although NAAT results may not be available before transplantation, the detection of HEV RNA in graft tissues could be used to guide posttransplant management of recipients. However, in resource-limited settings, the decision to implement universal organ donor HEV screening would pose substantial difficulties. Furthermore, even if the organ donor is HEV negative, recipients remain vulnerable to HEV infection through dietary and blood product transfusion routes. Ultimately, the decision to screen organ donors will depend on trends in HEV incidence in the general population. Screening organ donors in areas of low HEV prevalence may not add much value to transplant safety.

In our study, for all 5 organ recipients, hepatitis developed within a mean of 52 days after transplantation. The kidney, heart, and lung recipients showed no symptoms of hepatitis, but the liver recipient had severe hepatitis. This discrepancy is probably the consequence of an inflammatory response to high HEV antigenic load in the liver graft. Although the hepatitis in the liver graft recipient was temporarily suppressed by an increased dosage of immunosuppressants, graft dysfunction progressed to portal hypertension and refractory ascites, eventually requiring treatment with ribavirin.

HEV IgG is considered to provide cross-genotypic protection against HEV infection (*29*,*30*). In this outbreak, both ELISA and the Western blot results indicated that 4 of

Table 2. Summary of studies describing HEV-4 infections in immunocompromised patients*

Reference	Patient age, y/sex	Underlying immunosuppressive condition/treatment	Peak ALT, U/L	Progression to persistent HEV infection	Ribavirin treatment/ response	Patient outcome
(*37*)	4/M	Acute lymphoblastic leukemia	585	Spontaneous clearance, relapsed 20 mo later	No	Spontaneous clearance of relapse; resolution of hepatitis
(*36*)	68/M	Liver transplantation/tacrolimus	149	Yes	Yes/yes	Required retransplantation because of accelerated liver fibrosis; patient died of hemorrhage
(*35*)	47/F	Liver transplantation/tacrolimus	Not reported	Yes	Yes/no	Progressive cholestasis; patient died of sepsis
(*11*)	52/M	Renal transplantation/prednisolone, cyclosporin A, and everolimus or sirolimus	230	Yes	Yes/no	Ribavirin resistance; progressive cirrhosis
	55/M		456	Yes	Yes/yes	Resolution of hepatitis
	65/M		470	Yes	Yes/yes	Resolution of hepatitis
(*38*)	36/M	Renal transplantation/ tacrolimus	300	Yes	Yes/yes	Relapse after ribavirin withheld; progressive hepatitis

*ALT, alanine aminotransferase; HEV, hepatitis E virus.

5 patients were HEV IgG negative before transplantation, indicating absence of protective immunity from previous exposure. This finding is consistent with our previous findings of high HEV susceptibility among transplant recipients in Hong Kong (*31*).

The HEV strain in the outbreak reported here belonged to genotype 4b. Although some studies found that HEV-4 acute infection is more severe than HEV-3 infection (*32*,*33*), this finding has not been corroborated by a recent systematic review (*34*). Few data on the clinical characteristics of HEV-4 infection in immunocompromised patients are available (*11*,*35*–*38*). Of the 7 reported cases of HEV-4 infection in immunocompromised patients, 6 cases progressed to persistent infection; in 3 cases, infection did not respond to ribavirin or relapsed despite ribavirin (Table 2). All 5 patients in our study experienced persistent HEV-4 infection. Combined with the findings of our previous study (*11*), we note that persistent HEV-4 infection developed in 8 (89%) of 9 transplant recipients with hepatitis E in Hong Kong. In 4 patients from this study (case-patients 1–4) and 2 from our previous study (*11*), immunosuppression was reduced without any effect on HEV-4 viral load. This experience contrasts with findings of a previous seminal study in which HEV-3 infection cleared spontaneously even without reduction of immunosuppression in 34% of HEV-3 infected transplant recipients and cleared after reduction of immunosuppression in another 21% (*39*). The patients in our study were either taking tacrolimus or mTOR (mammalian target of rapamycin) inhibitors, which are risk factors for viral persistence (*39*,*40*). However, the effect of HEV genotype on persistence of infection requires further exploration.

All 4 surviving patients received ribavirin, which has been shown to be effective for treating persistent HEV-3 infections (*41*). At the time this article was written (≈2 months after patients started taking ribavirin), ALT levels had improved for all 4 patients, and viremia had cleared for 2 patients. Reduction of HEV viral load was slower for case-patient 3 and plateaued at the end of the second month of treatment. Further follow-up of this patient is needed to confirm whether this is ribavirin refractory disease, which we have previously described for HEV-4 infections (*11*).

Our study has several limitations. We were unable to ascertain the exact route of infection in the donor because of the long duration (≈6 months) between organ donation and recognition of the outbreak. Because archived blood samples obtained from recipients shortly after transplantation were not available, we were unable to comprehensively examine the viral load dynamics of HEV infection. The variable performance of HEV serologic assays and lack of a standard serologic test is a limitation of our study, which we tried to minimize by using 2 independent assay formats. Although some research findings suggest that the analytical sensitivity and specificity of the Wantai HEV IgG and IgM ELISAs are good, the work of Cattoir et al. shows that discordant results with other commercial assays are not uncommon (*42*,*43*). In our study, we found 100% concordance in HEV IgM detection between both formats, but the Western blot was able to detect HEV IgG in 1 more case-patient than the Wantai ELISA. Furthermore, the Wantai ELISA result indicated that case-patient 2 had HEV IgG in pretransplant serum, but this finding was not confirmed by Western blot.

We recognize that definitive conclusions on HEV-4 infections cannot be based on limited case series. To delineate intergenotypic differences in clinical characteristics, natural history, and ribavirin responsiveness, prospective studies of HEV-4 infection in cohorts of immunocompromised patients are needed.

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University-Based Outbreaks of Meningococcal Disease Caused by Serogroup B, United States, 2013–2018

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We reviewed university-based outbreaks of meningococcal disease caused by serogroup B and vaccination responses in the United States in the years following serogroup B meningococcal (MenB) vaccine availability. Ten university-based outbreaks occurred in 7 states during 2013–2018, causing a total of 39 cases and 2 deaths. Outbreaks occurred at universities with 3,600–35,000 undergraduates. Outbreak case counts ranged from 2 to 9 cases; outbreak duration ranged from 0 to 376 days. All 10 universities implemented MenB vaccination: 3 primarily used MenB-FHbp and 7 used MenB-4C. Estimated first-dose vaccination coverage ranged from 14% to 98%. In 5 outbreaks, additional cases occurred 6–259 days following MenB vaccination initiation. Although it is difficult to predict outbreak trajectories and evaluate the effects of public health response measures, achieving high MenB vaccination coverage is crucial to help protect at-risk persons during outbreaks of meningococcal disease caused by this serogroup.

Meningococcal disease, caused by the bacterium *Neisseria meningitidis*, is a severe, life-threatening illness with rapid onset and progression of symptoms. Case-fatality rates can be as high as 10%–20% among treated persons (*1*); 11%–19% of survivors develop major clinical sequelae, including loss of limbs, deafness, and seizures (*2*). In the United States, meningococcal disease incidence has steadily declined since 1995 (1.20 cases/100,000 persons) to a historic low of 0.11 cases/100,000 persons in 2017 (*3*).

Of the 4 meningococcal serogroups (B, C, W, Y) that cause most cases of the disease in the United States, serogroup B is currently the predominant serogroup overall and accounts for more than half of meningococcal disease cases among persons 16–20 years of age (*1*). Although the overall incidence is low, university students are at increased risk of meningococcal disease caused by serogroup B compared with other adolescents and young adults who do not attend university in the United States (*4*).

Vaccination is the primary strategy for prevention of meningococcal disease. Since 2005, the US Advisory Committee on Immunization Practices has recommended quadrivalent meningococcal conjugate vaccine covering serogroups A, C, W, and Y (MenACWY) for routine use in adolescents 11–18 years of age and other groups at increased risk for meningococcal disease, including unvaccinated college freshmen living in dormitories (*5*). In 2013, a serogroup B meningococcal (MenB) vaccine, MenB-4C (Bexsero; GlaxoSmithKline, https://www.gsk.com) (*6*), became available for outbreak response via a Centers for Disease Control and Prevention (CDC)–sponsored expanded access investigational new drug protocol. In 2014–2015, MenB-FHbp (Trumenba; Pfizer, https://www.pfizer.com) (*7*) and MenB-4C were licensed for use in the United States. Although these vaccines are not routinely recommended for all adolescents or college students, adolescents and adults 16–23 years of age may be vaccinated with a MenB series based on individual clinical decision-making (*8*). In addition, MenB vaccine is recommended for use in persons ≥10 years of age who are at increased risk for meningococcal disease caused by this serogroup, including during outbreaks (*9*). In outbreak settings, either a 2-dose series of MenB-4C (0, ≥1 month) or a 3-dose series of MenB-FHbp (0, 1–2, 6 months) is recommended (*10*).

Historically, most meningococcal disease outbreaks on university campuses in the United States were caused by serogroup C (*11*,*12*). However, serogroup B has caused all known US university-based outbreaks since 2011, likely in part because of high MenACWY coverage in adolescents (*13*). We summarize university-based outbreaks of meningococcal disease caused by serogroup B in the United States in the years following MenB vaccine availability (2013–2018) and describe the resulting MenB vaccination responses.

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Identifying University-Based Outbreaks

Outbreaks of meningococcal disease among university students are usually reported to CDC by state health departments as part of routine technical assistance. We also reviewed cases of meningococcal disease reported through the National Notifiable Diseases Surveillance System, supplemented since 2015 with data collected through enhanced meningococcal disease surveillance activities to improve completion of key variables, including association with an outbreak. However, this review did not identify any other previously unreported meningococcal disease outbreaks caused by serogroup B in university students. This analysis includes all university-based outbreaks in which ≥2 cases of genetically related meningococcal disease cases caused by serogroup B were reported during a 3-month period (*14*).

During January 2013–May 2018, a total of 10 university-based meningococcal disease outbreaks caused by serogroup B were reported in 7 states; these outbreaks resulted in a total of 39 cases and 2 deaths (5%) (Table 1). The median patient age was 19 years; 62% were male. Syndrome information was available for 38 (97%) cases: 63% were meningitis (with or without bacteremia) and 37% were bacteremia without focus. Only 1 case occurred in a student who previously received MenB vaccine; this student had been vaccinated with 1 dose of MenB vaccine 6 days before disease onset (*22*). The remaining cases occurred in persons believed to be unvaccinated with MenB vaccine. Thirty-six (92%) cases were in undergraduate students from 4-year degree-granting universities; 3 cases occurred in unvaccinated close contacts (*5*) of undergraduate students (*15*,*23*). In the 2017 Pennsylvania outbreak, the 2 cases were in close contacts on the same athletic team. In the remaining outbreaks, no direct epidemiologic links were identified between cases.

Outbreaks occurred at universities ranging from small private schools with 3,600–5,000 undergraduates to large public universities with up to 35,000 undergraduates. In the 2017–2018 Massachusetts outbreak, cases occurred at 2 universities in a college consortium in the same geographic area. The number of cases per outbreak ranged from 2 to 9 (median 3 cases), and the attack rate ranged from 10 to 134 cases/100,000 population (median 35 cases/100,000 population). Outbreak duration (time from onset date of first to last case) ranged from 0 to 376 days (median 34 days) (Figure 1).

Six of 10 outbreaks were caused by strains of *N. meningitidis* belonging to clonal complex (CC) 32 (Table 1), a relatively common CC among serogroup B cases in the United States. Three outbreaks in Oregon and California were caused by *N. meningitidis* strains belonging to multilocus sequence type (ST) 32; the 2 Oregon outbreak strains had the same outer membrane protein types and vaccine antigen variants and clustered into a single clade, indicating that both outbreaks may have been derived from the same strain (*30*). The 3 other outbreak strains belonging to CC32 were ST11910 (2016 California), ST11556 (2016 Wisconsin), and ST8758 (2017 Pennsylvania). Two of the outbreaks in the northeastern United States were caused by strains belonging to CC41/44, another CC commonly associated with serogroup B in the United States, although each was caused by a distinct sequence type (ST409 in New Jersey and ST41 in Massachusetts). The remaining 2 outbreaks were caused by ST11/CC11 (2016 New Jersey), a relatively common sequence type but most often associated with serogroups C and W, and ST9069 (2015 Rhode Island), a rare strain not yet assigned to a clonal complex and not previously reported as a cause of invasive disease (*31*).

Table 1. University-based outbreaks of meningococcal disease caused by serogroup B, United States, 2013–2018

State of university	Outbreak period	No. cases (deaths)	Approximate no. undergraduates	Clonal complex of outbreak strain	Sequence type of outbreak strain	References
New Jersey	2013 Mar–2014 Mar	9 (1)*	5,000	41/44	409	(*15*,*16*)
California	2013 Nov	4†	19,000	32	32	(*17*)
Rhode Island	2015 Jan–Feb	2	3,700	Unassigned	9069	(*18*–*21*)
Oregon	2015 Jan–May	7 (1)‡	20,000	32	32	(*22*,*23*)
California	2016 Jan–Feb	2§	5,000	32	11910	(*24*)
New Jersey	2016 Mar–Apr	2	35,000	11	11	(*25*)
Wisconsin	2016 Oct	3	30,000	32	11556	(*26*,*27*)
Oregon	2016 Nov–2017 Nov	5	25,000	32	32	(*28*)
Massachusetts¶	2017 Oct–2018 Feb	3	26,000	41/44	41	(*29*)
Pennsylvania	2017 Nov	2#	3,600	32**	8758**	

*Two of the cases occurred in close contacts of undergraduate students at the outbreak-affected university: 1 in a high school student who stayed in an undergraduate dormitory and 1 in a student at a different university.
†One additional associated case that occurred in March 2013 was identified after retrospective case review. An additional case occurred 2 years later in a close contact of an undergraduate student who may have been connected to this outbreak.
‡One case occurred in a close contact of undergraduate students at the outbreak-affected university.
§One additional suspected case with inconclusive lab results occurred in February 2016 in a student previously vaccinated with 2 doses of MenB vaccine and who received antimicrobial chemoprophylaxis the day before symptom onset.
¶Cases occurred at 2 universities in a college consortium in the same geographic area. The first 2 cases occurred at University 1 and the third case occurred at University 2.
#Cases were close contacts and members of the same athletic team.
**An isolate was available for only 1 of the 2 cases, so typing results refer to the single case isolate.

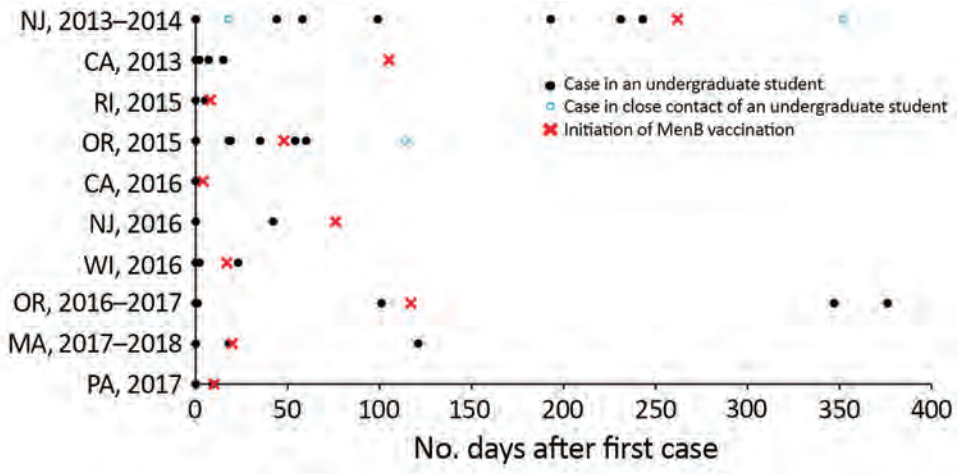

Figure 1. Timing of case onset dates and initiation of vaccination efforts during university-based outbreaks of meningococcal disease caused by serogroup B, United States, 2013–2018. MenB, serogroup B meningococcal vaccine.

In all outbreaks, chemoprophylaxis was provided to close contacts of case-patients; in some cases, chemoprophylaxis recommendations were expanded to include those in common social networks, such as athletic teams or social organizations. None of the serogroup B cases occurred in students who had received chemoprophylaxis before symptom onset; 1 person received ciprofloxacin chemoprophylaxis after symptom onset but before diagnosis (*24*).

MenB Vaccination Response

MenB vaccines were used in response to all 10 outbreaks (Table 2). Two of the outbreaks (New Jersey and California) occurred in 2013, before licensure of MenB vaccines; therefore, MenB-4C vaccine was provided through a CDC-sponsored expanded-access investigational new drug protocol (*15*,*17*). The remaining 8 outbreaks occurred following the licensure of MenB-4C and MenB-FHbp vaccines in the United States: 3 of the affected universities implemented MenB vaccination using primarily MenB-FHbp, and 5 universities primarily used MenB-4C. The choice of which MenB vaccine to use was driven largely by vaccine availability and procurement mechanisms at the time of the outbreak. In some instances, vaccine dosing schedule was also considered. In the 2016 New Jersey outbreak, a preferential recommendation was made for MenB-FHbp based on molecular and immunologic data (*25*).

MenB vaccination was implemented in a variety of ways. Mass vaccination campaigns were used in all but 2 outbreaks; most universities also made MenB vaccination available by appointment at the student health clinic. Some schools also encouraged students to seek vaccination through pharmacies and providers, 1 university integrated MenB vaccination into seasonal influenza vaccination clinics, and 2 universities instituted vaccination requirements.

Populations recommended for vaccination based on risk of exposure to the outbreak strain were similar at each university: all undergraduate students; graduate students living in undergraduate or graduate dormitories; and students, faculty, and staff with medical conditions putting them at increased risk for meningococcal disease. Some universities made additional efforts to target specific subpopulations of students for MenB vaccination, because of either the epidemiology of the cases or increased social mixing and close contact among specific student organizations, such as athletic teams or social organizations. Some universities also recommended vaccination for other small groups, such as spouses living with undergraduates in dormitories, persons in intimate physical relationships with undergraduates, and other staff or graduate students ≤25 years of age.

Even with mass vaccination campaigns, MenB vaccination coverage in response to university-based outbreaks was highly variable, ranging from an estimated 14% to 98% coverage for the first dose (median 67%) (Figure 2). In general, some small universities were able to achieve high coverage; however, 2 large universities with ≥25,000 undergraduates achieved high coverage as well. High first-dose coverage (67%) was achieved in response to the 2016 Wisconsin outbreak, driven by continued cases after vaccination efforts began and the use of federal funds to procure vaccine, thereby relieving hurdles related to vaccine financing and insurance coverage (*26*). In the 2016–2017 Oregon outbreak, an initial first-dose vaccination coverage of 8% was increased to 98% after continued cases prompted intensified vaccination efforts; eventually, the university opted to require proof of MenB vaccination as a prerequisite for registration (*28*). Vaccination efforts in response to the Massachusetts outbreak were ongoing at the time of publication, with coverage estimates expected to increase. In addition, several universities were able to track only vaccine doses administered on campus; because additional students received MenB vaccine from external providers and pharmacies, reported coverages are considered minimum estimates.

Table 2. Vaccination response to university-based outbreaks of meningococcal disease caused by serogroup B, United States, 2013–2018*

State of university	Primary MenB vaccine	Vaccination strategy	Vaccination coverage	Cases after vaccine implementation
New Jersey	MenB-4C†	Mass vaccination campaign	Dose 1, 95%; dose 2, 89%	1
California	MenB-4C†	Mass vaccination campaign	Dose 1, 51%; dose 2, 40%	0
Rhode Island	MenB-FHbp	Mass vaccination campaign	Dose 1, 94%; dose 2, 80%; dose 3, 77%‡	0
Oregon	MenB-FHbp§	Mass vaccination campaigns; student health; local pharmacies	Dose 1, 52%; dose 2, 40%; dose 3, 10%	3¶
California	MenB-4C	Mass vaccination campaign	Dose 1, 90%; dose 2, 90%#	0
New Jersey	MenB-FHbp	Local providers/pharmacies; student health; some vaccination campaigns	NA	0
Wisconsin	MenB-4C	Mass vaccination campaign	Dose 1, 67%; dose 2, >31%**	1¶
Oregon	MenB-4C	Mass vaccination campaign with targeted student groups; vaccine requirement	Dose 1, 98%; dose 2, 93%	2
Massachusetts††	MenB-4C	Mass vaccination campaign; student health; providers/pharmacies	Dose 1, 34%**,‡‡; dose 2, 16%	1
Pennsylvania	MenB-4C	Vaccinated athletic team; campus-wide recommendation for vaccination at student health	Dose 1, 14%; dose 2, 2%**	0

*MenB, serogroup B meningococcal; MenB-4C, Bexsero (GlaxoSmithKline, https://www.gsk.com); MenB-FHbp, Trumenba (Pfizer, https://www.pfizer.com); NA, vaccination coverage estimates not available.
†Vaccine available through a CDC-sponsored investigational new drug protocol.
‡Coverage data refer to 2015 campaign. Incoming freshmen and returning study abroad students were also vaccinated in 2015–2016.
§MenB-4C was also used in response to this outbreak; 21% of students completed either vaccine series. Presented coverage data for this university are as of July 2018.
¶One case in each of these outbreaks occurred 6 days after MenB vaccination efforts commenced, thus prior to any expected immunity.
#This coverage estimate represents the number of persons vaccinated in the second campaign, which likely included some first doses.
**Reported coverage reflects only vaccine doses given on campus; additional students received vaccine doses from providers or pharmacies at home or in other states.
††Cases occurred at 2 universities in a college consortium in the same geographic area. The first 2 cases occurred at university 1 and the third case occurred at university 2. Both universities began vaccination efforts following the second case.
‡‡Combined first-dose coverage data for both universities are presented in the table. First-dose coverage was 33% at university 1 (23,388 undergraduates) and 41% at university 2 (2,500 undergraduates) (Figure 2). Vaccination was ongoing at the time of publication; therefore, data should be considered preliminary.

In the 2 outbreaks in 2013, MenB vaccine was provided through an expanded access investigational new drug protocol (*15*,*17*). In the 2013 California outbreak, a vaccination campaign was initiated after 4 cases had occurred; no additional cases occurred following MenB vaccination implementation (Figure 1). In the 2013–2014 New Jersey outbreak, vaccination was implemented after 8 cases had occurred; an additional case occurred 90 days after the initiation of the vaccination campaign in an unvaccinated close contact of an undergraduate student. Among the 8 outbreaks that occurred following MenB vaccine licensure in the United States, 6 universities implemented MenB vaccination after 2 cases, 1 after 3 cases, and 1 after 4 cases. In the 6 outbreaks in which vaccination was implemented after the first 2 cases, 4 had no further outbreak-associated cases and 2 had 1 additional case each: 1 occurred 6 days after the initiation of a MenB vaccination campaign (2016 Wisconsin), and 1 occurred 101 days after MenB vaccination implementation (2017–2018 Massachusetts) but occurred at a nearby university in the same college consortium that had already begun vaccination efforts. The university that implemented vaccination after the first 3 cases (2016–2017 Oregon) had 2 additional cases occur, 230 and 259 days after the initiation of MenB campaigns. The university that implemented vaccination after the first 4 cases (2015 Oregon) had 3 additional cases occur, 6, 12, and 66 days after campaign initiation; only the case that occurred 6 days after campaign initiation was in a person who had been vaccinated (*22*). In total, 8 additional cases occurred after the initiation of MenB vaccination campaigns in 5 outbreaks: 3 cases within 2 weeks and 5 cases in 66–259 days (median 101 days), although 3 of these later cases occurred in persons outside the original university target population for vaccination.

In the United States, for the purposes of public health decision making, the risk of meningococcal disease may be considered to have returned to expected levels 1 year following the last case in a university-based outbreak (*14*). For this reason, or in response to additional cases, MenB vaccination efforts continued into the following school year at some universities, whether in the form of mass campaigns, offering MenB vaccine at student health, or continuing or instituting MenB vaccination recommendations or requirements.

Discussion

Although the incidence of meningococcal disease in the United States is low, university students are at increased risk of meningococcal disease caused by serogroup B and outbreaks (*4*). In 2013–2018, from 1 to 4 university-based

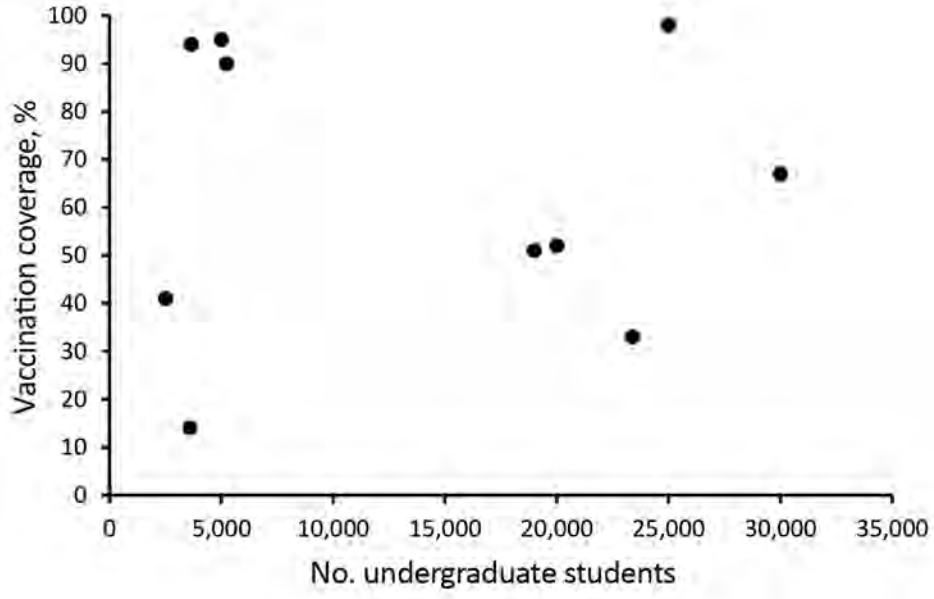

Figure 2. Association between university size and first-dose serogroup B meningococcal vaccine coverage in response to university-based outbreaks, United States, 2013–2018.

outbreaks occurred annually. These outbreaks varied in size and duration and affected both small and large universities. All the outbreaks occurred at residential 4-year degree-granting universities. Similar university-based disease outbreaks were reported in Canada in 2015 (2 cases, 1 death) (*32*) and the United Kingdom in 2017 (3 cases, 1 death) (*33*).

Although MenB vaccination coverage among all US adolescents and young adults is unknown, coverage with ≥1 dose of MenB vaccine among persons 17 years of age was estimated to be 14.5% in 2017 (*13*), and a recent survey demonstrated that only 2% of universities require MenB vaccination for students (*34*). With a mostly unvaccinated student population, most outbreak-affected universities implemented mass MenB vaccination campaigns to quickly increase vaccination coverage, requiring an immense mobilization of public health resources from the university, local and state health departments, and often CDC. Achieving high MenB vaccination coverage through mass vaccination campaigns during outbreaks has been most challenging for large universities, although 1 university achieved nearly 100% coverage after implementation and enforcement of a MenB vaccine requirement (2016–2017 Oregon).

In 2017, partly on the basis of the experiences of these recent university outbreaks, CDC revised its guidance for the evaluation and public health management of suspected outbreaks of meningococcal disease (*14*). In the revised guidance, the outbreak threshold for vaccine decision-making in an organization-based outbreak, such as at a university, is defined as 2–3 outbreak-associated cases within an organization during a 3-month period: "In most situations, 2 cases within an organization constitute an outbreak. However, in some situations, such as an outbreak within a large university (e.g., >20,000 undergraduate students) where no identifiable subgroup at risk within the population can be identified, it may be reasonable to declare an outbreak after 3 cases" (*14*). Decisions about whether, when, and how to offer MenB vaccine during an outbreak remain challenging and should be tailored to the unique epidemiology of each outbreak. At the same time as vaccination discussions and mobilization efforts begin, appropriate chemoprophylaxis, enhanced case detection, and communication efforts should be promptly initiated.

When dealing with university-based outbreaks, it is difficult to predict if or when additional cases may occur. In some outbreaks, cases occurred over a prolonged period of time, as in a 2008–2010 Ohio outbreak (*35*) (before MenB vaccine availability in the United States) and the 2013–2014 New Jersey outbreak (*15*). Half of the 10 studied outbreaks ended following implementation of MenB vaccination efforts, but additional cases occurred at the other 5 universities, mainly among unvaccinated students or close contacts. Factors that might contribute to the occurrence of new cases following implementation of a vaccination campaign include low vaccination coverage, resulting in a large number of students without direct vaccine protection, and lack of MenB vaccine impact on meningococcal carriage, precluding development of herd protection (*18*,*23*).

It is difficult to determine whether university-based outbreaks have increased in frequency in recent years, as reporting of outbreaks to CDC has historically been incomplete and routine national meningococcal disease surveillance began collecting standardized data on university attendance only in 2015. Enhanced detection and reporting, improved molecular methods to confirm strain relatedness, the recent availability of MenB vaccines

for outbreak response, and a low and declining overall disease incidence leading to greater awareness of outbreaks may have contributed to the increase in reported outbreaks among university students. Three university-based outbreaks were reported in the United States during 2008–2011 (*35*); however, the lack of routinely collected information on historical meningococcal disease outbreaks limits comparisons of outbreak size or duration before and after MenB vaccine availability.

Achieving high MenB vaccination coverage is necessary to help protect persons during outbreaks of meningococcal disease caused by serogroup B. Efforts are under way to better understand MenB vaccine effectiveness and duration of protection, MenB vaccine strain coverage, and current risk factors for meningococcal disease among university students. This information, along with lessons learned when implementing MenB vaccination campaigns, could help guide and improve responses to future outbreaks.

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Treatment Outcomes in Global Systematic Review and Patient Meta-Analysis of Children with Extensively Drug-Resistant Tuberculosis

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Evaluate the presentation of children from 11 countries who were managed between 1999 and 2013 for bacteriologically confirmed extensively drug-resistant tuberculosis (XDR TB), based on a global systematic review and individual patient meta-analysis
- Assess the treatment of children from 11 countries who were managed between 1999 and 2013 for bacteriologically confirmed XDR TB, based on a global systematic review and individual patient meta-analysis
- Determine the outcome of children from 11 countries who were managed between 1999 and 2013 for bacteriologically confirmed XDR TB, based on a global systematic review and individual patient meta-analysis.

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Extensively drug-resistant tuberculosis (XDR TB) has extremely poor treatment outcomes in adults. Limited data are available for children. We report on clinical manifestations, treatment, and outcomes for 37 children (<15 years of age) with bacteriologically confirmed XDR TB in 11 countries. These patients were managed during 1999–2013. For the 37 children, median age was 11 years, 32 (87%) had pulmonary TB, and 29 had a recorded HIV status; 7 (24%) were infected with HIV. Median treatment duration was 7.0 months for the intensive phase and 12.2 months for the continuation phase. Thirty (81%) children had favorable treatment outcomes. Four (11%) died, 1 (3%) failed treatment, and 2 (5%) did not complete treatment. We found a high proportion of favorable treatment outcomes among children, with mortality rates markedly lower than for adults. Regimens and duration of treatment varied considerably. Evaluation of new regimens in children is required.

Extensively drug-resistant tuberculosis (XDR TB) is a public health emergency that threatens global TB control. Multidrug-resistant TB (MDR TB) is caused by *Mycobacterium tuberculosis* that shows resistance to isoniazid and rifampin, and XDR TB includes additional resistance to any fluoroquinolone and a second-line injectable drug (*1*). In 2016, a total of 8,014 cases of XDR TB were reported to the World Health Organization (WHO) by 72 countries (*2*). Treatment success rates for XDR TB remain poor; only 30% of patients show cure or treatment completion, and costs for care far exceed those for drug-susceptible TB (*2*). There is an increasing awareness that children are also affected by MDR TB and XDR TB. Modeling studies estimated that as many as 2 million children currently have MDR TB globally, and MDR TB develops in an estimated 30,000 children <15 years of age each year (*3*,*4*). Estimates suggest that of children with MDR TB, 4.7% have XDR TB (*4*).

We recently completed a systematic review and individual patient data (IPD) meta-analysis commissioned by WHO that described clinical presentation, treatment, and outcomes for children treated for MDR TB (*5*). We reported data for 975 children with MDR TB from 18 countries; 731 (75%) had bacteriologically confirmed MDR TB, and 244 (25%) had clinically diagnosed MDR TB (*6*). Overall, 764 (78%) of 975 children had a successful treatment outcome (*6*), as defined by WHO guidelines (*7*). This meta-analysis provided information on pediatric aspects for the revised 2016 WHO drug-resistant TB treatment guidelines, and specific recommendations were subsequently made for treatment of MDR TB in children (*5*). Children with confirmed XDR TB were excluded because they were a distinct subgroup and insufficient evidence was available to make treatment recommendations for children with XDR TB at that time (*5*).

The management of XDR TB in children is challenging because of the limited availability of new drugs and appropriate treatment regimens. XDR TB treatment regimens for children have historically been individualized on the basis of mycobacterial drug-susceptibility testing (DST) of the organism of the child or the putative source case (*5*). There are limited data on the optimal combination of medications and the duration of treatment for XDR TB and major research gaps remain (*5*). Therefore, we aimed to describe clinical manifestations, routine treatment, and outcomes for children with confirmed XDR TB in the era preceding access to novel anti-TB drugs for children.

Methods

Data Collection

As part of a systematic review and IPD meta-analysis, we collected data from global collaborators on children <15 years of age treated for MDR TB as part of a defined treatment cohort (*6*). We identified published and unpublished data from retrospective and prospective studies by using a broad search strategy. Eligible studies were identified, and individual level patient data were requested from each author by using a standardized data collection tool. We requested demographics, clinical details, and outcomes on the basis of specified definitions. Additional interpretation of data was conducted by the study team, and the primary authors were contacted to resolve queries. Data were obtained for 1,012 children treated during 1999–2013 (*6*). Detailed methods and outcomes of IPD meta-analysis for 975 children with MDR TB and pre-XDR TB (MDR TB with additional resistance to either a fluoroquinolone

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or an injectable drug, but not both) was reported (*6*). We report on children identified through systematic review of drug-resistant TB in children who had bacteriologically confirmed XDR TB. Therefore, this analysis only included children who were investigated for TB, had presence of *M. tuberculosis* confirmed bacteriologically, had isolates tested for resistance to second-line anti-TB drugs, and received a treatment outcome during the episode of TB.

Definitions

We defined TB as pulmonary TB when disease was localized to the lungs or intrathoracic lymph nodes and as extrapulmonary TB when disease was found at site distant from the lungs, including pleural effusions and miliary TB. We classified pulmonary TB as severe or nonsevere by using adapted criteria of Wiseman et al. (*8*) on the basis of a review of reported chest radiographs by 2 independent reviewers; a third reviewer arbitrated discordance. Previous TB treatment history and type of treatment previously received (for drug susceptible TB or drug-resistant TB), was documented when known. We defined malnutrition as being underweight for age (weight-for-age z-score <–2) or per the report of the treating clinician.

Because the review spanned many years and sites, DST methods varied by region and period. Data collected for treatment of XDR TB varied, and sites inconsistently submitted data on individual drugs used and drug dose or duration of treatment. We report intensive phase and continuation phase as submitted, for which investigators defined these 2 stages according to their clinical practice. The intensive phase typically refers to initial months of treatment, which include more drugs and the use of an injectable drug. The continuation phase refers to a second phase of treatment generally with a reduction in the number of drugs.

We defined TB treatment outcomes by using standard 2014 WHO MDR TB outcome definitions as classified by treating clinicians: cure (treatment completed as recommended by the national policy without evidence of failure and ≥3 consecutive cultures taken at least 30 days apart were negative after the intensive phase of treatment); treatment complete (as for cure but without records of negative cultures); treatment failed (treatment stopped or requiring change of 2 drugs because of persistent positive cultures at end of the intensive phase or reversion to positive cultures in the continuation phase, or evidence of additional acquired resistance or adverse drug reactions); death (for any reason while receiving treatment); or loss to follow-up (treatment interruption for 2 consecutive months) (*9*). We defined favorable (cure and completed treatment) and unfavorable (treatment failure, death, or loss to follow-up) outcomes. Adverse events were inconsistently included in primary data and are not reported here.

Statistical Analysis

We completed descriptive statistics for demographic and clinical variables. Missing data were noted, and each analysis reflects the sample size used. Categorical variables are presented as frequencies and percentages, and continuous variables (duration of intensive or continuation phase) are presented as median and interquartile ranges (IQRs). Age of children was categorized as <2 years, 2–4 years, 5–9 years, or 10–14 years. We used logistic regression with a preset 95% level of significance and calculated odds ratios (ORs) and 95% CIs to estimate and predict unfavorable outcomes. Children with unknown outcomes or loss to follow-up were excluded from regression analyses. We analyzed by using SAS software version 9.4 (https://www.sas.com).

Ethical Approval

The study protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Stellenbosch University (reference no. X14/09/020). The oversight committee at the location institution of each contributor approved collection of data and submission to the collaborative systematic review.

Results

Of 1,012 children included in the larger systematic review of children with MDR TB, 37 children from 11 countries had bacteriologically confirmed XDR TB (Table 1). We also compiled demographic and clinical characteristics at baseline (Table 2). Median age was 11 years (IQR 6.0–13.1 years). Thirty-two (87%) children had pulmonary TB only. Among children with pulmonary TB and chest radiographic findings, 20 (65%) of 31 had markers of severity, including disseminated/extensive disease, airway compression with lobar collapse, miliary opacification, or cavitation. HIV status was documented for 29 (78%) children; of these, 7 (24%) were infected with HIV.

We also obtained previous TB treatment history for children with XDR TB (Figure 1). Among 33 children who had documented knowledge of previous treatment, 17 (52%) had been previously treated for TB. Of children previously treated, only 10 had known TB treatment outcomes; 7 (70%) had a history of treatment failure.

Data for microbiological investigations were limited. All 37 children had culture-confirmed XDR TB, but only 30 had smear results before treatment, and 10 (33%) were smear positive. Follow-up culture results were infrequently available. The DST pattern for this cohort (Figure 2) showed that all children had isolates with proven resistance to rifampin and isoniazid. Isolates from some children were tested against multiple injectable drugs and fluoroquinolones (some of which were susceptible), but all children in the cohort had isolates that were resistant to ≥1 second-line injectable drug

Table 1. Overview of 14 studies and 37 children with confirmed cases of extensively drug-resistant tuberculosis*

Reference	Country	No. persons in study	No. children with XDR TB	Study design
Amanullah, unpub. data	Pakistan	29	1	Retrospective cohort
Banerjee et al. (*10*)	USA	18	1	Retrospective cohort
Chiotan, unpub. data	Romania	17	1	Retrospective cohort
Drobac et al. (*11*)	Peru	36	4	Retrospective cohort
Gegia et al. (*12*)	Georgia	55	3	Retrospective cohort
Hicks et al. (*13*)	South Africa	82	5	Retrospective cohort
Isaakidis et al. (*14*)	India	8	2	Retrospective cohort
Kuksa et al. (*15*)	Latvia	53	4	Retrospective cohort
Smirnova et al. (*16*)	Russia	38	1	Retrospective cohort
Moore et al. (*17*)	South Africa	339	5	Retrospective cohort
Seddon et al. (*18*)	South Africa	88	5	Retrospective cohort
Seddon et al. (*19*)	South Africa	131	2	Prospective cohort
Skrahina, unpub. data	Belarus	5	2	Retrospective cohort
Swaminathan et al. (*20*)	Tajikistan	27	1	Retrospective cohort

*Each study population involved all consecutively presenting bacteriologically confirmed and clinically diagnosed patients. XDR TB, extensively drug-resistant tuberculosis.

(kanamycin, amikacin, or capreomycin), and ≥1 fluoroquinolone (moxifloxacin, levofloxacin, ofloxacin, or ciprofloxacin). DST for additional drugs was performed only for a limited number of children.

We also evaluated drugs used to treat these children (Figure 3). No children received bedaquiline or delamanid because both drugs became available only after the study period. The most commonly used drugs were an injectable (n = 27), a fluoroquinolone (n = 26), cycloserine/terizidone (n = 27), ethionamide/prothionamide (n = 24), and para-aminosalicylic acid (n = 25). Capreomycin was the most commonly used injectable drug (Figure 3); for some children, >1 injectable was used sequentially. Of 26 children who received a fluoroquinolone, 13 were given moxifloxacin alone, and 4 were switched between moxifloxacin and other fluoroquinolones. Limited information on timing and reason for changing drugs was available. Duration of use of individual drugs was recorded for a limited number of children, and estimates of individual treatment duration per drug could not be made. Median duration of the intensive phase for 26 children who completed treatment was 7 months (IQR 6.0–8.2 months), and median duration for 23 children who completed the continuation phase was 12.2 months (IQR 10.0–16.2 months).

Overall, 30 (81%) children had favorable treatment outcomes (Figure 4). Four (11%) children died during XDR TB treatment; 2 were HIV-infected and receiving antiretroviral therapy. One child (3%) had documentation of treatment failure (HIV infected when receiving antiretroviral therapy) and 2 (5%) were lost to follow-up during treatment (HIV uninfected). We obtained detailed demographic and clinical variables for the 7 children with unfavorable outcomes, including the DST pattern of the isolate and drugs used during treatment (Table 3).

We detected no associations between recorded variables and unfavorable treatment outcome by using univariate analyses. Although HIV-infected children had a 14.3 times (95% CI 1.2–174.8 times) greater odds of an unfavorable outcome than HIV-uninfected children, this logistic regression model used infected, uninfected, and unknown as 3 possible categories of HIV, and differences in the overall model were not significant (p = 0.098). Multivariable analysis was not completed because of lack of significance in all univariate analyses.

Discussion

Data for treatment of XDR TB in children are limited. Our group of 37 confirmed cases over 15 years represents a small sample yet was larger than those in previous reports, highlighting the serious underdiagnosis and underreporting of XDR TB in children in the published literature. We included data for clinical manifestations, treatment, and outcomes for these children. Overall, we describe encouraging rates of successful treatment outcomes in this small cohort of children despite the limited drug options available to treat children in most instances and the notable lack of access to novel drugs, such as bedaquiline and delamanid, at the time of treatment. All children had confirmed XDR TB; more work is needed to include children given a diagnosis of probable XDR TB in the absence of bacteriological confirmation. Most children were >5 years of age and had pulmonary TB and severe TB. Half of the children had been previously given treatment for TB, and of those children, half had been previously treated with a drug-susceptible TB treatment regimen.

Of 975 children described in the MDR TB IPD, only 37 had confirmed XDR TB, which might reflect the limited access to second-line DST. The older median age (11 years) observed in this group than that for a pediatric MDR TB IPD review (median age 7.1 years) (*6*) might indicate that younger children might not have been as likely to have been investigated and given a diagnosis of XDR TB. Because it has been estimated that 80% of childhood TB deaths occur in children <5 years of age

Table 2. Demographic and clinical characteristics of 37 children with bacteriologically confirmed extensively drug-resistant tuberculosis*

Characteristic	No. (%)
Age, y, median 11 y (IQR 6.0–13.1 y)	
<2	6 (16)
2–4	3 (8)
5–9 y	6 (16)
10–14 y	22 (60)
Sex	
F	23 (62)
M	14 (38)
Site of disease	
PTB	32 (86)
EPTB†	2 (5)
PTB and EPTB‡	3 (8)
Severe TB disease by chest radiography, n = 31§	
No	11 (35)
Yes	20 (65)
Documented adult TB source case, n = 28	
No	8 (29)
Yes	20 (71)
HIV status	
Uninfected	22 (59)
Infected	7 (19)
Unknown	8 (22)
Antiretroviral treatment	
Receiving treatment¶	7 (100)
Malnutrition, n = 30#	
No	18 (60)
Yes	12 (40)
Admitted to hospital for TB treatment, n = 29	
No	4 (14)
Yes	25 (86)
WHO TB treatment outcome	
Cured	23 (62)
Completed	7 (19)
Failed	1 (3)
Died	4 (11)
Loss to follow-up	2 (5)
Clinical outcome	
Favorable**	30 (81)
Unfavorable††	7 (19)

*EPTB, extrapulmonary tuberculosis; IQR, interquartile range; PTB, pulmonary tuberculosis; TB, tuberculosis; WHO, World Health Organization.
†Two cases of EPTB, 1 site specified as urogenital and 1 unspecified.
‡Three cases of PTB and EPTB. Sites of EPTB were peripheral lymph nodes and pleural TB in 1 child and abdominal TB in 2 children.
§Severity of TB based on grading of chest radiograph reports available only for 31 children; 2 children had EPTB only and no chest radiograph and 4.
¶Children had no chest radiograph despite documented PTB. Six children were receiving antiretroviral therapy at the start of TB treatment and 1 child started antiretroviral therapy during the course of TB treatment.
#Underweight for age (weight-for-age z-score <–2) or as per treating clinician's report.
**Includes all children who were cured or completed treatment.
††Includes all children who failed treatment, died, or were lost to follow-up during treatment.

(*21*), death before diagnosis and treatment initiation in the youngest children might also contribute to the older age of this cohort. Another potential explanation is that younger children might have been more likely to have been given treatment without microbiological confirmation (given a diagnosis based only on clinical findings) because of their typical paucibacillary disease and challenges in obtaining respiratory specimens. Finally, because older children are more likely to have adult-type TB with higher bacillary burden, bacteriological confirmation is more likely (*22*).

It is essential that empiric treatment for XDR TB in children be based on DST of the organism of the putative TB source case-patient, but the need for adequate sampling for TB bacteriology including gastric aspirates, or other samples, cannot be overstated (*23*–*25*). If one considers the risk for rapid disease progression (*26*), treatment initiation in children should not be delayed. Although DST for the XDR TB isolate might be used for development of a targeted treatment regimen, a good contact history with DST of the organism of the source case-patient might also be used where confirmation is not possible. In our study, 28 (76%) children had recorded information on source cases. Of these children, 20 (70%) had an identified source case during their episode of TB, but we did not have sufficient information to define the DST pattern of the source case. This finding highlights the need for contact investigation of source cases. Early screenings of households after diagnosis of TB is an opportunity to identify at-risk contacts. Symptom screening and sampling of child contacts could facilitate earlier management.

M. tuberculosis DST patterns in this cohort were limited because the review spanned 15 years starting in 1999. Determination of fluoroquinolone resistance varied and, for the definition we applied in this study, ciprofloxacin resistance was included. Data for additional testing of drugs was limited and did not enable us to make inferences about the DST pattern and the drug or regimen chosen or any changes in DST pattern during the course of treatment.

Although we were not able to evaluate the role of specific drugs because of the small number of children and the variability in treatment regimens, we found patterns of commonly used drugs. Most (n = 27) children received an injectable drug, and although more exact data were limited, the median duration used was 7 months. Isolates from all children had confirmed resistance to an injectable drug; thus, it is a concern that an injectable drug was used for so many children, particularly given the risk for adverse events, including permanent hearing loss (*27*,*28*). It is possible that clinicians expected incomplete cross-resistance within the injectable drug group, a rationale supported by the predominance of capreomycin use (*29*). However, cross-resistance within this group is relatively common (*30*). It is also possible that children might have been given these agents before second-line DST results became available. *M. tuberculosis* strains resistant to earlier-generation fluoroquinolones might retain susceptibility to a later-generation fluoroquinolone (*31*), which might have supported fluoroquinolone use

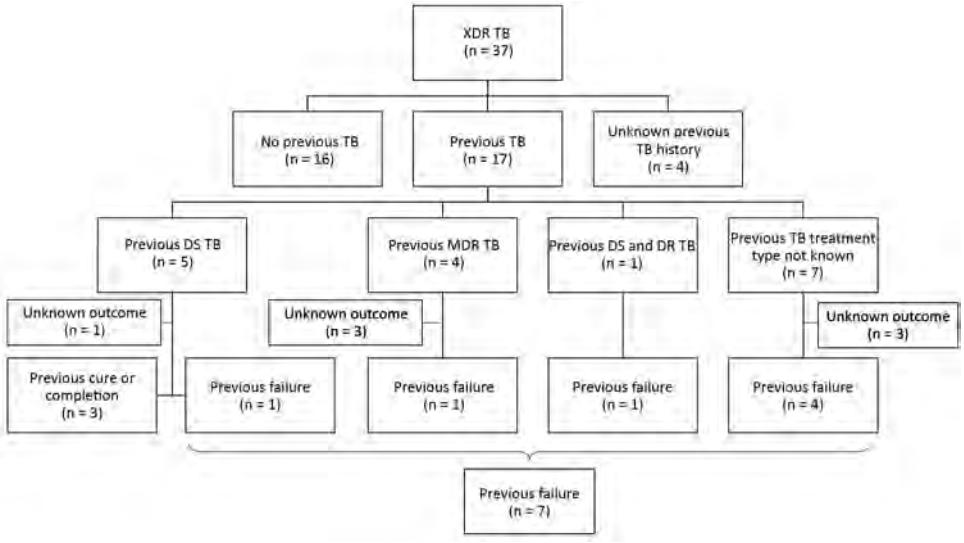

Figure 1. Flow chart for 37 children with confirmed XDR TB and details of TB treatment history, type of TB treatment, and treatment outcome. DR, drug-resistant; DS, drug-susceptible, MDR, multidrug-resistant; TB, tuberculosis; XDR TB, extensively drug-resistant TB.

despite documented resistance. Most (n = 26) children received a fluoroquinolone, and this drug was a later-generation treatment for >50% of case-patients. This finding might reflect the anticipation of clinicians for some activity of the drug, despite DST results and limited other treatment options.

Most children in our cohort were given treatment before availability of new drugs. Linezolid was only used for 3 children and clofazimine for 6 children, whereas bedaquiline and delamanid were not used. Para-aminosalicylic acid, ethionamide or prothionamide, and cycloserine or terizidone were used frequently, highlighting the limited drug options available and the necessity to use drugs with major toxicity and relatively poor efficacy. With new drugs, alternatives for children with drug-resistant TB are being explored. The WHO endorsement of delamanid for older children (>6 years of age) with MDR TB (*32*) and early reports on the use of bedaquiline in older children (≥10 years of age) (*33*) might be initial steps in finding the optimum treatment regimen for XDR TB in children.

Children with confirmed XDR TB had similar successful outcomes (81%) as children given treatment for clinically diagnosed and bacteriologically confirmed MDR TB (78%) over the same study period (*6*). Although loss to follow-up was lower (5.4% for XDR TB and 11.2% for MDR TB) (*6*), our cohort of children with XDR TB was smaller, and children with XDR TB might have had more intensive follow-up than children with MDR TB. WHO combined program data for 6,777 XDR TB case-patients (adults and children) reported from 52 countries during 2014 found treatment success in only 30%, with 28% deaths, 21% treatment failures, and 20% lost to follow-up (*2*). Overall mortality rates exceeded 40% in India and South Africa (*2*). High mortality rates for XDR TB in adults has been well documented

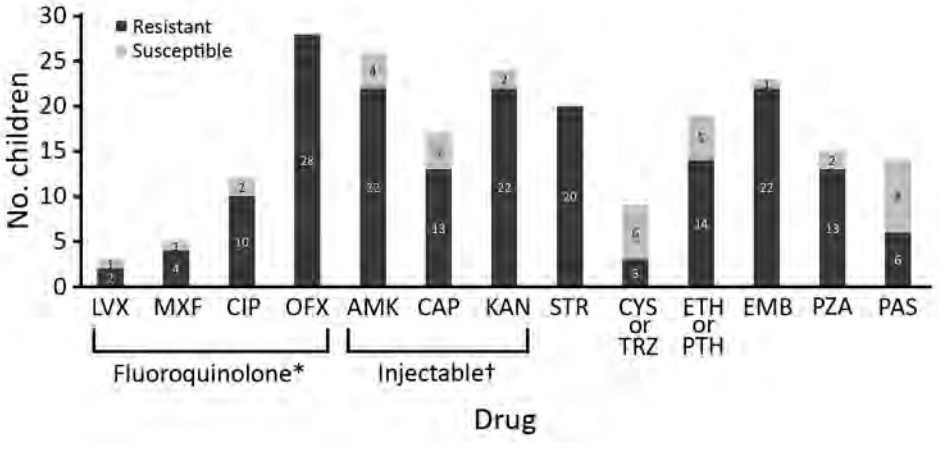

Figure 2. Mycobacterial drug susceptibility test pattern for children treated for extensively drug-resistant tuberculosis. All children had organisms that were resistant to rifampin and isoniazid. *Includes moxifloxacin, levofloxacin, ofloxacin, or ciprofloxacin. †Includes second-line injectable drugs kanamycin, amikacin, or capreomycin. AMK, amikacin, amikacin; CAP, capreomycin; CIP, ciprofloxacin; CYS, cycloserine; EMB, ethambutol; ETH, ethionamide; KAN, kanamycin; LVX, levofloxacin; MXF, moxifloxacin; OFX, ofloxacin; PAS, para-aminosalicylic acid; PTH, prothionamide; PZA, pyrazinamide; STR, streptomycin; TRZ, terizidone.

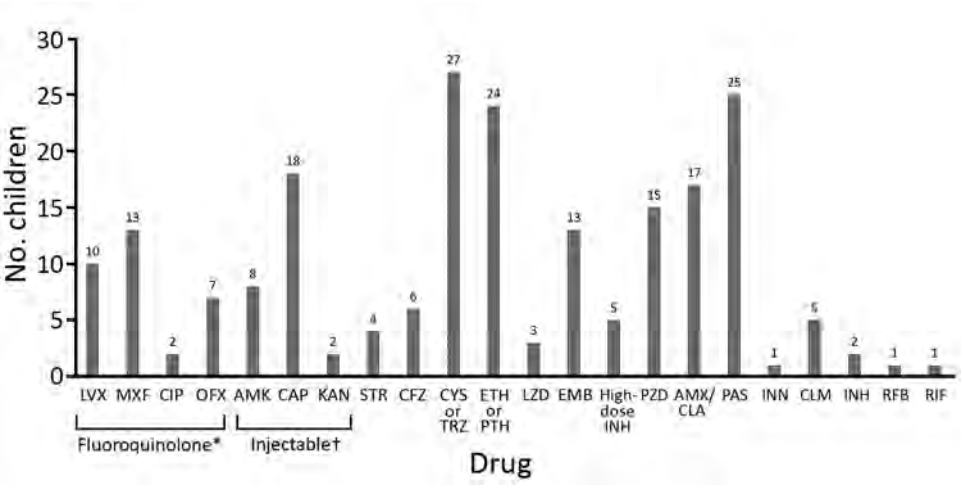

Figure 3. Drugs used for treatment of children with extensively drug resistant tuberculosis. *Includes moxifloxacin, levofloxacin, ofloxacin or ciprofloxacin. †Includes second-line injectable drugs kanamycin, amikacin, or capreomycin. AMK, amikacin, AMX, amoxicillin; CAP, capreomycin; CFZ, clofazimine; CIP, ciprofloxacin; CLA, clavulanic acid; CLM, clarithromycin; CYS, cycloserine; EMB, ethambutol; ETH, ethionamide; INH, isoniazid; INN, thioacetazone; KAN, kanamycin; LVX, levofloxacin; LZD, linezolid; MXF, moxifloxacin; OFX, ofloxacin; PAS, para-aminosalicylic acid; PTH, prothionamide; PZA, pyrazinamide; RFB, fifabutin; RIF, rifampin; STR, streptomycin; TRZ, terizidone.

(*34*–*37*). In our cohort of children with XDR TB, 4 (10.8%) died during treatment.

Considering the natural history of TB, we acknowledge that spontaneous cure in children occurs and might also contribute to the good outcomes seen. We note that none of the children included in our cohort had TB meningitis, which is well known to have poor outcomes (*38*), and this finding might contribute to the good outcomes we report. This lower mortality rate might be caused by this group representing a survival bias, only including children who were alive to make the diagnosis and initiate treatment. In addition, children tend to have lower organism load (paucibacillary), have less concomitant pathology, and be treated with more tailored individualized regimens compared with adults, which might improve survival. Diagnosis and treatment of XDR TB was historically limited to tertiary centers or specialized centers of excellence for TB care, providing more resources for care and improving diagnosis and treatment outcomes. Most (86%) children were admitted to a hospital and would have likely had good adherence support. We acknowledge that this cohort consisted of a small number of selected children and are therefore cautious about generalizability.

A further limitation of our study is poor reporting of adverse events, which could not be analyzed. Poor adverse event reporting has been identified by WHO as a problem (*2*). Systematic monitoring of TB drug safety for children is crucial, especially if one considers introduction of new drugs. Given the modest sample size and the limitations regarding the dose and exact duration of drugs, we could not analyze the effect of individual drugs, regimen combinations, or duration of treatment on final treatment outcomes. Newer drugs require evaluation because WHO has recommended use of delamanid for children and adolescents (*32*).

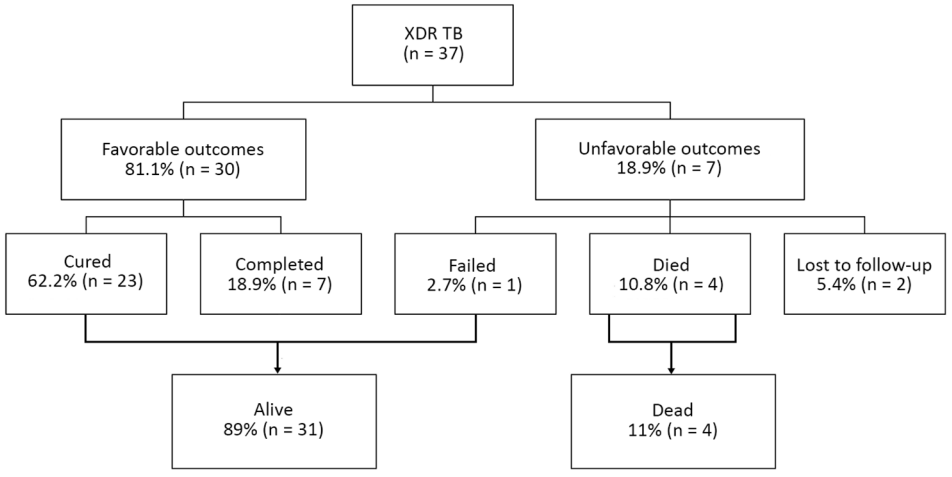

Figure 4. Treatment outcomes for 37 children treated for XDR TB. XDR TB, extensively drug-resistant tuberculosis.

Table 3. Demographic and clinical characteristics of 7 children with unfavorable treatment outcomes for XDR TB*

Child	Year of treatment	Age, y/sex†	Weight, kg, at start of XDR TB treatment	HIV status	History of TB	Previous TB outcome	Disease site	Chest radiograph result‡	Drugs to which isolate was resistant	Drugs used at any stage during XDR TB treatment	Outcome
1	2008	9/F	23	–	No	No	PTB	Normal	INH, RIF, OFX, AMK, STR, EMB	INH, CAP, EMB, PZA, ETH, PAS	Lost to follow-up
2	2008	14/F	41	–	U, DST	Failure	PTB	Nonsevere typical TB	INH, RIF, OFX, AMK, STR	Not specified	Lost to follow-up
3	2007	6/M	20	+	No	No	PTB	Nonsevere typical TB	INH, RIF, CIP, AMK, STR, EMB	AMK, EMB, PZA, ETH, CIP	Died
4	2007	14/M	42	+	DS TB	No	PTB, EPTB	Severe not typical TB	INH, RIF, OFX, KAN, STR, EMB, ETH, PAS	CAP, MXF, LVX, ETH, CYS, PAS, AMX, CLA	Died
5	2001	13/F	U	U	DR TB	Failure	PTB	Nonsevere typical TB	INH, RIF, LVX, CIP, KAN, AMK, CAP, STR, EMB, PZA, ETH, PAS	CAP, STR, LVX, CIP, ETH, CYS, PAS, AMX, CLA	Died
6	2009	12/F	19.5	+	Both	Failure	PTB	Nonsevere typical TB	INH, RIF, OFX, KAN	CAP, MXF, EMB, PZA, ETH, CYS, PAS, AMX, CLA, CLM	Failure
7	2013	13/F	34	–	U	No	PTB	U	INH, RIF, MXF, OFX, KAN, AMK, CAP, STR, EMB, PZA, ETH	CAP, LVX, PZA, ETH, CYS, PAS	Died

*Ethionamide or prothionamide was not differentially recorded; where ethionamide is stated, it implies that 1 of the 2 dugs was used. Cycloserine or terizidone was not differentially recorded; where cycloserine is stated, it implies that 1 of the 2 drugs was used. AMK, amikacin; AMX, amoxicillin; CAP, capreomycin; CIP, ciprofloxacin; CLA, clavulanic acid; CLM, clarithromycin; CYS, cycloserine; DS, drug sensitive; DR, drug resistant; DST, drug susceptibility testing; EMB, ethambutol; EPTB, extrapulmonary tuberculosis; ETH, ethionamide; INH, isoniazid; KAN, kanamycin; LVX, levofloxacin; LZD, linezolid; MXF, moxifloxacin; OFX, ofloxacin; PAS, para-aminosalicylic acid; PTB, pulmonary tuberculosis; PZA, pyrazinamide; RIF, rifampin; STR, streptomycin; TB, tuberculosis; U, unknown; XDR TB, extensively drug-resistant tuberculosis; –, negative; +, positive.
†Age at year of diagnosis.
‡By adapted Wiseman classification.

Access to newer TB drugs and effective shorter MDR TB regimens is improving. Systematic reviews of MDR TB or XDR TB have identified the benefits of linezolid, a larger number of effective drugs, the number of drugs used in each phase of treatment, and the duration of treatment (*39*–*42*). Although there might be some indication for use of more drugs during the intensive phase and continuation phase, with longer duration of treatment for XDR TB, this evidence is still limited (*41*). Studies to date have mainly included adults (*39*–*42*). Our findings highlight the need for more studies evaluating new drugs and treatment regimens in children with XDR TB.

In conclusion, we report treatment of XDR TB for children spanning 15 years. The limited number of children identified highlights a major gap in diagnosing and reporting XDR TB in children. The high proportion of favorable treatment outcomes and considerably lower mortality rates compared with those for adults is encouraging. We found considerable variability of regimens used and duration of treatment in children, but this review preceded availability and use of linezolid, clofazimine, bedaquiline, and delamanid. More collaborative, multicenter prospective cohorts are needed to collect better and more extensive data for children with drug-resistant TB. Evaluation of shorter effective and safe regimens for children with XDR TB is urgently needed.

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etymologia

Streptomycin [strep"to-mi'sin]

Ronnie Henry

In the late 1930s, Selman Waksman, a soil microbiologist working at the New Jersey Agricultural Station of Rutgers University, began a large-scale program to screen soil bacteria for antimicrobial activity. By 1943, Albert Schatz, a PhD student working in Waksman's laboratory, had isolated streptomycin from *Streptomyces griseus* (from the Greek *strepto-* ["twisted"] + *mykēs* ["fungus"] and the Latin *griseus*, "gray").

In 1944, Willam H. Feldman and H. Corwin Hinshaw at the Mayo Clinic showed its efficacy against *Mycobacterium tuberculosis*. Waksman was awarded a Nobel Prize in 1952 for his discovery of streptomycin, although much of the credit for the discovery has since been ascribed to Schatz. Schatz later successfully sued to be legally recognized as a co-discoverer of streptomycin.

Slide culture of a *Streptomyces* sp. bacteria, which produces the antibiotic streptomycin. Note the branching filamentous hyphae, abundant aerial mycelia, and long chains of small spores. Image: CDC/Dr. David Berd

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Bacillus Calmette-Guérin Cases Reported to the National Tuberculosis Surveillance System, United States, 2004–2015

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Release date: February 19, 2019; Expiration date: February 19, 2020

Learning Objectives

Upon completion of this activity, participants will be able to:

- Describe surveillance data, demographics, and epidemiology of bacillus Calmette-Guérin (BCG) cases and tuberculosis (TB) cases reported to the National TB Surveillance System (NTSS) during 2004 to 2015.
- Compare clinical characteristics and management of BCG cases and TB cases reported to NTSS during 2004 to 2015.
- Assess the clinical implications of these findings regarding BCG cases and TB cases reported to NTSS during 2004 to 2015.

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Mycobacterium bovis bacillus Calmette-Guérin (BCG) is used as a vaccine to protect against disseminated tuberculosis (TB) and as a treatment for bladder cancer. We describe characteristics of US TB patients reported to the National Tuberculosis Surveillance System (NTSS) whose disease was attributed to BCG. We identified 118 BCG cases and 91,065 TB cases reported to NTSS during 2004–2015. Most patients with BCG were US-born (86%), older (median age

75 years), and non-Hispanic white (81%). Only 17% of BCG cases had pulmonary involvement, in contrast with 84% of TB cases. Epidemiologic features of BCG cases differed from TB cases. Clinicians can use clinical history to discern probable BCG cases from TB cases, enabling optimal clinical management. Public health agencies can use this information to quickly identify probable BCG cases to avoid inappropriately reporting BCG cases to NTSS or expending resources on unnecessary public health interventions.

Countries with a high incidence of tuberculosis (TB) commonly use bacillus Calmette-Guérin (BCG), a live, attenuated strain of *Mycobacterium bovis*, as a vaccine to prevent disseminated tuberculosis disease among children. BCG is also used as first-line treatment for superficial bladder cancer (*1*). Rarely, BCG causes localized infection or disseminated disease with clinical features similar to those caused by *M. tuberculosis*, which causes most TB cases among humans (*2*,*3*). However, the primary diagnostic tests for TB (acid-fast bacillus [AFB] and smear or culture) do not distinguish BCG strains of *M. bovis* from other organisms in the *M. tuberculosis* complex. Nonetheless, the clinical and public health management of the diseases differ. Whereas treatment of TB is always indicated, and symptomatic BCG case-patients might require treatment with anti-TB drugs, asymptomatic BCG case-patients rarely, if ever, need treatment. Unlike typical TB cases caused by *M. tuberculosis*, BCG cases are primarily healthcare-associated, and a traditional TB contact investigation would be unnecessary for BCG cases without pulmonary involvement. For the purposes of this article, we will describe cases caused by non-BCG strains of the *M. tuberculosis* complex as TB disease and cases caused by BCG as BCG disease.

Difficulty distinguishing persons with BCG disease from persons with TB disease also has national surveillance implications. The Council of State and Territorial Epidemiologists (CSTE) collaborates with the Centers for Disease Control and Prevention (CDC) to establish national case definitions for public health notification and reporting purposes (*4*). The laboratory criteria for TB cases, as defined by CSTE, are isolation of *M. tuberculosis* complex from a clinical specimen, demonstration of *M. tuberculosis* complex from a clinical specimen by nucleic acid amplification test, or demonstration of AFB in a clinical specimen if neither culture nor nucleic acid amplification is available. CDC's Division of Tuberculosis Elimination, part of the National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, has issued additional guidance stipulating that cases caused by BCG strains, regardless of clinical manifestations, should not be reported as TB cases to the National Tuberculosis Surveillance System (NTSS) (*5*). Difficulty in distinguishing BCG cases from other organisms in the *M. tuberculosis* complex, as well as confusion between the CSTE case definition, which does not specifically exclude positive BCG cultures, and CDC guidance, which does, might explain why some BCG cases are reported as verified TB cases every year. We analyzed characteristics of these BCG cases and the circumstances under which reporting areas decided to report the cases to NTSS.

Methods

We used genotyping data from the National Tuberculosis Genotyping Service to identify BCG cases reported by the 50 US states and the District of Columbia to NTSS during 2004–2015. We defined a BCG case as a report with a positive laboratory result related to *M. bovis* BCG: spoligotype 676773777777600 and x, y, or z in the second locus of the mycobacterial interspersed repetitive unit–variable-number tandem-repeat analysis (*5*).

By using the standard case report information reported to NTSS, we described the demographic characteristics of persons with BCG. We also compared the clinical characteristics of BCG cases with those of TB cases reported during the same 12-year period.

To clarify the possible clinical spectrum of BCG cases, we more closely examined a convenience sample of locally stored public health records for a subset of 13 such cases reported by Texas and Florida during 2014–2015. We used a standardized medical record abstraction form to collect demographic, clinical, and treatment data for each case. We also conferred with public health practitioners who reported these 13 cases to gather additional information about the clinical manifestations, the subsequent clinical and public health management, and the circumstances under which the public health authority classified these cases as verified TB cases.

Analyses of data collected during routine TB surveillance were determined to be exempt from Institutional Review Board review at CDC. Waivers of consent were obtained in 2 states for the 13 patients who are described in this article because more clinical data were necessary to consider whether these reported BCG cases represented asymptomatic persons with BCG-related laboratory results or persons with symptomatic BCG disease.

Results

Surveillance Data

Twenty-six US states reported 118 BCG cases during 2004–2015. In the same period, the 50 US states and the District of Columbia reported 91,065 TB cases. Reporting of BCG cases ranged from a median of 6 reports/year during 2004–2007 to 12 reports/year during 2008–2015.

Of reported BCG cases, 86% occurred among US-born persons. Patients with BCG disease were almost exclusively male (93%) and non-Hispanic white (81%). The patients' median age was 75 years (interquartile range [IQR] 66–81 years),

but 6 cases occurred among children <5 years of age. One of these children was US-born; the others were born in Mexico (n = 2), Ethiopia (n = 1), Japan (n = 1), and Ukraine (n = 1). All of the children had exclusively skeletal sites of disease.

Eighty-seven percent of BCG cases had an extrapulmonary disease site; skeletal and genitourinary sites were most commonly reported (Table 1). Only 20 (17%) of the 118 BCG cases had any pulmonary involvement, including 4 with positive sputum smear results and 3 with cavitary lesions identified through chest radiography. Public health authorities might have considered these BCG cases to be infectious and to warrant contact investigations to prevent transmission of the infection to others, although the attenuated nature of BCG makes it less likely that disease would result even if secondary infections occurred in contacts of the patient. In contrast, most (84%) TB cases involved the lungs.

Social factors and underlying conditions among BCG patients differed from those for TB patients. Diabetes (21% vs. 9%) and residence in a long-term care facility at the time of diagnosis (9% vs. 2%) were more prevalent among BCG patients than TB patients.

Individual BCG Case Reviews

When we collected additional data for the subset of 13 BCG cases reported by Texas and Florida during 2014–2015, we learned that all 13 had occurred among men with a history of bladder cancer treated with intravesical BCG instillations (i.e., instillation of BCG into the bladder) (Table 2). One of the 13 men was asymptomatic when BCG was isolated from his urine within 6 months of his cancer therapy; he did not require treatment with anti-TB drugs. Two other men were considered to have local infections (i.e., cystitis only) related to their recent BCG instillations. The remaining 10 cases had evidence of BCG dissemination beyond the bladder; we describe 4 of these cases in further detail.

Case Report A

A non–US-born, non-Hispanic white man in his 50s sought care at an emergency department because of fever, hematuria, and dysuria a few days after a traumatic catheterization during BCG instillation for bladder cancer treatment. AFB were visualized on urine microscopic examination, and the patient was prescribed levofloxacin and isoniazid to be taken as an outpatient. Symptoms persisted throughout the following month. Although no abnormal findings were documented on physical examination, AFB were visualized on microscopic examination of a blood specimen. Subsequent chest radiography demonstrated multiple inflammatory nodules (≈15 mm) scattered throughout both lung fields, suggestive of miliary TB. Public health authorities

Table 1. Characteristics of BCG-related cases and TB cases reported, National Tuberculosis Surveillance System — United States, 2004–2015*

Characteristic	BCG cases, no. (%)	TB cases, no. (%)
Total cases reported, no. (%)	118 (100)	91,065 (100)
Median age (interquartile range), y	75 (66–81)	47 (31–62)
Age group, y		
0–4	6 (5)	954 (1)
5–14	0	856 (0.9)
15–24	2 (2)	10,398 (11)
25–44	3 (3)	30,236 (33)
45–64	16 (14)	28,327 (31)
≥65	91 (77)	20,289 (22)
Sex		
M	110 (93)	56,800 (62)
F	8 (7)	34,265 (38)
White, non-Hispanic	95 (81)	14,330 (16)
US-born	101 (86)	35,215 (38.7)
Pulmonary disease only	15 (13)	67,033 (74)
Extrapulmonary disease only	98 (83)	14,659 (16)
Both pulmonary and extrapulmonary disease	5 (4)	9,336 (10)
Extrapulmonary sites of disease†		
Bones and joints	37 (36)	2,953 (12)
Genitourinary	30 (29)	1435 (6)
Lymphatic system	4 (4)	9,088 (38)
Peritoneal	4 (4)	1,473 (6)
Pleural	3 (3)	5,428 (3)
Meningeal	0	1,203 (5)
Other extrapulmonary sites‡	30 (29)	3,453 (14)
History of long-term care facility residency	11 (9)	1,988 (2)
Diabetes§	15 (21)	7,864 (17)

*BCG, Bacillus Calmette-Guérin; TB, tuberculosis.
†Denominators for percentages are based on the preceding 2 rows of all cases with any extrapulmonary involvement (i.e., with or without pulmonary involvement).
‡Include blood, urinary bladder, subcutaneous tissues, and bone marrow.
§Diabetes comorbidity data only available starting in 2009; therefore, percentages are based on different denominators (45,786 TB cases and 72 BCG cases).

Table 2. Clinical characteristics, treatment duration, and outcomes of 13 male patients treated with BCG instillations for bladder cancer and subsequently reported to the National Tuberculosis Surveillance System, 2014–2015*

Patient	Type of complication	Time from BCG instillation into bladder to diagnosis	Site of culture specimen	Duration of treatment	Outcome
1	Asymptomatic	<6 mo	Urine	Not treated	Alive
2	Cystitis	<1 mo	Urine	Not treated	Alive
3	Cystitis	4 mo	Urine	3 weeks	Alive
4	Epididymo-orchitis	ND	Abscess	9 mo	Alive
5	Disseminated	ND	Urine	2 wks	Died
6	Left hip involvement	ND	Abscess	1 mo	Died
7	Parotiditis (case report C)	ND	Abscess	4 mo	Died
8	Prosthetic joint infection (case report B)	9 mo	Synovial fluid	ND	Alive
9	Prosthetic joint infection (case report D)	4 y	Abscess	9 mo	Alive
10	Septicemia	1 y	Blood	ND	ND
11	Septicemia, military (case report A)	<1 mo	Blood	9 mo	Alive
12	Spondylodiscitis	ND	Abscess	1 mo	Died
13	Spondylodiscitis	10 y	Abscess	1 mo	Died

*BCG, Bacillus Calmette-Guérin; ND, not documented in the medical and public health records that were available during the case reviews.

isolated the patient at home, but a contact investigation did not identify any infected contacts. The patient completed 9 months of isoniazid, rifampin, and ethambutol under directly observed therapy. Public health and medical staff confirmed they were aware of the patient's bladder cancer diagnosis, history of intravesical BCG therapy, and the possibility that the positive *M. tuberculosis* complex culture was BCG-related before genotyping results were available.

Case Report B

A US-born, non-Hispanic white man in his 80s with a history of bladder cancer sought care from his primary physician because of left hip pain. Five months before the patient's hip pain began, he had undergone intravesical BCG instillation for treatment of bladder cancer without complications. A month later, he underwent bilateral total hip replacement. *M. bovis* was isolated from culture of synovial fluid from the patient's left hip joint. A chest radiograph displayed a nodular infiltrate in the right upper lung lobe. At the time of the case review, the patient was receiving isoniazid, rifampin, and ethambutol under directly observed therapy. Public health and medical staff confirmed they were aware of the patient's bladder cancer diagnosis, history of intravesical BCG, and the possibility that the positive *M. bovis* culture was BCG-related before genotyping results were available.

Case Report C

A US-born, non-Hispanic white man in his 70s experienced left parotid swelling, weight loss, night sweats, and poor appetite. He had a history of bladder cancer treated with intravesical BCG instillations. Nucleic acid amplification test on the left parotid tissue specimen was positive for *M. tuberculosis* complex, and culture grew *M. tuberculosis* complex. Drug-susceptibility testing indicated resistance to pyrazinamide, which is suggestive of *M. bovis* infection. The patient's healthcare provider interpreted chest radiography performed at that time as normal. The patient began isoniazid, ethambutol, and rifampin under directly observed therapy; he died 4 months later. Whether or when the healthcare providers suspected BCG as the etiology of the swelling was uncertain.

Case Report D

A US-born, non-Hispanic white man in his 80s had a history of bladder cancer treated with intravesical BCG for 6 weeks. That same year, he also received a left knee replacement. Four years later, the patient visited his health care provider because of left knee pain of 3 months' duration. A review of symptoms noted an absence of fever or chills. *M. bovis* was subsequently isolated from culture of a left knee aspirate. The patient received isoniazid, rifampin, and ethambutol under directly observed therapy for 9 months. Public health and medical staff confirmed they were aware of the patient's bladder cancer diagnosis, history of intravesical BCG, and the possibility that the positive *M. tuberculosis* complex culture was BCG-related before genotyping results were available.

Discussion

Although CDC advises health departments not to report conditions caused by BCG strains of *M. bovis* to NTSS, 26 states reported 118 BCG cases during 2004–2015. Apart from a few cases occurring among non–US-born young children, BCG cases occurred primarily among US-born older white men. These demographics mirror the primary populations who receive BCG: young children born in TB-endemic countries and older men with bladder cancer (*6*,*7*).

Regardless of age distribution, these patients with BCG typically had extrapulmonary disease, whereas TB patients had pulmonary involvement. This difference probably reflects distinct routes of transmission: *M. bovis* BCG transmission can occur by either intradermal vaccination or intravesical therapy for bladder cancer, whereas *M. tuberculosis* transmission is typically airborne. Non-BCG *M. bovis* typically results from a foodborne transmission route and reportedly has a propensity for extrapulmonary disease (*8*,*9*).

Discerning between actual TB cases and medical complications of BCG therapy is crucial because their clinical and public health management differs. TB treatment is not indicated when BCG is isolated from otherwise asymptomatic persons (*10*,*11*). *M. bovis* BCG acquisition is a rare healthcare-associated event that might require a different type of investigation from the traditional TB contact investigation (*12*). To make that differentiation, clinicians can ask about a history of BCG vaccination or bladder cancer. They can also examine the organism's drug-susceptibility results; similar to other *M. bovis* strains, BCG is naturally resistant to pyrazinamide. Genotyping results, available through the National Tuberculosis Genotyping Service, can also help health departments identify BCG strains.

Healthcare providers should ask any adult with newly diagnosed TB and a history of bladder cancer detailed questions about previous cancer therapy, even if therapy occurred years earlier. One of the older male patients we describe had been treated for cancer a decade before, and others have reported BCG complications up to 17 years later (*13*).

Although the 13 cases that we reviewed in detail (Table 2) were a convenience sample, they illustrate a wide spectrum of complications of intravesical BCG therapy for bladder cancer. Other studies have reported a preponderance of disseminated over localized BCG complications (*2*,*14*). In contrast, isolation of BCG from the urine in the absence of clinical signs or symptoms might not require treatment because BCG can be isolated from the urine of asymptomatic patients for up to 16.5 months after completion of the instillation course (*15*). Basing TB treatment decisions on such specimens, without consideration of the clinical picture and patient history, can lead to misdiagnosis and unnecessary treatment.

Numerous published case reports have documented complications induced by BCG vaccination among children that can lead to diverse manifestations, including skeletal complications, although complications rarely occur among immunocompetent children (*16*). Six of the 118 BCG cases we describe here involved children ≤5 years of age. All had skeletal complications. Most were born in countries where BCG vaccination is routine for all newborns (*17*). Because BCG is not a routine childhood vaccine in the United States, these results support our expectation that vaccination would be the etiology for few BCG cases in NTSS.

This review has certain limitations. NTSS collects limited information about medical conditions other than TB; therefore, we cannot determine whether children with positive BCG cultures had a history of BCG vaccination or whether adults with positive BCG cultures had received treatment for bladder cancer. We also did not know for all 118 BCG cases whether a patient's BCG therapy had unintentionally led to disseminated disease complications or whether a positive BCG culture was simply incidental (e.g., from the urine of an otherwise asymptomatic person). For this reason, we collected additional data for a subset of 13 reported BCG cases. Although we learned that all of these 13 case-patients had been treated with intravesical BCG, we could not ascertain whether the other 99 adults with BCG disease also had been treated with intravesical BCG for bladder cancer.

Because CDC TB surveillance instructions discourage reporting cases with positive BCG cultures to NTSS and 24 states did not report any BCG cases during 2004–2015, additional BCG cases might have been diagnosed but not reported. Therefore, the case-patients we describe might not represent the epidemiology of BCG disease in the United States. Furthermore, the actual extent of BCG-related complications might be higher.

In conclusion, our review reveals the importance of bladder cancer treatment, rather than BCG vaccination, in the epidemiology of BCG cases in the United States. To avoid misclassification of TB disease, CDC instructions exclude BCG cases from national surveillance for TB. To further clarify that BCG cases should not be reported to NTSS, this exclusion could be explicitly added to the national TB surveillance case definition. Regardless of CSTE case definitions or CDC guidance, adverse events related to BCG or any other medical device or medication should still be reported to the US Food and Drug Administration's MedWatch (*18*). Nonetheless, distinguishing medical complications of BCG therapy from actual cases of TB disease enables optimal clinical and public health management for individual patients. In addition, early distinction of probable BCG cases from TB cases by public health agencies could prevent unnecessary use of public health resources to respond to these cases.

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Epidemiology of Extrapulmonary Tuberculosis among Inpatients, China, 2008–2017

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We investigated the epidemiology of extrapulmonary tuberculosis (TB) among patients admitted to Beijing Chest Hospital, Beijing, China, during January 2008–December 2017. Of 19,279 hospitalized TB patients, 33.4% (6,433) had extrapulmonary TB and 66.6% (12,846) had pulmonary TB. The most frequent forms of extrapulmonary TB observed were skeletal TB (41.1%) and pleural TB (26.0%). Younger, female patients from rural areas were more likely to have extrapulmonary TB. However, patients with diabetes mellitus were less likely to have extrapulmonary TB compared with patients without diabetes. A higher proportion of multidrug-resistant (MDR) TB was observed among patients with extrapulmonary TB than among patients with pulmonary TB. We observed a large increase in MDR TB, from 17.3% to 35.7%, for pleural TB cases. The increasing rate of drug resistance among extrapulmonary TB cases highlights the need for drug susceptibility testing and the formulation of more effective regimens for extrapulmonary TB treatment.

Tuberculosis (TB) is a major public health concern worldwide (*1*). The World Health Organization (WHO) estimated 10.4 million incident cases of TB and 1.67 million TB deaths in 2017 (*1*). Although TB most commonly affects the lungs, it also can affect other sites, a form known as extrapulmonary TB (*2*). The most common anatomic sites affected by extrapulmonary TB are lymph nodes, pleura, bones, and joints, urogenital tract, and meninges (*3*). Several types of extrapulmonary TB, such as tuberculous meningitis and miliary TB, cause substantial rates of illness and death in various populations (*4*). Of the 6.3 million new TB cases recognized by WHO in 2017, 16% were extrapulmonary TB cases; incidence rates ranged from 8% in the Western Pacific Region to 24% in the Eastern Mediterranean Region (*1*). In the past few decades, studies from high-income countries have shown that extrapulmonary TB cases comprise an increased proportion of total TB cases (*5*,*6*). Despite these data, research on extrapulmonary TB is limited, possibly because extrapulmonary TB is less transmissible than pulmonary TB (*6*–*8*).

Because extrapulmonary TB can affect virtually any organ, it produces a wide spectrum of clinical manifestations that pose challenges to effective disease diagnosis and management (*3*,*9*). In general, extrapulmonary TB affects persons with diabetes and HIV, as well as young children (<15 years of age) and older adults (>65 years of age) (*10*). Recent studies have revealed that women and persons who migrate from areas of high TB incidence are at greater risk for extrapulmonary TB (*10*–*12*). In addition, extrapulmonary TB anatomic sites exhibit variability related to patient geographic location, population group, and a wide variety of host factors (*13*,*14*). Previous studies conducted on extrapulmonary TB have been in high-income countries, thus indicating the need to understand extrapulmonary TB in low- and middle-income countries.

China has the second highest number of TB cases in the world, accounting for ≈9% of global TB incidence (*1*). Nationwide survey data indicated that China has reduced smear-positive TB prevalence by >50%, from 170 cases/100,000 population in 1990 to 59 cases/100,000 population in 2010 (*15*). Despite past success in controlling pulmonary TB, limited available epidemiologic information indicates that extrapulmonary TB incidence may be increasing in China (*16*). In this study, we retrospectively reviewed the clinical manifestations of extrapulmonary TB in hospitalized patients at Beijing Chest Hospital (Beijing, China) during January 2008–December 2017. Our aim was to analyze the proportions of various extrapulmonary TB forms and to identify independent risk factors associated with the occurrence of extrapulmonary TB.

Materials and Methods

Data Sources and Collection

We performed a descriptive analysis of demographic and clinical data of inpatients treated for extrapulmonary TB

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at Beijing Chest Hospital from January 1, 2008, through December 31, 2017. Beijing Chest Hospital, designated a National Clinical Center on Tuberculosis, is a 900-bed hospital that delivers specialized treatment for TB and thoracic tumors. This hospital provides tertiary care for TB patients from Beijing municipality and parts of surrounding regions and accepts severe TB case referrals for patients originating from other regions of China. In addition, the hospital, under the oversight of the hospital authority, ensures compliance with strict protocols for patient care.

Patients who come to the hospital with TB symptoms and who are presumed to have TB disease are asked to provide 2 sputum samples for laboratory testing. Samples are routinely tested for pulmonary and extrapulmonary TB, including microscopic examination, mycobacterial culture, or molecular testing. In addition, tissue specimens also are analyzed in the histology laboratory. Active TB disease is diagnosed by meeting ≥1 of the following criteria: 1 smear-positive or culture-positive specimen; positive histology (i.e., the presence of acid-fast bacilli in Zeihl-Neelsen–stained histological section); or TB history and strong clinical and radiographic evidence supporting the presence of active TB. The diagnosis of extrapulmonary TB followed the definitions and categories defined in the national guidelines for diagnosis of extrapulmonary TB in China (*17*).

During the timeframe of our analysis, 20,534 patients meeting the criteria for active TB were hospitalized at Beijing Chest Hospital. Hospitalization criteria included TB requiring surgery, severe or complicated TB, and admission at patients' request. Cases are categorized by major disease anatomic site and reported as either pulmonary TB or extrapulmonary TB. For our study, we excluded patients who were co-infected with pulmonary TB and extrapulmonary TB (1,255/20,534) from our descriptive statistics and risk factor analyses because their illnesses were not distinctly classifiable as either pulmonary TB or extrapulmonary TB (*5*). According to classification schemes described in previous reports, the extrapulmonary TB group included any extrapulmonary disease forms (i.e., pleural, lymphatic, skeletal, genitourinary, meningeal, and others) (*5*). In our study, disseminated TB refers to active TB detected in >1 noncontiguous anatomic site, miliary TB, or *Mycobacterium tuberculosis* isolated from blood samples.

The hospital's electronic patient record system documented inpatient illness, treatment, and care over time. We collected multiple demographic and clinical variables from electronic patient records to conduct comparative analyses between extrapulmonary TB and pulmonary TB groups, including sex, age, ethnicity, place of residence, previous TB episode, and concurrent conditions. Beijing Chest Hospital screens all patients for diabetes, according to national guidelines. These guidelines stipulate a fasting blood glucose test be performed by using venous plasma and a biochemical analyzer; a fasting blood glucose value ≥7.0 mm (126 mg/dL) was deemed a positive result for diabetes (*18*).

The hospital's clinical laboratory tested all positive mycobacterial cultures for drug susceptibility by using an absolute concentration method, according to WHO guidelines (*19*). Because clinical laboratories have performed routine drug-susceptibility testing for first- and second-line drugs since the 1990s, we compared extrapulmonary TB and pulmonary TB cases on the basis of previously established in vitro drug-susceptibility profiles for multidrug-resistant TB (MDR TB, defined as resistant to both rifampin and isoniazid) and extensively drug-resistant TB (XDR TB, defined as MDR with additional resistance to any fluoroquinolone and second-line injectable drug).

Statistical Analysis

We tabulated numbers and proportions of cases along with various demographic and clinical factors contributing to TB incidence and used univariable and multivariable logistic regression models to investigate factors associated with extrapulmonary TB. Multivariable models were built by using forward stepwise logistic regression procedures (with inclusion if $p<0.05$). For each anatomic site of disease, we tabulated the proportion of TB cases also by the year that a patient sought treatment. To analyze trends in the proportion of cases by anatomic site of disease, we used the χ^2 trend test. We performed all calculations by using SPSS version 17.0 for Microsoft Windows (SPSS Inc., http://www.spss.com.hk). For p values <0.05, differences in distribution of categorical variables across various classifications were evaluated for statistical significance. A p value <0.05 for the χ^2 trend test indicated that there was a significant change in the proportions of pulmonary TB and extrapulmonary TB over the duration of the study period.

Ethics Statement

This study was approved by the ethics committee of Beijing Chest Hospital (grant no. 2016-29), which is affiliated with Capital Medical University. This study used data collected from patient records while maintaining patient anonymity. Because this study presented no more than minimal risk of harm to patient subjects, the institutional review board approved a waiver of patient informed consent.

Results

Extrapulmonary TB Cases

During 2008–2017, a total of 20,534 patients with TB were hospitalized in Beijing Chest Hospital (Table 1). Of these patients, 62.6% (12,846) had pulmonary TB, 31.3% (6,433) had extrapulmonary TB, and 6.1% (1,255) had concurrent extrapulmonary TB and pulmonary TB. We excluded patients with concurrent extrapulmonary TB and pulmonary

Table 1. General characteristics of pulmonary TB and extrapulmonary TB patients, China, 2008–2017*

Characteristic	No. (%) patients			Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
	Pulmonary TB	Extrapulmonary TB	Total				
Sex							
M	8,757 (70.1)	3,736 (29.9)	12,493	Referent	–	Referent	–
F	4,089 (60.3)	2,697 (39.7)	6,786	1.55 (1.45–1.65)	<0.01	1.37 (1.27–1.47)	<0.001
Age group, y							
<25	2,024 (56.4)	1,563 (43.6)	3,587	2.51 (2.28–2.77)	<0.01	1.72 (1.53–1.93)	<0.001
25–44	3,539 (62.7)	2,102 (37.3)	5,641	1.93 (1.77–2.11)	<0.01	1.55 (1.40–1.73)	<0.001
45–64	4,078 (69.6)	1,782 (30.4)	5,860	1.42 (1.30–1.56)	<0.01	1.30 (1.17–1.44)	<0.001
≥65	3,205 (76.5)	9,86 (23.5)	4,191	Referent	–	Referent	–
Residence							
Urban	9,789 (68.3)	4,537 (31.7)	1,4326	Referent	–	Referent	–
Rural	3,057 (61.7)	1,896 (38.3)	4,953	1.34 (1.25–1.43)	<0.01	1.32 (1.22–1.43)	<0.001
Treatment history							
New case	10,967 (64.2)	6,118 (35.8)	17,085	Referent	–	Referent	–
Retreated case	1,879 (85.6)	315 (14.4)	2,194	0.30 (0.27–0.34)	<0.01	0.24 (0.20–0.27)	<0.001
Diabetes							
No	12,502 (66.3)	6,352 (33.7)	18,854	Referent	–	Referent	–
Yes	344 (80.9)	81 (19.1)	425	0.46 (0.36–0.59)	<0.01	0.54 (0.41–0.70)	<0.001
Culture determination							
Negative	6,256 (54.8)	5,152 (45.2)	11,408	Referent	–	Referent	–
Positive	6,080 (88.9)	758 (11.1)	6,838	0.15 (0.14–0.17)	<0.01	0.16 (0.15–0.18)	<0.001
Smear determination							
Negative	8,343 (60.0)	5,570 (40.0)	13,913	Referent	–		
Positive	4,000 (95.8)	174 (4.2)	4,174	0.07 (0.06–0.08)	<0.01		
DST							
MDR	684 (89.2)	83 (10.8)	767	1.25 (0.97–1.61)	0.084		
XDR	322 (89.2)	39 (10.8)	361	1.25 (0.88–1.77)	0.216		
Other	3,716 (91.1)	361 (8.9)	4,077	Referent	–		

*DST, drug susceptibility testing; MDR, multidrug-resistant; OR, odds ratio; TB, tuberculosis; XDR, extensively drug-resistant.

TB from our analyses because the primary site for TB infection could not be determined. Of the 6,433 extrapulmonary TB cases included in our study, the most frequent forms were skeletal TB (41.1% [2,643]) and pleural TB (26.0% [1,673]). Additional forms of extrapulmonary TB were meningeal TB (6.8% [440]), disseminated TB (6.6% [427]), and lymphatic TB (5.2% [333]) (Figure 1).

Demographic and Risk Factor Characteristics of Extrapulmonary TB Cases

We summarized characteristics of extrapulmonary TB patients compared with pulmonary TB patients (Table 1). More women were afflicted with extrapulmonary TB (39.7% [2,697/6,786]; adjusted odds ratio [aOR] 1.37, 95% CI 1.27–1.47]) than with pulmonary TB (29.9% [4,089/6,786]; aOR 1.37, 95% CI 1.27–1.47]). The distribution of extrapulmonary TB also differed among age groups. Using patients >65 years of age as a control group, we found that young persons (<25 years of age) were more likely to have extrapulmonary TB (aOR 1.72, 95% CI 1.53–1.93) and that patients exhibited decreasing extrapulmonary TB risk with increasing age (aOR 1.55, 95% CI 1.40–1.73 for patients 25–44 years of age; aOR 1.30, 95% CI 1.17–1.44 for patients 45–64 years of age). In addition, patients from rural areas had significantly higher odds of having extrapulmonary TB compared with those from urban areas (aOR 1.32, 95% CI 1.22–1.43). Extrapulmonary TB patients also were less likely to have a previous TB episode than did pulmonary TB patients (aOR 0.24, 95% CI 0.20–0.27). Patients with diabetes had lower risk for extrapulmonary TB than did patients without diabetes (aOR 0.54, 95% CI 0.41–0.70).

TB diagnosis was confirmed by a positive culture in 12.8% (758/5,910) of extrapulmonary TB cases, which was significantly lower than the percentage for pulmonary TB cases (49.3%, 6,080/12,336; p<0.01). Low rates of positive cultures were noted for several extrapulmonary TB forms, especially lymphatic TB cases (2.1%, 6/292) and meningeal TB cases (4.5%, 17/379). The highest rates of positive cultures were observed for pleural TB cases (12.5%, 198/1,581) and disseminated TB cases (23.9%, 88/368). Of 758 culture-positive extrapulmonary TB cases, 483 (63.7%) cultures yielded drug susceptibility results. Of note, we identified MDR TB in 17.2% (83/483) and XDR TB in 8.1% (39/483) of extrapulmonary TB cases. These rates were both significantly higher than the corresponding rates for pulmonary TB cases (14.3% for MDR TB, 6.8% for XDR TB; p<0.01).

We further summarized associations of extrapulmonary TB with various demographic and clinical characteristics (Tables 2, 3). Of the 6,433 extrapulmonary TB cases included in our study, women were more likely than men to have skeletal TB (aOR 1.64, 95% CI 1.49–1.81), disseminated TB (aOR 1.68, 95% CI 1.36–2.08), lymphatic

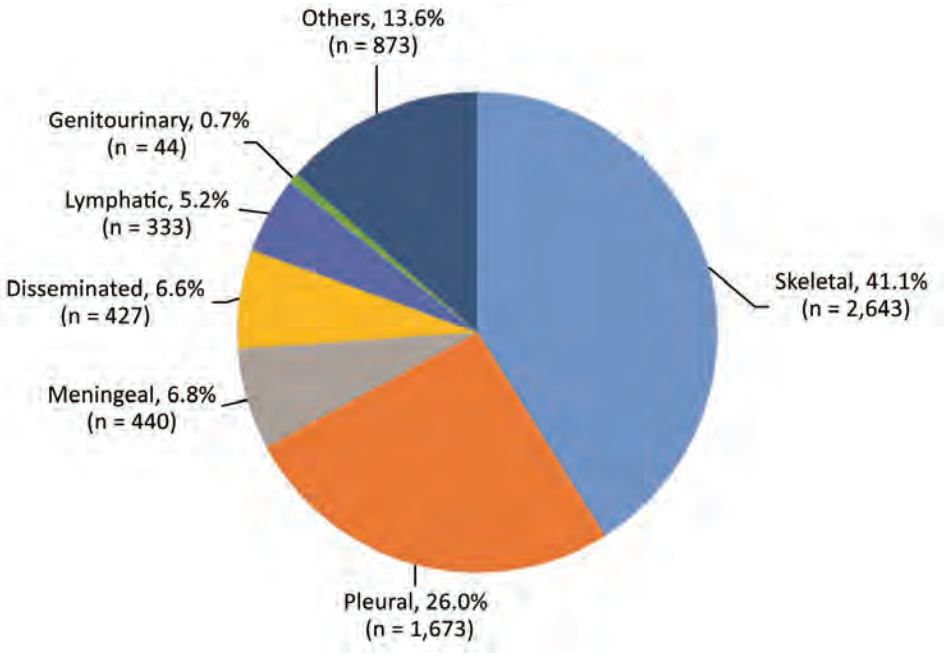

Figure 1. Extrapulmonary tuberculosis disease sites among 6,433 patients in China, 2008–2017.

TB (aOR 4.11, 95% CI 3.17–5.34), and genitourinary TB (aOR 5.74, 95% CI 2.77–11.90) but were less likely to have pleural TB (aOR 0.76, 95% CI 0.67–0.86). Patients from urban areas had a higher frequency of lymphatic TB (aOR 1.92, 95% CI 1.35–2.73) than patients from rural areas. By contrast, patients from rural areas were more likely to have skeletal TB (aOR 0.63, 95% CI 0.56–0.70), meningeal TB (aOR 0.68, 95% CI 0.54–0.86), and disseminated TB (aOR 0.69, 95% CI 0.54–0.87) than patients from urban areas. Of note, concurrent diabetes decreased pleural TB risk (aOR 0.29, 95% CI 0.16–0.54) but not risks for contracting other types of extrapulmonary TB.

Trends of Different TB Forms

We further analyzed trends by anatomic TB infection site from 2008 through 2017 (Figure 2). During the past decade, the proportion of extrapulmonary TB cases significantly increased from 29.8% to 31.4% ($p<0.01$) (Figure 2, panel A). Among extrapulmonary TB cases, the largest increase was seen in pleural TB, where the proportion of total extrapulmonary TB cases increased from 17.3% to 35.7% ($p<0.01$). A significant decrease in percentage of lymphatic TB within the total pulmonary TB case burden was observed (8.1% in 2008 vs. 3.2% in 2017; $p<0.01$). Meanwhile, we found no considerable differences in relative proportions of other anatomic sites in extrapulmonary TB disease between 2008 and 2017 (Figure 2, panel B).

Discussion

We describe the epidemiologic and clinical characteristics of extrapulmonary TB patients in a hospital in northern China. Our data show that skeletal TB is the predominant form of extrapulmonary TB among inpatients in this region, accounting for ≈41% of all extrapulmonary TB cases. Previous reports have indicated significant differences in relative frequencies of anatomic sites of infection by geographic region. For instance, lymph nodes are the most common site of extrapulmonary TB in the Netherlands (39%), the United States (40%), and the United Kingdom (37%) (*2*,*5*,*9*), whereas pleural TB is the most prevalent form of extrapulmonary TB in Poland (36%) and Romania (58%) (*3*). We suggest two possible reasons for the disparity in the predominant site of extrapulmonary TB in China compared with reports from other countries. First, bacillus Calmette-Guérin (BCG) immunization provides differential protective efficacy against various forms of TB but is not widely administered in many countries (*20*,*21*). Therefore, we hypothesize that nationwide BCG immunization in China may be associated with different affected sites of extrapulmonary TB compared with nations in which BCG is not administered. Second, surveillance studies conducted in the European Union and Benin have shown that lymphatic TB is more frequently observed in children <15 years of age (*6*,*8*). Such age-related differences in lymphatic TB may partially be explained by factors involved in cellular immune system development and maturation (*22*). However, the lower frequency of lymphatic TB observed in this study may be because of the small sample size of younger patients compared with those of other age groups.

Consistent with other studies, our study found an association between extrapulmonary TB and either female sex or absence of previous TB episode (*2*,*5*). We also found the prevalence of extrapulmonary TB substantially decreases

Table 2. Distribution of extrapulmonary tuberculosis by demographic and clinical characteristics, China, 2008–2017

Characteristics	Skeletal, %	Pleural, %	Meningeal, %	Disseminated, %	Lymphatic, %	Genitourinary, %	Others, %
Sex							
M	1,446 (38.7)	1,178 (31.5)	263 (7.0)	219 (5.9)	101 (2.7)	10 (0.3)	519 (13.9)
F	1,197 (44.4)	495 (18.4)	177 (6.6)	208 (7.7)	232 (8.6)	34 (1.3)	354 (13.1)
Age groups, y							
<25	520 (33.3)	470 (30.1)	128 (8.2)	154 (9.9)	84 (5.4)	6 (0.4)	201 (12.9)
25–44	736 (35.0)	561 (26.7)	170 (8.1)	139 (6.6)	162 (7.7)	17 (0.8)	317 (15.1)
45–64	914 (51.3)	362 (20.3)	102 (5.7)	72 (4.0)	62 (3.5)	18 (1.0)	252 (14.1)
≥65	473 (48.0)	280 (28.4)	40 (4.1)	62 (6.3)	25 (2.5)	3 (0.3)	103 (10.4)
Residence							
Rural	941 (49.6)	385 (20.3)	128 (6.8)	130 (6.9)	47 (2.5)	13 (0.7)	252 (13.3)
Urban	1,702 (37.5)	1,288 (28.4)	312 (6.9)	297 (6.5)	286 (6.3)	31 (0.7)	621 (13.7)
Treatment history							
New case	2,585 (42.2)	1,631 (26.7)	417 (6.8)	406 (6.6)	304 (5.0)	42 (0.7)	733 (12.0)
Retreated case	58 (18.4)	42 (13.3)	23 (7.3)	21 (6.7)	29 (9.2)	2 (0.6)	140 (44.4)
Diabetes							
No	2,591 (40.8)	1,660 (26.1)	437 (6.9)	423 (6.7)	332 (5.2)	44 (0.7)	865 (13.6)
Yes	52 (64.2)	13 (16.0)	3 (3.7)	4 (4.9)	1 (1.2)	0	8 (9.9)
Culture determination							
Negative	2,204 (42.8)	1,383 (26.8)	362 (7.0)	280 (5.4)	286 (5.6)	35 (0.7)	602 (11.7)
Positive	277 (36.5)	198 (26.1)	17 (2.2)	88 (11.6)	6 (0.8)	4 (0.5)	168 (22.2)

with advancing age, whereas the reverse is true for pulmonary TB. These opposing trends may be related to the dynamic changes in immunity during aging (*23*). Previous investigations have demonstrated functional decline of monocytes and macrophages during aging, whereas the production of proinflammatory cytokines by mononuclear cells in older persons (mean age 80.8 ±2.1 years) is higher than in young persons (mean age 26.8 ±0.8 years) (*24*). Nevertheless, our findings indicate that the mechanism by which *M. tuberculosis* affects extrapulmonary sites might involve different processes in extrapulmonary TB compared with those in pulmonary TB. Further research is needed to investigate the role of various immune responses to *M. tuberculosis* infection within different anatomic sites.

In addition, we found an association between diabetes and increased pulmonary TB incidence but not increased

Table 3. Multivariate analysis of risk factors for 7 types of extrapulmonary TB compared with pulmonary TB, China, 2008–2017*

	Odds ratio (95% CI)						
Patient characteristic	Skeletal, n = 2,643	Pleural, n = 1,673	Meningeal, n = 440	Disseminated, n = 427	Lymphatic, n = 333	Genitourinary, n = 44	Others, n = 873
Sex							
M	Referent	Referent	Referent	Referent	Referent	Referent	Referent
F	**1.64 (1.49–1.81)**	**0.76 (0.67–0.86)**	1.15 (0.93–1.43)	**1.68 (1.36–2.08)**	**4.11 (3.17–5.34)**	**5.74 (2.77–11.90)**	**1.27 (1.09–1.48)**
Age group, y							
<25	1.14 (0.97–1.33)	**2.26 (1.88–2.70)**	**3.24 (2.17–4.86)**	**2.32 (1.65–3.26)**	**3.54 (2.14–5.85)**	2.26 (0.46–11.70)	**1.92 (1.45–2.54)**
25–44	1.08 (0.93–1.25)	**1.86 (1.57–2.20)**	**3.06 (2.09–4.49)**	1.38 (0.98–1.93)	**4.93 (3.09–7.87)**	3.48 (0.76–15.93)	**2.06 (1.59–2.66)**
45–64	**1.33 (1.16–1.52)**	1.01 (0.85–1.21)	**1.65 (1.11–2.46)**	0.78 (0.54–1.12)	**2.09 (1.26–3.46)**	**5.85 (1.33–25.78)**	**1.83 (1.42–2.36)**
≥65	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Residence							
Rural	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Urban	**0.63 (0.56–0.70)**	0.94 (0.82–1.07)	**0.68 (0.54–0.86)**	**0.69 (0.54–0.87)**	**1.92 (1.35–2.73)**	0.91 (0.45–1.85)	**0.83 (0.70–0.98)**
Treatment history							
New case	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Retreated case	**0.06 (0.04–0.09)**	**0.14 (0.10–0.20)**	**0.24 (0.14–0.40)**	**0.31 (0.19–0.49)**	**0.36 (0.22–0.58)**	0.29 (0.07–1.20)	0.95 (0.77–1.17)
Diabetes							
No	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Yes	0.84 (0.61–1.16)	**0.29 (0.16–0.54)**	0.25 (0.06–1.03)	0.62 (0.23–1.68)	0.18 (0.02–1.30)	–	**0.34 (0.15–0.77)**
Culture determination							
Negative	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Positive	**0.13 (0.12–0.15)**	**0.15 (0.13–0.18)**	**0.05 (0.03–0.08)**	**0.36 (0.29–0.47)**	**0.02 (0.01–0.06)**	**0.14 (0.05–0.38)**	**0.31 (0.26–0.37)**

*The pulmonary TB group was set as a reference for each comparison in multivariate analysis. Odds ratios were adjusted for all variables used in this model. Bold indicates significance. TB, tuberculosis.

extrapulmonary TB incidence. Our findings are consistent with the results of studies from Brazil and Taiwan (*7*,*25*), but opposing results have been reported in the United States, where diabetes appears to increase extrapulmonary TB risk (*26*). Previous studies have shown that extrapulmonary TB may be associated with immunosuppression more than is pulmonary TB (*27*,*28*). However, concurrence of diabetes and TB might result in abnormalities of innate immune function, thus leading to increased extrapulmonary TB incidence within this population group, as seen in studies of patients with concurrent HIV and TB infections (*10*). An additional recent report from the southeastern United States revealed that immunosuppression was strongly and positively associated with risk for TB infection at meningeal and disseminated sites and less likely associated with risk for pleural TB (*29*). In view of these observations, the contradictory conclusions regarding the association between extrapulmonary TB and diabetes in previous studies may be related to geographic factors that influence the relative proportions of various extrapulmonary TB forms in patients living in different regions.

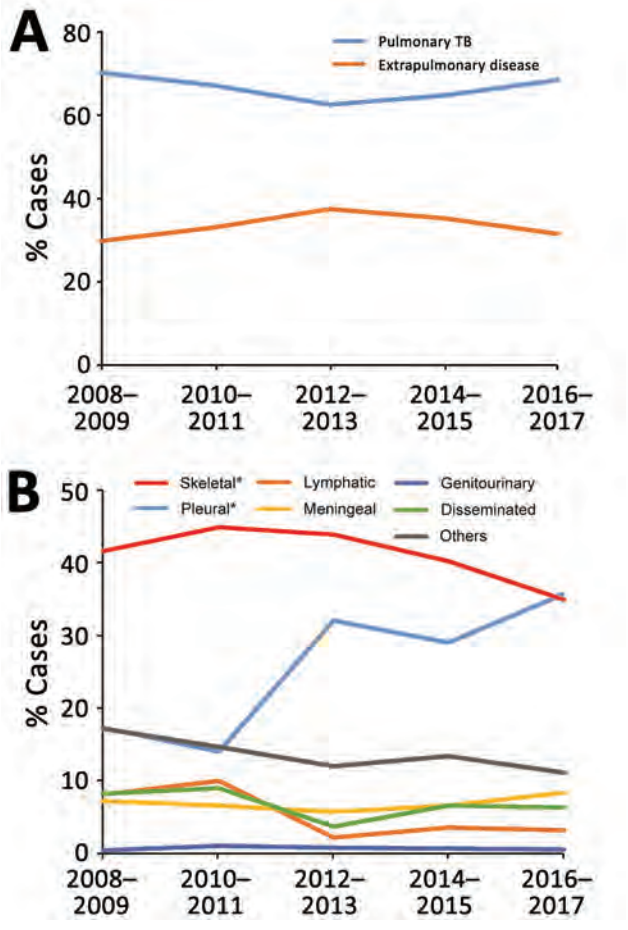

Figure 2. Trends in extrapulmonary TB and pulmonary TB, China, 2008–2017. A) Relative rates of extrapulmonary TB and pulmonary TB. B) Relative rates of different extrapulmonary TB forms. *p<0.01 compared with pulmonary TB group. Cases reported in 2-year periods. TB, tuberculosis.

The increasing trend in pleural TB observed during the past decade should also be discussed here. In a previous study conducted in San Francisco, California, USA, Ong et al. found that pleural TB differs from other forms of extrapulmonary TB and exhibits the highest clustering rate of all forms of TB, suggesting that pleural TB clustering could be an indicator of recent transmission (*30*). The increasing epidemic of pleural TB recently observed in China may be driven by primary transmission. In view of this point, our data emphasize the importance of studying pleural TB and understanding the implications of this disease for achieving TB control in China.

In contrast to studies from countries with low TB incidence (*5*,*6*), we observed a higher proportion of MDR TB in extrapulmonary TB cases than in pulmonary TB cases in China. Numerous epidemiologic studies have documented that Beijing genotype strains, especially MDR TB, are strongly associated with drug resistance, suggesting increased bacterial fitness (*31*). We speculate that the higher frequency of MDR TB among extrapulmonary TB cases might be attributed to the current epidemic of the Beijing genotype in China. Despite a lack of experimental evidence, several studies regarding the molecular characteristics of extrapulmonary TB strains in China demonstrated the presence of the Beijing genotype in 88% of skeletal TB cases and in 80.0% of meningitis TB cases, rates that are higher than the average rate (62%) of the Beijing genotype among pulmonary TB cases in China (*32*,*33*). Because of the challenges of diagnosing and obtaining positive cultures for extrapulmonary TB, treating patients for this disease has been mainly empirical rather than based on drug susceptibility patterns of infecting strains. Such empirical treatment for patients with extrapulmonary TB no doubt delays effective treatment and may too often lead to a poor prognosis. Recently, Xpert MTB/RIF (Cepheid, http://www.cepheid.com), an automatic molecular assay, has been endorsed by the WHO based on a systematic review demonstrating its excellent performance for detecting *M. tuberculosis* and rifampin resistance in various types of specimens (*34*). Further investigation validates its use for diagnosis of extrapulmonary TB (*35*). In view of the high prevalence of drug resistance in extrapulmonary TB, our data suggest that the application of Xpert MTB/RIF is essential for the formulation of appropriate treatment regimens for patients with extrapulmonary TB in China.

Our study is subject to some limitations. First, our retrospective research only collected data of extrapulmonary TB cases from Beijing Chest Hospital rather than from national surveillance data, possibly limiting the overall relevant scope of our findings. Unfortunately, because extrapulmonary TB does not contribute substantially to

the transmission of TB, it has been neglected by China's National TB Control Program, and consequently the acquisition of national surveillance data has been difficult. Further surveys on the basis of data from TB hospitals located in different regions will be essential for obtaining improved extrapulmonary TB estimates in China. Second, only 12.8% (758/5,910) of patients with extrapulmonary TB yielded positive bacterial cultures, compared with 49.3% (6,080/12,336) of patients with pulmonary TB. Thus, most extrapulmonary TB was diagnosed only from clinical symptoms. Extrapulmonary TB can manifest with a variety of clinical symptoms that can mimic symptoms of other pathogens. Therefore, the lack of laboratory verification of extrapulmonary TB cases might lead to diagnostic delays, misdiagnoses, resistant strains, and increased mortality rates (*3*). Our data highlight the urgent need for a more accurate test, such as Xpert MTB/RIF or loop-mediated isothermal amplification, to diagnose various forms of extrapulmonary TB. Third, use of new molecular diagnostics, such as Xpert MTB/RIF, likely would have improved extrapulmonary TB patient detection and possibly affected the analysis results. Although Xpert MTB/RIF is endorsed for testing specific specimens from patients suspected of having extrapulmonary TB, the diagnostic criteria of extrapulmonary TB remained unchanged during the study period because Beijing Chest Hospital was unable to obtain a license from the Chinese Food and Drug Administration for its use in smear-negative specimens. Fourth, our study included only inpatients in the final analysis. Rates of extrapulmonary TB obtained for this report might be affected by the higher rates of hospitalization for some types of extrapulmonary TB, such as skeletal and meningeal TB, due to severe clinical symptoms. Fifth, HIV infection has been considered an additional risk factor for extrapulmonary TB. However, patients co-infected with TB and HIV were referred to other hospitals that specialize in HIV treatment. The absence of patients co-infected with HIV and TB in this study precluded analysis of associations between extrapulmonary TB and HIV infection trends in China.

In conclusion, this study describes the epidemiologic and clinical characteristics of patients with extrapulmonary TB in a hospital in China. Our data show that the most frequent forms of extrapulmonary TB are skeletal TB and pleural TB. Women, young persons (<25 years of age), and persons living in rural regions are at high risk for developing extrapulmonary TB, whereas patients with diabetes have lower extrapulmonary TB risk compared with patients without diabetes. We also observed that most extrapulmonary TB is diagnosed from clinical symptoms, suggesting a high likelihood of diagnostic delays and misdiagnosis of extrapulmonary TB cases. In addition, the increased trend of drug-resistant TB among extrapulmonary TB highlights the importance of drug susceptibility testing in successful development of effective treatment regimens for patients with extrapulmonary TB in China.

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Reassortments among Avian Influenza A(H5N1) Viruses Circulating in Indonesia, 2015–2016

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Highly pathogenic avian influenza (HPAI) A(H5N1) viruses have been circulating since 2003 in Indonesia, with major impacts on poultry health, severe economic losses, and 168 fatal laboratory-confirmed human cases. We performed phylogenetic analysis on 39 full-genome H5N1 virus samples collected during outbreaks among poultry in 2015–2016 in West Java and compared them with recently published sequences from Indonesia. Phylogenetic analysis revealed that the hemagglutinin gene of all samples belonged to 2 genetic groups in clade 2.3.2.1c. We also observed these groups for the neuraminidase, nucleoprotein, polymerase, and polymerase basic 1 genes. Matrix, nonstructural protein, and polymerase basic 2 genes of some HPAI were most closely related to clade 2.1.3 instead of clade 2.3.2.1c, and a polymerase basic 2 gene was most closely related to Eurasian low pathogenicity avian influenza. Our results detected a total of 13 reassortment types among HPAI in Indonesia, mostly in backyard chickens in Indramayu.

Highly pathogenic avian influenza viruses (HPAI) continue to be a major global problem for both animal and human health. Since the first outbreak of HPAI A(H5N1) in Guangdong, China, in 1996, these viruses have caused outbreaks in various species of birds globally. HPAI H5N1 is endemic in multiple countries and causes a major impact on poultry health and severe economic losses. In addition, >860 laboratory-confirmed human cases of HPAI H5N1 have been reported to the World Health Organization (WHO). In Indonesia, 200 laboratory-confirmed human cases of avian influenza A(H5N1) have been reported, with a case-fatality rate of 84%, which is higher than the current global case-fatality rate of 53% (*1*). The zoonotic potential of HPAI is a global public health concern, particularly in preventing a potential pandemic (*2*,*3*).

In Indonesia, HPAI H5N1 has been endemic in poultry since 2003 and continues to cause major economic losses to both poultry industry and backyard farms. The disease has been reported in 32/34 provinces, resulting in the death of millions of birds (*4*,*5*) and the closure of many farms in high-incidence areas (*6*). While HPAI H5N1 viruses continuously circulated among poultry in Indonesia during 2003–2010, the hemagglutinin (HA) genes evolved from clade 2.1 into multiple subclades, according to the unified nomenclature system for the HA gene of HPAI H5N1 virus (*7*). In 2012, a new virus classified as clade 2.3.2.1 was detected in ducks, suggesting a new incursion of HPAI H5N1 viruses in Indonesia from other parts of Southeast Asia (*7*–*9*). Vaccination programs have been applied to control the spread of HPAI H5N1 but have not prevented it because of low vaccination coverage and use of unlicensed vaccines. These problems have led to the emergence of antigenically distinct HPAI H5N1 virus clades in Indonesia (*10*). In addition to the continuous circulation of HPAI H5N1 viruses in poultry, transmission to humans has been reported in Indonesia since 2005 (*1*).

Clarifying the epidemiology of HPAI H5N1 requires more intense monitoring of outbreaks of HPAI in Indonesia and performing genetic and phylogenetic analysis on viruses detected during these outbreaks. However, recent information on the genetic divergence of HA, and in particular on other gene segments, is very limited (*8*,*11*–*13*), and samples are often collected in a nonsystematic way. Therefore, the aim of this study was to perform genetic and phylogenetic analysis on recent HPAI H5N1 viruses that

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were obtained from poultry during active searches for outbreaks in West Java, a province of Indonesia. West Java was selected for this study because it has a high poultry density, multiple different farming systems and live-bird markets, and several environmental components that all form risk factors for HPAI H5N1 virus transmission. Moreover, because a high percentage of the land in this region is paddy fields and water sources, free-ranging ducks and chickens undermine the effectiveness of prevention and control measures, resulting in the continuous circulation of the virus (*14*,*15*).

Materials and Methods

Sample Collection

During April 2015–October 2016, district animal health officers of the West Java Animal Health Authority collected samples from birds in 6 districts of West Java Province: Subang, Indramayu, Tasikmalaya, Purwakarta, Sukabumi, and Bandung (Figure 1). The districts were chosen on the basis of the history and reoccurrence of HPAI outbreaks. In addition, these districts have multiple sectors of poultry farms using various production systems and a high density of poultry farms that have ≥50 birds/farm (*4*,*16*).

The samples were collected after detection of clinical signs in or increased mortality of birds. The criteria for increased mortality was set at >5% of the population in birds vaccinated against H5N1 and 10% in those unvaccinated for 2 consecutive days. When the criteria were met, oropharyngeal and cloacal samples were collected from 5 sick birds and pooled into viral transport medium containing brain–heart infusion broth and antimicrobial drugs according to European Union instructions (http://extwprlegs1.fao.org/docs/pdf/eur65757.pdf). The specimens were kept chilled and shipped by overnight courier to the 2 collaborating veterinary laboratories, Disease Investigation Center (DIC) Subang and West Java Animal Health Laboratory Cikole.

Sample Screening

We tested the collected samples in veterinary laboratories using a national protocol for influenza A screening

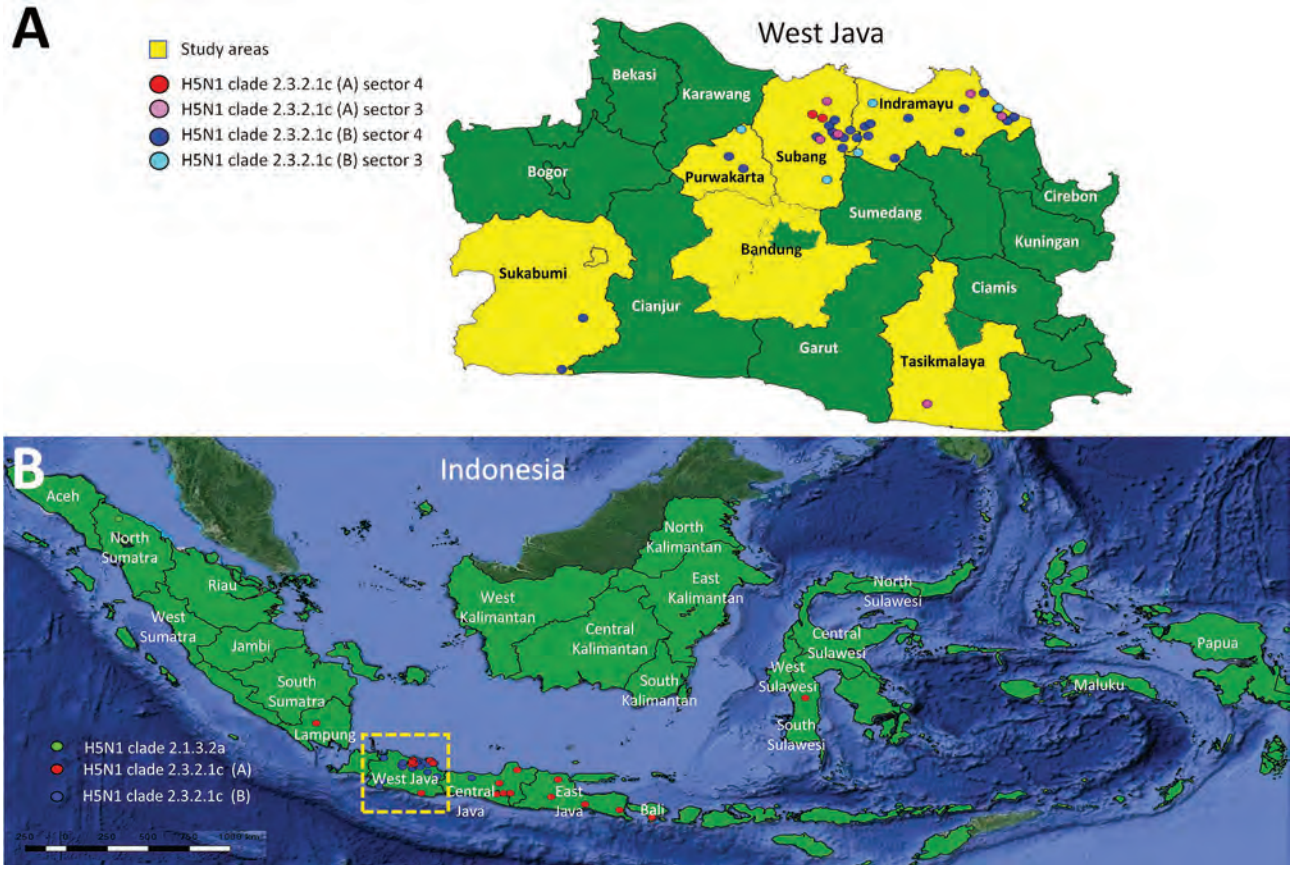

Figure 1. Locations of sampling areas and of different hemagglutinin (HA) clades in study of avian influenza A(H5N1) viruses circulating in Indonesia, 2015–2016. A) West Java Province; B) location of province in Indonesia (box). Data were compiled from this study and additional sequence data of Directorate General for Livestock Services, the Indonesian Ministry of Agriculture, and submitted to GenBank (accession nos. EPI1009273–463).

developed from a real-time reverse transcription PCR (RT-PCR) targeting the matrix gene. Specimens with a cycle threshold value <30 were inactivated using binding buffer of High Pure Viral RNA kit (Roche Applied Science, http://www.roche.com), and transported to the Eijkman Institute for Molecular Biology in Jakarta for Sanger sequencing. Two additional HPAI H5N1–positive samples, collected in 2016 and obtained from the Animal Health Laboratory (AHL) Cikole of West Java, were also inactivated and included in this study for Sanger sequencing.

Sequencing

At the Eijkman Institute, we rescreened the specimens and extracted RNA in accordance with the protocol of the manufacturer and synthesized cDNA by Invitrogen Super Script III First-Strand Synthesis SuperMix (Thermo Fisher Scientific, http://www.thermofisher.com) with Uni12 primer (*17*). On specimens that tested positive in this PCR, we performed additional PCRs to amplify other gene segments present in the samples. We performed amplification of the full genomes of HPAI H5N1 viruses using a 2-step RT-PCR TaKaRa Z-Taq DNA Polymerase (Takara Bio, http://www.takarabio.com) or Toyobo KOD FX Neo (Toyobo, http://www.toyobo-global.com) if the genomes were not successfully amplified using the Takara product.

The primers used were primarily designed by Wageningen Bioveterinary Research. We obtained additional primer sequences from the Australian Animal Health Laboratory and from scientific literature (*17*,*18*) and applied them to unsuccessfully sequenced gene fragments that could not be amplified by standard primers. We purified the amplified PCR products with Roche High Pure PCR Product Purification Kit (Roche) or Zymoclean Gel DNA Recovery Kit (Zymo Research, https://www.zymoresearch.com) for PCR products for which gel separation was necessary, and subsequently sequenced them using a BigDye Terminator v3.1 Cycle Sequencing Kit in an ABI 3130 Genetic Analyzer (both from Thermo Fisher).

Genetic and Phylogenetic Analysis

We assembled and edited sequences with Lasergene SeqMan Pro version 12 (DNASTAR, http://www.dnastar.com) and aligned them by using MUSCLE (*19*). We initially determined HA clade of sequenced HPAI H5N1 viruses using the Highly Pathogenic H5N1 Clade Classification Tool of the Influenza Research Database (https://www.fludb.org) and confirmed results through further phylogenetic analysis (*20*). We estimated phylogenetic inference using the maximum-likelihood method with 1,000 bootstrap replicates (Figure 2; Appendix 1 Figure 1, https://wwwnc.cdc.gov/EID/article/25/3/18-0167-App1.pdf). We chose the most suitable substitution rates and pattern model based on the lowest Akaike information criterion for each alignment. Evolutionary distances were computed using average pairwise distance (APD) between and within sequence groups.

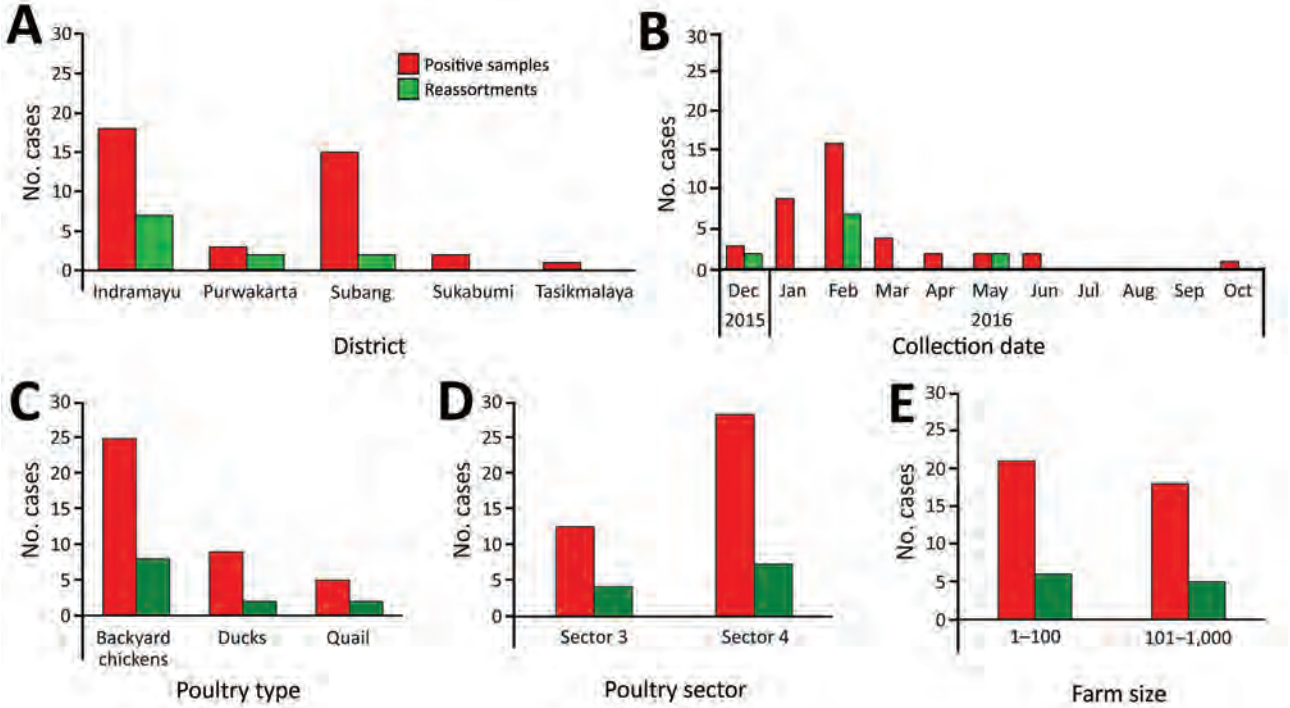

Figure 2. Number of samples in study of avian influenza A(H5N1) viruses circulating in Indonesia, 2015–2016, by district (A), time (B), poultry type (C), poultry sector (D), and farm size (E) from which the complete HPAIV H5N1 genome could be obtained.

Evolutionary analyses and APD were conducted in MEGA6 (*21*).

We aligned the sequences of HPAI H5N1 gene segments collected during this study with reference sequences from GenBank (Appendix 1 Figures 1–8) using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We included in the analysis sequences obtained from viruses detected during other recent outbreaks in Indonesia (2014–2016). These viruses had been collected via passive outbreak surveillance by the Disease Investigation Centres (DIC) in Medan, Sumatra; Wates, Central Java; and Denpasar, Bali, under the Directorate General for Livestock and Animal Health Services and the Indonesian Ministry of Agriculture (DGLAHS-MoA). Viruses were submitted by DIC Wates of DGLAHS-MoA to GenBank, and then downloaded to GISAID (https://www.gisaid.org; accession nos. EPI1009273–463) (Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/25/3/18-0167-App2.xls). For sequencing, we used mainly viruses from original material, as well as some isolates obtained after 1–2 passages in embryonated chicken eggs. We deduced reassortment events on the basis of deviant location of sequences in maximum-likelihood trees of different gene segments.

We used deduced HA amino acid sequences to calculate estimated antigenic distances of viruses based on 27 aa residues in HA, as described previously (*22*). We measured the antigenic distances with 3 HPAI H5N1 strains that are or were routinely used to vaccinate poultry in Indonesia: A/chicken/Legok/2003 (clade 2.1.1); A/chicken/West Java/PWT-WIJ/2006 (clade 2.1.3.2); and A/duck/Sukoharjo/BBVW-1428–9/2012 (clade 2.3.2.1c). We used a *t*-test to estimate the significance of the comparison between the 2 averages of antigenic distances.

Results

Detection and Sequencing of HPAI Viruses

A total of 76 pooled samples were collected from various districts of West Java, Indonesia (Figure 1). We observed the highest number of outbreaks in Indramayu in February 2016. During April 2015–October 2016, a total of 56 of the samples tested positive for influenza A virus by real-time RT-PCR with a cycle threshold value <30. We obtained the complete genome from 37 oropharyngeal samples and 2 swab specimens of the 55 samples and used these sequences in the analysis. Positive samples with complete genomes were mostly collected in Indramayu (46.15%, 95% CI 30.5%–61.8%) and Subang (38.46%, 95% CI 23.2%–53.7%); the highest peak came in February 2016 (41.3%, 95% CI 25.6%–56.5%), and most positive samples came from backyard chickens (69.23%, 95% CI 54.74%–83.71%). The positive samples were primarily from sector 4 (69.23%, 95% CI 52.4%–83%), from farms with <100 birds/farm (53.85%, 95% CI 37.2%–70%) (Figure 2). Sequences comprising the whole genome were submitted to GISAID (Appendix 2 Table 1).

Phylogenetic Analysis of HPAI H5N1 Genes

Analysis of obtained hemagglutinin (HA) and neuraminidase (NA) nucleotide and deduced amino acid sequence data confirmed that viruses in our samples were HPAI H5N1 with polybasic cleavage motif (Q-R-E-R-R-R-K-R-G-L-F) and (Q-R-E-K-R-R-K-R-G-L-F). Phylogenetic analysis showed that the HA genes of the HPAI H5N1 viruses in our study samples all belong to clade 2.3.2.1c. In-depth analysis revealed that Indonesia 2015–2016 HPAI H5N1 clade 2.3.2.1c has evolved into 2 putative new subgroups, A and B. The APD between the 2 subgroups within clade 2.3.2.1c was >1.5% (3.3% ± 0.4%); the bootstrap value was >60%; and the APDs within the 2 groups within clade 2.3.2.1c were <1.5% (0.9% ± 0.1% for subgroup A and 1.9% ± 0.2% for subgroup B). One sample collected by DIC Medan in 2016 from Sumatra Island was identified as clade 2.1.3.2a (Appendix 1 Figure 1).

We observed the evolution of clade 2.3.2.1c of Indonesia 2015–2016 HPAI H5N1 viruses into putative new subgroups (A and B) for the polymerase basic 1 (PB1), polymerase (PA), nucleoprotein (NP), and neuraminidase (NA) genes, as became apparent from comparing respective phylogenetic trees of these genes (Appendix 1 Figures 2–5). The APDs of the PB1, PA, NP, and NA genes were computed, although APD for these genes has not been used yet for HPAI nomenclature. The APD between the 2 different subgroups A and B within clade 2.3.2.1c viruses was 2.3% ±0.3% for PB1, 2.4% ±0.3% for PA, 2.1% ±0.3% for NP, and 3.4% ±0.3% for NA; and the APDs within the 2 different subgroups of clade 2.3.2.1c were 0.8% ±0.1% (A) and 1.6% ±0.2% (B) for PB1, 0.7% ±0.1% (A) and 1.3% ±0.1% (B) for PA, 0.6% ±0.1% (A) and 1.1% ±0.1% (B) for NP, and 0.7% ±0.1% (A) and 1.9% ±0.2% (B) for NA.

We identified 4 different variants of PB2 in HPAI H5N1 cases from Indonesia in 2015–2016, whereas MP and NS consisted of 3 variants. One of the 4 variants in the PB2 gene of HPAI H5N1 viruses collected by DIC from poultry outbreaks in Central and East Java in 2016 was similar to PB2 of LPAI from Asia (Appendix 1 Figures 1, 7, 8).

Detection of Possible Reassortments

Analysis of obtained sequence data by the maximum-likelihood method revealed the presence of multiple reassortments of HPAI H5N1 virus gene segments of different viruses circulating in Indonesia, using viruses of clade 2.3.2.1c, 2.1.3.2a, and Asia LPAI as parent strains (Figure 3). Based on the complete genome sequences of 37 positive samples, we identified the district with the most reassorted

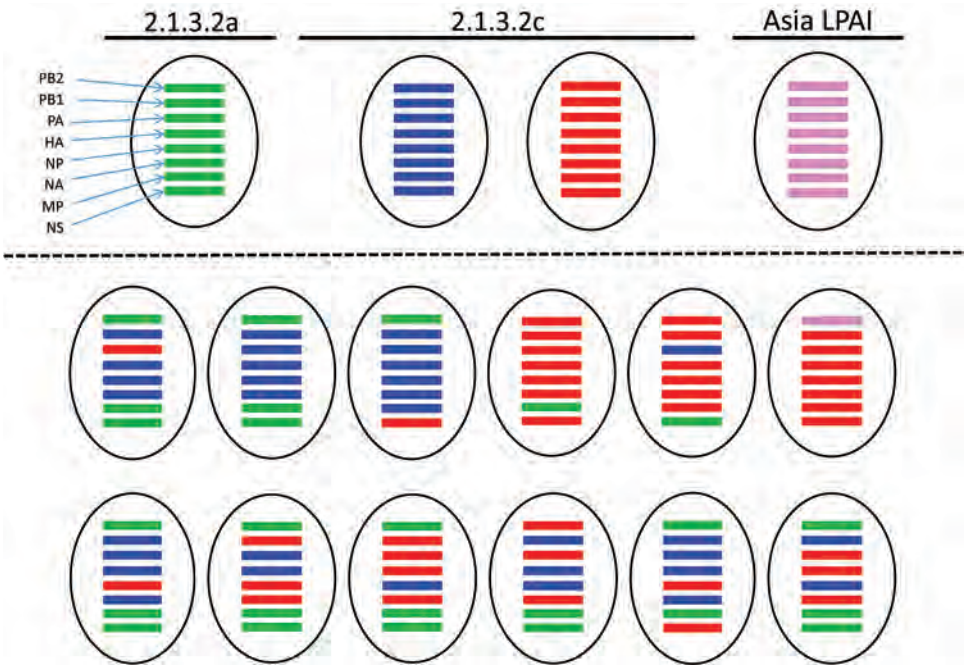

Figure 3. Reassortment events of avian influenza A(H5N1) viruses in samples from Indonesia, 2015–2016, some of which were confirmed using maximum-likelihood analysis with parent strains clade 2.3.2.1c, 2005-11 (clade 2.1.3.2a), and Asia low pathogenicity avian influenza virus. Parent strains appear above the dotted line and 13 detected reassortment types below the dotted line. HA, hemagglutinin; LPAI, low pathogenicity avian influenza virus; MP, matrix protein; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural protein; PA, polymerase; PB1, polymerase basic 1; PB2; polymerase basic 2. Green bars indicate clade 2.1.3.1a; blue, clade 2.1.3.2c subgroup A; red, clade 2.1.3.2c subgroup B; violet, Asia LPAI.

viruses as Indramayu (20.5%, CI 95% 9.3%–36.5%). The month with the largest proportion of infections was February 2016 (18%, 95% CI 7.5%–33.5%), and the type of poultry with the largest proportion of infections was backyard chickens (15.4%, 95% CI 5.9%–30.5%). We identified ≈18% (95% CI 7.5%–33.5%) of reassorted viruses in poultry sector 4; 15.4% (95% CI 5.95%–30.5%) were in farms with ≤100 birds/farm (Figure 3).

Antigenic Distance Based on Genetic Distance

It has been demonstrated recently that genetic distances in 27 selected amino acid residues of the HA of HPAI H5 viruses correlate with antigenic distances (*22*). These 27 positions correlate closely with antigenicity and are close to receptor binding sites (*23*,*24*). We observed amino acid changes in the HA of the HPAI H5N1 viruses analyzed in our study at 19/27 selected residues: N72D, D97N, Q115H, S129L, S133A, P136S/P136L, L138Q, S140N, P141S, N154D/N154S, R162K, S163G/S163N/S163T, D183N, E184G, A185G, T188I, K189R/K189M, R212K, M226I (Appendix 2 Table 4).

Results show that the estimated average antigenic distance of HPAI H5N1 viruses from subgroup A was slightly smaller than from subgroup B to the most recent seed virus vaccine, A/duck/Sukoharjo/BBVW-1428-9/2012. Not surprisingly, these average antigenic distances were lower than to older seed vaccine strains of different clades (A/chicken/Legok/2003, A/chicken/West clade 2.1, and Java/PWT-WIJ/2006 clade 2.1.3.2). In all cases, the distance difference between subgroup A or B and the 3 seed vaccine strains was significant ($p<0.05$) (Appendix 2 Tables 4, 5).

Discussion

We performed genetic and phylogenetic analysis on 39 complete genomes of HPAI H5N1 viruses obtained from recent outbreaks in West Java, Indonesia. The results of genetic analyses of the samples indicated that H5N1 clade 2.3.2.1c viruses are currently circulating predominantly in West Java and Sumatra. The finding of a single clade 2.1.3.2a virus, however, showed that this clade is still present in Indonesia. More systemic surveillance is required to confirm the prevalence of HA clade 2.1.3.2a viruses in Sumatra and Java. Of interest, we detected 2 new subgroups HA within clade 2.3.2.1c. These subgroups are candidate subclades; they share a common node, monophyletic grouping with bootstraps values ≥60, and APD between groups of >1.5% and within groups of <1.5%, fulfilling the criteria designed by the World Health Organization/World Organisation for Animal Health/Food and Agriculture Organization (WHO/OIE/FAO) H5 Evolution Working Group (*7*).

The diversity we detected in the HA subgroups of HPAI viruses in Indonesia in 2015–2016 we also detected in gene segments PB1, PA, NP, and NA, as was apparent by determination of the APD. However, although the APD between the groups was >2%, not all bootstrap values were >60. At the least, the calculated APD of PB1, PA, NP, and NA suggests that genetic variation of these genes is similar to that in HA.

The antigenic distances we deduced of the differences of 27 aa that determine antigenicity vaccination effectiveness of West_Java/PWT-Wij/2006 vaccines are expected to be lower against clade 2.3.2.1c than against clade 2.1.3.2a. Whether immunity induced by routine vaccination practices

actually did facilitate (*25*–*28*) the replacement of 2.1.3.2a viruses by clade 2.3.2.1c after its incursion in Indonesia in 2012 needs further investigation. Whether vaccination also played a role in the emergence of subgroup B viruses is less likely; the difference in the antigenic distance between subgroup B and the vaccine virus A/duck/Sukoharjo/BBVW-128-9/2012, which came into use after 2012, is rather small and only just significant. Additional studies of other variables that might have affected the evolution of H5N1 virus in Indonesia, such as transmission efficiency of the viruses in different hosts, are required to prove or reject a possible role of vaccination. In all cases, the observed genetic variation combined with its effect on antigenicity illustrates the need for continued intense surveillance and prompt genetic characterization. Calculating antigenic distances based on the 27 aa of HA could greatly speed up the process of seed virus selection because serologic analyses, antigenic cartography, and experimental vaccination-challenge experiments are time-consuming and costly processes. However, such studies are still crucial to confirm the validity and reliability of this antigenic distance method for seed selection.

We observed the evolution of clade 2.3.2.1c into 2 subgroups in 2 different locations. One subgroup within this clade (A) was observed mostly in West Java, whereas another subgroup (B) was seen in diverse regions of Indonesia (Figure 1; Appendix 1). Additional studies are needed to confirm that there are indeed geographic differences between subgroups A and B and to elucidate possible causes, such as differences in vaccination strategies and differences in trade connections (*29*).

We identified reassortment events in West Java, mostly in backyard chickens in Indramayu. The high poultry density, the presence of different poultry types, and the frequent contacts between poultry farms and between domestic poultry and wild birds may have led to reassortment in West Java (*14*). A parallel study on contacts of different poultry sectors revealed that backyard chicken farms have the highest contact rate (*30*), which may have facilitated reassortment in West Java. Of interest, a recent study described reassortant HPAI H5N1 viruses in samples collected from live-bird markets associated with suspected human HPAI H5N1 cases in Indonesia (*13*). More intense surveillance programs are required to confirm the prevalence and distribution of the clade 2.1.3.2a and 2.3.2.1c subgroups and its reassortments and to be able to unveil the transmission of HPAI from different sectors, vaccination practices, and regions.

Reassortments between influenza viruses can only occur when a host cell is infected by ≥2 viruses with discrete genomes and when mixing within the host cells produces a hybrid genotype from segments of different parental strains. Because such events are dependent on simultaneous infections with multiple viruses, reassortments are more likely to occur at hotspots such as live-bird markets where different types of birds originating from many different farms, and potentially infected with different viruses, come together (*29*,*31*). Some computational methods have recently been developed to identify a putative reassortment event (*32*,*33*). In this study, the events were identified by maximum-likelihood phylogeny and genetic distance-based methods; we reconfirmed selected reassortments by Graph Incompatibility based on Reassortment Finder using Markov chain Monte Carlo computational methods (data not shown).

Phylogenetic analysis of PB2, M, and NS indicated reassortment between viruses circulating in Indonesia. The detection of 3 different variants of M and NS, and 4 different variants of PB2 suggests that reassortment occurs frequently in HPAI viruses in West Java, Indonesia. Of interest, 1 variant of PB2 was highly similar to LPAI from nearby countries: Malaysia (H5N2), Korea (H7N7, H3N8), Japan (H1N1), and Mongolia (H7N1); viruses that until recently had not been detected in Indonesia (*31*). A similar PB2 and putative reassortants with other LPAI viruses were recently reported (*13*). These results suggest that many more LPAI viruses are likely to circulate in Indonesia but are not detected because active surveillance in wild birds or poultry is not performed. Also, diagnostic procedures that solely focus on the detection of H5N1 viruses may contribute to missing influenza viruses of other subtypes.

The presence of multiple reassortants of HPAI viruses should be an alert to the regional and international community to strengthen mitigation action plans to prevent the further reassortment and genetic drift of the viruses. Preventing virus transmission between poultry flocks, stringent biosecurity measure in (wild) bird markets, and keeping poultry separated from wild birds will help to prevent introduction, adaptation, and reassortment of LPAI viruses to a possibly novel zoonotic HPAI virus as currently observed in China and other countries (*18*,*34*,*35*).

Structured, active surveillance in combination with genetic and phylogenetic analysis are urgently needed to reveal these viruses' mutations and potential zoonotic effects, as the viruses rapidly and continually evolve with frequent reassortment (*36*). Also, adequate interventions at live poultry markets, such as separate markets for different poultry types with higher biosecurity and restructuring of the poultry chain, are crucial to prevent further loss from novel reassortant HPAI H5N1 viruses (*29*,*37*,*38*).

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Mycobacterium avium in Community and Household Water, Suburban Philadelphia, Pennsylvania, USA, 2010–2012

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Attention to environmental sources of *Mycobacterium avium* complex (MAC) infection is a vital component of disease prevention and control. We investigated MAC colonization of household plumbing in suburban Philadelphia, Pennsylvania, USA. We used variable-number tandem-repeat genotyping and whole-genome sequencing with core genome single-nucleotide variant analysis to compare *M. avium* from household plumbing biofilms with *M. avium* isolates from patient respiratory specimens. *M. avium* was recovered from 30 (81.1%) of 37 households, including 19 (90.5%) of 21 *M. avium* patient households. For 11 (52.4%) of 21 patients with *M. avium* disease, isolates recovered from their respiratory and household samples were of the same genotype. Within the same community, 18 (85.7%) of 21 *M. avium* respiratory isolates genotypically matched household plumbing isolates. Six predominant genotypes were recovered across multiple households and respiratory specimens. *M. avium* colonizing municipal water and household plumbing may be a substantial source of MAC pulmonary infection.

Nontuberculous mycobacteria (NTM) are opportunistic human pathogens. Several species of NTM, including members of the *Mycobacterium avium* complex (MAC), can cause potentially life-threatening pulmonary infections that are difficult to treat (*1*,*2*). In 1989, Prince et al. described MAC pulmonary disease in persons without predisposing conditions (*3*). That study consisted of 21 patients from 2 hospitals in greater Philadelphia, Pennsylvania, USA, 1 of which was Lankenau Medical Center, located in Montgomery County. In 2012, Adjemian et al. identified Montgomery County as 1 of 7 US counties associated with a high risk for MAC lung disease (*4*). The reason for this risk was not apparent.

A recognized source of NTM infections is the environment (*5*–*7*). Many species of NTM are found in drinking water distribution systems (*8*), buildings (*9*), and household plumbing (*7*,*10*). Pulmonary NTM disease often recurs, even after completion of prolonged courses of therapy and periods of NTM-free sputum cultures (*11*). In a molecular epidemiologic study that confirmed transmission of *M. avium* from potable water to patients, the same strain of *M. avium* was found in 2 groups of patients with AIDS and in the recirculating hot water systems of the 2 hospitals at which they had been treated (*12*). To prevent NTM infection and reinfection of vulnerable populations, identification and elimination of environmental reservoirs is crucial. Remarkably, however, establishing epidemiologic links between clinical NTM isolates and specific environmental reservoirs has been difficult, partly because of the absence of robust tools for comparison of NTM isolates.

Newer molecular techniques, including variable-number tandem-repeat (VNTR) genotyping and whole-genome sequencing (WGS), provide greater discrimination of MAC isolates than previous methods (*13*–*15*). In this study, we used VNTR and WGS to test the hypothesis that household plumbing is a reservoir for NTM and is responsible for some cases of pulmonary MAC infection.

Materials and Methods

Study Setting and Population

Lankenau Medical Center is a community-based, academic medical center in Montgomery County, Pennsylvania,

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adjacent to Delaware and Philadelphia Counties. We prospectively identified and randomly chose study participants from patients at Lankenau Medical Center for whom MAC pulmonary infection was newly diagnosed during 2010–2012. Because the study was designed to investigate MAC pulmonary disease among women, we excluded male patients from the study. Chest computed tomography images were reviewed by the principal investigator (L.L.) and by a chest radiologist experienced in bronchiectasis (*16*). All patients had evidence of nodular disease and bronchiectasis. Potential participants for whom ≥2 sputum cultures or 1 bronchoscopic culture were positive for MAC were investigated further. Patients who met the microbiological, radiographic, and clinical criteria for MAC as outlined by the 2007 American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) Statement on Nontuberculous Lung Disease (*2*) were offered study enrollment. Clinical and demographic information was obtained through chart review and from questionnaires that were filled out by patients with the aid of study personnel (Table 1).

The study protocol was approved by the Main Line Health Institutional Review Board, and informed consent was obtained from participants before study inclusion. Microbiology components of the study performed at partner institutions were deemed exempt from institutional review board review, and patient identifiers were removed from cultures or DNA samples before evaluation.

Table 1. Characteristics of patients with *Mycobacterium avium* complex infection and study controls, Philadelphia, Pennsylvania, USA, 2010–2012*

Characteristic	Patients, n = 26	Controls, n = 11
Age, y		
40–49	1	4
50–59	2	0
60–65	4	3
66–69	3	1
70–75	3	2
76–79	2	1
>80	11	0
Median (range)	77 (44–90)	64 (40–77)
Race		
White	25 (97)	11 (100)
Black	1 (3)	0
Tobacco use		
Lifetime nonsmoker	15 (58)	8 (73)
Current smoker	0	0
Former smoker	11 (42)	3 (27)
Pack-years, no.		
5–10	4	0
11–20	4	2
21–30	1	1
31–40	0	0
>40	2	0
Preexisting lung disease†		
Present	3 (12)	3 (27)
Absent	23 (88)	8 (73)

*Values are no. (%) except as indicated. Only female patients were included in the study.
†Lung disease recognized before diagnosis of nontuberculous mycobacteria infection.

Control Population

For controls, we included 11 geographically matched persons from households serviced by the same municipal water system as the patients. Control participants were healthy neighbors of MAC patients and persons with bronchiectasis who had negative MAC culture results. All controls volunteered to have samples from their homes cultured (Figure).

Isolation and Identification of MAC

Isolation and identification of MAC from household biofilms were performed as described by Falkinham et al (*6*,*7*). We chose biofilm samples because the numbers of NTM are higher in biofilms than in water (*7*). Swab samples were vortexed in a tube for 60 s, then 0.1 mL of the suspended cells was spread in triplicate on Middlebrook 7H10 agar (Becton Dickinson and Company, https://www.bd.com) containing 0.5% glycerol and 10% oleic acid–albumin, sealed with Parafilm (Bemis NA, http://www.bemis.com), and incubated at 37°C. At weekly intervals, the plates were examined for evidence of mycobacterial colonies. Putative mycobacterial colonies were picked and streaked on M7H10 agar, and identical colonies on the original isolation M7H10 agar plates were counted to calculate CFUs per square centimeter of surface. After growth, colonies were acid-fast stained, DNA was isolated, and colonies were identified by PCR amplification and restriction digestion fragment pattern analysis of the *hsp-65* gene. MAC were identified to species level by partial 16S rRNA gene sequencing, as previously described (*13*,*17*). *M. avium* isolates were subjected to PCR for IS*901*, and isolates negative for IS*901* were classified as *M. avium* subsp. *hominissuis* (*18*,*19*).

Respiratory Samples

Respiratory samples were processed at the Lankenau Medical Center Microbiology Laboratory by use of standard methods, including a commercial hybridization assay (AccuProbe; Hologic, Inc., http://www.hologic.ca). For each patient for whom respiratory culture was positive, 1 MAC isolate was subcultured and sent for further analysis to the University of Texas Health Science Center (Tyler, TX, USA) and Virginia Polytechnic Institute and State University (Blacksburg, VA, USA) (Table 2). Species identification was performed by the same methods used for biofilm isolates.

Household Samples

Samples for mycobacterial culture were collected from all patient and control households (Table 2). Study personnel

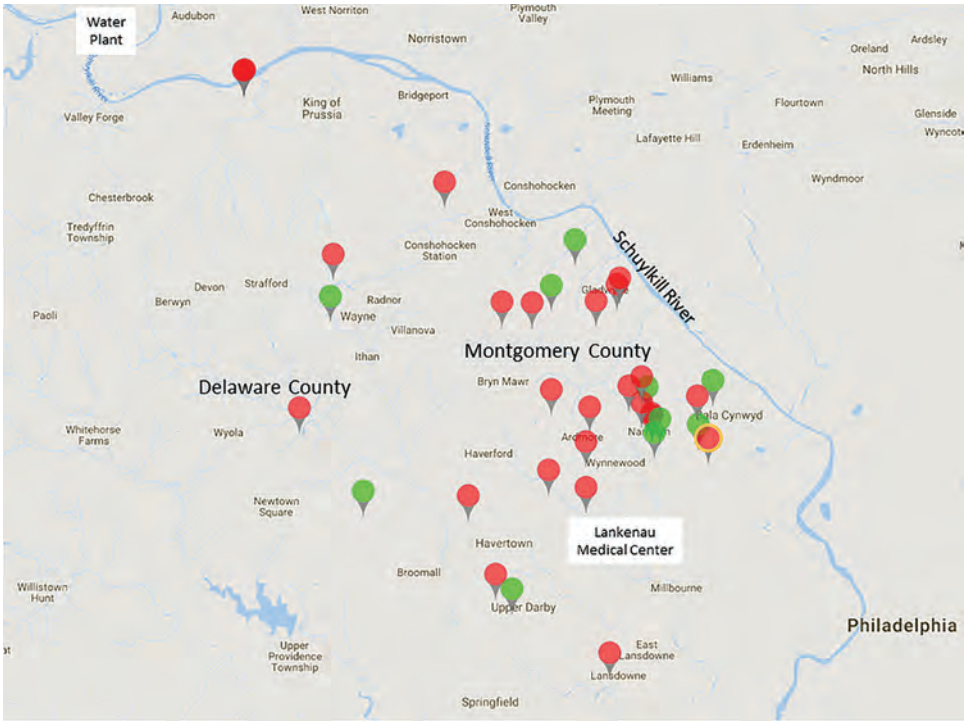

Figure. Area of study of *Mycobacterium avium* in community and household water, Philadelphia, Pennsylvania, USA, 2010–2012. The 26 patients (red tags) and 11 controls (green tags) lived in suburban Philadelphia (Montgomery and Delaware Counties). Darket red symbol in upper left indicates 3 patients who lived in the same apartment building. Red symbol with yellow border indicates patient and control households in very close proximity. All households were supplied by water that came from the Schuylkill River and was processed by the same water treatment plant.

used sterile swabs to sample surfaces in contact with water, including kitchen plumbing (sink faucets, refrigerator ice and water dispensers), bathroom plumbing (shower pipes, showerheads, tub and sink faucets, toilet water tanks), and household humidifiers attached to central heating units. After sampling, each swab was placed in 3 mL of water from the source being tested, sealed in a sterile conical tube, and sent to the Falkinham Lab at Virginia Polytechnic Institute and State University. When cultures from a sample included colonies of diverse morphology, those colonies were counted, and a representative colony of each morphotype suspected of being an NTM was selected for analysis.

Genotyping of *M. avium* Isolates

VNTR genotyping of *M. avium* isolates was performed by using 6 previously characterized *M. avium* tandem-repeat sequences (MATR1, MATR2, MATR3, MATR7, MATR13, MATR14) (*20*) plus mycobacterial interspersed repetitive unit locus 3 (*21*). Internal transcribed spacer (ITS) sequencing (*17*,*22*) was also performed for all *M. avium* isolates. Results were compared with an in-house database that included genotyping results for 416 *M. avium* subsp. *hominissuis* isolates from 121 patients and 80 biofilm samples (*18*). Genotypes were assigned as previously published for *M. avium* subsp. *hominissuis* (*13*,*18*,*23*). New numbers were assigned to VNTR/ITS combinations not previously encountered (R.J. Wallace, Jr., unpub. data). VNTR 37a strains were further subtyped by using 3′ *hsp65* sequencing (*24*) (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/25/ 3/18-0336- App1.pdf).

Household Water Sources

We collected addresses for all participants and recorded them on a regional map (Figure). All patient and control households were located in southern Montgomery County or adjacent regions of Delaware County, Pennsylvania. According to the local water utility company, these areas of Montgomery and Delaware Counties are serviced by 1 water treatment plant, which processes surface water from the Schuylkill River and its tributaries (http://www.montcopa.org/DocumentCenter/View/4342). The age of the water pipes leading to the households ranged from 65 to 115 years. Most (65%) homes were >50 years old.

WGS

To assess genomic diversity within the most common VNTR types and the genetic similarity of respiratory and plumbing isolates from individual households, we performed WGS. We selected 40 *M. avium* isolates, representing genotypes 14a, 36, 22, and 37a (subtypes B1 and B2) (Appendix Table 2). Data associated with this study have been registered in the National Center for Biotechnology Information database as BioProject ID PRJNA339271 (https://www.ncbi.nlm.nih.gov/bioproject/339271). *M. avium* was isolated from the respiratory tract of 17 participants, from the respiratory tract and >1

Table 2. Description of MAC isolates from respiratory and household samples, Philadelphia, Pennsylvania, USA, 2010–2012*

Patient	Case no.	Respiratory sample MAC species (VNTR type)	Household (biofilm) sample			VNTR match between patient's respiratory and own household samples	VNTR match between patient's respiratory and community household samples
			No. sites sampled	No. (%) sites positive for *M. avium*	VNTR type(s)		
1	P1	*M. chimaera*	13	6 (46.1)	22 and 37a[B9]	NA	NA
2	P2	*M. avium* (15)	11	1 (9.1)	47	No	No
3	P4	*M. avium* (14a)	8	1 (12.5)	14a	Yes	Yes
4	P5	*M. avium* (7)	15	0	NA	NA	No
5	P6	*M. avium* (36)	6	4 (66.7)	14a	No	Yes
6	P7	*M. avium* (37a[B1])	13	2 (15.3)	14a	No	Yes
7	P8	*M. avium* (14a)	8	4 (50.0)	14a	Yes	Yes
8	P9	*M. avium* (36)	11	2 (18.1)	14a	No	Yes
9	P10	*M. avium* (22)	13	8 (61.5)	14a, 22, and 37a[B1]	Yes	Yes
10	P11	*M. avium* (36)	10	8 (80.0)	14a, 31a, 36, and 37a[B2]	Yes	Yes
11	P12	*M. intracellulare*			NA	NA	NA
12	P13	*M. avium* (14a)	7	2 (28.5)	14a and 37a[B1]	Yes	Yes
13	P14	*M. avium* (14a)	10	3 (30.0)	14a	Yes	Yes
14	P18	*M. avium* (37)	11	4 (36.3)	14a, 31a, 36 and 37	Yes	Yes
15	P19	*M. avium* (14a)	6	1 (16.7)	31a	No	Yes
16	P20	*M. intracellulare*	6	3 (50.0)	37	NA	NA
17	P21	*M. avium* (14a)	8	1 (12.5)	14a	Yes	Yes
18	P22	*M. intracellulare*	10	3 (30.0)	38a	NA	NA
19	P23	*M. avium* (14a)	5	5 (100)	31a and 36	No	Yes
20	P24	*M. avium* (14a)	8	2 (25.0)	14a	Yes	Yes
21	P27	*M. avium* (37)	13	3 (23.0)	36	No	Yes
22	P28	*M. avium* (37a[B2])	6	2 (33.3)	30 and 36	No	Yes
23	P30	*M. avium* (55)	14	0	NA	No	No
24	P32	*M. chimaera*	15	0	NA	NA	NA
25	P33	*M. avium* (14a)	4	1 (25.0)	14a	Yes	Yes
26	P34	*M. avium* (14a)	19	5 (26.3)	14a	Yes	Yes
Control							
27	P3	No MAC	9	0	NA	NA	NA
28	P17	No MAC	5	4 (80.0)	14a and 36	NA	NA
29	P26	NA	14	4 (28.5)	36	NA	NA
30	P29	No MAC	10	3 (30.0)	31a and 36	NA	NA
31	P31	NA	3	0	NA	NA	NA
32	P35	NA	4	1 (25.0)	19	NA	NA
33	P36	NA	11	5 (45.4)	31a and 36	NA	NA
34	P37	NA	6	3 (50.0)	36	NA	NA
35	P38	NA	9	1 (11.1)	30	NA	NA
36	P40	NA	9	3 (33.3)	14a and 37a[B1]	NA	NA
37	P41	NA	4	0	NA	NA	NA
Total			334	95 (28.4)			

*ID, identification; MAC, *Mycobacterium avium* complex; NA, not applicable; VNTR, variable-number tandem-repeat.

household plumbing source for 12 of these 17, and from household plumbing only of the other 4 participants. A final participant was represented by 2 subcultures of a single respiratory isolate. These 2 biological replicates were processed separately and included to assess genomic variation that might be introduced during strain manipulation (e.g., subculture, DNA extraction, library preparation, and sequencing). Methods for WGS and bioinformatic analyses have been described (*25*). In brief, we generated paired-end libraries with the NEBNext Ultra DNA library prep kit and NEBNext Multiplex Oligos for Illumina (New England BioLabs, https://www.neb.ca). WGS was performed on an Illumina MiSeq platform by using the MiSeq reagent version 3 kit (600 cycle) according to the manufacturer's guidelines (Illumina, Inc., https://www.illumina.com). High-quality core single-nucleotide polymorphism comparison was performed by using SNPhyl version 1.0.1 (*26*). Isolates from the same cluster were considered epidemiologically related if they differed by <15 single-nucleotide variants (SNVs) (*27*).

Results

Patient Characteristics

All 26 patients were female, had nodular bronchiectasis confirmed by computed tomography, and had MAC lung

disease as defined by the ATS/IDSA criteria (*2*). At enrollment, patient median age was 77 years (range 44–90 years), 97% (25/26) were white, 42% (11/26) were former smokers, and 88% (23/26) had no previous or co-occurring lung disease other than bronchiectasis (Table 1). All patients lived within 20 miles of each other (Figure): 20 in Montgomery County and 6 in Delaware County.

Control Characteristics

All 11 control participants were female; median age was 64 years (range 40–77 years). Eight lacked any history or signs of pulmonary NTM disease (i.e., no cough, dyspnea, fatigue, weight loss, or recurrent respiratory infections). Three participants had bronchiectasis and ≥2 sputum samples that did not grow NTM on culture. Controls lived within 20 miles of each other and the patients: 7 in Montgomery County and 4 in Delaware County (Figure).

Respiratory Samples

MAC respiratory isolates were recovered from 26 patients during the study period. The positive cultures were obtained from expectorated sputum and bronchoscopic sampling. We identified 3 species of MAC: *M. avium* subsp. *hominissuis* (21/26, 80.8%), *M. intracellulare* (3/26, 11.5%), and *M. chimaera* (2/26, 7.7%) (Table 2).

Household Samples

We collected 334 environmental biofilm samples, including 250 samples from 26 MAC patient households (range 4–19 samples from 3–13 sites in each household) and 84 samples from the 11 control households (range 3–14 samples from 3–11 sites in each household). A total of 95 *M. avium* isolates were recovered; 30/37 (81.1%) households were positive for *M. avium*, including 22/26 MAC patient households, 19/21 *M. avium* patient households, and 8/11 control households (Table 2).

M. avium was recovered from the kitchen sink faucet in 21/37 (56.8%) households and from 20/37 (54.1%) of the primary bathroom sites sampled, including 15/36 (41.7%) bathroom sink faucets, 13/35 (37.1%) showerheads, and 11/29 (37.9%) shower pipes (Appendix Table 3). Among households with humidifiers connected to the central heating units (with household plumbing as their water source), 7/17 (41.1%) were positive for *M. avium.* Among other sample sites, 2/14 (14.2%) refrigerator ice/water dispensers were positive, and 0 sampled toilet tanks were positive.

M. avium Genotypes

For 11/21 (52.4%) patients with *M. avium* disease, the genotype isolated from their respiratory sample was identical to the genotype recovered from their household plumbing sample (Table 2). For 18/21 (85.7%) *M. avium* patients, the respiratory isolate was the same genotype as that of plumbing biofilm isolates from ≥1 households within the same community. For 7/21 (33.3%) *M. avium* patients, the respiratory isolate genotype did not match that of a plumbing isolate from their own household but did match that of plumbing isolates from neighboring households. Overall, the 21 respiratory and 95 household isolates of *M. avium* encompassed 15 genotypes (Appendix Table 1). Six genotypes were broadly distributed across multiple households and respiratory specimens, and 2 genotypes were isolated from multiple household sources but not from respiratory specimens. In 11 households, the *M. avium* populations were heterogeneous, and 2–4 genotypes were recovered.

Whole-Genome Sequences and SNV–Based Phylogeny

SNVPhyl analysis of *M. avium* samples identified 26,871 variable sites across the core set of 4.3 million nt positions per isolate (Appendix Table 4). WGS was performed on 8 pairs of VNTR-matched respiratory and household isolates. For all 8 paired samples, the distance between respiratory and household isolates of *M. avium* was 4–51 SNVs; 5 pairs were separated by ≤15 SNVs (Table 3). The respiratory isolate from patient 13 differed from an isolate from her own kitchen sink by 37 SNVs and by <15 SNVs from plumbing biofilm isolates from households of patient 14 (9 SNVs) and patient 4 (11 SNVs). These patients did not know one another and had not visited each other's homes.

M. avium patients 9, 10, and 11 resided in different apartments in the same high-rise building (Table 4). Across the 3 apartments, 32 sites were sampled and 17 *M. avium* cultures, representing 6 different genotypes, were recovered. The respiratory isolate from patient 9 was type 36, which was not found in her apartment but was the same as the respiratory isolate from patient 11 and was cultured from the apartment of patient 11. The respiratory isolate from patient 10 was type 22, which was the most common

Table 3. Whole-genome sequencing of selected respiratory and household *Mycobacterium avium* isolates with matched genotypes by VNTR, Philadelphia, Pennsylvania, USA, 2010–2012*

Case no.	Source	Genotype	SNV distance between respiratory and household isolates
P4	Kitchen sink	14a - I	51
P8	Shower pipe	14a - I	8
P10	Shower pipe	22	4
P13	Kitchen sink	14a - I	37
P14	Shower pipe	14a - I	8
P24	Refrigerator tap	14a - II	12
P24	Central humidifier	14a - II	14
P33	Kitchen sink	14a - I	24
P34	Bathroom sink	14a - I	8
P34	Kitchen sink	14a - I	11
P34	Shower pipe	14a - I	16

*SNV, single-nucleotide variant; VNTR, variable-number tandem-repeat.

Table 4. *Mycobacterium avium* culture results for patients living in different apartments within the same apartment building, Philadelphia, Pennsylvania, USA, 2010–2012*

	Culture results (VNTR type)		
Sampling site	Patient 9	Patient 10	Patient 11
Respiratory samples	*M. avium* (36)	*M. avium* (22)	*M. avium* (36)
Household samples			
Kitchen			
Sink faucet	*M. avium* (14a)	*M. avium* (14a)	*M. avium* (37a[B2])
Primary bathroom			
Sink faucet	No NTM	*M. chimaera*	*M. avium* (14a)
Showerhead	*M. avium* (14a)	*M. avium* (22) and *M. chimaera*	*M. avium* (37a[B2])
Shower pipe	No NTM	*M. avium* (37a[B1])	*M. avium* (37a[B2])
In-line shower filter	Not determined	Not determined	*M. avium* (31a)
Secondary bathroom			
Sink faucet	Not determined	*M. avium* (22)	*M. avium* (36)
Showerhead	Not determined	*M. avium* (37a[B1])	Not determined
Shower pipe	Not determined	*M. avium* (22)	Not determined
Humidification system			
Water inflow	No NTM	*M. avium* (22)	*M. avium* (14a)
Water in system	No NTM	*M. avium* (22)	*M. avium* (14a)
Water drainage	No NTM	Not determined	No NTM

*NTM, nontuberculous mycobacteria; VNTR, variable-number tandem-repeat.

genotype recovered from her apartment (5/8 sites) and matched (i.e., 4 SNVs different) an isolate from her shower. Comparison of 5 type 14a strains isolated from sites across all 3 apartments revealed only minimal differences (25–63 SNVs). Detailed results of the WGS and SNVPhyl analyses are provided in the Appendix.

Discussion

Determining the environmental source of infection for persons with pulmonary MAC disease has proven to be remarkably difficult. We used VNTR typing, targeted gene sequencing, and core-genome SNV analysis to compare *M. avium* isolates recovered from household plumbing biofilms with respiratory isolates from patients with MAC disease. We chose biofilm sampling of household plumbing, as opposed to direct water sampling, to enhance isolation rates of mycobacteria, which concentrate in biofilm. All households were serviced by the same water filtration plant and shared a common water source. MAC was widespread; 95 *M. avium* isolates were recovered from 22/26 (84.6%) MAC patient households (including 19/21 *M. avium* patient households) and 8/11 (72.7%) control households.

Respiratory and household isolates for 11/21 (52.4%) patients with *M. avium* nodular bronchiectatic pulmonary disease were genotypically matched. For 8 patients, the matched isolates were of the same genotype (14a). VNTR type 14a isolates were recovered from 10 (38.5%) respiratory and 16 (43.2%) household samples, and type 36 isolates were recovered from 4 (15.4%) respiratory and 10 (27%) household samples.

A 2016 report by Iakhaeva et al. (*18*) characterized 416 *M. avium* isolates from 120 patients and 80 environmental biofilms by using the methods that we used in this study and identified 49 VNTR types/subtypes. Since then, ≈100 additional isolates have undergone VNTR typing. Despite their predominance in our study, genotypes 14a and 36 were not identified in any previous studies (*18*; R.J. Wallace Jr., unpub. data). Our previous screening of additional patients and households in nearby areas that share the same primary water source (the Schuylkill River) but different water treatment plants found VNTR types 14a and 36 in those samples as well (R.J. Wallace Jr., unpub. data.)

VNTR genotyping is reproducible, portable, and discriminatory to the extent that isolates with different VNTR patterns are considered unrelated. However, as illustrated by the 37 and 37a subtypes found in this study, pseudoclustering can occur, such that isolates with identical VNTR patterns can have different ITS and *hsp65* sequences (*18*). Previous work with *M. tuberculosis* also demonstrates that a VNTR match is not always proof of strain identity or an epidemiologic link (*28*,*29*). To better assess the relationship between VNTR-matched isolates, we turned to core-genome SNV analysis, which included ≈4.3 million nt positions; for phylogenetic comparisons, we used 26,871 variable sites. For the most common genotype identified in this study, type 14a, strains were split into 3 distinct subclusters (Appendix Figure). Isolates from the same subcluster were similar, differing by <100 of 26,871 SNVs, even though the sequenced strains were collected over 2 years and were recovered from diverse clinical and environmental sources. This widespread distribution and persistence is consistent with extensive colonization of the local water system with ≥1 *M. avium* strains endemic to Montgomery and Delaware Counties. For VNTR-matched respiratory and household isolates, the maximum distance was <60 SNVs. In 5 households, <15 SNVs separated the patient respiratory isolate from a plumbing biofilm isolate, suggesting an epidemiologic association between *M. avium* infection and household water. An additional patient respiratory

isolate was <15 SNVs distant from biofilm strains recovered from neighboring households. This finding suggests that MAC exposure may occur outside the patient's home but is more likely to represent incomplete recovery of respiratory strains, household strains, or both. Consistent with incomplete recovery, respiratory isolates from 7/21 (33.3%) *M. avium* patients had genotypes that were not present in their own households but did match isolates from neighboring households. Overall, 18/21 (85.7%) *M. avium* respiratory isolates matched ≥1 household biofilm isolates in the same community.

Our study had several limitations. The study population was relatively small and included only women from a specific region of Pennsylvania. To assess the universality of our findings, a larger, geographically diverse cohort is required. Because of the labor-intensive nature of the study, sampling was not comprehensive for all patients or households, and it is probable that because of undersampling some patient-household matches were missed. Longitudinal studies show that over time, patients with nodular bronchiectasis usually become infected with multiple genotypes of MAC (*30*), but we evaluated only 1 respiratory sample per patient.

The WGS component was conceived as a proof-of-principle experiment and limited to 40 isolates. The results of reference-based WGS comparisons are influenced by the analysis pipeline and choice of reference genome. Stringent pipeline filters intended to eliminate mapping errors can also suppress identification of true variants (*31*). Similarly, if a divergent genome is used as a reference, only a small subset of sequencing reads will be mapped and epidemiologically relevant SNVs may be missed (*32*). However, in our study, the core genome comprised >88% of the reference genome (≈4.3 million nt) despite the inclusion of genotypically diverse strains and the conservative filters used by the SNVPhyl pipeline.

Because an epidemiologic threshold for *M. avium* has not been definitively established, it remains unclear what SNV difference constitutes a definite genetic match. In a genomic analysis of *M. abscessus*, genetic relatedness was considered probable for isolates differing by <20 SNVs and possible for isolates differing by 20–38 SNVs (*27*). A cutoff of <12–25 nt for epidemiologically related isolates has been used in other studies of mycobacteria (*33*,*34*), but determining the most suitable threshold for *M. avium* will require additional isolates and analyses. In our WGS analysis of 8 pairs of VNTR-matched respiratory and household isolates, 5 pairs were separated by ≤15 SNVs, suggesting unequivocal genetic relatedness. In the other 3 pairs, distances were 24, 37, and 51 SNVs, which may also represent genetic relatedness but cannot be definitively established. In addition, the isolate from patient 13 was <15 SNVs distant from isolates from plumbing biofilm in the households of patients 14 (9 SNVs) and 4 (11 SNVs). These patients lived in separate towns that were 3–4 miles apart and serviced by the same water company, suggesting clonal infection from a common municipal water source. With MAC disease, there is often a delay between acquisition of infection and diagnosis, during which time mutations will accumulate in the environmental reservoir and the clinical isolates. For most cases in our study, infection probably preceded sample collection by several years. The estimated mutation rate for *M. avium* ssp. *paratuberculosis* is >0.5 substitutions/genome/year (*35*), but rates may be higher for *M. avium* subsp. *hominissuis* and have shorter doubling times. Rates may also be influenced by environmental and host factors. We recently sequenced serial isolates of *M. intracellulare* from patients receiving antimicrobial drug therapy and observed mutation rates that were 25-fold higher than for *M. avium* ssp. *paratuberculosis* (0.6–1 substitutions/mo) (*25*).

Our study identified household plumbing biofilms as a reservoir for *M. avium* and suggests that local rates of MAC lung disease may be influenced by mycobacterial colonization of municipal water. We cannot rule out other reservoirs, such as soil and dust, but thus far, recovery of *M. avium* from those sites and subsequent matches to patient isolates have been minimal (*36*). Moreover, because of innate resistance to chlorine and other disinfectants typically used in water treatment (*37*), *M. avium* has a survival advantage over most waterborne pathogens. In any geographic region, the prevalence of a particular *Mycobacterium* species probably affects disease rates. A recent study in Hawaii found that *M. chimaera* was the most common NTM recovered from household plumbing and the most common NTM recovered from patients in Hawaii (*10*). The value of understanding environmental sources of MAC infection have been highlighted by recent experiences with *M. chimaera* contamination of heater–cooler units (*38*).

Proof of an environmental source of *M. avium* has broad implications regarding prevention of recurrent infection in existing patients as well as prevention of new disease in susceptible persons. Additional studies are needed to establish effective methods for eliminating environmental reservoirs of *M. avium* and other problematic NTM. Tackling this challenging problem will require engagement of water utility workers, plumbers, environmental scientists, and engineers.

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SNP-IT Tool for Identifying Subspecies and Associated Lineages of *Mycobacterium tuberculosis* Complex

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The clinical phenotype of zoonotic tuberculosis and its contribution to the global burden of disease are poorly understood and probably underestimated. This shortcoming is partly because of the inability of currently available laboratory and in silico tools to accurately identify all subspecies of the *Mycobacterium tuberculosis* complex (MTBC). We present SNPs to Identify TB (SNP-IT), a single-nucleotide polymorphism–based tool to identify all members of MTBC, including animal clades. By applying SNP-IT to a collection of clinical genomes from a UK reference laboratory, we detected an unexpectedly high number of *M. orygis* isolates. *M. orygis* is seen at a similar rate to *M. bovis*, yet *M. orygis* cases have not been previously described in the United Kingdom. From an international perspective, it is possible that *M. orygis* is an underestimated zoonosis. Accurate identification will enable study of the clinical phenotype, host range, and transmission mechanisms of all subspecies of MTBCin greater detail.

Mycobacterium tuberculosis complex (MTBC) encompasses a group of organisms that cause tuberculosis (TB) in humans and animals. TB in humans is caused mainly by *M. tuberculosis* but also by other members of MTBC, including the less well understood animal-associated subspecies *M. bovis*, *M. caprae*, *M. pinnipedii*, *M. suricattae*, *M. orygis*, *M. microti*, and *M. mungi* (*1*–*6*). The global burden of zoonotic TB is thought to be both underestimated and increasing (*7*); however, accurate assessment of prevalence is made difficult by a lack of clinical diagnostic tools and surveillance (*8*).

Efforts to differentiate members of MTBC and study the phylogeny of the complex have thus far included analysis of large genomic deletions (*9*), variable-number tandem-repeats (VNTR), spacer oligonucleotide typing (spoligotyping), multilocus sequence typing, and, more recently, single-nucleotide polymorphism (SNP)–based phylogenies (*10*). Numerous tools now exist that make in silico predictions of lineages within the complex from whole-genome sequencing (WGS) data using a variety of approaches, including the detection of single SNPs from both unassembled and mapped genomes, comparison of de Brujin graphs, and MinHash-based comparisons (*11*–*14*). None of these tools has yet been calibrated to reliably differentiate among all subspecies, particularly the animal-associated ones, whose incidence and clinical significance are likely to be underestimated as a result.

The host ranges of the various MTBC subspecies differ, which has serious implications for contact investigations and source case finding. For example, *M. microti* is found in wild cats and rodents and causes human infection, usually in association with rodent contact (*15*). In contrast with infections caused by *M. bovis,* most *M. microti* infections have been reported to cause pulmonary TB, which raises the possibility of onward transmission, although such transmission has not yet been reported (*16*). *M. pinnipedii*, which causes TB in seals, is sometimes transmitted to humans during outbreaks in zoos or wildlife parks (*17*). Although isolated mostly from gazelle species, *M. orygis* has also been seen in humans in recent years, although how humans contract this bacterium is still unclear (*2*). *M. mungi* causes

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disease in banded mongooses, the dassie bacillus causes disease in rock hyraxes, and *M. suricattae* causes disease in meerkats, but none of these bacteria is currently known to cause disease in humans (*3*,*5*).

The spectrum of clinical phenotypes associated with human infection by MTBC animal lineages is largely unknown, partly because the identification of these organisms is currently difficult. Accurate identification of the causative subspecies in all cases would enable characterization of disease associated with animal lineages and of diversity in clinical phenotypes, which would contribute to better disease management. A higher level of knowledge on the spread and host range of the subspecies would also provide a better basis on which to study the history of the evolutionary development of the complex as a whole. Now that routine WGS is being performed by Public Health England (PHE) for all MTBC isolates, we sought to use these data to estimate the burden of animal-associated TB in England. Therefore, we identified a broad panel of SNPs that define each subspecies, lineage, and sublineage within the MTBC and assessed them using a new SNP-based tool, SNPs to Identify TB (SNP-IT).

Materials and Methods

Calibration Set

We defined a set of isolates (N = 323) from which to identify SNPs associated with subspecies, lineages, and sublineages within the MTBC (Figure 1). We identified isolates from the collection of the National Institute for Public Health and the Environment (RIVM; Bilthoven, Netherlands) using a combination of spoligotyping patterns, SNPs, restriction fragment length polymorphism (RFLP) patterns, the hybridization patterns in the HAIN Genotype MTBC assay, polymorphic GC-rich repeat sequence (PGRS) profiles, and VNTR patterns in accordance with current and previous standard practice (Appendix Table 1, http://wwwnc.cdc.gov/EID/article/25/3/18-0894-App1.pdf). We identified isolates from a whole-genome sequencing archive held at the University of Oxford (Oxford, UK) using the SNP typing system of Stucki et al. (*18*) for Mtb lineages 1–4 and by clustering on a maximum likelihood tree with the isolates from the Netherlands for lineages 5 and 6. In addition, we used published strains (n = 5) to be able to include *M. suricattae, M. mungi*, and the dassie bacillus, which were not present in the Oxford or RIVM collections. The nomenclature we adopted for this study is summarized in Appendix Table 2.

Bioinformatics

We applied parallel bioinformatics approaches to assess applicability across pipelines. As such, we independently mapped reads from Illumina platforms (https://www.illumina.com) to 2 different versions of the H37Rv reference genome. We mapped reads to NC000962.3 with Breseq version 0.28.1 (http://barricklab.org/twiki/bin/view/Lab/ToolsBacterialGenomeResequencing), using a minimum

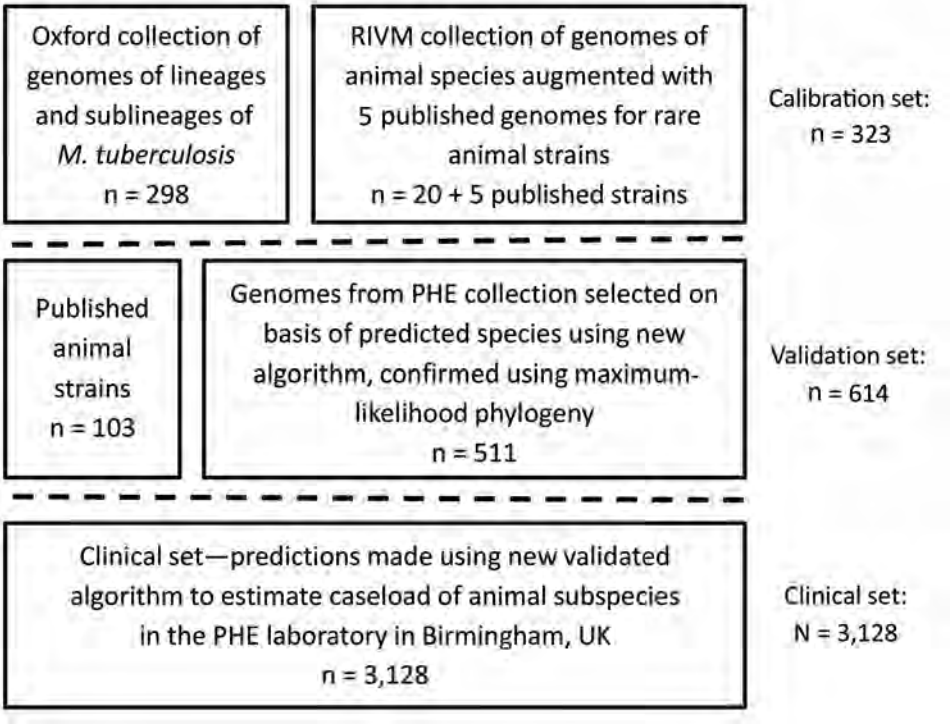

Figure 1. Description of the *Mycobacterium tuberculosis* complex datasets used in the 3 stages of calibration, validation, and application to a clinical set of the new SNPs to Identify TB tool. PHE, Public Health England (Birmingham, UK); RIVM, Netherlands National Institute for Public Health and the Environment (Bilthoven, the Netherlands); SNP, single-nucleotide polymorphism.

allele frequency of 80% and minimum coverage of 5 times for SNP calls. Separately, we mapped reads again to NC000962.2, for which we used Snippy version 3.1 with default settings (minimum coverage 10 times, minimum allele frequency 90%) (*19*,*20*). We extracted all SNPs shared exclusively by isolates of each subspecies, lineage, and sublineage identified by both pipelines. The lineage-defining positions for lineage 4 are not variants with respect to the reference, itself lineage 4, but are uniquely conserved positions. We therefore identified these positions by mapping a core SNP alignment to a maximum-likelihood tree using Mesquite version 3.30 (*21*). These nucleotide loci were added to the catalog of phylogeny-determining SNPs.

All newly sequenced genomes are available from the National Center for Biotechnology Information under project accession no. PRJNA418900. The SNP-IT tool, including all relevant reference libraries used for this study, is available as an open-source package online (https://github.com/samlipworth/snpit).

Validation Set

To validate the algorithm, we compiled an independent collection of genomes (N = 511) using clinical isolates sequenced by PHE Birmingham, UK, identified as MTBC and not included in the calibration set (Figure 1). We augmented them with data from the European Nucleotide Archive and the National Center for Biotechnology Information Sequence Read Archive to increase the representation of animal subspecies (N = 103; Appendix Table 2). To maximize inclusion of animal isolates from the public archives, we used the new Colored Bloom Graph (CBG) software (*22*). Using CBG, we searched a snapshot of the Sequence Read Archive (to December 2016, N = 455,632) with our new set of reference kmers for Mykrobe predictor (see Comparison with Existing Tools).

We compared FASTA files of the whole genome (https://www.ebi.ac.uk/Tools/sss/fasta) to the catalog of phylogeny-determining SNPs to make predictions for PHE isolates, whereas for isolates downloaded from the nucleotide archive, we created only limited variant calling format files using Snippy (only SNPs with respect to the reference genome were included to increase computational efficiency). To ensure that genomic loci defining lineage 4 were included, we used a mutated reference genome to create these limited variant calling format files. We compared SNPs in the query sample with reference libraries of lineage-specific SNPs for each clade. We assigned query genomes to particular subspecies or lineages if ≥10% of lineage- or subspecies-specific SNPs were detected in the strain in question. We assessed all predictions against the maximum-likelihood phylogeny. For *M. mungi*, we could locate only 1 genome in the public sequence libraries, so we could not validate this subspecies.

Clinical Isolates

To assess the caseload across the different members of the MTBC seen by the PHE laboratory in Birmingham, we applied the algorithm to 3,128 MTBC genome sequences from consecutively obtained clinical isolates. H37Rv is routinely sequenced by the laboratory on WGS plates; these isolates were not removed, and their identification served as an internal control.

Comparison with Existing Tools

We first compared strain characterization by our new SNP-IT tool with those of KvarQ (https://github.com/kvarq/kvarq), TB-Profiler (http://tbdr.lshtm.ac.uk), and Mykrobe predictor (http://www.mykrobe.com/products/predictor) on default settings and then after integrating our updated SNP library. To enable our new data to be integrated with published SNP libraries (*23*) and for practical reasons when modifying existing tools, we created a minimal SNP dataset. We filtered our larger SNP catalog for synonymous SNPs that occurred in coding regions (as annotated by SnpEff version 4.3 [*24*]) and selected 1 representative SNP for each subspecies, lineage, and sublineage at random. We then modified the existing software packages to include reference SNPs (or kmers for Mykrobe predictor) for the subspecies, lineages, or sublineages that they initially failed to identify.

Results

Calibration and Validation

In total, we identified 13,893 SNPs (median of 229 SNPs per group, interquartile range 296) as predictive of taxonomic and phylogenetic groups of interest (Appendix Table 3). The greatest number of phylogenetic SNPs was seen in *M. canettii* (n = 6,837) and the fewest in *M. bovis* (n = 23). Subspecies that arise from common deep branches, such as *M. microti* and *M. pinnipedii* (Figure 2), have lower numbers of unique phylogenetic SNPs (n = 128 for *M. microti* and n = 301 for *M. pinnipedii*) than those that do not, such as *M. orygis* (n = 781). All predictions made by SNP-IT across all the subspecies, lineages, and sublineages were consistent with the maximum-likelihood phylogeny for all isolates in the validation set (Table 1).

Determining Prevalence of Animal Subspecies in a Collection of Clinical Isolates

We retrospectively applied SNP-IT to clinical isolates sequenced as part of the routine PHE diagnostic workflow in Birmingham to estimate the prevalence of the animal subspecies among MTBC samples. Of 3,128 samples from 2,106 patients for which there was a whole-genome sequence available, we identified 24 as *M. orygis*, 3 as *M. microti*, 34 as *M. bovis*, and 1 as *M. caprae* (Table 2). In

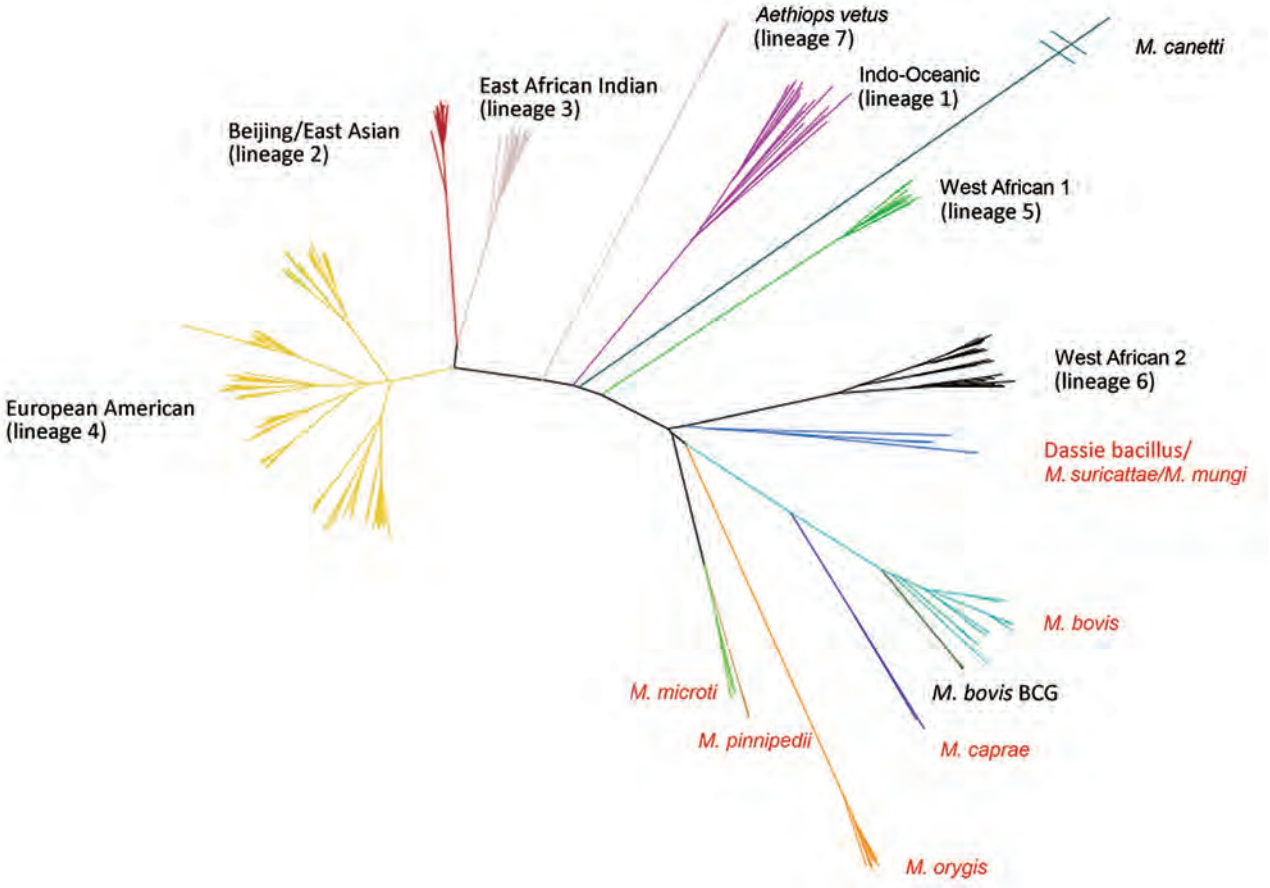

Figure 2. Maximum-likelihood tree built from 70,144 informative positions from whole-genome sequences of all 323 *Mycobacterium tuberculosis* complex samples in the calibration set for the new SNPs to Identify TB tool. Lineages are of *Mycobacterium tuberculosis*. Underlined text denotes animal subspecies. BCG, bacillus Calmette–Guérin; SNP, single-nucleotide polymorphism.

the case of *M. orygis*, we further investigated whether there was any genomic signal of possible person-to-person transmission. We identified 2 such instances, 1 in which the pairwise genetic distance between 2 patients was 0 SNPs and a second in which it was 6 SNPs (Appendix Figure 1).

Phylogenetic SNPs in Drug Resistance–Associated Genes

Using a previously published list of drug resistance–associated genes for *M. tuberculosis* (*25*), we searched all subspecies for phylogenetic SNPs in drug resistance–associated genes (Appendix Table 4). All subspecies contain unique phylogenetic SNPs (N = 95 in total) in these genomic regions, but on the basis of our data, we were unable to determine whether any of these mutations are linked to lineage-specific resistance because we did not have the corresponding phenotypic drug susceptibility testing data.

Comparison with Existing Software

Compared with SNP-IT for the clinical set of isolates, Mykrobe predictor reported *M. orygis* as *M. tuberculosis* West African lineage and *M. pinnipedii* as *M. microti*. KvarQ identified all animal-associated subspecies only as "animal lineage." TB-Profiler was unable to delineate among animal subspecies, which were all reported as *M. bovis/M. tuberculosis* West African lineage.

After we modified the KvarQ, TB-Profiler, and Mykrobe predictor databases with our minimal SNP catalog, all systems agreed on the identity of all the MTBC isolates in the clinical set. SNP-IT was unable to identify 10 samples because <10% of type-specific SNPs were present in these strains. This result was because our pipeline made no call at the lineage informative sites because of the presence of a minor allele, most likely the result of contamination or a mixture of 2 strains in the sample. However, TB-Profiler and Mykrobe predictor were both able to identify 2 of these isolates as mixed Beijing lineage/*M. orygis* using our new minimal SNP dataset.

Discussion

SNP typing is a powerful method for discriminating among members of MTBC, which are often not discernible

Table 1. Comparison between speciation calls for 614 MTBC samples in validation set made by SNP-IT and position on maximum-likelihood phylogenetic tree*

Species (lineage)	SNP-IT typing calls	Maximum-likelihood calls	% Correct
Mycobacterium bovis BCG	22	22	100%
M. bovis	15	15	100%
M. orygis†	31	31	100%
M. microti†	18	18	100%
M. canettii†	34	34	100%
M. pinnipedii†	8	8	100%
M. caprae†	15	15	100%
Dassie bacillus†	2	2	100%
M. suricattae†	2	2	100%
M. tuberculosis			
Indo Oceanic (lineage 1)	44	44	100%
Beijing/East Asian (lineage 2)	42	42	100%
East African Indian (lineage 3)	46	46	100%
European American (lineage 4)‡	18	18	100%
Lineage 4 sublineages			
Ghana (4.1)	12	12	100%
X-type (4.1.1)	45	45	100%
Haarlem (4.1.2.1)	45	45	100%
Ural (4.2.1)	19	19	100%
Tur (4.2.2.1)	25	25	100%
LAM (4.3)	37	37	100%
S-type (4.4.1.1)	45	45	100%
Uganda (4.6.1)	18	18	100%
Cameroon (4.6.2.2)	32	32	100%
West African 1 (lineage 5)	13	13	100%
West African 2 (lineage 6)	11	11	100%
M. aethiops vetus (lineage 7)	15	15	100%

*Numbers in parentheses represent the lineage numbering scheme of Coll for the major lineages 1–7 and Stucki for the lineage 4 sublineages. BCG, bacillus Calmette–Guérin; MTBC, *Mycobacterium tuberculosis* complex; SNP, single-nucleotide polymorphism; SNP-IT, SNPs to Identify TB.
†Validation set for subspecies augmented with published strains.
‡No sublineage call made.

by conventional laboratory methods. The SNP databases of Stucki, Coll, and Comas are currently used as the knowledge base for KvarQ, TB-Profiler, and Mykrobe predictor (*18*,*23*,*26*). None of these databases, however, provides adequate resolution for the animal subspecies. In contrast, SNP-IT was able to assign subspecies, lineages, and sublineages to all samples in the validation set with 100% accuracy compared with maximum-likelihood phylogeny. Implementing this fine-resolution algorithm into a routine diagnostic workflow would be a major step toward understanding the epidemiology and pathogenicity of the less common members of MTBC. All 3 existing systems tested (KvarQ, Mykrobe predictor, and TB-Profiler) were identical in performance when given the same SNP reference database, demonstrating that the clinically meaningful differences highlighted in a recent review are easily ameliorated (*27*).

By applying SNP-IT to a clinical dataset, we discovered an unexpectedly high number of animal subspecies among MTBC isolates, particularly *M. orygis*, from humans in the United Kingdom. This recently described member of the complex has a host range that includes waterbucks, gazelles, rhesus monkeys, cows, and rhinoceri (*2*,*28*,*29*). Several human cases have been described in patients in the Netherlands of South/Southeast Asian origin (*2*), but no cases have been described in the United Kingdom. Human-to-animal transmission has been described in 1 case in New Zealand (*30*). Given that zoonotic TB is associated with higher rates of extrapulmonary disease and may be less likely to grow in culture (*31*,*32*), retrospective interrogation of WGS libraries, as in this study, is likely to underestimate the true burden of disease.

Given the large amount of resources aimed at controlling bovine TB, it is noteworthy that another zoonosis is seen at a similar rate in this collection of clinical isolates. This finding raises questions about the host range and transmission of *M. orygis*, with potential implications for TB control both in animals and humans. To recognize the particulars of the clinical phenotype, epidemiology, and optimal management strategy of *M. orygis* infection, it is first crucial to accurately distinguish these cases from *M. tuberculosis* West African lineages (*5*,*6*). This discernment is currently not possible by either the Hain Genotype MTBC molecular probe or existing SNP-based platforms. We identified 2 pairs of nearly identical *M. orygis* isolates that could be compatible with either person-to-person transmission or, possibly, common exposure to the same infected animal. From an international perspective, the role of *M. orygis* in zoonotic transmission in Africa, Asia, and other high-prevalence settings with extensive animal contact is poorly understood and may warrant further investigation.

Table 2. Speciation predictions for collection of 3,128 clinical MTBC isolates from 2,106 patients using SNP-IT*

Species and subspecies (lineage)	No. isolates (no. patients)
Mycobacterium tuberculosis	
Indo-Oceanic (lineage 1)	240 (208)
Beijing/East Asian (lineage 2)	242 (175)
East African Indian (lineage 3)	775 (644)
European American (lineage 4); no sublineage call made†	512 (+ 368 H37Rv‡) (336)
Lineage 4 sublineages	
Ghana (4.1)	5 (4)
X-type (4.1.1)	197 (159)
Haarlem (4.1.2.1)	266 (213)
Ural (4.2.1)	43 (34)
Tur (4.2.2.1)	41 (36)
LAM (4.3)	213 (159)
S-type (4.4.1.1)	60 (45)
Uganda (4.6.1)	13 (12)
Cameroon (4.6.2.2)	40 (32)
West African 1 (lineage 5)	4 (2)
West African 2 (lineage 6)	11 (9)
No call made	10 (10)
M. bovis BCG	26 (20)
M. bovis	34 (28)
M. orygis	24 (19)
M. microti	3 (2)
M. caprae	1 (1)
Total	3,128 (2,106)

*Numbers in parentheses represent the lineage numbering scheme of Coll for the major lineages 1–7 and Stucki for the lineage 4 sublineages. BCG, bacillus Calmette-Guérin; MTBC, *Mycobacterium tuberculosis* complex; SNP, single-nucleotide polymorphism; SNP-IT, SNPs to Identify TB.
†These samples belong to lineage 4 but not to one of the named sublineages.
‡SNP-IT correctly identified 880 isolates as being lineage 4; however, 368 of these were identified as being H37Rv when we unblinded ourselves to their laboratory records.

All the animal subspecies had phylogenetic SNPs in drug resistance–associated genes. When these genes are not known to be associated with drug resistance, they can be helpfully annotated as such by diagnostic algorithms and excluded for the purpose of predicting susceptibility. An unavoidable weakness of any SNP-based approach is its vulnerability to null-calls as a result of minor alleles at informative positions or a lack of coverage. SNP-IT uses the entire library of subspecies/lineage/sublineage-defining SNPs such that this weakness is not an issue unless it occurs at most of these positions. An additional limitation is that, although we have sought to calibrate SNP-IT using the most diverse collection of samples available to us, it may not be able to correctly identify isolates that originate from deeper phylogenetic branches than those in our calibration set.

In conclusion, in this study we demonstrate a higher-than-expected burden of zoonotic TB in a large collection of clinical isolates from the United Kingdom. The SNP-IT tool we have developed will help researchers to examine the epidemiology of zoonotic TB in a global context, as well as optimizing the disease's clinical management. As more healthcare systems begin to routinely use WGS, there is an opportunity to accurately diagnose the causative subspecies of TB in all cases, which will enable identification of previously underrecognized zoonoses and reverse zoonoses and implementation of control interventions in the interests of One Health.

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Use of Genomics to Investigate Historical Importation of Shiga Toxin–Producing *Escherichia coli* Serogroup O26 and Nontoxigenic Variants into New Zealand

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Shiga toxin–producing *Escherichia coli* serogroup O26 is an important public health pathogen. Phylogenetic bacterial lineages in a country can be associated with the level and timing of international imports of live cattle, the main reservoir. We sequenced the genomes of 152 *E. coli* O26 isolates from New Zealand and compared them with 252 *E. coli* O26 genomes from 14 other countries. Gene variation among isolates from humans, animals, and food was strongly associated with country of origin and *stx* toxin profile but not isolation source. Time of origin estimates indicate serogroup O26 sequence type 21 was introduced at least 3 times into New Zealand from the 1920s to the 1980s, whereas nonvirulent O26 sequence type 29 strains were introduced during the early 2000s. New Zealand's remarkably fewer introductions of Shiga toxin–producing *Escherichia coli* O26 compared with other countries (such as Japan) might be related to patterns of trade in live cattle.

Shiga toxin–producing *Escherichia coli* (STEC) is an important public health pathogen, capable of causing hemorrhagic diarrhea and life-threatening kidney failure, particularly in children (*1*). STEC is primarily transmitted through the fecal–oral route, and ruminants are important reservoirs of this zoonotic pathogen (*2*).

Initial research focused on STEC serotype O157:H7 as the main STEC pathogen involved in hemolytic uremic syndrome (*3*). However, STEC serogroup O26 has become an increasingly common cause of human disease. STEC O26 is the second most frequently detected serogroup causing STEC illness in New Zealand (*4*), the United States (*5*), and Europe (*6*).

Whole-genome sequencing (WGS) offers high-resolution identification of related bacterial isolates, while helping to direct source attribution investigations and interventions (*7*). The large amount of sequence data produced by such initiatives as GenomeTrakr (*8*) provides an opportunity to interpret the evolution and transmission of organisms across national boundaries.

New Zealand is a geographically isolated island nation that offers an opportunity to interpret the effects of importation and biosecurity measures on the control and transmission of zoonotic diseases (*9*). New Zealand has a relatively high incidence of notified human disease caused by STEC compared with other countries where the disease is notifiable; 11.9 STEC cases per 100,000 population were reported in New Zealand in 2017 (*10*), compared with 2.85 cases per 100,000 population in 2016 in the United States (*11*). A case–control study in New Zealand identified contact with animal manure and the presence of cattle in the local area as significant risk factors for human infection (*12*). After this case–control study, a New Zealand–wide cross-sectional study using

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Table 1. Summary of 404 *Escherichia coli* serogroup O26 isolates in an investigation of the bacterium's historical importation into New Zealand

	stx Profile				Sequence type			Source			
Country	*stx1*	*stx2*	*stx1* and *stx2*	No *stx*	ST21	ST29	Other	Human	Bovine	Food	Other animal
Australia, n = 1	1	0	0	0	1	0	0	1	0	0	0
Belgium, n = 24	20	1	2	1	20	1	3	16	8	0	0
Continental Europe,* n = 21	3	13	2	3	6	13	2	19	2	0	0
Japan, n = 94	70	8	11	5	88	5	1	77	16	0	1
New Zealand, n = 152	104	0	0	48	136	16	0	32	120	0	0
Other North America,† n = 4	3	0	0	1	3	0	1	2	1	1	0
United Kingdom, n = 29	10	7	8	4	25	3	1	28	1	0	0
United States, n = 79	60	9	5	5	66	10	3	45	27	4	3
Total, n = 404	271	38	28	67	345	48	11	220	174	5	5

*Denmark, n = 1; France, n = 9; Germany, n = 6; Italy, n = 1; Norway, n = 2; Poland, n = 1; Switzerland, n = 1.
†Canada, n = 3; Mexico, n = 1.

a culture-independent test found STEC O26 in 7.2% of young dairy calves sampled (*13*).

Our objective was to compare genomes of *E. coli* serogroup O26 isolates from human clinical cases and cattle in New Zealand with genomes of bacterial isolates from non–New Zealand sources, examining the genetic diversity and population structure, evolution, time to most recent common ancestor (tMRCA), antimicrobial resistance, and virulence genes. These data can be used to infer the probable importation, transmission, and evolution of STEC O26, which can inform risk management decisions with regard to movement of reservoir animals, as well as potential interventions for public health. This research received Massey University Ethics approval (Notification No. 4000016530).

Methods

New Zealand Bacterial Isolates: Selection, DNA and Library Preparation, and Sequencing

We conducted random stratified selection, by year, region, farm, and source, of 152 serogroup O26 bacterial isolates from New Zealand human sources (32 isolates) and bovine sources (120 isolates) from 1985 to 2016. We previously analyzed a subset of 66 bovine isolates as part of a cross-sectional study of STEC prevalence on dairy farms (*13*). We obtained human isolates from the Institute of Environmental Science and Research (Wallaceville, New Zealand) and bovine isolates from the Hopkirk Research Institute at Massey University (Palmerston North, New Zealand). We extracted DNA from a single colony picked

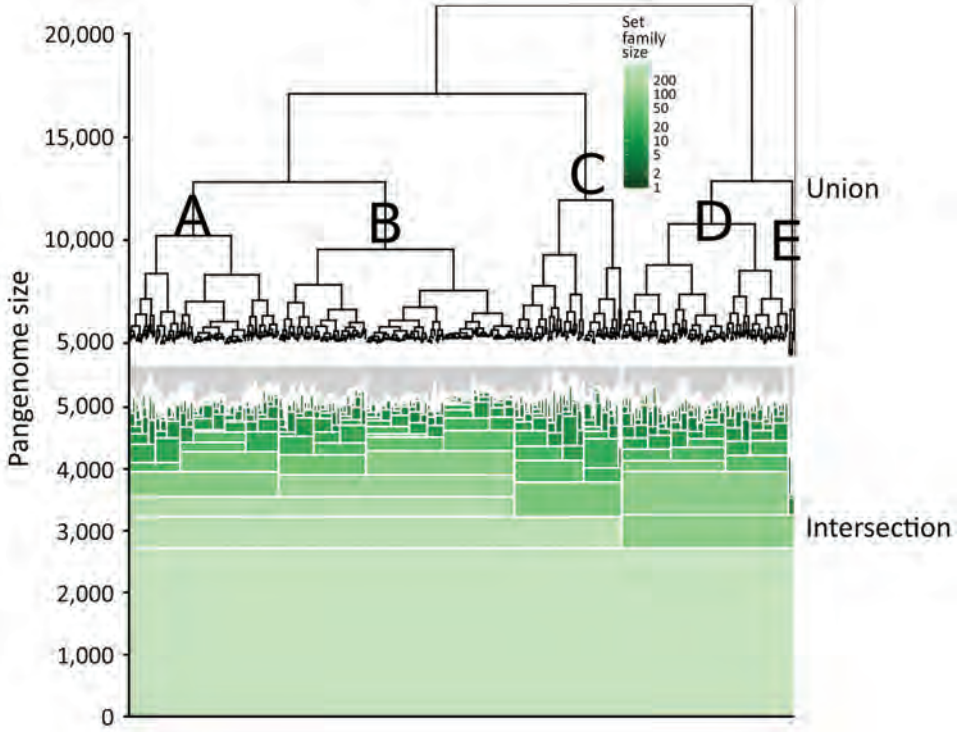

Figure 1. Hierarchical set analysis of 404 *Escherichia coli* serogroup O26 isolates in investigation of historical importation of Shiga toxin–producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand, with a hierarchical set RaxML pangenome tree (top of figure) and shared gene groups visualized in green (bottom of figure). This figure illustrates shared gene groups after pangenome analysis. The union portion represents the pangenome relatedness between bacterial isolates. A–E indicate clades.

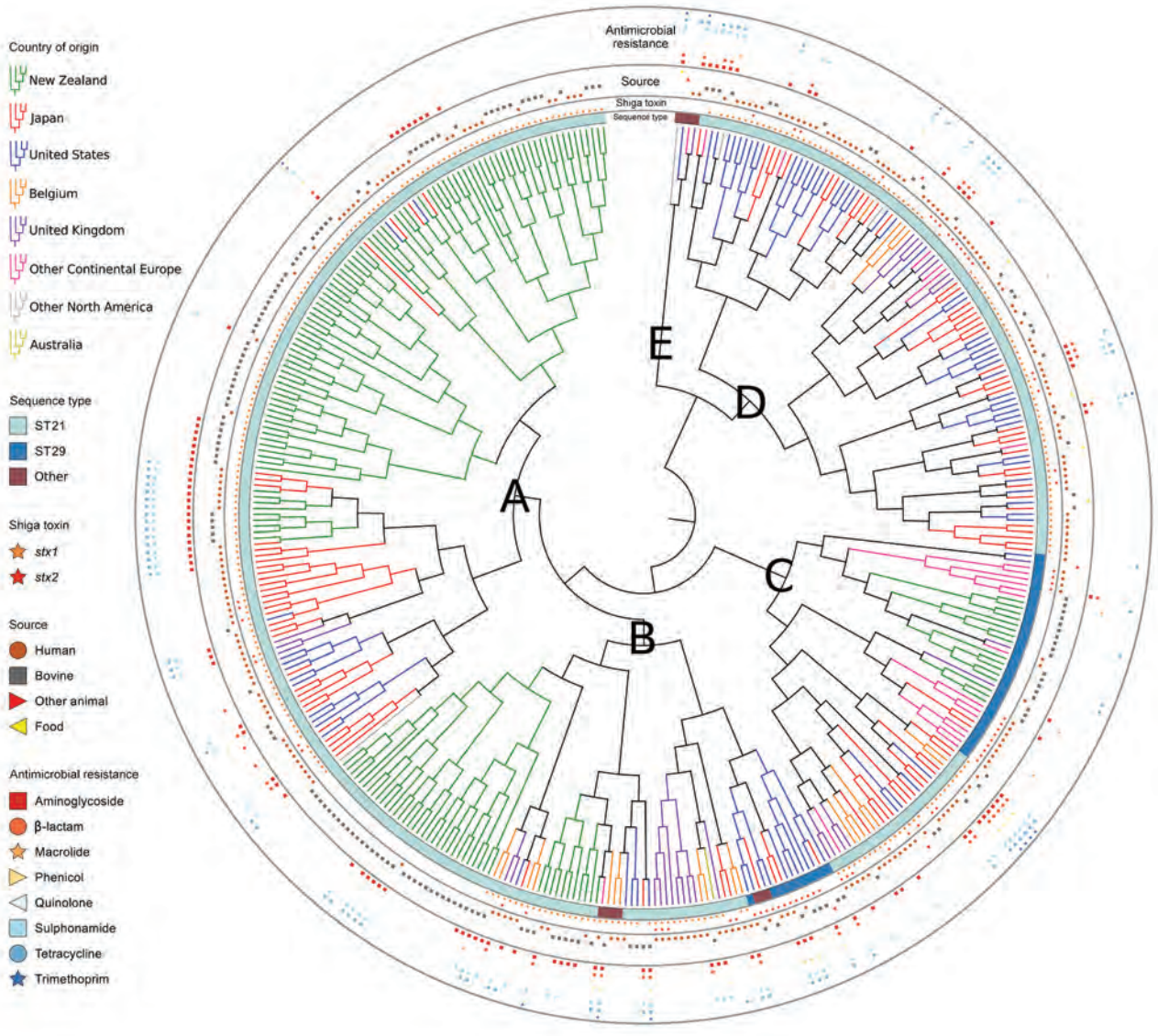

Figure 2. Hierarchical set RaxML tree of pangenome elements of 404 *Escherichia coli* serogroup O26 isolates in investigation of historical importation of Shiga toxin–producing *E.* serogroup O26 and nontoxigenic variants into New Zealand. A–E indicate clades, which are annotated. ST, sequence type.

from Columbia Horse Blood Agar (Fort Richard Laboratories, http://www.fortrichard.com) using the QIAamp DNA MiniKit (QIAGEN, https://www.qiagen.com) and prepared sequencing libraries using the Nextera XT DNA Library Preparation Kit (Illumina, https://www.illumina.com). Prepared libraries were submitted to New Zealand Genomics Limited (University of Otago, https://www.otago.ac.nz/genomics/index.html), which performed sequencing using Illumina MiSeq 2 × 250 bp PE or Illumina HiSeq 2 × 125 bp PE v4.

Processed reads are publicly available on the National Center for Biotechnology Information Sequence Read Archive under BioProject ID PRJNA396667. Metadata are stored under BioSample accession nos. SAMN07430747–SAMN07430900 (Appendix 1 Table 1, https://wwwnc.cdc.gov/EID/article/25/3/18-0899-App1.pdf).

Selection and Retrieval of Publicly Available *E. coli* Serogroup O26 Raw Sequence Data

To standardize the downstream genomics comparison pipeline, we included only bacterial isolates with raw sequencing data in this study. Isolate selection was stratified by country, year, and isolation source; we randomly selected up to 4 isolates from the same country, year, and

Table 2. PERMANOVA analysis of *Escherichia coli* serogroup O26 pangenome genes and virulence genes in an investigation of the bacterium's historical importation into New Zealand*

Dataset, variable, no. genes	df	p value	Component of variation, %
Pangenome, n = 21,399			
Sequence type	5	0.0001	33
Country	14	0.0001	18
Isolation source	3	0.358	<0.01
stx profile	3	0.0001	6
Virulence genes, n = 192			
Sequence type	5	0.0001	83.7
Country	14	0.01	1.9
Isolation source	3	0.07	0.3
stx profile	3	0.0001	6.2

*This method determines whether the variation of the dataset is significantly associated with a particular variable. Residual variation: pangenome (43.0%), virulence genes (7.8%).

isolation source. Raw beef samples were classified as bovine, whereas the food classification indicated nonmeat samples (e.g., spinach, flour). All potential sequences for this study were selected in December 2017, and 2 corresponding authors of publicly available assembled genomes (*14*,*15*) provided unpublished raw sequence data (S. Dellanoy, French Agency for Food, Environmental and Occupational Health & Safety, pers. comm., 2017 Jan 12; C. Gabrielsen, St. Olavs University Hospital, pers. comm., 2017 Jan 18). All 252 publicly available serogroup O26 sequences selected for this study are listed in Appendix 1 Table 2.

Assembly, Annotation, and Initial Analyses of WGS Data

We used the Nullarbor pipeline in the accurate mode (*16*) to evaluate, assemble, and annotate the WGS data and ECTyper to identify somatic (O) and flagellar (H) antigens (O:H serotype) (https://github.com/phac-nml/ecoli_serotyping). We identified virulence and resistance genes using ABRicate (https://github.com/tseemann/abricate), which bundles multiple databases for gene queries (Resfinder, CARD, ARG-ANNOT, NCBI BARRGD, NCBI, EcOH, PlasmidFinder, Ecoli_VF and VFDB). Identified attributes, metadata, virulence genes, and resistance genes for all genomes are provided in Appendix 2 (https://wwwnc.cdc.gov/EID/article/25/3/18-0899-App2.xlsx).

We performed pangenome analysis with the FindMyFriends package in the RStudio environment (*17*), which groups genes into orthologous clusters by implementing the cd-hit clustering algorithm (*18*), followed by a cluster refinement based on k-mer similarity. We examined pangenome composition using the HierarchicalSets package (*19*), estimating the similarity of isolates based on the number of shared (core) and characteristic (accessory/pan) genes. Gamma heterogeneity (the ratio of the number of core genes [intersect] to the number of pan genes [union]) was calculated for each group of genomes, and isolates are hierarchically clustered to minimize total heterogeneity, producing a dendrogram representation of genomic similarity.

We generated RaxML maximum-likelihood trees using a general time-reversible model from the concatenated alignment of all core genes outputted by FindMyFriends (*20*). Then, we created a dissimilarity matrix with the virulence gene output, based on the presence or absence of virulence genes between pairs of isolates, and used it to create neighbor-joining trees.

We evaluated the pangenome similarity matrix, as well as a dissimilarity matrix of the 192 virulence genes, with PERMANOVA (PRIMER-E; Quest Research Limited, https://www.primer-e.com) by using sequence type (ST), country, isolation source, and *stx* profile as independent factors. Phylogenetic figures were created using the iTOL (Interactive Tree of Life) software (*21*), and further amended using Inkscape open source software version 0.92.2 (https://inkscape.org).

Single-Nucleotide Polymorphism Core Gene Alignment and tMRCA Analyses

We created a core gene alignment from the FindMyFriends package using DECIPHER (*22*). Two core alignments were performed for 2 STs: ST21 (345 isolates) and ST29 (48 isolates). Recombinant regions and identical isolates were removed using Gubbins 2.3.1 (*23*), resulting in 344 ST21 isolates and 48 ST29 isolates in the final analysis.

We determined the tMRCA using BEAST2 (*24*). The temporal signal was evaluated with BactDating (*25*) and found to be significant for both ST21 and ST29 data. Model evaluation of a combination of substitution, clock, and population models was performed using a method-of-moments estimator (*26*), and evaluation of log files using Tracer version 1.6.1 (http://tree.bio.ed.ac.uk/software/tracer/) led to a preferred model selection with the lowest AICM (Akaike's information criterion for Markov chain Monte Carlo) estimates and consistent tracer line. General time-reversible substitution models were used to estimate tMRCAs with a coalescent extended Bayesian skyline model and relaxed molecular clock (*27*). tMRCA analysis was calibrated by tip dates (ST21, 1947–2017; ST29, 1952–2017); decimal dates were rounded to the middle of the month or year if an exact date was not available within the month or year. Effective sample size exceeded 100 for all models evaluated. Maximum-clade credibility trees were created using TreeAnnotator version 2.4.7 with a 10% burn-in (*24*). We determined the substitution rate for each ST, multiplying the substitution rate estimated by BEAST2 by the number of analyzed single-nucleotide polymorphisms (SNPs) and dividing the product by the mean genome size of the isolates analyzed.

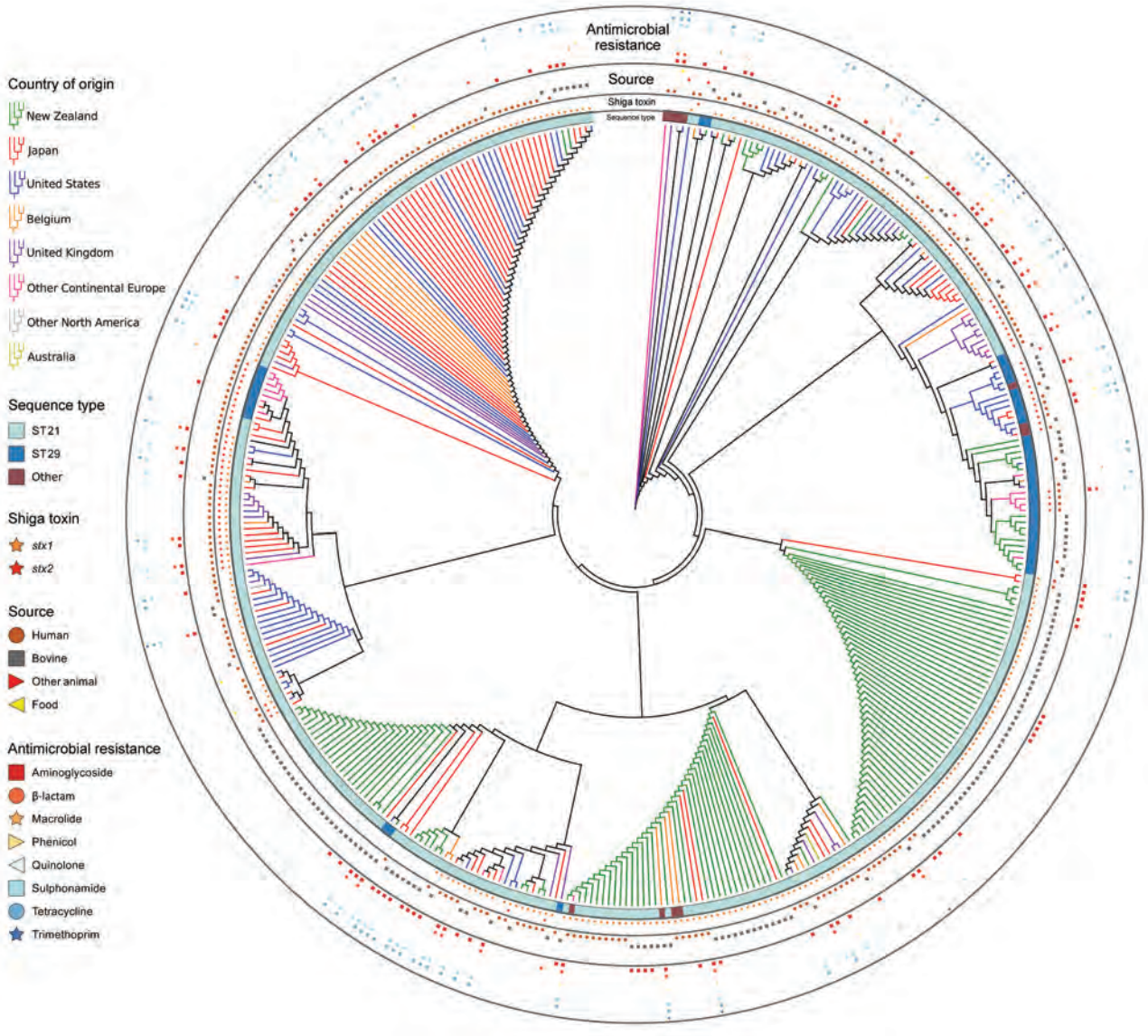

Figure 3. Neighbor-joining tree of 192 virulence genes of 404 *Escherichia coli* serogroup O26 isolates in investigation of historical importation of Shiga toxin–producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand. Branch lengths are ignored to better illustrate the country of origin of each isolate; therefore, closely spaced trellis-like branches have identical virulence profiles. ST, sequence type.

Cattle Importation Data

New Zealand cattle importation data were combined from a previous publication of historical importations of cattle into New Zealand (*28*) and Food and Agriculture Organization data from 1961 to 2013 (*29*). Live cattle imports into Japan from 1961 to 2013 were obtained from Food and Agriculture Organization data to enable us to compare them with New Zealand live cattle imports (*29*).

Results

Most genomes were obtained from New Zealand (152 genomes), Japan (94 genomes), and the United States (79 genomes) (Table 1). Most isolates were ST21 (345 isolates) and ST29 (48 isolates); multiple *stx* gene profiles were represented (*stx1*, *stx2*, *stx1*, *stx2*, and no *stx*); and the source of isolates fell into 4 groups: human, bovine, food, and other animal.

Evolutionary Dynamics of *E. coli* Serogroup O26

Gene clustering analyses identified 2,718 core genes, 8,904 accessory genes, and 9,777 singleton genes, for a pangenome size of 21,399 genes. The pangenome was open and had a Heaps' Law coefficient of 0.35. Hierarchical clustering based on pangenome composition enabled the definition of 5 independent classes of isolates (A–E) for comparative purposes; group E contained

Table 3. Detection of antimicrobial resistance genes of 404 *Escherichia coli* serogroup O26 isolates in an investigation of the bacterium's historical importation into New Zealand*

	Antimicrobial resistance, %							
Factor evaluated	Aminoglycoside	β-lactam	Macrolide	Phenicol	Quinolone	Sulphonamide	Tetracycline	Trimethoprim
Country								
Australia, n = 1	100	0	0	100	0	100	0	0
Belgium, n = 24	67	25	4	17	0	67	42	17
Continental Europe, n = 21	29	14	10	5	0	24	14	0
Japan, n = 94	32	13	1	6	1	32	24	3
New Zealand, n = 152	26	1	1	0	0	12	12	1
Other North America, n = 4	50	25	25	0	25	50	100	0
United Kingdom, n = 29	24	21	0	4	0	29	25	4
United States, n = 79	13	13	0	4	0	4	14	3
Source*								
Human, n = 220	28	12	<1%	4	<1%	27	21	3
Bovine, n = 175	27	6	3	4	0	16	15	2
Food, n = 5	20	20	0	0	20	20	20	20
Total isolates, n = 404	30	10	2	4	1	23	19	3

*No antimicrobial resistance genes were detected in other animals (n = 5).

non-O26:H11 strains (Figure 1); clades A–D each share ≈4,000 core genes. A pangenome hierarchical set tree was annotated with country, ST, isolation source, and antimicrobial resistance gene class (Figure 2), with a real branch length figure available (Appendix 1 Figure 1). Multiple strains have circulated globally and are present in many countries.

PERMANOVA analysis for the pangenome and virulence genes revealed that gene variation among isolates was mostly explained by ST (pangenome, 33%; virulence genes, 84%), country of origin (pangenome, 18%; virulence genes, 2%), and *stx* profile (pangenome, 6%; virulence genes, 6%). Isolation source was not a significant factor (Table 2).

Pathogenicity and Antimicrobial Resistance of *E. coli* O26

A neighbor-joining tree based on a distance matrix of the presence and absence of virulence genes detected (n = 192) (Figure 3) shows that a large number of New Zealand isolates had identical virulence profiles from human and bovine sources; a large clade from Japan, the United States, and Belgium also has identical profiles. We compiled a real branch length figure (Appendix 1 Figure 2), the name and function of all 192 detected virulence genes (Appendix 1 Table 3), and the virulence genes detected for each genome (Appendix 2).

We detected resistance genes for 8 classes of antimicrobial drugs (Table 3; Appendix 2 Table). Resistance genes were detected in 252 (62.4%) bacterial isolates.

tMRCA Analysis and Inferred Global Importation and Transmission of *E. coli* O26

A core gene alignment of the 344 serogroup O26 ST21 isolates generated 9,702 SNPs, and the 48 ST29 isolates generated 4,686 SNPs. In the tMRCA estimates for ST21 isolates (Figure 4) and ST29 isolates (Figure 5), important convergence dates were annotated with a 95% highest posterior density (HPD) interval (Appendix 1 Figures 3, 4). The calculated substitution rate for ST-21 was 1.4×10^{-7} substitutions/site/year (95% CI $1.1–1.7 \times 10^{-7}$ substitutions/site/year), and the substitution rate for ST29 isolates was 3.2×10^{-7} substitutions/site/year (95% CI $2.3–3.9 \times 10^{-7}$ substitutions/site/year).

Four New Zealand ST21 monophyletic clades indicate tMRCA estimates from the 1920s through the 1990s (Figure 4). Individual New Zealand monophyletic clades show evidence of importation from Europe (95% HPD interval 1958–1982) and more recently from the United States (95% HPD interval 1971–1992). Paraphyletic clades are visible from European sources, particularly from US and Japan isolates, which create a panmictic community, indicating frequent transmission between these countries. Two New Zealand ST29 monophyletic clades show tMRCA estimates from the late 1960s to the early 21st century (Figure 5). Japanese strains of 4 *stx2*-positive ST29 isolates appear to be closely related to strains from the United States and from Europe. Minimal evidence exists of transmission of New Zealand strains to the other countries evaluated in this study (Figures 4, 5).

Most live cattle imported into New Zealand arrived during the 1860s (Figure 6), and importations increased during the 1950s–1990s. New Zealand imported fewer cattle than Japan for all years examined; since 1991, New Zealand has consistently imported <100 live cattle per year, whereas Japan has imported >10,000 per year (Figure 7).

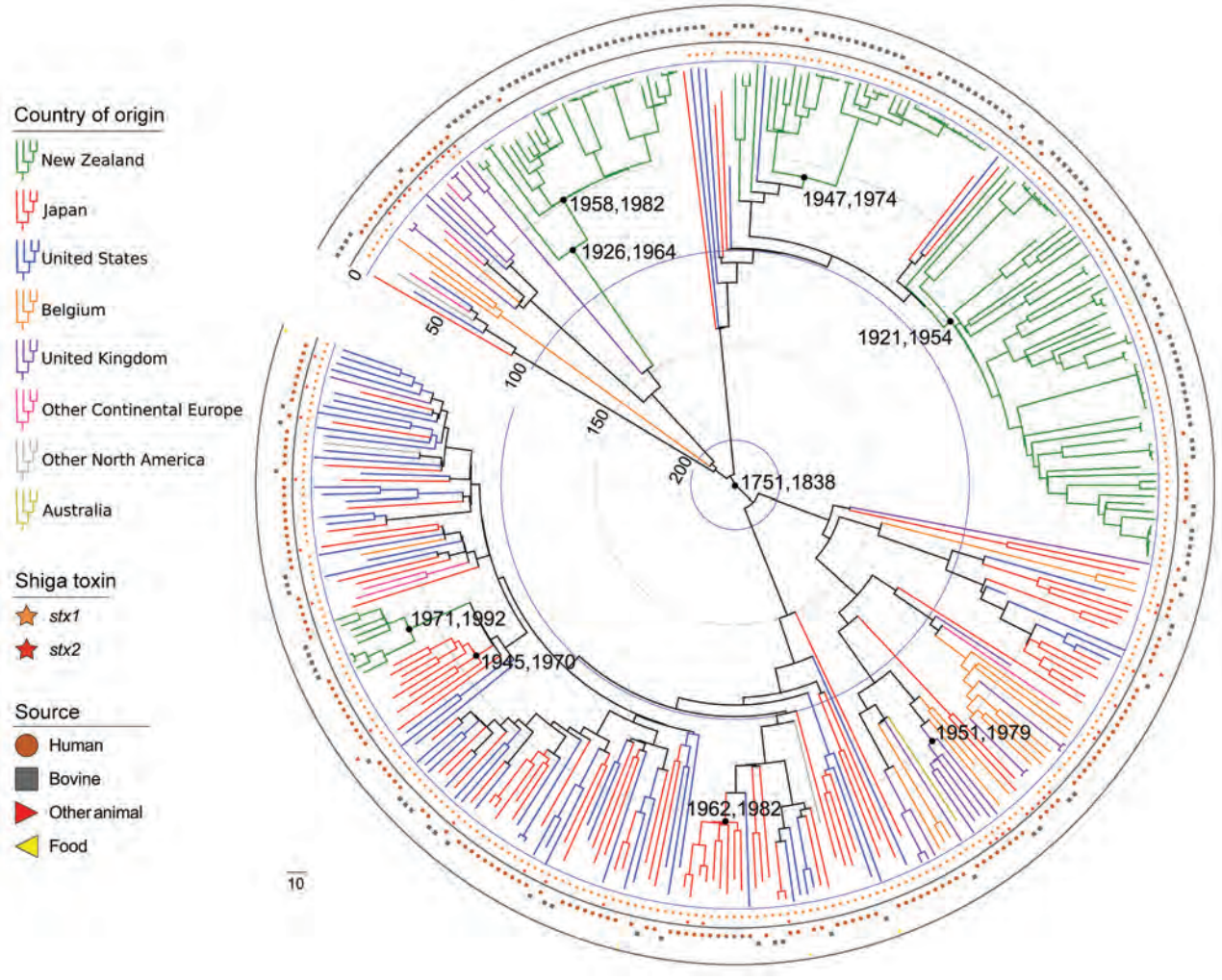

Figure 4. Maximum clade credibility tree of time of most recent common ancestor analysis of 344 *Escherichia coli* serogroup O26 sequence type 21 isolates in investigation of historical importation of Shiga toxin–producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand. Key convergence dates are annotated with 95% highest posterior density intervals, and the concentric circles indicate earlier time periods (blue, 100 years; gray, 50 years) from the age of the newest isolate (2017.5 in decimal years).

Discussion

We used WGS to compare New Zealand *E. coli* serogroup O26 bacteria with isolates from around the world. Our analyses demonstrated contrasting patterns of global panmixia and local isolation between different lineages of the O26 serogroup. Based on these patterns, we suggest that global O26 exchange most likely is linked to the import/export of live cattle; periods of between-country transmission occurred mainly during the 20th century. In New Zealand, several lineages have unique virulence profiles, each of which is likely to have been introduced at a single point in the recent past, and subsequently undergone local expansion and diversification. This country-specificity contrasts with other strains, notably from the United States and Japan, that appear to have been exchanged between geographic locations on multiple occasions over the same time.

Pangenome and virulence gene PERMANOVA analysis (Table 2) indicated that variation was best explained by multilocus subtype, country of origin, and *stx* profile. The lack of isolation source as a significant factor for pangenome or virulence gene analysis (Table 2) suggests that serogroup O26 isolates from humans, cattle, food, and other animals are not genetically differentiated and zoonotic transmission of this bacteria occurs frequently.

WGS analysis of STEC O157:H7 isolates in New Zealand showed findings similar to ours, where the within-country isolate diversity was unique and not related to the source of the isolate (*30*). As in our study, bovine and human New Zealand STEC O157:H7 isolates were closely related by genotyping, compared with isolates from the United States and Australia (*30*). Genes classified as virulent for humans are involved in the intestinal colonization of cattle

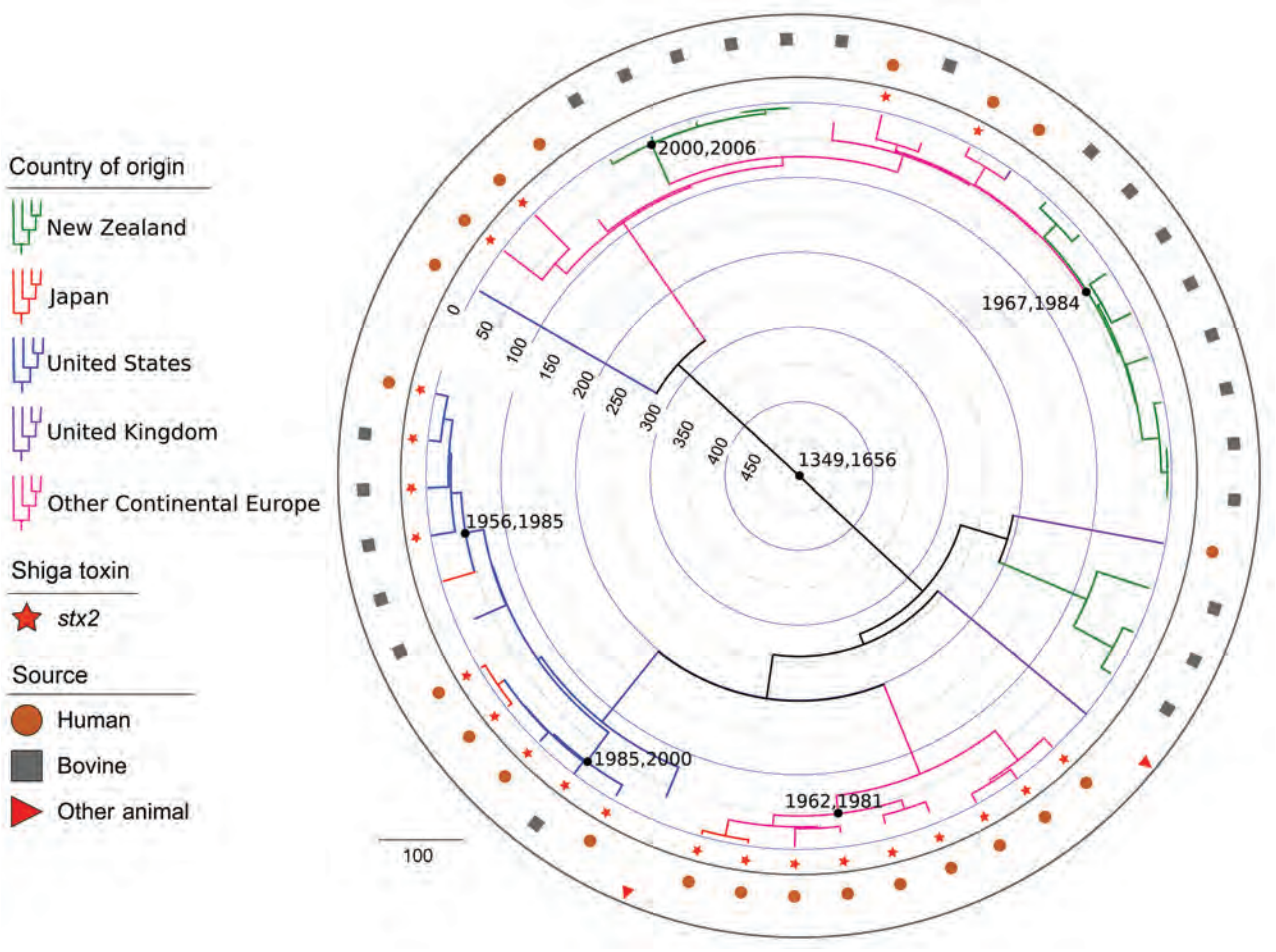

Figure 5. Maximum clade credibility tree of time of most recent common ancestor analysis of 48 *Escherichia coli* serogroup O26 sequence type 29 isolates in investigation of historical importation of Shiga toxin–producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand. Key convergence dates are annotated with 95% highest posterior density intervals, and concentric circles indicate prior time periods (blue, 100 years; gray, 50 years) from the age of the newest isolate (2017.0411 in decimal years).

(*31*,*32*); therefore, the contrasting virulence attributes associated with isolates from different countries might indicate separate niche adaptation and advantages for colonization of local host populations.

We observed that the *E. coli* O26 genome is open (Heaps' Law coefficient 0.35), meaning that the suite of genes possessed by each isolate is highly variable. These genes will be picked up from their local environments. The Public Goods Hypothesis proposes that the horizontal exchange of widely available DNA sequences is the primary driver for local bacterial evolution (*33*). This horizontal exchange of DNA will influence virulence profiles and other phenotypic traits, such as antimicrobial resistance. Applying this hypothesis to the evolution of *E. coli* O26 implies continued adaptation of the bacterial strains in local environments as they further acquire and share genes, which ultimately could lead to the emergence of new pathogenic lineages of STEC.

Evidence of relatively recent acquisition of *stx2* virulence within STEC O26 ST29 is a cause of concern (*14*). Non-STEC ST29 strains are present in cattle in New Zealand, but at the time this article was written, no STEC O26 with the *stx2* virulence gene had been reported there. The emergence of highly pathogenic strains that harbor the *stx2* toxin gene has led to an increase in hemolytic uremic syndrome related to the O26 serogroup (*14*,*34*). Serogroup O26 ST29 *stx2* isolates also have been identified in Japan (*35*), and 2 separate clades of *stx2* ST29 isolates from Japan may have been imported from the United States and Europe (Figure 5). The lack of highly pathogenic ST29 *stx2* isolates in New Zealand might be due to few live cattle importations, as well as no major horizontal genetic transfer events of *stx2* to *E. coli* O26 in New Zealand.

The resistance profiles form distinct combinations of resistance genes in isolates from particular countries (Figures 2, 3). Antimicrobial drugs are not usually prescribed

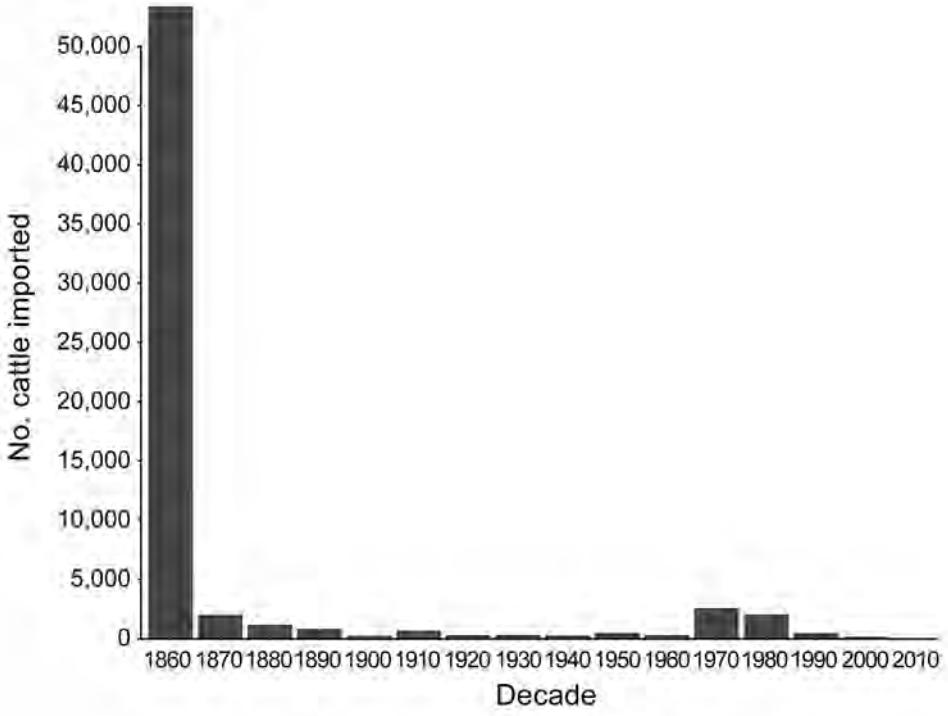

Figure 6. Historical importations of live cattle into New Zealand, 1860–2010.

for human STEC infections (*36*); however, selection pressure from antimicrobial drug use in livestock and humans with undiagnosed diarrheal illness may influence the evolution of resistance. Antimicrobial resistance from all human isolates was higher or equal to that of bovine isolates (Table 3), with the exception of that to macrolides.

The tMRCA of New Zealand *E. coli* O26 clades suggests several separate importations of strains that appear to coincide with cattle importation events (Figures 4–6). Phylogenetic analyses suggest certain New Zealand clades are more associated with specific geographic areas (e.g., United States or continental Europe), indicating that transmission pathways are likely to exist through live animal imports. The estimated substitution rates for ST21 and ST29 isolates in the present study are similar to previous estimates for serogroup O26 ($2.8–4.3 \times 10^{-7}$ substitutions/site/year) (*37*) and O157:H7 (*38*). The tMRCA of all ST21 (1751–1838) (Figure 4) is similar to tMRCA estimates of ≈213 years ago for a large ST21 clade evaluated by Ogura et al. (*37*).

We observed a remarkable difference between 2 island nations with the most bacterial isolates analyzed: New

Figure 7. Comparison of live cattle imported (log_{10} scale) into New Zealand and Japan during 1961–2013. Japan, black; New Zealand, gray.

Zealand (n = 152) and Japan (n = 94). In the Hierarchical-Sets pangenome (Figure 2), virulence gene (Figure 3), and BEAST2 ST21 (Figure 4) analyses, New Zealand isolates show monophyletic clades, whereas Japan isolates are paraphyletic with US isolates. The historically larger number of live cattle importations into Japan than into New Zealand (Figure 7) might explain this difference. Japan was a major importer of US live cattle during the second half of the 20th century, until the detection of bovine spongiform encephalopathy led to a ban on all live cattle from the United States in 2003; current importations come from Australia (*39*,*40*). The relatively large number of imported live cattle in Japan could explain the different population structure of *E. coli* O26 in New Zealand and Japan.

Our dataset enables minimal interpretation of open border areas, such as the European Union or countries in the North American Free Trade Agreement (Mexico, Canada, and the United States), but our results from New Zealand suggest the introduction of serogroup O26 bacterial strains occurred during periods of intensive cattle importation. In cattle, STEC is a commensal bacterium and is shed intermittently (*41*); therefore, testing cattle before transportation is unrealistic. Our tMRCA and phylogenetic analyses suggest that minimal exchange of strains has occurred between countries in the 21st century; however, continued movement of cattle across international borders is likely to continue to influence the spread and genetic diversity of STEC around the world.

The results of our study are subject to several limitations. The quantity and diversity of *E. coli* O26 isolates from other countries were variable. More *E. coli* O26 isolates from Australia would have enabled us to better compare the effect of importation of cattle into New Zealand because Australia was the source of many historical cattle importations (*28*). Sequence data were more common from the past few years, and mostly human isolates were available. Although we randomly selected our New Zealand isolates from human and bovine isolates spanning >30 years from a diverse geographic range in New Zealand, some isolates were from the same farm (Appendix 1 Table 1), leading to a potential bias. Although non-STEC and STEC strains of the same serogroup are commonly of different lineages (*42*), our focus on a defined O surface antigen (O26) to classify bacterial isolates and evaluate evolutionary and phylogenetic relationships is consistent with other studies (*30*,*37*).

Our results suggest worldwide dissemination of multiple strains of ST21 and ST29 STEC and nontoxigenic serogroup O26 lineages occurred during the 20th century. Close genetic similarities between *E. coli* O26 isolated from multiple different sources indicates common transmission pathways among animals, food sources, and humans. The limited introductions of *E. coli* O26 strains into New Zealand are most likely linked to minimal importations of live cattle.

Further sequencing of historical isolates from multiple sources will improve evolutionary and epidemiologic studies. Full use of the genomic information of STEC will require a coordinated international approach to sequencing, data curation, analysis, and interpretation of those data (*43*). Although it is difficult to directly attribute transmission and emergence of STEC strains based on global historical events, interpreting evolutionary genomic data against economic and sociopolitical factors can help determine the drivers of pathogen emergence and dissemination, and inform future public health policy.

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About the Author

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Utility of Whole-Genome Sequencing to Ascertain Locally Acquired Cases of Coccidioidomycosis, Washington, USA

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Coccidioidomycosis is an emerging fungal infection in Washington, USA, and the epidemiology of the disease in this state is poorly understood. We used whole-genome sequencing to differentiate locally acquired cases in Washington on the basis of the previously identified phylogeographic population structure of *Coccidioides* spp. Clinical isolates from coccidioidomycosis cases involving possible Washington soil exposure were included. Of 17 human infections with epidemiologic evidence of possible local acquisition, 4 were likely locally acquired infections and 13 were likely acquired outside Washington. Isolates from locally acquired cases clustered within the previously established Washington clade of *C. immitis*. Genetic differences among these strains suggest multiple environmental reservoirs of *C. immitis* in the state.

Coccidioidomycosis, also known as Valley fever, is a disease of growing public health concern and is caused by 2 closely related fungal species, *Coccidioides immitis* and *C. posadasii*. *Coccidioides* spp. are dimorphic, forming mycelia in soil and arthroconidia capable of infecting humans and certain other mammals, and spherules in mammalian tissue (*1*). This mycosis causes a wide spectrum of conditions, ranging from asymptomatic infection and mild pulmonary disease to severe pulmonary and disseminated disease, which can be life-threatening (*1*,*2*). Infection is generally caused by inhalation of pathogenic arthroconidia from disturbed soil or dust, such as through occupational or recreational activities or weather events that raise dust (*2*–*7*).

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Coccidioides spp. are endemic to warm, arid regions of the Western Hemisphere that have low rainfall (*1*,*8*). The 2 species have colonized different geographic locations: *C. posadasii* is largely found in Arizona, Texas, Mexico, and Central and South America; and *C. immitis* is primarily found in central and southern California. Coccidioidomycosis causes a major burden of disease in many of these areas; the annual incidence in Arizona is >75 cases/100,000 population (*9*). Case counts have recently increased in California; >5,300 cases were reported in 2016 (13.7 cases/100,000 population (*10*). Climate suitability projections predict expansion of the suitable environment for coccidioidomycosis and increasing incidence in areas that already sustain *Coccidioides* growth (*11*).

During 2010–2011, three cases of coccidioidomycosis were identified and reported in southeastern Washington (*7*). Subsequently, *Coccidioides* DNA and viable propagules were isolated from soil at a location of suspected exposure for 1 of these cases (*12*,*13*). Whole-genome sequencing (WGS) analysis of the recovered isolates demonstrated near identical genetic identity between soil and clinical isolates, further confirming endemic presence of infectious *C. immitis* in Washington (*13*).

Public health surveillance for coccidioidomycosis was implemented statewide in Washington in April 2014. Before 2014, cases of coccidioidomycosis were reported sporadically, but no standard reporting procedure existed. Since 2014, the number of reported cases of coccidioidomycosis has increased each year; reported cases in Washington suspected to be locally acquired have higher rates of hospitalization and death compared with cases from other disease-endemic regions (*14*).

Because most persons infected with *Coccidioides* spp. are asymptomatic or have only mild illness, and most illness self-resolves, we believe that only the most severe cases of coccidioidomycosis with exposure in Washington are being identified and reported to public health

authorities. In addition, *Coccidioides* spp. infections might be asymptomatic or only manifest as subclinical disease until a reactivation or complication develops later. Also, most residents of Washington have some travel history to historically disease-endemic areas, such as Arizona, California, or Mexico. These findings create a challenge in differentiating between autochthonous and imported cases of coccidioidomycosis, which is a useful distinction for public health surveillance in Washington.

To better determine the nature of exposure for reported cases of coccidioidomycosis in Washington, the Washington Department of Health, in collaboration with the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) and the Translational Genomics Research Institute (Flagstaff, AZ, USA), initiated enhanced surveillance by incorporating WGS of clinical isolates aimed at determining the geographic origins of *Coccidioides* spp. strains. This approach is based on previous population structure studies providing evidence that *C. immitis* and *C. posadasii* have well-defined geographic structures (*15*–*19*). Subsequently, Engelthaler et al. demonstrated that isolates can generally be assigned to the specific geographic populations on the basis of WGS analysis (*20*). This approach is consistent with a general trend in molecular epidemiology that uses WGS and a One Health approach to ascertain clusters or outbreaks of bacterial, fungal, viral, and parasitic diseases (*21*–*23*).

We report WGS analyses of isolates from 17 recent coccidioidomycosis cases in Washington, and demonstrate that 13 (76%) cases involved coccidioidomycosis most likely acquired outside Washington; 4 cases (24%) likely involved local acquisition. We discuss the utility of the WGS method to enhance epidemiologic surveillance.

Materials and Methods

Cases and Isolates

We identified coccidioidomycosis cases through passive reporting to local health jurisdictions from healthcare providers in Washington and laboratories performing testing for residents of Washington. Confirmed cases, as classified according to the Council of State and Territorial Epidemiologists case definition (*24*), reported during 2014–2017 were included in the analysis. Reported case-patients were interviewed by local health jurisdictions to determine clinical course, travel history, and any potential soil or dust exposures. Medical records were requested when possible for complete data abstraction to case reporting forms. When the exposure was suspected to have occurred in the disease-endemic area of Washington or exposure history was unknown, available clinical isolates were sent to CDC for confirmation.

At CDC, all isolates were grown on brain heart infusion (BHI) agar at 25°C for 10 days. Genomic DNA was extracted by using the DNeasy Blood and Tissue kit (QIAGEN, https://www.qiagen.com/us) according to the manufacturer's recommendations. Genomic DNA was stored at –20°C until further use. The Washington State and CDC Institutional Review Boards determined this project to be enhancing surveillance for a notifiable condition and did not require human subjects review.

Genome Sequencing, Assembly, and Analyses

We sequenced genomes of 18 isolates from 17 patients using Illumina (https://www.illumina.com) HiSeq and MiSeq sequencing platforms, as described (*20*,*25*). We prepared DNA samples for paired-end sequencing by using the Kapa Biosystems (https://www.kapabiosystems.com) Library Preparation Kit protocol with an 8-bp index modification and sequenced to a read length of 250 bp on the Illumina HiSeq or 250 bp on the Illumina MiSeq. All WGS data files have been deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject PRJNA472461.

We performed genome assembly and analyses as described (*20*). In brief, the Washington-1(B10637) strain was used as the reference for *C. immitis* single-nucleotide polymorphism (SNP) matrices, and the B10813_Tx strain was used as a reference in the *C. posadasii* SNP matrix. We aligned Illumina read data against the respective reference assemblies by using Burrow-Wheeler Aligner version 0.7.7 (http://bio-bwa.sourceforge.net) and identified SNP variants by using SamTools version 0.1.19 (http://samtools.sourceforge.net). We filtered SNP calls by using a publicly available SNP analysis pipeline (*26*) (http://tgennorth.github.io/NASP), as described, to remove positions with <10 times coverage with <90% variant allele calls, or identified by using Nucmer (http://nebc.nox.ac.uk/bioinformatics/docs/nucmer.html) as being within duplicated regions in the reference (*20*,*25*). We constructed a maximum-parsimony phylogeny on the basis of SNP matrices by using MEGA7 (http://www.megasoftware.net), including 17 described strains from various geographic areas for comparison; we determined bootstrap support for the tree by using 1,000 reiterations (*20*).

We extracted candidate SNPs that distinguish Washington strains from non-Washington strains (Appendix Table, https://wwwnc.cdc.gov/EID/article/25/3/18-1155-App1.pdf) from whole-genome assemblies by using Samtools version 1.8, EMBOSS version 6.5.7, and NCBI-toolkit version 7.0.0 (*27*–*29*). We chose SNPs if their positions were consecutive or no more than 1 nt apart and had ≥10 times coverage. In brief, we extracted an ≈1-kb region surrounding candidate SNP positions from all draft assemblies, aligned it to Washington-1(B10637) reference, and designed degenerate primers by using Primer3 version 3.0 (*30*). We compared regions with those of *C. immitis* strain H538–4 to determine genomic loci annotation.

Results

During 2014–2017, a total of 167 confirmed cases of coccidioidomycosis were reported in Washington. For these cases, 18 isolates (Table) from 17 cases were available. Travel and exposure histories suggested local acquisition in Washington (9 case-patients) or were unknown at the time of specimen collection (8 case-patients).

We speciated 14 isolates as *C. immitis* and 4 as *C. posadasii* and subjected these isolates to WGS with 25–160 times sequencing coverage. A total of 68,717 SNPs were identified by comparison with the reference isolate. Phylogenetic analysis and comparison with sequenced isolates of *C. immitis* demonstrated that 5 isolates from 4 patients in Washington were highly genetically related to each other and to isolates from the identified Washington clade that contained human and soil isolates (*13*). No more than 354 SNPs differentiated any 2 isolates within the Washington cluster, which was also supported by 100% bootstrap values (Figure). In contrast, >10,000 SNPs differentiated the Washington cluster from its nearest neighbor, an isolate from another case-patient in Washington. This case-patient (B12496) reported travel history to disease-endemic regions in California and Mexico. Tens of thousands of SNPs differentiated Washington cluster isolates from those from other states. No more than 3 SNPs were identified among multiple soil isolates collected 4 years apart from a single sampling site, and 4 SNPs were identified between isolates collected 2 years apart from 1 patient in Washington who had a chronic infection. However, 234–324 SNPs were identified in pairwise comparisons between isolates from different patients in the Washington clade (Figure, panel B).

Three of the 4 case-patients infected with Washington clade strains reported no travel history outside Washington. Conversely, 8 of 9 *C. immitis* isolates that did not cluster with the Washington clade were isolated from patients who reported travel to California.

Washington strains can be distinguished from non-Washington strains in 3 genomic regions on the basis of annotations from *C. immitis* strain H538-4; locus DS016982: adipocyte-derived leucine aminopeptidase; locus DS01702: hypothetical protein; and locus DS016985: glyoxal oxidase (Appendix). The DNA fragment from locus DS016982 contains SNP GG at positions 94460–94461 for Washington strains and SNP CA for non-Washington strains and can be amplified by using forward primer 5′-GGTACGTCACAAGTCCCCAG-3′ and reverse primer 3′-AAGAGTACTCGCGAAGGAAGC-5′. For locus DS017021, the DNA fragment amplified with forward primer 5′-CTTGACTGTGCAGGGCCTTA-3′ and reverse primer 3′-ACCGGCCTAACTCCATGGTA-5′ encompasses SNPs GGT for Washington strains and TGC for non-Washington strains at positions 105566–105568) The fragment of locus DS016985 can be amplified by using primer pairs 5′-TTCCGCTTGATGGCTGAAGT-3′/3′-TGTGGCCCTCCTATTGCTTG-5′ and contains consecutive SNP CC for Washington strains and SNP GA for non-Washington strains (positions 232442–232443).

We identified 4 case-patients who had *C. posadasii* infections by using internal transcribed spacer sequencing and confirmed by WGS. Isolates from the 4 case-patients clustered with described *C. posadasii* populations from Arizona (data not shown). Three of those case-patients had traveled to Arizona, and 1 case-patient had an unknown travel history.

Table. Characteristics of 18 clinical isolates subjected to whole-genome sequencing analysis from patients with coccidioidomycosis, Washington, USA, 2014–2017*

CDC-ID	Travel history of patient	*Coccidioides* species	Collection date	Specimen source site	Phylogenetic population
B10917	Unknown	*C. posadasii*	2014	Sputum	Non-Washington
B10918	AZ, MT, SD, OR	*C. posadasii*	2014	Sputum	Non-Washington
B11036	AZ	*C. posadasii*	2015	Lung	Non-Washington
B11034	AZ	*C. immitis*	2014	Lung	Washington
B11019	None	*C. immitis*	2014	BAL	Washington†
B11035	CA	*C. immitis*	2015	Cerebrospinal fluid	Non-Washington
B11080	Mexico, CA	*C. immitis*	2015	Neck	Non-Washington
B11198	Mexico, CA	*C. immitis*	2015	Tissue	Non-Washington
B11299	AZ	*C. posadasii*	2015	Bronchial washings	Non-Washington
B11517	CA	*C. immitis*	2016	Throat	Non-Washington
B11518	None	*C. immitis*	2016	Testicle	Washington†
B11587	CA	*C. immitis*	2016	Tissue	Non-Washington
B11863	HI, Costa Rica	*C. immitis*	2016	Sputum	Non-Washington
B11873	CA	*C. immitis*	2016	Bronchial washings	Non-Washington
B12398	None	*C. immitis*	2016	Blood	Washington
B12495	CA	*C. immitis*	2016	Cerebrospinal fluid	Non-Washington
B12496	Mexico, CA	*C. immitis*	2016	Knee wound	Non-Washington
B13956	None	*C. immitis*	2017	BAL	Washington

*BAL, bronchoalveolar lavage; CDC, Centers for Disease Control and Prevention; ID, identification.
†These isolates were from the same patient.

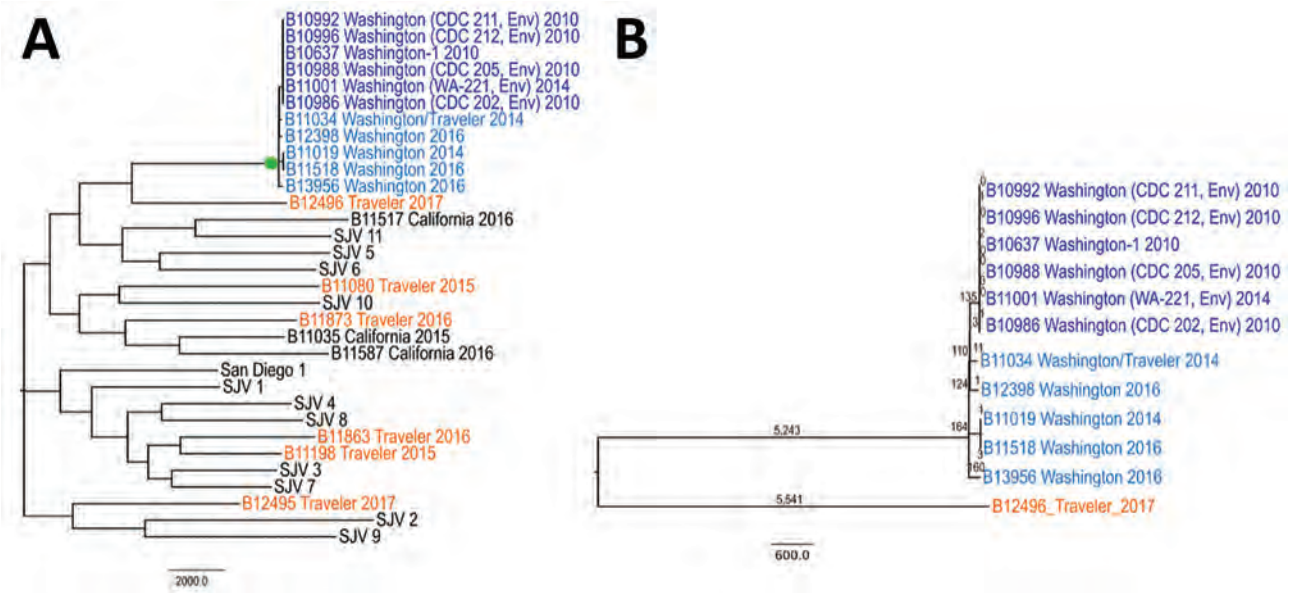

Figure. Genetic relationships among *Coccidioides immitis* isolates. A) Isolates from patients in Washington, USA, compared with isolates from other locations. B) Genetic relationships among *C. immitis* isolates from the Washington clade; single-nucleotide polymorphism numbers are shown above the branches. Dark blue indicates previously described environmental and human isolates (*13*) from Washington, light blue indicates isolates from new cases that were likely acquired in Washington, and orange indicates isolates from cases that were likely acquired outside Washington. Isolates from patients with the documented travel histories are indicated as Traveler. Travel history is detailed in the Table. Isolates with SJV names are from the San Joaquin Valley, California, as described (*20*). Bootstrap values shown in green circle indicate 100% support. Scale bars indicate nucleotide substitutions per site. CDC, Centers for Disease Control and Prevention; Env, environment.

Discussion

Determining the potential sources of exposure and geographic distribution of *Coccidioides* in areas where coccidioidomycosis is uncommon is a surveillance challenge because patients often report travel histories to other disease-endemic areas. To address this problem, we implemented WGS to better determine the likely geographic origin of isolates obtained from patients in Washington who had suspected local acquisition or unknown exposure histories. This method is based on the observation that *Coccidioides* populations have well defined geographic structure, and most isolates can be assigned to specific geographic areas on the basis of their WGS genotypes, such as Arizona, Texas, Mexico, central California, or Washington (*20*). We demonstrated the utility of this tool for investigating the epidemiology of coccidioidomycosis in Washington.

Of 18 isolates from 17 patients included in this study, 5 (28%) clustered with strains isolated from soil in Washington, which was consistent with local exposure. The remaining 13 (72%) isolates clustered with populations from other areas, such as Arizona, California, or Mexico, which was generally consistent with reported travel histories of patients. The only discordance between WGS and epidemiologic data was observed for isolate B11863, which clustered with *C. immitis* isolates from California. The patient from whom this isolate originated reported travel out of state but also reported extensive soil exposure in a county in Washington that had previously reported autochthonous cases. Specifically, the only reported travel was to Hawaii 2 months before disease onset and to Costa Rica 1 year before disease onset, with a layover in California during which the patient did not leave the airport. In addition, the patient reported 1 trip to southern California 6 years before disease onset. This discordance was an unexpected finding and might represent more diversity among *Coccidioides* spp. in Washington than previously understood, reactivated infection because of previous travel, or infection from a fomite or other carrier source of *C. immitis*. Further investigation and surveillance data are needed to determine which hypothesis is most plausible.

Of the 4 patients infected with the local strains from Washington, 3 patients reported no travel history outside Washington. The fourth patient reported travel to Arizona 1 year before the onset of coccidioidomycosis but also reported extensive exposure to local soils in Washington and dust during hiking and gardening. This result indicates that compatible travel history alone does not exclude the possibility of local exposure and demonstrates the added value of genomic approaches to determining exposure location. Although isolates in

the Washington clade were genetically related (Figure, panel A), considerably higher genetic diversity was observed for new strains compared with that detected for the original isolates from Washington identified in 2013 (*13*). Specifically, 245–299 pairwise SNPs were identified among the newly identified strains from Washington, compared with <5 SNPs detected among previously described isolates from Washington from the same exposure site (Figure, panel B). This small but apparent genetic divergence of the new isolates indicates that new infections were acquired from different local *C. immitis* populations, likely at different sites, and suggests the presence of multiple environmental loci for *Coccidioides* spp. in Washington. The divergent isolates were collected from soil in a location ≈70 miles from the suspected exposure location of 1 of the patients. However, in sharp contrast to the populations of *Coccidioide*s spp. in Arizona or California, where isolates are differentiated by thousands of SNPs, the overall low genetic variability in the Washington population is consistent with a relatively small population size, strongly suggesting a recent common ancestry of Washington strains and a relatively recent expansion of *C. immitis* to Washington, (Figure).

Our study has 2 main limitations. First, there might be additional genetically diverse populations of *Coccidioides* in Washington that have not yet been discovered or proven; therefore, some genotypes that have been deemed as acquired outside Washington might have been acquired locally. For example, 1 isolate from a patient who was highly suspected of having local acquisition clustered with isolates from California. Likewise, there might be genetically similar populations of *Coccidioides* spp. in Oregon, California, or other nearby states that have not yet been documented. These genetically similar populations might add complexity to determination of location of exposure. However, to date, no clinical or environmental isolates with strong epidemiologic links to locations outside Washington that cluster with isolates from Washington have been identified. This finding is consistent with those of previous studies that demonstrated that *C. immitis* and *C. posadasii* have well-defined geographic structures (*15*–*19*).

Second, all patients in our study were given diagnoses of rather atypical cases of coccidioidomycosis that were either asymptomatic (diagnosed during biopsy), cutaneous, chronic, or unusually severe. In general, the acute primary pulmonary form of coccidioidomycosis constitutes the most common manifestation in highly endemic areas and often represents the earliest manifestation of the disease. Conversely, reactivation of latent infections can occur months to years after initial exposure, typically in immunosuppressed persons. Many complicated infections might follow a subacute or chronic disease progression. This lack of acute respiratory cases and preponderance of atypical disease in Washington indicates the likely gap between early identification and delayed diagnosis because of lack of awareness and might also explain the high prevalence of the out-of-state strains of *C. immitis* in our study. However, even in highly endemic regions, acute pulmonary disease is often missed or delayed in diagnosis for months (*31*,*32*), supporting the possibility that an underlying background of typical acute disease might remain unidentified in Washington (*33*).

Our results indicate that WGS is a useful tool to assist in determining exposure location in surveillance situations in which exposure histories are unclear or unknown. For patients in this study, coccidioidomycosis was more commonly associated with travel to other disease-endemic areas compared with local exposure; however, travel to endemic regions does not preclude local acquisition of the disease. Our results also indicate that coccidioidomycosis is likely to be underdiagnosed and underreported in Washington on the basis of atypical disease manifestations for locally acquired cases. More research is needed to determine the true prevalence of locally acquired coccidioidomycosis in Washington.

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Simplified Model to Survey Tuberculosis Transmission in Countries without Systematic Molecular Epidemiology Programs

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Systematic molecular/genomic epidemiology studies for tuberculosis surveillance cannot be implemented in many countries. We selected Panama as a model for an alternative strategy. Mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) analysis revealed a high proportion (50%) of *Mycobacterium tuberculosis* isolates included in 6 clusters (A–F) in 2 provinces (Panama and Colon). Cluster A corresponded to the Beijing sublineage. Whole-genome sequencing (WGS) differentiated clusters due to active recent transmission, with low single-nucleotide polymorphism–based diversity (cluster C), from clusters involving long-term prevalent strains with higher diversity (clusters A, B). Prospective application in Panama of 3 tailored strain–specific PCRs targeting marker single-nucleotide polymorphisms identified from WGS data revealed that 31.4% of incident cases involved strains A–C and that the Beijing strain was highly represented and restricted mainly to Colon. Rational integration of MIRU-VNTR, WGS, and tailored strain–specific PCRs could be a new model for tuberculosis surveillance in countries without molecular/genomic epidemiology programs.

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Tuberculosis (TB) control depends on rapid diagnosis, efficient therapy, and control of transmission. New genotyping strategies in *Mycobacterium tuberculosis* have led to development of molecular epidemiology strategies that enable clarification of TB transmission dynamics (*1*–*3*). PCR-based molecular epidemiology approaches, namely mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR), are being replaced by genomic epidemiology approaches. These approaches are based on the identification of single-nucleotide polymorphisms (SNPs) using whole-genome sequencing (WGS), which offers higher discriminatory power and precision in assigning transmission clusters (*4*–*6*).

The national TB-control programs of many resource-constrained countries with a high TB prevalence lack systematic molecular epidemiology analysis (*7*). These countries face insurmountable obstacles to availability of genomic epidemiology programs. Panama is an example of a country that, despite recently being classified in the upper-income category, has not implemented a systematic molecular/genomic epidemiology–based surveillance program (*7*). The lack of such a program, together with other limitations, means that Panama now has the second highest incidence of TB in Central America and the highest TB death rate (*8*). The recent expansion of the Panama Canal has attracted migrants into the country, which probably

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has affected host-country TB epidemiology, as reported in other settings (*8–10*).

The convergence of these circumstances made Panama ideal for evaluating an alternative simplified approach for optimized surveillance of TB transmission. We based our effort on a rational application of MIRU-VNTR, subsequent WGS analysis of clustered representatives, and final tailoring of allele-specific oligonucleotide PCR (ASO-PCR) for local prospective targeted surveillance of TB cases.

Materials and Methods

Study Sample

The retrospective study sample comprised 94 *M. tuberculosis* isolates from diagnostic specimens of patients with symptomatic respiratory disease (excluding those under treatment) from 19 health centers and hospitals in Panama and Colon provinces in 2015. This convenience sample comprised 80 isolates from Panama and 14 from Colon. These 2 provinces provided 660 TB cases in 2015; thus, the convenience sample represented 14.4% of TB cases from that year. Among the 91 isolates with susceptibility data, 84 (89.4%) were pansusceptible, 6 (6.4%) were monoresistant to isoniazid (2.1%) and rifampin (4.3%), and the remaining 1 (1.1%) was multidrug-resistant (MDR) (Table). We also included additional prospective isolates from Colon and Panama (January–August 2018), selected following the same selection criteria as the retrospective strain collection.

Table. Study population, drug resistance, and genotypes of *Mycobacterium tuberculosis*, Colon and Panama provinces, Panama, 2015

Characteristic	Result*
Province	
Panama, n = 80	80 (85)
Colon, n = 14	14 (15)
Antimicrobial drug susceptibility	
Susceptible	84 (89.4)
Monoresistant	6 (6.4)
Isoniazid	2 (2.1)
Rifampin	4 (4.3)
Multidrug-resistant	1 (1.1)
No data	3 (3.2)
Lineage/sublineage	
Lineage 1	3
East African Indian	3
Lineage 2	7
Beijing	7
Lineage 4	82
Latin-American-Mediterranean	31
Haarlem	28
H37Rv-like	18
Cameroon	3
X	2
Lineage 5	1
West_African_1	1
Lineage 6	1
West_African_2	1
Distribution of clustered isolates	
Cluster A (Beijing)	7 (15)
Cluster B (Haarlem)†	14 (30)
Cluster C Latin-American-Mediterranean†	9 (19)
Cluster D (H37Rv-like)	12 (26)
Cluster E Latin-American-Mediterranean†	3 (6)
Cluster F (H37Rv-like)	2 (4)

*Values are no. (%) isolates or % isolates.
†One strain of this cluster was monoresistant to rifampin.

Drug Susceptibility Testing

Drug susceptibility was determined at the National Tuberculosis Reference Laboratory in Gorgas Memorial Institute (Panama City, Panama) using GenoType MTBDRplus (Hain Lifescience, https://www.hain-lifescience.de/en/). We confirmed drug susceptibility results by the proportion method on Löwenstein–Jensen medium against the first-line drugs isoniazid, rifampin, streptomycin, and ethambutol. Second-line resistance was not tested in this strain collection.

DNA Extraction

DNA was extracted from Löwenstein–Jensen cultures using double-distilled water protocols from the GenoType Kit (Hain Lifescience) for first-line drug susceptibility testing. DNA for MIRU-VNTR and WGS was extracted using the QIAamp DNA Mini Kit (QIAGEN, https://www.qiagen.com) according to the manufacturer's protocol or the cetyl trimethylammonium bromide–based standard purification.

MIRU-VNTR Analysis

We conducted genotyping using multiplex PCR based on 24-locus MIRU-VNTR (*11*). DNA extraction and MIRU-VNTR typing of the retrospective sample were performed at Instituto de Investigaciones Científicas y Servicios de Alta Tecnología Laboratories (City of Knowledge, Panama). PCR products were sized using capillary electrophoresis in a 3130 Genetic Analyzer (Applied Biosystems, https://www.thermofisher.com). For the Beijing isolates, we added the 4 previously recommended additional hypervariable locus set (1982, 3232, 3820, and 4120) to the 24 loci MIRU-VNTR set (*12*). We assigned TB lineage and sublineages from MIRU-VNTR data using a lineage prediction tool (TBminer, http://info-demo.lirmm.fr/TBminer) (*13*).

WGS

WGS was performed in Hospital Gregorio Marañón (Madrid, Spain) as detailed elsewhere (*14*) on 2–3 representative isolates of each cluster. We generated DNA libraries following the Nextera XT Illumina protocol (Nextera XT Library Prep kit [FC-131–1024]; Illumina, https://www.illumina.com). Libraries were run in a MiSeq device (Illumina) by applying a paired-end reading procedure. We mapped the reads for each strain by using the ancestral *M. tuberculosis* complex genome, which was identical to H37Rv in terms of structure

but with the ancestral alleles inferred by using a maximum-likelihood approach (*15*). SNP calls were made with SAMtools (http://samtools.sourceforge.net/) and VarScan (http://varscan.sourceforge.net/) (coverage of at least 20-fold, mean SNP mapping quality of 20) (*14*,*16*). From the variants detected, we kept only the homozygous calls (those present at a specific position in ≥90% of the reads). Moreover, to filter out potential false-positive SNPs attributable to mapping errors, we omitted the variants detected in repetitive regions, phages, and Pro-Glu (PE)– and Pro-Pro-Glu (PPE)–rich regions. In addition, we omitted SNPs close to insertion/deletions (10-bp window) and those in areas with an anomalous accumulation of variants (≥3 SNPs in 10 bp). Alignments and SNP variants were visualized and checked in IGV version 2.3.59 (http://software.broadinstitute.org/software/igv). A cutoff of ≤12 SNPs was used to consider 2 isolates as clustered due to recent transmission, as defined in Walker et al. (*6*). We deposited the sequences obtained in EMBL-EBI (http://www.ebi.ac.uk [accession nos. PRJEB23681 and PRJEB29408] and http://bioinfo.indicasat.org.pa).

Design of ASO-PCR

For MIRU-VNTR–defined clusters A, B, and C, we identified the strain-specific SNPs after comparing WGS data with a database containing 4,598 sequence genomes from strains circulating throughout the world (A. Chiner-Oms, unpub. data). We selected 3 strain-specific SNPs for cluster A and 4 for clusters B and C to be targeted by a multiplex ASO-PCR (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1593-App1.pdf). We designed these ASO-PCRs to ensure the presence of amplification patterns, regardless of the strain analyzed. For cluster A, we designed 1 selective primer (SNP2) to target the allele found in the cluster A strain, whereas the 2 remaining primers (SNP1 and SNP3) targeted the alternative alleles, as expected in any other non-A strain. For clusters B and C, 2 primers (SNP1/SNP4 and SNP3/SNP4, respectively) targeted the alleles found in strains B and C, whereas the remaining SNPs targeted the alternative alleles. The size of the amplicons was calculated to rule out overlap and led to different band patterns in each case (Appendix Table 1). We fixed the final PCR protocols (Appendix Tables 1, 2) after evaluating multiple experimental conditions until the expected patterns for the strains surveyed and a set of different control strains were confirmed.

The ASO-PCR design and optimization were conducted in Hospital General Universitario Gregorio Marañón (Madrid, Spain). The data were transferred to be applied at the National Tuberculosis Reference Laboratory in Gorgas Memorial Institute in Panama City.

Results

MIRU-VNTR–Based Cluster Analysis

In MIRU-VNTR analysis, 47 (50.0%) of the *M. tuberculosis* retrospective isolates from Panama and Colon provinces grouped into 6 clusters, A–F. Four clusters (A–D) comprised 7–14 isolates each; the remaining 2 were smaller (E, 3 isolates, and F, 2 isolates) (Appendix Figure). Most (87%) isolates corresponded to lineage 4; 7.4% corresponded to lineage 2. Sublineages for the largest clusters corresponded to Beijing (cluster A) and Haarlem (cluster B), Latin-American-Mediterranean (cluster C), and H37Rv-like (cluster D) (Table). Clusters A and F consisted mostly of cases from Colon; the remaining 4 clusters consisted mostly of cases from Panama. Clusters A, D, and F corresponded exclusively to pansusceptible strains; clusters B, C, and E each included 1 isolate that was monoresistant to rifampin.

WGS Analysis

Representative isolates (2 each from clusters A and B and 3 from cluster C) were available for a more in-depth analysis by WGS in Madrid, Spain. The sequences obtained offered a depth of coverage of 20×–55×, with a Phred score >20. The short pairwise distances (1–3 SNPs) for cluster C were consistent with recent transmission. However, 19 SNPs were found between the isolates in cluster A, and 20 SNPs were found between the isolates in cluster B, which was more consistent with the higher diversity expected for clusters involving long-term prevalent strains.

ASO-PCRs for Targeted Surveillance of Selected Clusters

Once we identified the coexistence of 1 cluster due to active recent transmission with 2 clusters that were more likely to be prevalent, ASO-PCRs were designed in Madrid with 2 objectives: 1) prioritize the targeted surveillance of the actively transmitted cluster C; and 2) facilitate capture of new cases involved in clusters A and B with the aim of completing the phylogenetic reconstruction of long-term clusters. We designed 3 multiplex ASO-PCRs to target 3 SNPs for cluster A, 4 for cluster B, and 4 for cluster C (Appendix Tables 1, 2). We used all representatives for clusters A and C and 12 of 14 of those in cluster B as positive controls, and we detected the expected amplification patterns. Forty-nine Beijing isolates other than A strain, 46 Haarlem isolates other than B strain, and 36 Latin-American-Mediterranean isolates other than C strain were also used as negative controls of the strains targeted. In all cases, the expected patterns were obtained for the non–A–C control isolates (Figure 1).

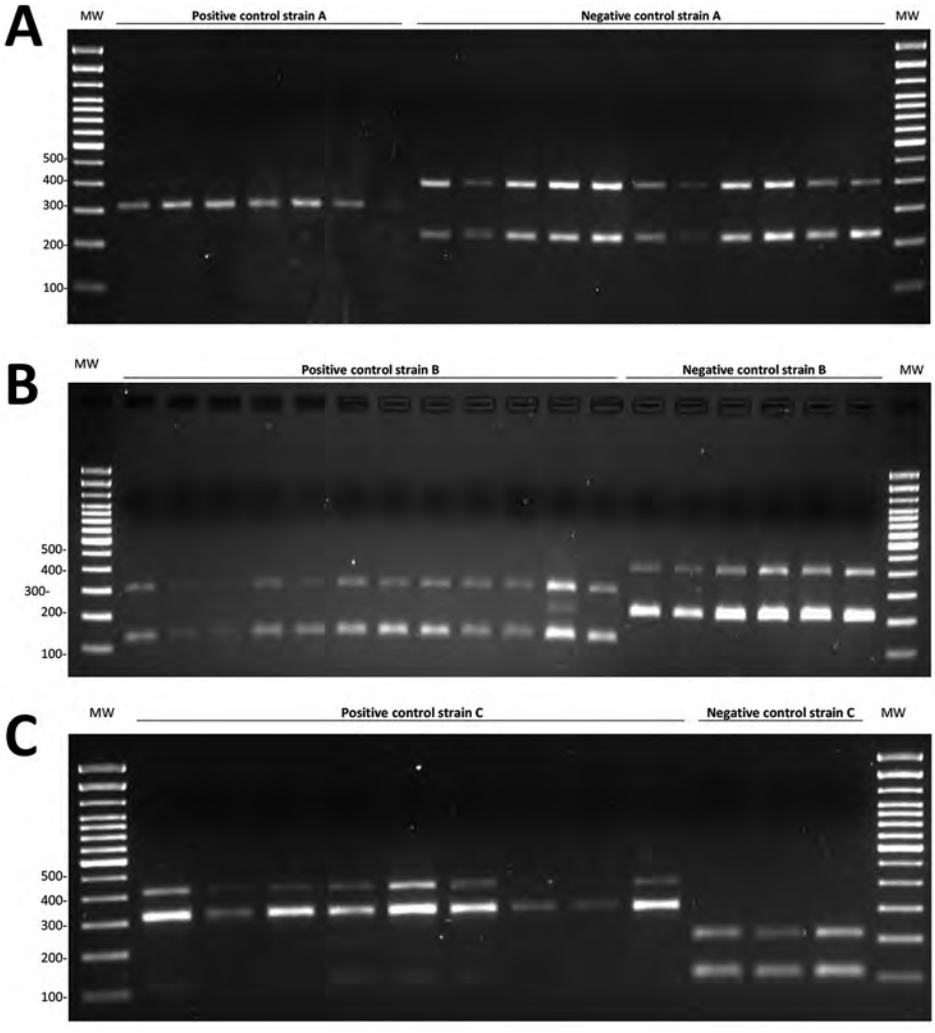

Figure 1. PCR products for allele-specific oligonucleotide PCRs for *Mycobacterium tuberculosis* cluster A, cluster B, and cluster C strains on a selection of representative strains A, B, and C and a selection of non-A, non-B, and non-C controls. Testing was for a simplified model to survey tuberculosis transmission using data from patients and controls in Panama and Colon provinces, Panama, 2015. A) 308-bp PCR product (single-nucleotide polymorphism [SNP] 2) for strain A and 400-bp and 228-bp products (SNP1/SNP3) for non-A strain. B) 127-bp and 297-bp PCR products (SNP1/SNP4) for strain B and 209-bp and 406-bp products (SNP2/SNP3) for non-B strain. C) 307-bp and 413-bp PCR products (SNP3/SNP4) for cluster C strain and 103-bp and 207-bp products (SNP1/SNP2) for non-C strain. MW, molecular weight (100-bp DNA ladder).

Prospective Implementation of the ASO-PCR–Based Strategy

We used 51 prospective isolates from Colon and Panama collected during 2018 to evaluate the performance in situ of the 3 sets of cluster A–C–specific ASO-PCRs. The amplification patterns obtained in Madrid in the optimization step were now reproduced exactly in Panama. Cluster A PCR revealed 13 (25.5%) patients infected by Beijing strain A of the total sample, 11 from Colon, and 2 from Panama. Cluster B PCR identified 1 (1.9%) patient infected by strain B and 2 (3.9%) patients infected by strain C; all 3 of these patients were from Panama province.

All the isolates not labeled as corresponding to strains A, B, or C by the strain-specific PCRs were genotyped by VNTR. None showed a pattern corresponding to the strains targeted (they showed differences in >3 loci). This finding enabled us to rule out that we could be missing some strains related to the clusters targeted.

We used WGS to analyze all cases (except 2 for strain A) captured by the ASO-PCRs to determine the network of relationships between the isolates (Figure 2). As expected, we observed lower SNP-based diversity acquired along a linear topology for cluster C, consistent with a sequential host-to-host recent-transmission nature for this cluster. In contrast, we found more SNPs, acquired along different branches, with several nonsampled nodes (median vectors, mv; Figure 2) for clusters A and B, more likely corresponding to prevalent strains that acquired higher diversity. In fact, for cluster A, this diversity was also detected by MIRU-VNTR; 4 isolates captured by the ASO-PCR showed single-locus variations.

Discussion

Molecular epidemiology strategies enable us to investigate TB transmission dynamics in a given population. However, to capture a true snapshot of TB transmission, these strategies require a universal, long-term fingerprinting scheme. In other words, it is necessary to ensure that transmission links are not missed because of incomplete sampling of all the TB cases in a complete population. Unfortunately, few

countries have implemented universal systematic genotyping. Many countries with more limited resources and a high TB prevalence can afford only fragmented molecular epidemiology studies based on convenience samples.

An additional concern is the recent process of substituting molecular epidemiology studies with more precise genomic epidemiology studies based on WGS analysis (*4*,*6*). If we failed to implement standard systematic molecular epidemiology in many high-prevalence areas, the gap between low- and high-income countries would widen in terms of genomic epidemiology. Therefore, we must define new strategies to offer an alternative to fill this gap.

In this study, we propose a model based on 4 stages: 1) updating the identification of strains responsible for the largest clusters in a population, 2) application of WGS to obtain more in-depth knowledge of clusters, 3) design of ASO-PCRs to identify specific strains, and 4) implementation of a simplified TB-control scheme based on targeted surveillance of predominant strains. To evaluate whether our approach could simplify detection of TB transmission in countries with no systematic molecular epidemiology programs, we applied it in Panama, a country with high TB-related mortality (*8*). No systematic universal genotyping is available in Panama, and the only data available are from studies of cases from a single clinic and of MDR isolates (*17*,*18*). These studies were performed before the recent expansion of the Panama Canal, whose socioepidemiologic effect (*19*) can be considered similar to that of the construction of the interoceanic railroad and the Panama Canal in the mid-19th century, when large numbers of workers from Africa, India, and China arrived in Panama. High rates of malaria- and yellow fever–related deaths were associated with work on the Panama Canal (*20*). Regarding TB, the rates of latent infection were higher for migrant students than for US students living in the canal area (*21*).

The first step in our model revealed an unexpectedly high percentage of MIRU-VNTR–clustered cases. Half of the cases collected in Panama and Colon provinces grouped into 6 clusters. Fortunately, none of these clusters corresponded to resistant/MDR strains, despite the high clustering rates for MDR strains reported during 2002–2004 (*17*). Four of the 6 clusters identified in our study were responsible for nearly half of all cases analyzed.

Ours was a limited strain collection, and thus some transmission links might be missing because of the limited number of samples we were able to analyze, which covered only 1 year and 2 local settings, leading us to expect that the true figures for clustered cases are higher. In a previous study of a convenience pansusceptible *M. tuberculosis* strain collection

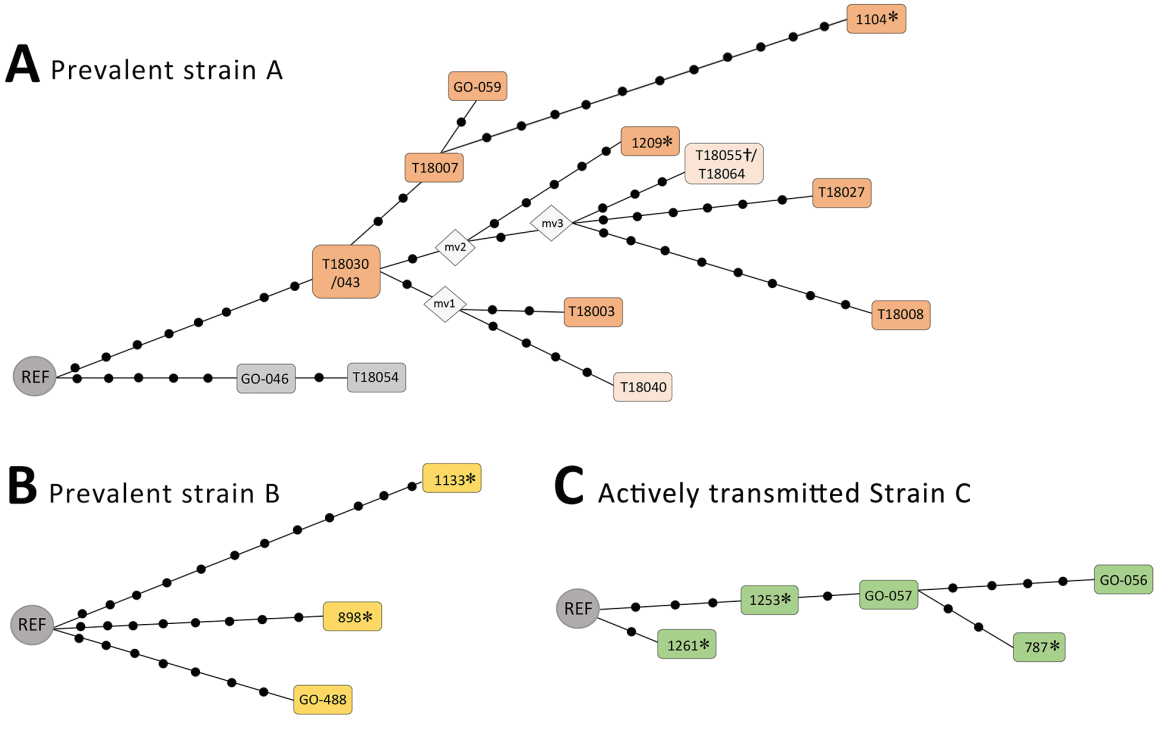

Figure 2. Networks of relationships based on whole-genome sequencing data of prevalent clusters (A, B) and active transmission cluster (C) in testing of a simplified model to survey tuberculosis transmission in Panama and Colon provinces, Panama, 2015. Each dot corresponds to a single-nucleotide polymorphism (SNP). In panel A, when 2 isolates are included in the same box, they showed no SNPs between them; the isolates within boxes with different colors show mycobacterial interspersed repetitive units–variable number of tandem repeats patterns with single-locus variations between them. mv, median vector corresponding to nonsampled nodes; REF, reference. *Isolates from 2015 used to design the allele-specific oligonucleotide–PCRs; †strain identified in an additional analysis in Madrid (out of the prospective study applying the strain-specific PCRs in Panamá). A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/25/3/18-1593-F2.htm).

from 2005 (*18*), only 21% (13/62) were found to be clustered. Direct comparisons between the figures from that study and our study cannot be made because of marked differences in genotyping approaches applied. In any case, it seems that the clustering snapshot is now different, possibly because our sampling was restricted to a more precise geographic location.

Despite the short distance (74 km) between Panama and Colon provinces, which are connected by the best highway in Panama, clusters are rich in cases from one or the other province, thus alerting us to the epidemiologic singularities of each population, with some strains more restricted to specific geographic niches. This finding is especially relevant for the Beijing sublineage (associated with high transmissibility), which seems to be better represented in Colon province.

The second step in our model focused on applying WGS to the MIRU-VNTR clusters. This analysis revealed that 2 different phenomena were co-occurring in the MIRU-VNTR–defined large clusters A, B, and C, as follows: 1) recent transmission in cluster C, robustly determined by the low diversity identified by WGS; and 2) more SNPs in clusters A and B. The higher number of SNPs within clusters A and B (more than the 12 SNPs accepted as a threshold for inferring recent transmission from WGS data) suggests that these clusters involve prevalent strains that had accumulated higher diversity after circulating for longer periods. Since it was first determined (*6*), the SNP-based threshold for recent transmission has been proven correct in many studies (*22*–*25*). This threshold was robust even in circumstances that could lead to greater accumulation of diversity, such as prolonged host-to-host transmission and the simultaneous involvement of reactivation and recent transmission in the same event (*16*).

Robustly defined MIRU-VNTR clusters corresponded to different magnitudes of SNP-based diversity between the clustered isolates; that is, clusters caused by recent transmission coexisted with other clusters not associated exclusively with recent transmission but with prevalent strains that had circulated for long periods. This observation obliges us to reinterpret the load of true recent transmission in high-prevalence settings. According to our WGS findings, our initial estimation of 50.0% of cases from recent transmission, which was based on MIRU-VNTR analysis, must be reduced. This bias in the estimation of recent transmission in MIRU-VNTR–defined clusters has been discussed (*26*–*28*). We also demonstrated how MIRU-VNTR failed to differentiate between migrants who had acquired TB by recent transmission after arrival in Spain from other patients who had imported TB from their country of origin (*28*). Both shared identical MIRU-VNTR patterns, and only WGS succeeded in differentiating them based on the magnitude of SNP diversity (*28*). From our data, it can be deduced that WGS is necessary in high-prevalence settings to differentiate MIRU-VNTR–defined clusters that genuinely result from recent transmission from those with the involvement of long-term prevalent strains.

Because the systematic application of WGS in low-resource settings is not realistic, our tailored ASO-PCR model for targeting marker SNPs for the strains previously identified to be responsible for a large percentage of TB cases remains an affordable strategy. Our proposal reconciles the high discriminatory power of WGS (targeted SNPs are obtained from the WGS analysis) with the low-cost and easy implementation of PCR-based tools. Based on the initial MIRU-VNTR analysis, application of these 3 multiplex ASO-PCRs would have enabled us to cover 32% of all retrospective TB cases in our sample. This coverage justified the prospective evaluation to assess their usefulness and precision, the last step in the implementation of our model. Because our multiplex ASO-PCRs were designed based on WGS data from a limited size convenience sample from Panama and Colon provinces, the PCRs should be used only for surveillance in these 2 major provinces.

The limited sample of isolates obtained in 2015, which were used to tailor the surveillance strain-specific PCRs, could make the PCRs useless when moving forward because the epidemiology of TB and the composition of circulating strains changes over time. However, the application in Panama of the set of 3 ASO-PCRs on the prospective samples from Panama City and Colon enabled us to determine the strain involved in 31.4% of the cases, a figure close to the 32% of the isolates belonging to the 3 surveyed strains in 2015. This finding suggested a rather stable composition of strains in this setting.

Initially, the quality of the DNA obtained from the isolates in 2015 for the fourth predominant cluster (cluster D) was not suitable for WGS. Just recently, the VNTR analysis from the 2018 isolates not belonging to the A/B/C clusters enabled us to identify new representative cases for this cluster, which will be analyzed by WGS to tailor a new PCR to complete and extend the coverage of the surveillance panel.

Once our strategy has proved to be useful, a country-wide genotyping effort will be necessary to determine highly transmitted strains nationwide and develop new ASO-PCR tools for surveillance of the entire country. The strategy will be evaluated periodically. It will mean updating the composition of circulating strains by MIRU-VNTR analysis as soon as we detect a reduction in the proportion of incident isolates that can be labeled by the strain-specific PCRs already implemented. From the VNTR analysis, we will select the new clusters to be targeted, according either to their magnitude or to the speed in which the involved strain is transmitted. Three representative isolates from each cluster selected will be analyzed by WGS to identify the strain-specific SNPs to tailor new PCRs to target them. Therefore, the panel of PCRs applied as a surveillance tool will be constantly updated according to the changing epidemiology of the setting.

The networks of relationships obtained from WGS data of the representatives of clusters A–C captured by the ASO-PCRs were consistent with the different nature (either prevalent strains A/B or recently transmitted strain C) of the clusters analyzed. A pattern of closely related isolates (below the 12-SNP cutoff determined as similarity threshold) (*6*) distributed along a simple linear topology was found for cluster C. However, more complex branched topologies and a higher diversity between the isolates were observed for the prevalent clusters A and B.

A high proportion (21.5%) of patients in Colon were infected by the Beijing strain (strain A). This finding constitutes a challenging alert for the national TB-control program because this lineage is involved in long-term outbreaks, which eventually become uncontrollable. In Gran Canaria Island, Spain, the importation of a Beijing strain in the 1990s caused a major outbreak that could not be controlled. Three years after importation, 27% of TB patients on the island were infected by this strain (*29*). Three decades later, the strain remains highly prevalent, not only on the island to which it was imported but also on neighboring islands (*30*). The marked presence for the Beijing lineage in Colon province seems to be an emergent issue because the lineage accounted for only 3.7% of the cases in 2005 (*18*). Those authors raised the alert about the effect of the expansion of the Panama Canal on the likely modification of the composition of circulating strains. Application of only 1 of our PCRs (the one targeting the Beijing strain) would have enabled us to characterize half of all current newly diagnosed TB cases in Colon; that is, persons infected by a highly transmissible strain. This PCR could constitute a key tool for optimizing the national TB-control programs to avoid future uncontrollable situations, such as the one reported for the Canary Islands.

In summary, our study evaluated the feasibility and usefulness of a new model for simplifying and optimizing surveillance of transmission of TB in countries with no systematic molecular or genomic epidemiology program. Preliminary MIRU-VNTR analysis alerted us to a high percentage (50%) of clustered TB cases and revealed a differential distribution of clustered cases in 2 provinces within Panama. Subsequent WGS analysis showed that some of the MIRU-VNTR clusters were the result of active recent transmission, whereas others more likely resulted from prevalent long-term strains. We applied the WGS data to tailor 3 ASO-PCRs, which covered most cases in the population. The prospective application of only 3 ASO-PCRs enabled us to identify the strains infecting 31.4% of incident cases. This strategy optimized tracking of actively transmitted *M. tuberculosis* strains and capture of new cases involving long-term prevalent strains, thus making it possible to reconstruct the global phylogeny of their clusters. Our strategy also made it possible to identify the alarming presence of the Beijing sublineage in the incident cases in Colon province. A design based on a rational integration of MIRU-VNTR, WGS, and ASO-PCR could provide a new model for surveillance of TB transmission in countries without universal molecular/genomic epidemiology programs.

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Multicenter Study of *Cronobacter sakazakii* Infections in Humans, Europe, 2017

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Cronobacter sakazakii has been documented as a cause of life-threating infections, predominantly in neonates. We conducted a multicenter study to assess the occurrence of *C. sakazakii* across Europe and the extent of clonality for outbreak detection. National coordinators representing 24 countries in Europe were requested to submit all human *C. sakazakii* isolates collected during 2017 to a study center in Austria. Testing at the center included species identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, subtyping by whole-genome sequencing (WGS), and determination of antimicrobial resistance. Eleven countries sent 77 isolates, including 36 isolates from 2017 and 41 historical isolates. Fifty-nine isolates were confirmed as *C. sakazakii* by WGS, highlighting the challenge of correctly identifying *Cronobacter* spp. WGS-based typing revealed high strain diversity, indicating absence of multinational outbreaks in 2017, but identified 4 previously unpublished historical outbreaks. WGS is the recommended method for accurate identification, typing, and detection of this pathogen.

Cronobacter sakazakii is a motile, gram-negative, rod-shaped opportunistic pathogen of the family *Enterbacteriaceae* (*1*). In 2007, organisms previously classified as *Enterobacter sakazakii* were reassigned to the new genus *Cronobacter*, which now consists of 7 species: *C. sakazakii*, *C. condimenti*, *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. turicensis*, and *C. universalis* (*2*,*3*). *C. sakazakii* has been isolated from various environments (e.g., domestic environments and manufacturing plants), clinical sources (e.g., cerebrospinal fluid, blood, and sputum), food (e.g., cheese, meat, and vegetables), and animals (e.g., rats and flies) (*4*,*5*).

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Most reported cases of illness caused by *C. sakazakii* are in infants <2 months old (*6*,*7*). Premature infants and infants with underlying medical conditions are at the greatest risk for illness. Numerous outbreaks caused by *C. sakazakii* have been traced to contaminated powdered infant formula (*8*). Powdered infant formula is not a sterile product, and the ability of *C. sakazakii* to tolerate dry conditions enables it to survive for long periods in the final powdered product (*9*).

The screening of food (particularly powdered formula) was proposed to reduce the risk to neonatal and infant health (*10*,*11*). The most common syndromes of foodborne infection in infants include necrotizing enterocolitis (NEC), bacteremia, and meningitis (*12*,*13*). Examples of outbreaks of illness in hospital neonatal units caused by *C. sakazakii* associated with powdered infant formula have been compiled by Iversen and Forsythe (*6*) and by Lund (*8*).

A few cases of illness (usually nongastrointestinal) in adults caused by *C. sakazakii* have been reported. In most of these cases the adults had underlying diseases, and no evidence of foodborne transmission was reported (*14*,*15*).

We performed a multicenter study of *C. sakazakii* infections in humans (EUCRONI) to determine the occurrence of *C. sakazakii* in clinical microbiology laboratories across Europe. We also assessed the extent of clonality for human *C. sakazakii* isolates.

Material and Methods

Study Design

EUCRONI consisted of national coordinators (EUCRONI study group members) from 24 countries in Europe. Coordinators had to actively approach all medical microbiology laboratories to collect human *C. sakazakii* isolates (1 per patient) in their respective countries during 2017. Human historical isolates (with isolation dates before 2017) were also accepted. The 24 participating countries were arbitrarily chosen to reflect a wide geographic and

[1]Members of the EUCRONI study group are listed at the end of this article.

socioeconomic range (Figure 1). Isolates were transferred to the study center (Austrian Agency for Health and Food Safety, Vienna, Austria) for whole-genome sequencing (WGS), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) analysis, and antimicrobial drug susceptibility testing. We submitted data capture forms to national coordinators to collect the following demographic data: patient age and sex, patient status (colonized or infected), specimen source, type of healthcare facility requesting the microbiologic culture, and date of specimen collection.

Species Identification and DNA Extraction

We cultured isolates on Columbia blood agar plates (bioMérieux, http://www.biomerieux.com/) overnight at 37°C. We performed species identification by using MALDI-TOF Biotyper (Bruker, https://www.bruker.com) and MBT Compass IVD 4.1.60 (Bruker) according to the manufacturer's instructions. We conducted isolation, quantification, and WGS of genomic DNA according to methods described by Lepuschitz et al. (*16*). We used Sequencing Coverage Calculator (http://www.illumina.com) for calculation of a desired mean coverage of ≥80-fold.

WGS Data Analysis

We de novo assembled raw reads by using SPAdes version 3.9.0 (*17*) and processed them in SeqSphere+ (Ridom GmbH, https://www.ridom.de) for bacterial typing. We deposited the genome sequences in the PubMLST *Cronobacter* database (https://pubmlst.org/Cronobacter) under accession nos. 2403 and 2495–2552. To determine the core genome multilocus sequence type (cgMLST) gene set, we performed a genome-wide gene-by-gene comparison by using the MLST+ target definer function of SeqSphere+

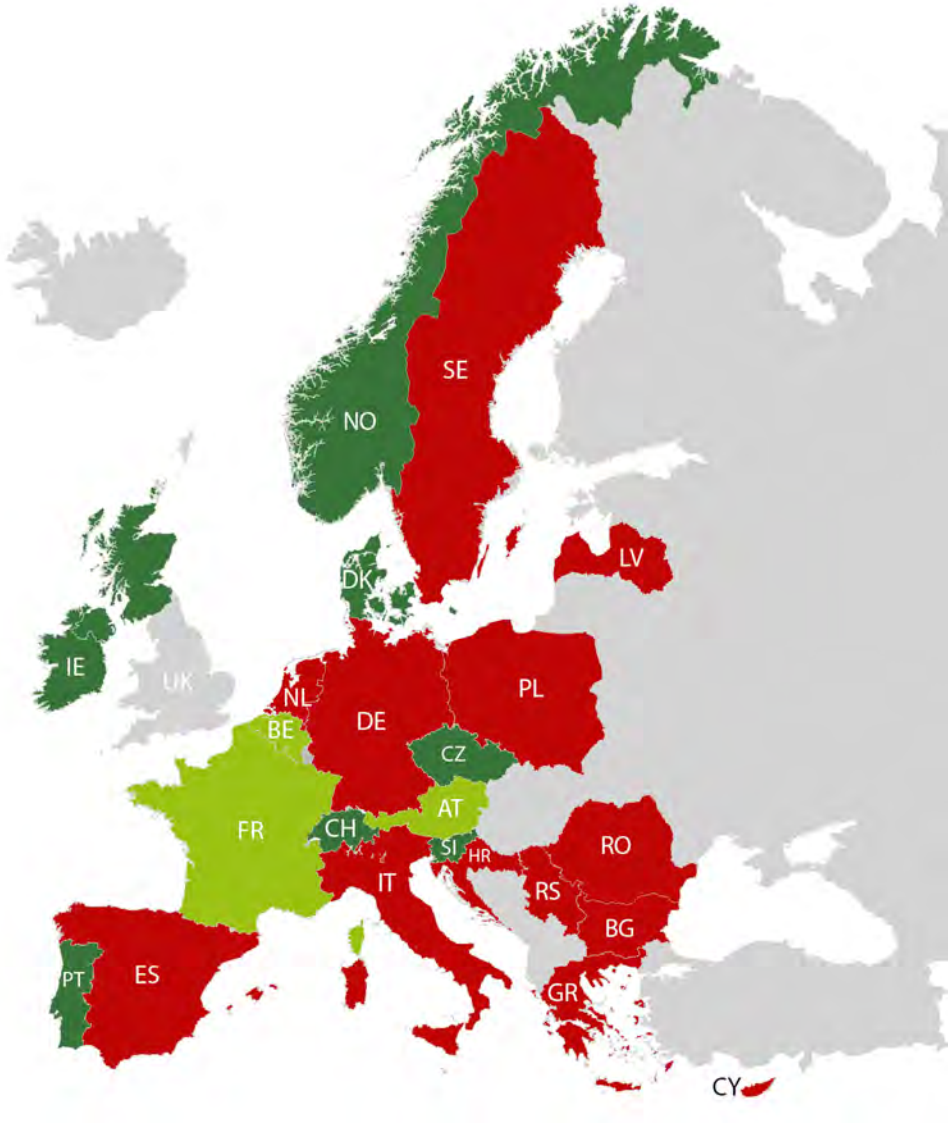

Figure 1. Countries participating in a multicenter study of *Cronobacter sakazakii* infections in humans, Europe, 2017. Dark green indicates the 8 countries that sent *C. sakazakii* isolates to the study center in Austria; light green indicates the 3 countries where historical outbreaks were detected; and red indicates the 13 countries that participated but did not provide isolates. AT, Austria; BE, Belgium; BG, Bulgaria; CH, Switzerland; CY, Cyprus; CZ, Czech Republic; DE, Germany; DK, Denmark; ES, Spain; FR, France; GR, Greece; HR, Croatia; IE, Ireland; IT, Italy; LV, Latvia; NL, Netherlands; NO, Norway; PL, Poland; PT, Portugal; RO, Romania; RS, Serbia; SE, Sweden; SI, Slovenia; UK, United Kingdom.

as described previously (*18*) with default parameters and the complete genome of *C. sakazakii* strain ATCC BAA-894 (*19*) as reference genome, all complete *C. sakazakii* genomes available at GenBank, 8 isolates retrieved from whole-genome shotgun sequencing projects, and 4 *C. sakazakii* isolates sequenced at the Austria study center as query genomes. We extracted sequences of the 7 genes comprising the allelic profile of the classical MLST scheme and queried them against the *C. sakazakii* MLST database (*1*), assigning classical sequence types (STs) in silico. We obtained additional species confirmation by using JSpeciesWS (*20*) and ribosomal MLST (*21*). We included 23 *C. sakazakii* historical isolates from 4 different outbreaks (F. Allerberger, 2016; F. Barbut, 2010–2016; G. Feierl, 2009; D. Piérard, 1997–1998, all unpub. data; Appendix Table 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1652-App1.xlsx) and 3 reference strains, ATCC BAA-894 (*19*), ATCC29544 (PRJNA224116), and NCTC 8155 (PRJNA224116), to determine the level of microevolution.

Antimicrobial Resistance Testing

We performed in vitro susceptibility testing with the VITEK 2 Compact System (bioMérieux) and interpreted the VITEK 2 AST196 card according to European Committee on Antimicrobial Susceptibility Testing criteria for *Enterobacteriaceae* (Clinical Breakpoint Tables version 8.0, http://www.eucast.org/ast_of_bacteria/previous_versions_of_documents). For detection of antibiotic resistance genes, we used the Comprehensive Antibiotic Resistance Database (*22*) with default settings "perfect" and "strict" for sequence analysis. We tested isolates in SeqSphere+ for *Cronobacter*-specific variant *ampC* (e.g., CSA-1, CSA-2, CMA-1, and CMA-2) (*23*).

Results

Strain Collection and Primary Species Identification

During the study period, 11 of 24 national coordinators (Figure 1) provided 77 presumptive *C. sakazakii* isolates previously identified by conventional biochemical testing, local MALDI-TOF MS analysis (Bruker Biotyper and VITEK MS), locally performed *Cronobacter* genus- and species-specific PCRs, or 16S rRNA gene sequence analysis. These 77 isolates consisted of 36 human isolates from 2017 and 41 historical human isolates obtained during 1964–2016. The participating laboratories, using local conventional phenotypical methods or local MALDI-TOF MS analysis, incorrectly identified 18 (23.4%) of 77 human isolates as *C. sakazakii*.

MALDI-TOF MS analysis in the study center identified 69 of 77 isolates as *C. sakazakii;* 1 isolate from 2017 yielded low-confidence identification (log[score] value 1.70–1.99). We assigned 7 clinical isolates from 2017 and 1 historical clinical isolate from 2005 to other species (Table 1). The WGS-based species identification using JSpeciesWS and rMLST confirmed MALDI-TOF MS identification results in all but 10 of the 69 isolates. WGS indicated that 5 isolates were *C. malonaticus*, 2 were *C. turicensis*, 1 was *C. dublinensis*, 1 was *C. universalis*, and 1 was *Siccibacter turicensis* (Table 1; Appendix Table 1).

Human *C. sakazakii* Isolates Collected in 2017

In total, 21 *C. sakazakii* isolates from 21 patients were collected in 2017 in 9 participating countries in Europe. Case-fatality ratio (within 30 days after specimen collection) was 2 of 21 case-patients (Table 2).

Molecular Typing of Bacterial Isolates

The defined cgMLST gene set consisted of a total of 2,831 core and 1,017 accessory targets. Of 77 sequenced isolates, 59 isolates were confirmed as *C. sakazakii*. These isolates had on average 99.4% of good core genome targets (97.7% to 99.9%) (*18*) and revealed in total 17 different sequence types (STs) (Table 3).

Core genome comparison of 59 *C. sakazakii* isolates and the 3 reference strains revealed an average allelic difference of 2,402 and a maximum allelic difference of 2,724 (Figure 2). Isolates clustered in the minimum-

Table 1. Comparison of MALDI-TOF mass spectrometry and whole-genome sequencing results for 77 isolates submitted as *Cronobacter sakazakii* in a multicenter study of *C. sakazakii* infections in humans, Europe, 2017*

MALDI-TOF	Whole-genome sequencing	Total no. isolates	Human isolates detected in 2017	Historical human isolates
C. sakazakii	*C. sakazakii*	59	21	38
C. sakazakii	*C. dublinensis*	1	1	–
C. sakazakii	*C. malonaticus*	5	4	1
C. sakazakii	*C. turicensis*	2	1	1
C. sakazakii	*C. universalis*	1	1	–
C. sakazakii	*Siccibacter turicensis*	1	1	–
Enterobacter aerogenes	*Kluyvera intermedia*	1	1	–
E. asburiae	*E. cloacae*	2	2	–
E. asburiae	*E. asburiae*	1	–	1
Klebsiella oxytoca	*Klebsiella oxytoca*	1	1	–
Kosakonia cowanii	*Kosakonia cowanii*	2	2	–
Paenibacillus pasadenensis	*Paenibacillus pasadenensis*	1	1	–

*MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; –, no isolates detected.

Table 2. Characteristics of patients enrolled and *Cronobacter sakazakii* isolates collected in a multicenter study of *C. sakazakii* infections in humans, Europe, 2017*

Sample ID	Country of origin	Patient age, y/sex	Specimen source	Death within 30 d	MLST
802520	Austria	73/F	Stool	No	630
7750-17	Austria	<1/M	Blood	Yes	4
16862-17	Austria	77/F	Blood	No	37
808921	Austria	69/F	Stool	No	21
56487-17	Austria	78/M	Urine	No	17
101807-17	Austria	77/M	Blood	No	1
9929-17	Austria	5/M	Stool	No	17
EUCRONI016	Belgium	61/M	Urine	No	13
EUCRONI012	Belgium	78/M	Wound	No	31
1481-17	Czech Republic	80/F	Rectal swab	No	8
436-17	Czech Republic	31/M	Rectal swab	No	4
10965-17	Czech Republic	74/M	Rectal swab	No	4
D97986	Denmark	85/F	Sputum	No	1
17007483	Denmark	69/M	Urine	No	58
423410	Ireland	65/M	Blood	No	12
170215-0130	Norway	87/M	Blood	Yes	17
M732000	Portugal	60/M	Urine	No	40
80357408-17	Scotland	73/F	Stool	No	33
80363028-17	Scotland	71/M	Urine	No	4
07_2005	Slovenia	54/M	Tracheal aspirate	No	184
2017C1	Switzerland	55/F	Cervix uteri	No	40

*MLST, multilocus sequence type.

spanning tree to their respective MLST. Eight isolates belonging to ST1 included 2 stool isolates from neonates with a common epidemiologic link in Austria in 2009; these 8 isolates showed 1 allelic difference and were most closely related (203 alleles difference) to reference ATCC BAA-894, an isolate collected from powdered formula in the United States in 2001. That outbreak affected 2 neonates with necrotizing enterocolitis (both male, age 10 days and 12 days) hospitalized in the same neonatal intensive care unit.

Twelve isolates belonged to ST4, of which 3 were confirmed isolates from infants. Two infant isolates belonged to an outbreak cluster with a common epidemiologic link detected in Austria in 2016; these isolates shared the same cgMLST profile and showed a maximum of 47 allelic differences to the historical reference strain NCTC 8155 (from milk, United Kingdom, 1950). This outbreak again affected 2 neonates (neonate A: female, age 22 days, positive blood culture, fatal outcome; neonate B: male, age 16 days, positive respiratory tract specimen) hospitalized in the neonatal intensive care unit of another hospital in Austria. The third infant isolate was a 2017 ST4 isolate from a case in Austria with a fatal outcome and was most closely related (302 allelic differences) to a historical strain from Denmark isolated in 2003.

Six clinical isolates assigned to ST8 consisted of 2 historical human isolates from Canada (date of isolation unknown). These 6 isolates shared the identical core genome profile and had 1 allelic difference to reference strain ATCC29544 (from an infant, United States, 1970).

Nine human isolates assigned to ST21 comprised historical outbreak cluster from France collected during 2010–2016. The outbreak included 3 female patients (mean age 62 years) and 5 male patients (mean age 68 years); initial specimens were abscess material from the digestive tract (n = 1), ascites fluid (n = 1), respiratory tract specimens (n = 2), and rectal swab specimens (n = 4). Eight of these 9 isolates showed the same core genome genes, and 1 yielded 1 allelic difference.

All 10 isolates assigned to ST155 belonged to a historical outbreak among infants in Belgium during 1997–1998; the isolates originated from blood cultures (n = 2), stool specimens (n = 2), rectal swab specimens (n = 4), and respiratory tract specimens (n = 2). The first positive sample was collected in November 1997; the remaining 9 specimens were obtained during August–September 1998. Eight

Table 3. In silico evaluation of MLSTs for *Cronobacter sakazakii* strains in a multicenter study of *C. sakazakii* infections in humans, Europe, 2017*

MLST	Total no. isolates	Human isolates detected in 2017	Historical human isolates
1	7	2	5
12	1	1	–
13	1	1	–
148	1	–	1
155	10	–	10
17	3	3	–
184	1	1	–
21	10	1	9
31	2	1	1
33	1	1	–
37	1	1	–
4	11	4	7
40	2	2	–
50	1	–	1
58	1	1	–
630	1	1	–
8	5	1	4

*MLST, multilocus sequence type; –, no isolates detected.

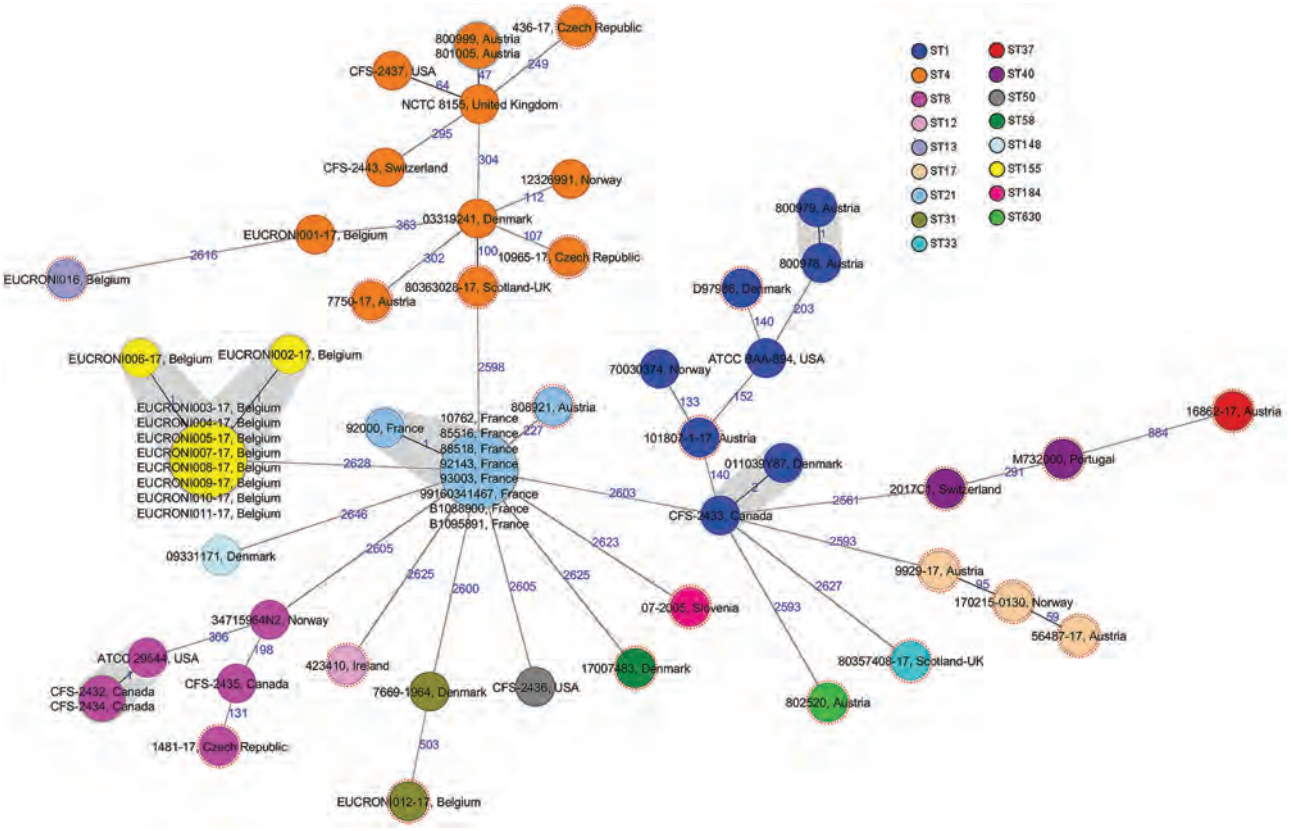

Figure 2. Minimum-spanning tree of 59 *Cronobacter sakazakii* isolates, including 21 human isolates from 2017 and 38 historical human isolates, from 11 countries in Europe. Each circle represents isolates with an allelic profile based on the core genome multilocus sequence type, which consists of 2,831 alleles. Blue numbers indicate the allelic differences between isolates; isolates with closely related genotypes are shaded in gray. Isolates were colored according to classical multilocus sequence type, labeled with the country of isolation and the respective sample identification. Nodes encircled with a dotted red line were collected in 2017. Ireland additionally provided 7 historical isolates originating from Canada (n = 4), United States (n = 2), and Switzerland (n = 1). For comparison, sequence data of reference strains ATCC BAA-894 (United States, ST1), ATCC29544 (United States, ST8), and NCTC 8155 (United Kingdom, ST4) were included. ST, sequence type.

of the 10 isolates shared the same cgMLST profile, and 2 had 1 allelic difference.

In total, 27 of 38 historical isolates were most closely related (≤1 allelic difference) to other historical isolates; 11 were singletons. All 21 isolates collected in 2017 were singletons, and no close relatedness was evident (≥100 allelic differences) between historical isolates and isolates from 2017.

In Vitro and In Silico Antimicrobial Resistance Analysis

In vitro susceptibility testing of 21 human *C. sakazakii* isolates from 2017 revealed 20 *C. sakazakii* isolates that were susceptible to all 14 tested antibiotics (Appendix Table 2). One isolate was resistant to ampicillin, cefotaxime, gentamicin (intermediate), moxifloxacin, and trimethoprim/sulfamethoxazole.

Of 21 *C. sakazakii* isolates, 12 isolates carried the efflux genes *emrB*, *msbA*, *patA*, regulatory systems modulating antibiotic efflux *CRP*, *marA*, *emrR*, *marR*, H-NS, antibiotic target protection gene *msrB*, and the determinant of fosfomycin resistance *glpT*. Seven isolates had in addition the antibiotic protection gene *vgaC*. One isolate had also the efflux gene *norB*, the antibiotic inactivation gene *fosX*, and the antibiotic target alteration gene *mprF*. One isolate had the additional antibiotic inactivation genes *aac(6')-Ib-cr*, *aadA16*, *aadA2*, *ant(2'')-Ia*, *arr-3*, *catB3*, *CTX-M-9*, *OXA-1*, the antibiotic target protection gene *qnrA1*, and the antibiotic target replacement gene *sul1*.

The presence of variant *ampC* was confirmed for all 21 isolates. Seventeen isolates harbored CSA-2, and 4 isolates harbored CSA-1 (Appendix Table 2).

Discussion

The aim of our 2017 *C. sakazakii* study was to assess the occurrence of this opportunistic pathogen in countries of Europe, characterize the isolates, and recognize possible multinational outbreaks. Our finding that only 59 of 77 presumptive *C. sakazakii* isolates had the species-identification

C. sakazakii confirmed at the central study center shows that correct identification of *Cronobacter* spp. is still a challenge for many routine laboratories.

The prevalence of reported *C. sakazakii* cases was low, with only 11 (45.8%) of 24 participating countries submitting *C. sakazakii* isolates. Clinical isolates from 2017 showed high genetic diversity, indicating that neither multinational nor national outbreaks occurred in 2017 in the 24 countries studied. However, characterization of the historical isolates obtained during this study confirmed occurrence of 4 previously unpublished historical outbreaks: 2 outbreaks from 2009 and 2016 in Austria, 1 from Belgium during 1997–1998, and 1 from France during 2010–2016. Hospitals affected by nosocomial *C. sakazakii* outbreaks might still be reluctant to publish possibly food-related outbreaks or nosocomial infections, especially in the case of affected infants and particularly in the case of related fatalities.

Strain typing using classical MLST identified a total of 17 STs among 59 sequenced *C. sakazakii* isolates. Our addition of a new ad hoc cgMLST scheme consisting of 2,831 core target genes provides more discriminative power for outbreak investigation and source tracking than the standard 7-loci MLST scheme.

The dominant STs found among our clinical *C. sakazakii* isolates from 2017 were ST4, ST17, ST1, and ST40, a distribution consistent with results from other studies (*1*). The medical literature often links *C. sakazakii* ST4 with powdered infant formula–associated outbreaks in infants (*3*). In our study, the sole strain (7750-17) affecting an infant (a 3-month-old baby boy who died) was ST4, isolated from a blood culture.

Antibiotic treatment is essential in the care of a patient with a confirmed *Cronobacter* infection. The traditional antibiotic regimen for *Cronobacter* spp. was ampicillin in combination with either gentamicin or chloramphenicol. In view of claimed resistance to ampicillin and most first- and second-generation cephalosporins, it has been suggested that carbapenems or third-generation cephalosporins be used with an aminoglycoside or trimethoprim/sulfamethoxazole (*24*). In our study, antimicrobial resistance testing showed susceptibility to all tested antibiotics for 20 of 21 human isolates from 2017. In comparison to other members of the family *Enterobacteriaceae*, *Cronobacter* strains seem to be more susceptible against so-called "key access antibiotics" of the World Health Organization's Model List of Essential Medicines (*25*), such as ampicillin, aminoglycosides, chloramphenicol, and third-generation cephalosporins (the last is included in the List of Essential Medicines only for specific, limited indications) (*26*). For all isolates, we confirmed the presence of 1 of 4 tested *ampC* β-lactamase variants, which confer phenotypic resistance exclusively to first-generation cephalosporins (e.g., cephalothin) but not to ampicillin (*23*). A few studies have reported *Cronobacter* isolates conferring multidrug resistance (*26*), a phenomenon observed in our study only for 1 strain from Slovenia.

Correct species identification within the *Cronobacter* group was a major challenge for 7 of 11 participating laboratories. This identification problem is consistent with numerous misidentifications reported in the literature (*27*,*28*). The discrepancies in correct *Cronobacter* spp. identification on a genus and species level between the study center in Austria and the primary testing laboratories using MALDI-TOF MS is probably attributable to outdated databases used by primary testing laboratories. Nevertheless, our study showed that the overall MALDI-TOF MS performance for *Cronobacter* spp. identification on the species level is insufficient and misleading. The databases contained data for *C. sakazakii* only, and therefore all 7 species of the genus *Cronobacter* were identified as *C. sakazakii*. In addition, although a database comment indicated that *Cronobacter* could only be identified on the genus level, the MALDI-TOF MS result simulated the highest identification score for *C. sakazakii*. This shortfall should be corrected by an update of the MALDI-TOF MS databases to enable accurate *Cronobacter* identification at the species level. In comparison, WGS-based species identification represents a major improvement to conventional identification methods and MALDI-TOF MS (*29*). Therefore, we recommend the use of WGS-based identification tools and databases for identification of species within the *Cronobacter* group.

Adults were the main affected age group in our study. All but 2 of the isolates from 2017 originated from adults. This finding confirms the results from previous recent studies (*14*,*30*) and contradicts statements in numerous medical textbooks, postulating that infants are more often affected than adults (*8*,*31*–*33*).

Our study has some limitations. Lack of information (e.g., detailed epidemiologic and clinical patient data) and misidentification on genus and species levels, might have played a role in underestimating the real prevalence rate; 13 of the 24 participating countries did not find or did not submit *C. sakazakii* isolates.

In conclusion, this *C. sakazakii* study in Europe revealed a high strain diversity, which points to highly diverse infection sources and an absence of national or multinational outbreaks in 2017. Correct identification of *C. sakazakii* still poses a diagnostic challenge to many laboratories, and the use of such imperfect detection systems might explain the low prevalence of reported clinical *C. sakazakii* isolates found in this study. WGS data must be used for accurate species identification and high-resolution strain typing. We recommend the inclusion of *C. sakazakii* as a notifiable organism by public health authorities.

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The institutional review board of the city of Vienna studied the protocol and decided on July 28, 2016, under EK 16-161-VK-NZ that the study did not require formal ethics review.

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Ms. Lepuschitz studied molecular biology with a focus on molecular medicine and is currently a PhD student focusing on infectious diseases at the Vienna University of Technology and the Austrian Agency for Health and Food Safety, Vienna, Austria. Her primary research interests include the characterization and epidemiology of clinical, foodborne, and environmental pathogens.

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Window Period Prophylaxis for Children Exposed to Tuberculosis, Houston, Texas, USA, 2007–2017

Andrea T. Cruz, Jeffrey R. Starke

In this retrospective study, we assessed the safety of window period prophylaxis and proportion of tuberculin skin test (TST) conversions in children <5 years of age who were exposed to an adult with tuberculosis disease during 2007–2017. Children included in this study had unremarkable examination and chest radiograph findings and negative test results for TB infection. In total, 752 children (41% cohabitating with the index patient) received prophylaxis during the window period, usually directly observed therapy with isoniazid. Hepatotoxicity and tuberculosis disease did not develop in any child. TST conversion occurred in 37 (4.9%) children and was associated with the index patient being the child's parent (odds ratio 3.2, 95% CI 1.2–8.2). TST conversion was not associated with sputum smear results, culture positivity, or cohabitation. Thresholds for initiation of window prophylaxis in exposed young children should be low given the safety of medication and difficulties with risk stratification.

In 2017, tuberculosis (TB) disease was diagnosed in >9,000 persons in the United States (*1*). Each time a person (the index patient) receives a diagnosis of potentially contagious TB, health departments query that patient to determine recent contacts; this practice enables active surveillance and identification of persons who would benefit from evaluation and possibly treatment. Young children have a high rate of TB disease development shortly after infection (*2*). Prechemotherapy studies show that disease develops in 40%–50% of recently infected children <1 year of age and 25% of those 1–2 years of age, and miliary or meningeal TB develops in 25% of the children with TB disease <1 year of age and 20% of those 1–2 years of age. The rates of disease development gradually decreased with age among 3–5-year-olds (*2*).

US guidelines recommend that all children <5 years of age who are exposed to a person with potentially contagious TB be assessed for TB disease. This evaluation should include symptom screening, physical examination, and chest radiography, regardless of symptoms. In addition, children should receive a test of infection (either a tuberculin skin test [TST] or the interferon-γ release assay [IGRA]). If children have an examination and chest radiograph with unremarkable results and an initial negative test of infection, treatment is offered during what is termed the window period (i.e., the time it can take for a TST or IGRA to become positive after a person has shared air with another person with contagious TB). This window prophylaxis therapy is continued until a second test of infection performed 8–12 weeks after the last exposure determines definitively if infection has occurred (*3*).

Given the safety profile of TB medications in children (*4*,*5*) and the risk for serious disease rapidly developing, treatment during this window is considered highly beneficial for young children. However, although window prophylaxis has been used for decades, its safety and effectiveness have not been assessed. The goals of this study were to evaluate safety and tolerability of window prophylaxis in young children and determine the proportion and predictors of developing TB infection or disease in these exposed children.

Methods

We performed a retrospective cohort study of children <5 years of age referred to pediatric TB clinics in Houston, Texas, USA, during 2007–2017. Our clinic (Children's Tuberculosis Clinic at Texas Children's Hospital, Houston, Texas, USA) is the main referral source for children seen in 12 counties of the greater Houston metropolitan area (population ≈7 million), where TB incidence is twice the national average (*6*). We included all infants <6 months of age who had recent contact with an index TB patient, as well as all other children <5 years of age who had recent contact with an index patient and initial negative diagnostic test results (TST <5 mm or negative IGRA), no symptoms of TB disease, and an

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unremarkable 2-view chest radiograph and who were started on window prophylaxis. We excluded children whose contacts were later determined to not have TB or to have solely extrapulmonary TB.

Children are within the window period until 8–12 weeks after contact with the index patient has ceased, either by physical separation or effective treatment of the index patient (generally reflected by 3 consecutive negative results on acid-fast bacilli [AFB] sputum smear testing) (*7*). Health department workers performed a TST or obtained blood for IGRAs in the home of the child as soon as the child's exposure was recognized and 8–12 weeks after contact was broken. In the interim, isoniazid window prophylaxis was offered to exposed children twice weekly (20–30 mg/kg/dose, maximum 900 mg/d) under directly observed preventive therapy (DOPT) in the child's home by health department workers, as previously described (*8*). If the index case isolate was resistant to isoniazid or isoniazid was contraindicated, healthcare workers administered rifampin daily (10–20 mg/kg/dose, maximum dose 600 mg/d). Children were seen in the clinic at the initiation of therapy and every 1–2 months thereafter. We abstracted medical records for demographic variables, epidemiology and microbiology results for the index patient, medication regimens, and adverse events (AEs). We obtained institutional review board approval from Baylor College of Medicine (Houston, Texas, USA).

The primary outcome was the proportion of families reporting AEs for their children while they were on window prophylaxis. All children were assessed for signs and symptoms of hepatotoxicity at each DOPT medication visit. Children with abdominal pain, vomiting, anorexia, icterus, or weight loss had their medications withheld pending clinical and laboratory evaluation. Otherwise healthy asymptomatic children did not have baseline or serial liver testing performed. We used the National Cancer Institute guidelines to grade AE severity (*9*). The secondary outcomes were the proportion of infants with an initially negative test of infection in which TB infection or disease subsequently developed while on treatment and the epidemiologic factors associated with TST or IGRA conversion. We considered TST results of ≥5 mm of induration and QuantiFERON (QIAGEN, https://www.qiagen.com) results ≥0.35 international units/mL positive (*10*,*11*). We performed a priori analyses to evaluate test conversion in children <2 years and ≥2 years of age because children in the first 2 years of life are at the highest risk for disease progression with untreated TB infection (*2*). We compared proportions using odds ratios (ORs) with 95% CIs and expressed continuous variables as medians with interquartile ranges (IQRs). We used Stata 14 (StataCorp, https://www.stata.com) for analyses.

Results

Demographics

Pediatric Demographics

During the study period, 841 children from 12 health departments in the greater Houston area were seen for TB exposure. In total, 89 (10.6%) children were not started on window prophylaxis; 76 of these children had already had 2 negative tests of infection separated by 8–12 weeks, 8 (1%, 8/841) had familial refusal, 4 immediately moved out of the area, and 1 child had an index case that was multidrug-resistant (MDR) TB. The remaining 752 (89.4%) children initiated window prophylaxis (Table 1). No differences were found between children who did and did not receive window prophylaxis regarding age, race/ethnicity, or sex of the child or AFB sputum smear positivity of the index patient.

Index Patient Demographics

A total of 483 unique index patients (median age 44 [IQR 30–59] years) had preschool-aged contacts. In 311 (41.4%) of 752 instances, the index patient lived in the same home as the child. The most common relationship of the index patient to the child was grandparent or great-grandparent

Table 1. Characteristics of 752 children exposed to index tuberculosis patients, index patients, and index case *Mycobacterium tuberculosis* isolates, Houston, Texas, USA, 2007–2017

Characteristic	Value*
Demographics	
Age, y, median (interquartile range)	2.4 (1.2–3.6)
Sex	
F	380 (50.5)
M	372 (49.5)
Race/ethnicity	
Hispanic	493 (65.6)
Black	141 (18.8)
Asian	78 (10.4)
White	30 (4.0)
Biracial	10 (1.3)
Residing in home of index patient	
Yes	311 (41.4)
No	441 (58.6)
Index patient microbiology	
Acid-fast bacilli smear positive	513 (68.2)
Acid-fast bacilli culture positive	680 (90.4)
Index isolate drug susceptibilities, n = 680	
Isoniazid and rifampin susceptibility	635 (93.4)
Isoniazid resistance†	32 (4.7)
Isoniazid and rifampin resistance	13 (1.9)
Rifampin monoresistance	1 (0.1)
Index patient sample used for culture	
Sputum	637 (84.7)
Bronchoalveolar lavage	93 (12.4)
Lung biopsy	11 (1.5)
Pleural fluid	11 (1.5)

*Values are no. (%), except where specified. Percentages might not sum up to 100% because of rounding.
†Seven isolates were resistant to isoniazid and streptomycin, and 5 were resistant to isoniazid and ethambutol; the remainder of isolates had isoniazid monoresistance.

(37.8%, 284), followed by aunt or uncle (25.8%, 194), parent (15.8%, 119), cousin (6.5%, 49), sibling (0.9%, 7), other relative (2.4%, 18), babysitter (1.6%, 12), and other nonrelative (9.2%, 69).

Regimens

The most common medication used was isoniazid (730/752, 97.1%), followed by rifampin (20/752, 2.7%); 2 children whose index patient had MDR TB received ethambutol and pyrazinamide. For 17 (2.3%) children, therapy was changed after drug susceptibilities became available for the source case isolate; in these cases, the drug was changed to rifampin for 8 children and ethambutol and pyrazinamide for 2 children. For 7 children, isoniazid was stopped and no other drug begun because the second TST result was by then available and negative. These regimen changes occurred a median of 6 (IQR 2–8) weeks into therapy. Two (0.3%) children did not complete therapy: 1 child whose family refused medications and 1 child whose family moved out of the area before the second TST was performed.

Safety

No child had hepatotoxicity or TB disease progression while receiving window prophylaxis. The presence of any AE was rare (7/752, 0.9%). Rash developed in 2 patients; emesis in 2 patients; and diarrhea, weakness, and angioedema each in 1 patient. All 7 of those patients were taking isoniazid. Two AEs (angioedema, weakness) were grade 2, and the remainder were grade 1. The median time to development of an AE was 5 (IQR 2–6) weeks. TB treatment for the 7 children with AEs was changed to rifampin, which was well tolerated for the remainder of window prophylaxis. Children received therapy almost exclusively (744/752, 98.9%) under direct observation and for a median of 9 (IQR 7–12) weeks.

Conversion of Test of Infection

TSTs were used for initial and subsequent testing in 749 (99.6%) children. The median time between the first and definitive TST was 73 (IQR 63–90) days. This time interval did not differ between children residing (77 days) and not residing (78 days) in the same household as the index patient ($p = 0.47$) or between children younger (83 days) or older (78 days) than 6 months ($p = 0.09$).

Tests of infection converted from negative to positive in 37 (4.9%) children a median of 11 weeks after the initial test of infection. No differences were observed in TST conversion between children <2 years and ≥2 years of age ($p = 0.11$). The median TST induration in these 37 children was 12 (IQR 10–15) mm. TST conversion was more common when the index patient was young or the child's parent. No microbiological parameters (e.g., AFB smear and culture status) or epidemiologic factors (e.g., residence in same or different household) were associated with TST conversion (Table 2).

In total, 35 (94.6%) of 37 children with TB infection completed therapy; for 1 child, the family opted to stop isoniazid treatment after 2 months, and for the other, whose source case was MDR TB, treatment with ethambutol and pyrazinamide was stopped after abdominal pain developed (hepatic transaminases for this patient were within reference ranges). To the best of our knowledge, TB disease did not develop in any child with TB exposure or with a TB infection with a total of 4,466 (median 5.7 [IQR 3.7–7.6]) person-years of follow-up.

Discussion

We found that most families accepted their young children being treated for TB exposure. Therapy was initiated and completed for most children, most of whom were treated with twice-weekly DOPT with isoniazid. This treatment was safe; no child developed hepatotoxicity. Progression from exposure to infection was uncommon and not associated with epidemiologic factors typically associated with transmission of *M. tuberculosis*. Health department workers predominantly used TSTs for testing and isoniazid for window prophylaxis and only infrequently used IGRA and rifamycin-based regimens.

Prior studies have shown that up to 50% of children do not begin or complete isoniazid therapy for TB infection when children or other family members administer the drug (*5*,*12*,*13*). In contrast, families of TB-exposed children felt comfortable directly observing the administration of medication to their young, asymptomatic children who had unremarkable examination findings and chest radiograph results and negative tests of infection. Their acceptance of this therapy might reflect them having known a symptomatic contact (*14*) but, we hope, was also supported by our counseling. Directly observed therapy (DOT), which removes barriers to medication acquisition and administration, is often used to optimize adherence (*13*) and enables close monitoring for drug toxicities. We had a low threshold to change drug regimens in the event of any AE to facilitate continuation of window prophylaxis.

In this study, we did not find an association between TST conversion and AFB sputum smear status or cohabitation with the index patient, both found to be risk factors for acquisition of TB infection in other studies (*15*,*16*). Several factors could help explain this discrepancy. First, baseline smear positivity is not an accurate proxy of the degree of infectiousness. Other factors, such as intensity of exposure, room air exchanges, radiographic findings, sputum viscosity, and aerosolization of viable bacteria, also affect transmission (*17*,*18*). In fact, some data suggest that adults with high concentrations of bacteria in their smears might not transmit TB efficiently because they are too weak to

Table 2. Association between epidemiologic and microbiologic variables and tuberculin skin test conversion among children exposed to index tuberculosis patients, Houston, Texas, USA, 2007–2017

Variable	Tuberculin skin test conversion*		Value†
	Converted, no. (%), n = 37	Not converted, no. (%), n = 715	
Child demographics			
Age, y			
0–<1	4/154 (2.6)	150/154 (97.4)	Referent
1–<2	8/164 (4.9)	156/164 (95.1)	1.9 (0.6–6.5)
2–<5	25/434 (5.8)	409/434 (94.2)	2.3 (0.8–6.7)
Sex			
F	21/380 (5.5)	359/380 (94.5)	Referent
M	16/372 (4.3)	356/372 (95.7)	0.8 (0.4–1.5)
Race/ethnicity			
Hispanic	24/493 (4.9)	469/493 (95.1)	Referent
Black	5/141 (3.5)	136/141 (96.5)	0.7 (0.3–1.9)
Asian	4/78 (5.1)	74/78 (94.9)	1.1 (0.4–3.1)
Other	4/40 (10)	36/40 (90)	2.1 (0.7–6.6)
Index patient demographics			
Age, y	37	46	p = 0.002
Relationship to child			
Grandparent	8/284 (2.8)	276/284 (97.2)	Referent
Parent	10/119 (8.4)	109/119 (91.6)	3.2 (1.2–8.2)
Other relative	13/255 (5.1)	242/255 (94.9)	0.7 (0.3–1.8)
Nonrelative	6/94 (6.4)	88/94 (93.6)	0.9 (0.3–2.8)
Household member of child			
No	17/441 (3.9)	424/441 (96.1)	Referent
Yes	20/311 (6.4)	291/311 (93.6)	1.7 (0.9–3.3)
Index patient microbiology			
Sample type cultured			
Nonsputum‡	3/115 (2.6)	112/115 (97.4)	Referent
Sputum	34/637 (5.3)	603/637 (94.7)	2.1 (0.6–7.0)
Acid-fast bacilli stain result			
Negative	8/239 (3.3)	231/239 (96.7)	Referent
Positive	29/513 (5.7)	484/513 (94.3)	1.7 (0.8–3.8)
Acid-fast bacilli culture result			
Negative	3/72 (4.2)	69/72 (95.8)	Referent
Positive	34/680 (5.0)	646/680 (95.0)	1.2 (0.4–4.0)
Isoniazid-susceptible isolate§	30/635 (4.7)	605/635 (95.3)	Referent
Isoniazid-resistant isolate§	4/45 (8.9)	41/45 (91.1)	2.0 (0.7–5.9)

*Percentages reflect within-row frequencies and might not sum up to 100% because of rounding.
†Values are odds ratios (95% CI) except where indicated.
‡Bronchoalveolar lavages, lung biopsies, and pleural fluid cultures.
§Isoniazid resistance documented only in microbiologically confirmed cases.

produce a vigorous cough (*19*). Second, infected persons are not equally contagious throughout the natural history of infection, and discerning when during the index patient's course of infection the exposure to the child occurred is often difficult. Third, children were defined as household contacts if they resided in the same home as the index patient. However, some children spent most of their waking hours in another home cared for by a nonparent index patient, in which case, the child was defined as a nonhousehold contact. A rigid definition of what constitutes a household contact lacks epidemiologic meaning (*20*). Another finding from our study was that epidemiologic and microbiological factors were inadequate to risk stratify the children at higher risk of progressing from TB exposure to infection. As such, we feel that all exposed young children need to be evaluated with a physical examination, chest radiograph, and a test of infection and that all families should be offered window prophylaxis for their children. Of note, a TB exposure risk scoring system has been validated for children in settings of TB hyperendemicity (*21*). Although this system was not validated in low-incidence settings, such as the United States, discussing some of the scoring system variables associated with increased risk for exposure (maternal TB, sleep proximity, and duration of exposure) with families reluctant to initiate therapy might be useful.

Our primary diagnostic test for exposed children was TSTs, and treatment was primarily with isoniazid. The decision to use this testing and treatment strategy was driven by several considerations: a paucity of data on IGRAs and other treatment regimens in young children, ease of administration, and cost. The ability to place and read a TST in the home, rather than referring a child to a laboratory for an IGRA, has cost and convenience advantages for families. Given the large number of children evaluated for TB annually, using lower cost tests of infection and medications enabled health departments to provide services to more patients. Video DOT (i.e., the secure uploading and transmission of videos to the health department showing the child

taking medication) enables remote monitoring of adherence and toxicity and is less costly than standard DOT (*22*). Rifampin is considerably more expensive than isoniazid, and data do not support giving rifampin less frequently than daily, making DOPT with this drug difficult and expensive. The safety and efficacy of giving window prophylaxis with either medication by DOPT or by family member administration has not been compared in any studies.

Routine use of rifampin for window prophylaxis is reasonable in certain communities where isoniazid-resistant *M. tuberculosis* isolates are highly prevalent. In the United States, ≈8% of isolates are isoniazid resistant and rifampin susceptible (*6*). In our clinic, efficient communication with our health department partners facilitated prompt changing of regimens on the basis of source case drug susceptibility results. Another possible use for rifampin may be for exposed young infants, where concern about TST anergy can result in children receiving therapy for much longer than 8–12 weeks. For these children, 4 months of rifampin might be provided before physicians feel comfortable relying on the definitive TST.

For the children whose test of infection converted to positive, indicating TB infection, we chose to continue twice-weekly isoniazid and complete the 9-month regimen. Considering the data on safety, effectiveness, and adherence to 4-month regimens of daily rifampin (among children) and 3-month regimens of weekly isoniazid and rifapentine (for children ≥2 years of age) (*4*,*23*), changing to 1 of these shorter regimens seems reasonable. Whether the duration of either of these treatments could be shortened in the event of previous isoniazid window prophylaxis is unclear, but giving the infected child the full course of either regimen is most reasonable.

This study had some limitations. Not all variables were documented for all children. Children could have moved outside the area and TB disease could have developed thereafter. Our study was not powered to identify exposure risk factors for TST conversion; with only a 4.9% conversion rate, we might not have had enough cases to adequately assess the influence of certain putative risk factors. Almost all children had serial TSTs and not IGRAs; thus, we cannot rule out the possibility of boosting in the children who had TST conversions. Radiographic findings for the index patient were not uniformly available from local health departments; as such, we could not correlate cavitary disease or other forms of multibacillary disease with the risk for infection in children. Quantifying the duration of exposure was impossible; instead, we used living within the same or different household as a proxy. Timing of when contact was broken for children who were no longer around the contagious person was subject to recall bias. The study was not powered to evaluate efficacy of treatment. However, given the age of the children who had TST conversions (*2*), we estimate that 4–5 children would have progressed to TB disease if untreated. Last, because window prophylaxis is a recommended intervention, performing a randomized controlled trial comparing treatment and no treatment would have been unethical. The administration of window prophylaxis potentially could have prevented the establishment of TB infection in some children; thus, our study potentially underestimates the effect of window prophylaxis in the prevention of infection and disease.

In conclusion, ≈5% of TB-exposed children given prophylaxis progressed to TB infection, and available epidemiologic or microbiological data could not accurately identify which children might receive more benefit from window prophylaxis. However, given the safety of the medications and the acceptability of treatment by families, the benefits of therapy greatly outweigh the risks in this vulnerable patient population.

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About the Author

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Increased Risk for Invasive Group A *Streptococcus* Disease for Household Contacts of Scarlet Fever Cases, England, 2011–2016

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The incidence of scarlet fever in England and Wales is at its highest in 50 years. We estimated secondary household risk for invasive group A *Streptococcus* (iGAS) disease within 60 days after onset of scarlet fever. Reports of scarlet fever in England during 2011–2016 were matched by residential address to persons with laboratory-confirmed iGAS infections. We identified 11 iGAS cases in an estimated 189,684 household contacts and a 60-day incidence rate of 35.3 cases/100,000 person-years, which was 12.2-fold higher than the background rate (2.89). Infants and contacts ≥75 years of age were at highest risk. Three cases were fatal; sepsis and cellulitis were the most common manifestations. Typing for 6 iGAS cases identified *emm* 1.0 (n = 4), *emm* 4.0 (n = 1), and *emm* 12.0 (n = 1). Although absolute risk in household contacts was low, clinicians assessing household contacts should be aware of the risk to expedite diagnosis and initiate life-saving treatment.

During 2014, England and Wales had a sharp increase in the incidence of scarlet fever, which by 2016 had reached 33.2 cases/100,000 person-years, the highest rate in almost 50 years (*1*,*2*). An increase in disease incidence was similarly reported from 2009 onward in Vietnam, Singapore, Hong Kong, and mainland China but has not been reported elsewhere in Europe (*1*,*3*–*7*). The cause of this increase is unknown (*1*,*5*,*8*).

Scarlet fever was once a common cause of childhood death before incidence and deaths decreased dramatically during the 19th century (*1*,*9*). Although now typically a mild disease, scarlet fever remains statutorily reportable in England to enable prediction of periods of increased incidence of invasive group A *Streptococcus* (iGAS) infection given the temporal correlation between these two conditions (*1*,*2*). Genomic assessment of *Streptococcus pyogenes* has furthermore demonstrated that the same strains cause scarlet fever and iGAS infection (*10*,*11*). iGAS is statutorily reportable to make contact tracing easier, given the increased risk for secondary iGAS infection among household contacts (*4*,*12*,*13*).

This study was initiated as part of a coordinated public health response to determine the cause and effect of the increase in scarlet fever in the United Kingdom (*1*,*2*,*11*). We investigated whether there is an excess risk for secondary iGAS infection in households in which a person was given a diagnosis of scarlet fever to determine whether further public health actions are required to protect contacts.

Methods

Study Design, Population, and Definitions

We conducted a retrospective cohort study to compare the incidence of iGAS infection among household contacts of persons with scarlet fever with the background incidence of iGAS infection in England. The cohort comprised all scarlet fever case-patients resident in England who had disease onset during January 1, 2011–December 31, 2016. Suspected cases of scarlet fever are reported by clinicians on the basis of clinical signs consistent with the condition, with or without laboratory confirmation of GAS infection. iGAS infection was defined by isolation of GAS from a normally sterile site (including blood, joint aspirates, cerebrospinal/pericardial/peritoneal/pleural fluids, deep tissue or abscess at surgery or necropsy, and bone).

A scarlet fever–iGAS household cluster was defined as a household in which a person of any age received a diagnosis of scarlet fever and, within the next 60 days, a different member of the same household received a diagnosis of iGAS infection. Case-patients resident in institutional settings were excluded. A 60-day interval was

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selected on the basis of preliminary analysis of the interval between onset of scarlet fever and iGAS specimen date in address-matched pairs.

Data Sources

Demographic details of scarlet fever reports were obtained from the Public Health England (PHE) HP Zone (InFact UK, Ltd., http://hpzoneinfo.in-fact.com), a tool used nationally by health protection teams to assist case and incident management. Reports of iGAS infection were extracted from the PHE national laboratory surveillance database (Second Generation Surveillance System, https://sgss.phe.org.uk). Both datasets were sent to a National Health Service demographic batch tracing service to complete missing postcodes (typically corresponding to 15 addresses [*14*]), addresses, and patient identifiers. We sought missing postcodes for iGAS cases from the national reference laboratory database. Preliminary analysis indicated that all scarlet fever case-patients within address-matched pairs were ≤10 years of age. Therefore, data for the number of households in England with ≥1 child ≤10 years of age and the number of persons by single year of age in these households were provided by the Office for National Statistics Labour Force Survey (*15*) for use as denominators in risk calculations. Midyear resident population estimates for 2011 through 2016 were also obtained from the Office for National Statistics.

We obtained clinical information for cases within scarlet fever–iGAS pairs from HP Zone. National laboratory surveillance and reference laboratory data were used to identify co-infection and *emm* typing. We obtained the index of multiple deprivation decile score for each household on the basis of residential postcode (*16*). This index is a measure of relative socioeconomic deprivation based on 7 domains and provides a ranking at granular geographic level (≈1,500 residents) from the most to least deprived areas in England (*17*).

Data Analysis

Identification of Household Clusters

We cleaned and analyzed data by using R version 3.2.2 (https://cran.r-project.org/bin/windows/base/old/3.2.2). Records without a postcode were excluded. We matched scarlet fever cases to all iGAS reports with a specimen date during November 1, 2010–March 1, 2017, by residential postcode. This matching enabled capture of linked iGAS cases occurring within 2 months of the first and last scarlet fever case. Cases within postcode-matched pairs without full address were removed. Addresses of remaining matched pairs with an interval between onset of scarlet fever and iGAS specimen date ≤60 days were visually scrutinized to exclude institutional settings and confirm co-location.

The iGAS and scarlet fever datasets were deduplicated after matching to ensure that all sequential specimens were considered in identifying temporal links between cases. An interval ≥14 days between specimen dates was considered a new episode for iGAS and ≥30 days between onset dates for scarlet fever. To supplement household clusters identified from address matching, we reviewed GAS clusters and outbreaks recorded on HP Zone and the reference laboratory outbreak database over the same period.

Calculation of Risk

We estimated the average number of household contacts of scarlet fever cases by dividing the total number of persons living in households with a child ≤10 years of age in England during the study period by the number of households and subtracting 1 to account for the case-patient being a household member. We multiplied this figure by the number of scarlet fever cases to estimate the total number of contacts and calculated the person-years at risk over 60 days. We calculated the incidence of iGAS infection among scarlet fever household contacts by dividing the number of iGAS cases among these contacts by the number of person-years at risk and used Poisson distribution to define 95% CIs. The background rate of iGAS infection was based on the total number of iGAS cases in England. We repeated this analysis by year and age group. We conducted a sensitivity analysis to investigate the effect of increasing the average household size by up to 3 household members (3.8/household to 6.8/household).

Ethics Considerations

Ethics approval was not required because we analyzed only routinely collected data. PHE has the authority to collect and process confidential patient information for communicable disease surveillance and control under Section 251 of the National Health Service Act of 2006.

Results

A total of 73,344 scarlet fever cases were reported to PHE during 2011–2016. Of the 9,978 episodes of iGAS infection extracted for address matching to scarlet fever cases, 2.7% (269) were excluded due to a missing address; a higher proportion of cases before 2014 had a missing postcode than cases from 2014 onward (4.4% vs. 1.4%, χ^2 85.2, df 1, 95% CI 2.3–3.7; p<0.0001). We identified 991 scarlet fever–iGAS pairs with identical postcodes in any setting (including institutions); 1.8% (18) did not have a full address and were excluded from further analysis (onset interval range 93–1,893 days) (Figure 1). Of the remaining 973 pairs, 53 were resident in a private home and confirmed as being at the same address; iGAS cases occurred after scarlet fever onset for 28 of 53 pairs.

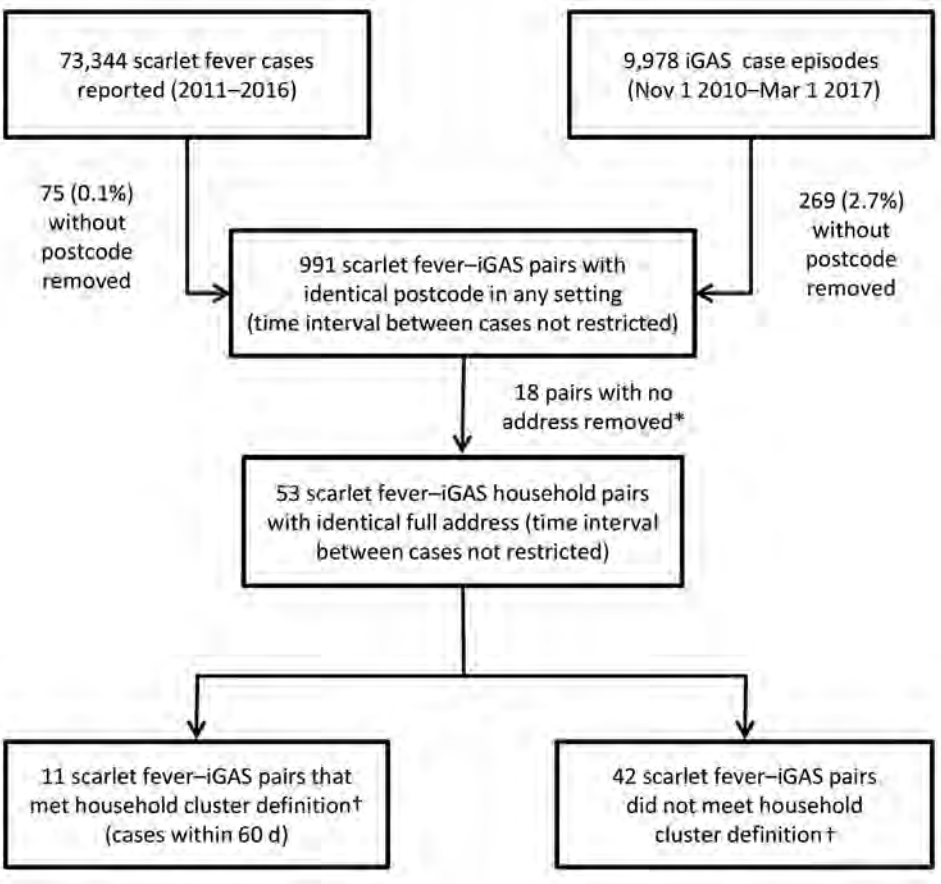

Figure 1. Summary of records included at each stage of the matching process of scarlet fever and iGAS cases, England 2011–2016. *Interval between excluded pairs was >60 days. †A household cluster was defined on the basis of a person being given a diagnosis of scarlet fever and a different member of the same household given a diagnosis of iGAS infection for which onset of iGAS symptoms occurred within 60 days after onset of scarlet fever. iGAS, invasive group A *Streptococcus* infection.

A pronounced increase in the number of pairs was evident within the first 100 days after onset of scarlet fever (Figure 2): 13 pairs identified, compared with an expected 1.5 (95% CI 0.2–7.2) iGAS cases based on background iGAS infection rates. All 13 pairs were within 60 days, and on review of case details, 11 met the household cluster definition. No clusters were identified through review of the national case management system or the reference laboratory database. Two of the 25 pairs in which iGAS occurred before scarlet fever had an interval between cases ≤60 days, a rate of 6.4 iGAS cases/100,000 person-years and twice the background rate (rate ratio [RR] 2.2, 95% CI 0.6–8.9). Fifty-one pairs with onset dates within 60 days had the same postcode but were resident in different private homes.

We identified 18 persons given a diagnosis of iGAS and scarlet fever. The median interval between onset of scarlet fever and iGAS specimen date was 155 days (range 5–1,488 days). Sixteen cases had an interval >100 days, and 7 cases had iGAS infection after a diagnosis of scarlet fever.

Characteristics of Household Clusters

All household clusters were composed of 1 scarlet fever case and 1 iGAS case and occurred after March 2014. The median interval between onset of scarlet fever and of iGAS infection was 18 days (range 3–54 days) (Figure 3). Five iGAS cases occurred in parents of children with scarlet fever and 4 in siblings; the relationship to the scarlet fever case-patient was not recorded for 2 clusters (iGAS case-patients 86 and 26 years of age) (Table 1). Five iGAS case-patients had sepsis and 3 had cellulitis. Five of the 11 iGAS case-patients had an underlying chronic condition, and 1 had an acute infection with influenza. Three of the iGAS case-patients died; 2 of them had predisposing conditions (arthritis, diabetes, and atypical mycobacterial infection). The median household size was 4 persons (range 4–6 persons). Seven of 11 households were in the 30% most deprived neighborhoods in England, and 3 were in the 30% least deprived.

Strain typing was available for 6 of the iGAS household cluster cases, 4 of which were *emm* 1.0 and 1 each were *emm* 4.0 and *emm* 12.0. Typing results for scarlet fever isolates were not available.

Calculation of Risk

All scarlet fever case-patients within clusters were ≤10 years of age. Therefore, we restricted analysis of risk for iGAS infection to household contacts of scarlet fever

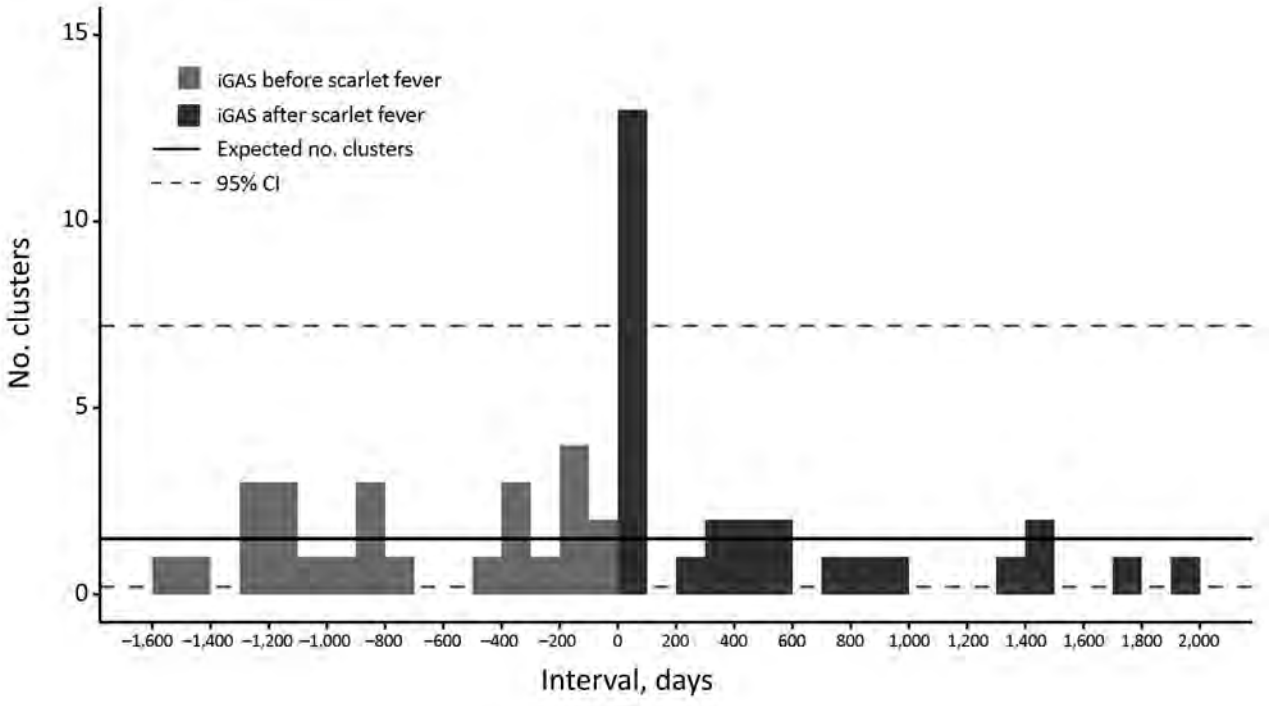

Figure 2. Distribution of time interval between onset of scarlet fever and iGAS within address-matched pairs (n = 53) and expected number of clusters, England 2011–2016. Exploratory analysis was used to identify the period of excess numbers of iGAS cases before review of case records; iGAS cases might be linked to >1 scarlet fever case episode in the same household. The background iGAS rate was 2.88 cases/100,000 person-years; 95% CI is based on 2 expected cases/100 days. There were 189,684 scarlet fever household contacts. iGAS, invasive group A *Streptococcus* infection.

case-patients ≤10 years of age (n = 66,191). We estimated that these case-patients had 189,684 household contacts (average household size 3.9 persons). We estimated the incidence of iGAS infection among these contacts to be 35.3 cases/100,000 person-years (95% CI 17.6–63.2 cases/100,000 person-years) compared with a background incidence of iGAS in England (all ages) of 2.9 cases/100,000 person-years (Table 2). Therefore, the rate of iGAS infection in household contacts of persons with scarlet fever was 12 times higher than the background rate in England over the same period (RR 12.2, 95% CI 6.7–22.1) (Table 2). The highest absolute rates were for infants (138 cases/100,000 person-years, 95% CI 16.7–496.8 cases/100,000 person-years) and persons ≥75 years of age (1,419 cases/100,000 person-years, 95% CI 35.9–7907.3 cases/100,000 person-years), although these rates were based on a small number of cases (Table 3). RR was highest for contacts 11–17 years of age (RR 43.9, 95% CI 6.1–313.7) and contacts ≥75 years of age (RR 139.2, 95% CI 19.6–988.5) (Table 3).

Sensitivity analysis, increasing the average household size from 3.8 to 6.8 members, reduced the RR for iGAS in scarlet fever household contacts relative to the background iGAS rate to 6 (95% CI 3.3–10.8). The rate of iGAS infection in scarlet fever household contacts before the period of increased scarlet fever incidence (2011–2013), 0 cases/100,000 person-years (95% CI 0.0–61.1), was not significantly different for the period of increased incidence (2014–2016), 43.7 cases/100,000 person-years (95% CI 21.8–78.4).

Discussion

Our study identified a low risk for iGAS infection among household contacts of scarlet fever cases (35.3 cases/100,000 person-years). However, this risk was increased when compared with the background risk. Eleven iGAS cases occurred among an estimated 189,684 contacts during the 60 days after scarlet fever onset; 1.5 cases would have been expected on the basis of a background rate of 2.9 cases/100,000 person-years. Small numbers of cases preclude robust subgroup analysis but excess risk was highest in contacts ≥75 and 11–17 years of age. Although the absolute risk was low, the effect of these infections was severe; 3 deaths were reported. These findings have implications for other countries reporting a high incidence of scarlet fever.

Half the secondary iGAS cases occurred in parents and one third occurred in siblings of scarlet fever case-patients.

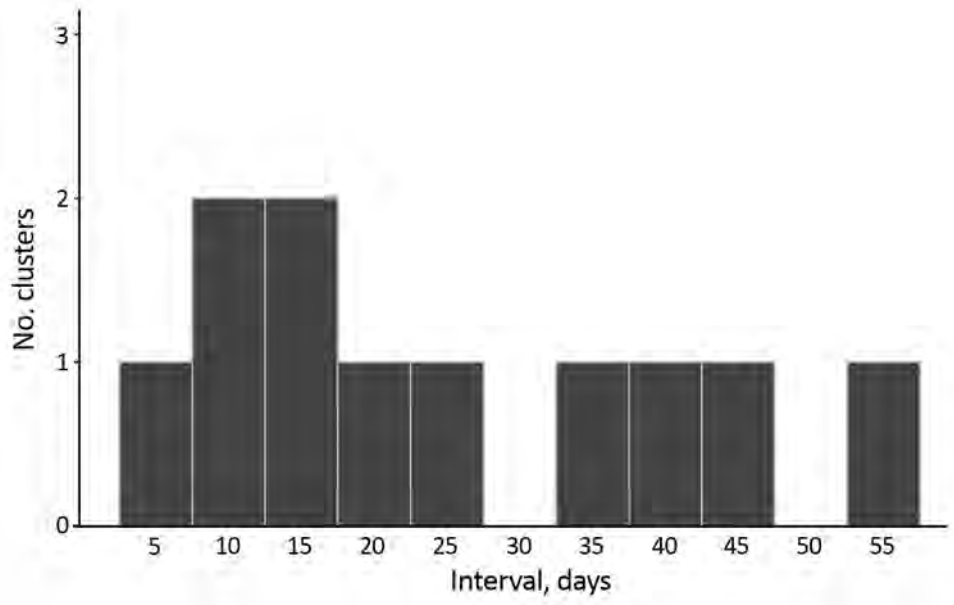

Figure 3. Distribution of time intervals between onset of scarlet fever and invasive group A *Streptococcus* infection within each pair meeting the household cluster definition (n = 11), England 2011–2016.

A previous study showed a slight excess in scarlet fever incidence in young adult women, possibly explained by caring responsibilities for children with the infection (*1*). We did not observe a similar pattern for iGAS; 3 of 5 cases in parents were in men. Contacts ≥75 years of age had the highest absolute risk for development of iGAS. Although the background rate for iGAS was highest in elderly persons, because there were only an estimated 429 household contacts of scarlet fever case-patients within this age group, 1 secondary iGAS case in this group translated into a high attack rate.

An estimated 5 million grandparents have regular childcare responsibilities in the United Kingdom (*18*). We were unable to assess the risk for grandparents not living in the same household and are likely missing a major group potentially at risk through contact with scarlet fever case-patients. Our identification of 51 postcode-matched pairs with different addresses suggests a possible increased risk for iGAS infection in the neighborhood of scarlet fever case-patients and warrants further assessment. Although a proportion of the observed secondary household iGAS risk might be caused by transmission in wider social networks, the fact that parents constituted most secondary iGAS cases suggests that transmission within the home underpins these clusters because parents are less likely than children to be exposed to scarlet fever outside the home.

Almost half the iGAS case-patients reported underlying chronic conditions, although diabetes, Crohn's disease, and arthritis are common, which limits the potential to target public health actions. A broad range of clinical initial manifestations were reported for iGAS infections within clusters, including skin and soft tissue and joint infections. Overcrowding is a known predisposing factor for *S. pyogenes* infections (*4*), but we did not find evidence of this factor in clusters who lived in average sized households (median 4 occupants), although this information was not available in public health records for 4 of 11 households.

Pharyngeal carriage of GAS among close contacts of persons with invasive infections has been demonstrated (*19*); person-to-person transmission of GAS occurs by respiratory droplets or skin contact (*4*). We do not assume that iGAS infection was necessarily acquired from the child with scarlet fever. Other members of the household (symptomatic or asymptomatic) might have been the source to either case-patient, particularly given the long period of risk (60 days) and that the scarlet fever case-patient probably received treatment.

We examined the risk for secondary iGAS infection before and after onset of scarlet fever, without preconceptions as to the length of identified period of excess risk. Transmission of GAS within the household for 60 days is plausible; back-and-forth transmission between household members is well described (*20*–*22*) and has been demonstrated to occur over a 10-month period (*21*). Transmission from an asymptomatic carrier can occur up to several weeks after acquisition although communicability is lower than from symptomatic cases (*19*,*23*). An ongoing study in London aims to assess GAS carriage in family members of scarlet fever case-patients (*24*).

Environmental reservoirs have been implicated in hospital (*25*,*26*), nursery (*27*), and care home outbreaks (*4*,*28*), but the duration of viability in the environment is unknown. Survival on dry surfaces has been demonstrated after several months; therefore, public health

Table 1. Characteristics of 11 iGAS case-patients within household clusters, England, 2011–2016*

Characteristic	No. case-patients		
	Total	Male sex	Female sex
Age, y			
<1	2	1	1
1–18	2	0	2
19–50	6	3	3
≥75	1	1	0
Total	11	5	6
Relationship to scarlet fever case-patient			
Parent	5	3	2
Sibling	4	1	3
Unknown	2	1	1
Acute health conditions at time of diagnosis			
Influenza A	1	NR	NR
Chronic health condition at time of diagnosis			
Arthritis	1	NR	NR
Crohn's disease	1	NR	NR
Premature birth	1	NR	NR
Diabetes	1	NR	NR
Atypical mycobacterial infection	1	NR	NR
Asplenia	1	NR	NR
Multiple unnamed conditions	1	NR	NR
No concurrent conditions	6	NR	NR
Died	3	NR	NR
Clinical manifestation			
Sepsis	5	NR	NR
Cellulitis	3	NR	NR
Septic arthritis	1	NR	NR
Other invasive infection (unspecified)	2	NR	NR
iGAS *emm* typing			
1.0	4	NR	NR
4.0	1	NR	NR
12.0	1	NR	NR
Untyped	6	NR	NR

*iGAS, invasive group A *Streptococcus* infection; NR, not reported.

messaging should include advice on infection control in households, particularly where there are susceptible persons (*29*,*30*).

Although it was not the focus of this study, we observed a slight excess risk for iGAS occurring before scarlet fever. Public health guidelines on the management of iGAS infection in the United Kingdom recommend that household contacts are advised to visit their general practitioner (GP) for assessment if they have symptoms of GAS infection in the 30 days after onset in the index case-patient. Therefore, these scarlet fever case-patients should have already been under surveillance, potentially increasing the likelihood of their diagnosis.

The number of contacts of scarlet fever case-patients was the main source of uncertainty in our risk estimation. If households that have scarlet fever case-patients differ in size from the average household with children, we could have underestimated or overestimated the risk. However, the risk for iGAS infection would still have been 6 times higher than background if the average household size was increased to 7 members. Coupled with our observation that household clusters had a median size of 4 (compared with 3.9 for all households in England), it is unlikely the uncertainty about numbers of contacts could account for the increase in risk observed.

Failure to match iGAS to scarlet fever cases could have occurred because of missing postcodes (3% of iGAS cases), errors in the postcode or address, or because the traced postcode represents the current address and might be different from that at the time of infection. Our finding that all clusters occurred after the increase in scarlet fever during 2014 was possibly influenced by this factor, given that postcode completion was higher in the later years of the study and enabled identification of clusters. We did not adjust for residence in a long-term care facility or hospitalization in the background risk calculation: 3.5% of iGAS cases in England (2009–2010) were estimated to be acquired in long-term care facilities, and 6% of these infections were estimated to be acquired in hospitals. Residents of long-term care facilities had a 6-fold higher risk for iGAS infection than community residents (*31*,*32*). Including institutionally acquired infections slightly increased the background iGAS risk in this study.

We used clinical reports of scarlet fever and recognized that a proportion of reported cases might have had other infections, which has the potential to influence the

Table 2. Risk for iGAS infection among household contacts of scarlet fever case-patients ≤10 years of age by year compared with background iGAS incidence, England, 2011–2016*

Year	No. scarlet fever cases	Estimated no. contacts	No. iGAS cases in contacts†	Attack rate/100,000 person-years (95% CI)	Background iGAS incidence/100,000 person-years	Rate ratio (95% CI)
2011	3,128	8,929	0	0.0 (0.0–251.5)	2.29	NA
2012	4,632	13,073	0	0.0 (0.0–171.8)	2.35	NA
2013	5,204	14,778	0	0.0 (0.0–152.0)	2.99	NA
2014	16,394	47,015	3	38.8 (8.0–113.5)	2.29	16.9 (5.5–52.6)
2015	18,022	51,454	3	35.5 (7.3–103.7)	3.48	10.2 (3.3–31.7)
2016	18,811	54,435	5	55.9 (18.2–130.5)	3.91	14.3 (5.9–34.7)
Total	66,191	189,684	11	35.3 (17.6–63.2)	2.89	12.2 (6.7–22.1)

*iGAS, invasive group A *Streptococcus* infection; NA, not applicable.
†During the 60 days after onset of scarlet fever in the household.

risk estimate in either direction. Although only ≈50% of scarlet fever consultations in primary care in England are formally reported (*1*), this finding would not influence the risk estimate because it was based on iGAS cases that occurred in contacts of only the reported cohort. We did not capture the burden of disease associated with severe noninvasive GAS infections (GAS isolated from a nonsterile site), although these infections are estimated to comprise only 1% of all severe GAS infections (*33*,*34*).

We assessed the risk for secondary iGAS infection for household contacts to be low. However, potential severity is high. The prodrome for iGAS infection can be nonspecific, and the disease can progress rapidly; therefore, increasing the index of suspicion in specific circumstances or groups at increased risk might expedite diagnosis and commencement of life-saving treatment (*4*,*35*). Offering antimicrobial drug prophylaxis to household contacts to eradicate carriage and treat incipient infection could reduce the risk for iGAS infection. However, the unintended consequences of large-scale increased use of antimicrobial drugs, heightened patient anxiety, the effect on GP workload, and the lack of evidence on effectiveness make this option disproportionate given the low overall risk estimated. Antimicrobial drug prophylaxis could be targeted to high-risk contacts, such as elderly persons and infants. However, there is considerable uncertainty for the risk estimate for these groups because of the small number of secondary iGAS cases. Providing information on signs and symptoms of iGAS infection to patients or parents at the point of scarlet fever diagnosis to accelerate self-referral for medical assessment could be effective but has the potential to increase anxiety for many persons and increase presentations of worried healthy persons to GPs and emergency departments at scale. Increasing awareness among frontline clinicians assessing patients of this increased risk to improve early identification and treatment of cases is perhaps the most proportionate and feasible response on the basis of available data. Information could also be made available to the public through patient-facing websites provided that messages are worded carefully so as not to increase anxiety.

We recommend repeating this analysis at regular intervals to monitor and increase precision around our estimated risk. Enhanced surveillance of iGAS patients should include questions on the number of contacts and recent scarlet fever infections in the household. This information would help address some of the methodological uncertainties around the number of contacts and enable assessment of the attributable risk in the context of other risk factors. Of ≈10,000 iGAS cases identified during our study, only 11 were associated with scarlet fever contact: as such, a proportionate response to further investigations is warranted. Although increases in iGAS infection have been observed during the latter period of the scarlet fever upsurge (2016 onward), these increases follow a longer-term trend of increasing iGAS infection in England, and the connection with increased scarlet fever activity remains unclear (*36*).

Table 3. Risk for iGAS among household contacts of 66,191 scarlet fever case-patients ≤10 years of age compared with background iGAS incidence by age, England, 2011–2016*

Age of contacts, y	Estimated no. contacts	No. iGAS cases in contacts†	Attack rate/100,000 person-years (95% CI)	Background iGAS incidence/100,000 person-years	Rate ratio (95% CI)
<1	8,853	2	137.5 (16.7–496.8)	6.42	21.4 (5.31–86.1)
1–10	28,660	1	21.2 (0.5–118.3)	2.84	7.5 (1.1–53.1)
11–17	22,209	1	27.4 (0.7–152.7)	0.58	43.9 (6.1–313.7)
18–50	122,801	6	29.7 (10.9–64.7)	1.69	18.4 (8.4–41.1)
51–74	6,733	0	0 (0–333.5)	3.59	0
≥75	429	1	1,419.2 (35.9–7.907.3)	10.20	139.2 (19.6–988.5)

*iGAS, invasive group A *Streptococcus* infection.
†During the 60 days after onset of scarlet fever in the household.

It is likely that contact with other superficial manifestation of GAS infection would also increase the risk for iGAS infection. However, the mixed etiology for these conditions and lack of microbiological testing make this potential risk difficult to assess. Nonetheless, with drives to reduce antimicrobial drug treatment for conditions such as pharyngitis to relieve selection pressure favoring antimicrobial drug resistance, understanding the possible repercussions for the patient and wider community are essential.

In conclusion, we identified an excess risk for iGAS among household contacts of scarlet fever case-patients, although we assessed the overall risk to be low. We recommend that frontline clinicians maintain heightened awareness of the risk for iGAS in scarlet fever contacts when assessing patients. Further research to tighten our risk estimates and improve our understanding of transmission patterns in households will inform future prevention strategies.

Acknowledgments

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About the Author

Ms. Watts is a field epidemiology training fellow with the Field Service, Public Health England, Liverpool, UK. Her research interests include surveillance and epidemiology of *S. pyogenes* infections.

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Tuberculosis Surveillance and Control, Puerto Rico, 1898–2015

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The World Health Organization recognizes Puerto Rico as an area of low tuberculosis (TB) incidence, where TB elimination is possible by 2035. To describe the current low incidence of reported cases, provide key lessons learned, and detect areas that may affect progress, we systematically reviewed the literature about the history of TB surveillance and control in Puerto Rico and supplemented this information with additional references and epidemiologic data. We reviewed 3 periods: 1898–1946 (public health efforts before the advent of TB chemotherapy); 1947–1992 (control and surveillance after the introduction of TB chemotherapy); and 1993–2015 (expanded TB control and surveillance). Although sustained surveillance, continued care, and use of newly developed strategies occurred concomitantly with decreased incidence of reported TB cases and mortality rates, factors that may affect progress remain poorly understood and include potential delayed diagnosis and underreporting, the effects of government debt and Hurricane Maria, and poverty.

Tuberculosis (TB) remains a major public health challenge. Worldwide in 2017, an estimated 10 million incident cases occurred (*1*); ≈1.3 million deaths were caused by TB, and another 300,000 deaths were caused by TB among HIV-positive persons. TB now ranks above HIV as the leading cause of death from an infectious disease (*1*). On September 26, 2018, the United Nations held its first high-level meeting on TB to accelerate efforts to end TB by reaching all affected persons with prevention and care.

Progress has been made toward global targets since the World Health Organization (WHO) declared a Global TB Emergency in 1993 (*2*). WHO has recognized 33 low-incidence countries and territories where the goal of TB elimination, defined as ≤1 case/1 million population, is possible by 2035 (*3*). Puerto Rico, a US territory, is one of the low-incidence areas. During 1993–2015, incidence of reported cases decreased from 7.1 to 1.5 cases/100,000 population (*4*). Still, challenges to surveillance and control remain.

To learn more about decreasing incidence of reported TB cases and mortality rates, provide key lessons learned, and discuss areas that may affect progress toward TB elimination, we reviewed the history of TB surveillance and control in Puerto Rico. We divided our review into 3 periods (Table 1; Figure 1): 1898–1946 (public health efforts before the advent of TB chemotherapy); 1947–1992 (control and surveillance after the introduction of TB chemotherapy, which in Puerto Rico was predominantly delivered through government-subsidized primary health services); and 1993–2015 (expanded TB control and surveillance, which followed renewed efforts in the United States to control TB and the introduction of Reforma de Salud de Puerto Rico, a government-run healthcare program managed through private insurance companies).

Study Design

Literature Review

On August 30, 2015, we searched publications on PubMed and the US Centers for Disease Control and Prevention (CDC) Primo (CDC-based electronic literature search engine that enables users to search for books, journal articles, CDC stacks, and digital objects) for the terms "Puerto Rico" and "tuberculosis." The search returned 240 articles, 18 of which we downloaded after reviewing titles and abstracts. Additional articles were added after a review of the article references (n = 28) and Spanish-language articles (n = 8), contextual literature (n = 26), and co-author literature databases (n = 4). We fully reviewed 84 articles and excluded 8 because of lack of relevance.

Epidemiologic and Population Data

Epidemiologic data were primarily sourced from the CDC TB annual reports for 1953–1992 (https://www.cdc.gov/tb/statistics/reports/default.htm) and CDC Online Tuberculosis

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[1]These authors were co–principal investigators.

Table 1. Summary of the evolution of TB surveillance, diagnosis, and treatment in Puerto Rico over 3 periods*

Period	Surveillance	Diagnosis	Treatment
1898–1946	Passive, relying on voluntary reporting; Bureau of TB established in 1924; TB becomes a part of vital statistics centrally compiled by PRDH in 1931	Primarily clinical diagnosis; TST progressively routinized after 1929; chest radiography and limited sputum examination in TB dispensaries since 1935	Pneumothorax procedures, bed rest in sanatorium, and isolation of patients with active TB
1947–1992	TB recording and reporting through PRDH TB centers and centralized at PRDH; private physicians, hospitals, and VA report to PRDH; case-level data collection and reporting to NTSS uses RVCT	TST and chest radiography routine for screening (e.g., medical cards); limited sputum AFB examination and ability to culture	Three-drug regimen of streptomycin, PAS, and isoniazid; free treatment in 3-mo courses for total treatment of ≥2 y; introduction of short-course regimens using rifampin in the 1970s
1993–2015	RVCT revised and expanded; CDC NTSS electronic registry launched; molecular testing for DST at PRDH laboratory and genotyping and molecular testing through CDC introduced	Diagnosis relies on sputum AFB examination and culture; screening with TST and chest radiography; few cases diagnosed clinically	DOT, 4-drug regimen of isoniazid, rifampin, pyrazinamide, and ethambutol; intensive and continuation phase for 6–8 mo; regimens individualized according to DST results

*AFB, acid-fast bacillus; CDC, Centers for Disease Control and Prevention; DOT, directly observed therapy; DST, drug-sensitivity testing; NTSS, National TB Surveillance System; PAS, para-aminosalicylic acid; PRDH, Puerto Rico Department of Health; RVCT, Report of Verified Case of Tuberculosis; TB, tuberculosis; TST, tuberculin skin testing; VA, Veterans Administration.

Information System (OTIS; https://wonder.cdc.gov/tb.html) for 1993–2015. We calculated mortality rates for Puerto Rico and the United States (excluding Puerto Rico) by using epidemiologic and population data sourced from the following databases: CDC WONDER (https://wonder.cdc.gov/mortSQL.html), Vital Statistics (https://www.cdc.gov/nchs/nvss/mortality_historical_data.htm), US Census Bureau (https://www.census.gov), and the United Nations Statistics Division (https://unstats.un.org) (Appendix, https://wwwnc.cdc.gov/EID/article/25/3/18-1157-App1.pdf).

Statistical Analyses

Using reported TB cases from 1993–1997 as a referent, we analyzed patient age distribution for reported TB cases during 2011–2015 by using the χ^2 test. We considered $p<0.05$ to be significant.

Results

Public Health Efforts before the Advent of TB Chemotherapy (1898–1946)

In 1898, Spain ceded Puerto Rico to the United States through the Treaty of Paris. Thereafter, health directives were implemented under US territorial administration. Building on nascent efforts, on March 14, 1912, law no. 81 created the Puerto Rico Department of Health (PRDH), charged with protecting public health on the island.

The annual reports of the Puerto Rico Commissioner of Health TB statistics included mortality rates. TB notification relied on voluntary reporting from private physicians and healthcare centers across the island. During the 1920s, the PRDH collected additional health-related data and compiled statistics, including those for TB.

During 1922–1923, the US Public Health Service (USPHS) conducted the first TB survey of Puerto Rico by using records from schools, the US Veteran's Bureau, Army enlistments, private practitioners, and hospitals (*5*). The USPHS concluded that estimating TB morbidity rates was not possible because of underreporting; an estimated 60% of deaths from TB were not recorded (*5*,*6*). In 1924, PRDH established a Bureau of Tuberculosis, which was responsible for recording TB statistics (*7*). By 1931, a centralized vital statistics system was implemented (*8*), and starting in 1932, Puerto Rico data were included in US annual mortality statistics (*9*). Recorded TB mortality rates subsequently increased, in part as an artifact of improved reporting, and peaked in 1933 at 333 deaths/100,000 population; the high mortality rate was linked to the destruction wrought by Hurricane San Ciprian, a Category 4 storm that traversed the island in September 1932 (*8*). By comparison, the estimated TB mortality rate for that year in the United States was 56.7/100,000 population (Figure 2). Sanatoria, including 1 sanatorium each in Rio Piedras (200 beds) and Ponce (30 beds), were unable to cope with the high volume of patients (*10*).

In addition to regular recording and reporting activities, several TB-related surveys were conducted, leading to public health action. For example, after a 1930 survey of 2,500 schoolchildren 8–10 years of age found that 68% had positive reactions to tuberculin skin testing (TST) (*6*,*8*), TST was used in epidemiologic studies and to screen children and adolescents. Additional surveys took 1 of 2 forms. First, evaluations of postmortem and pathology specimens characterized TB among the dead, finding a "large measure of tuberculization of the people" (*11*,*12*). Second, epidemiologic surveys were conducted in a variety of settings, including coastal and mountainous municipalities (*6*), urban (*13*) and rural (*14*) communities, and low-resource communities (*15*). Surveys made use of TST results, chest radiographs, patient histories,

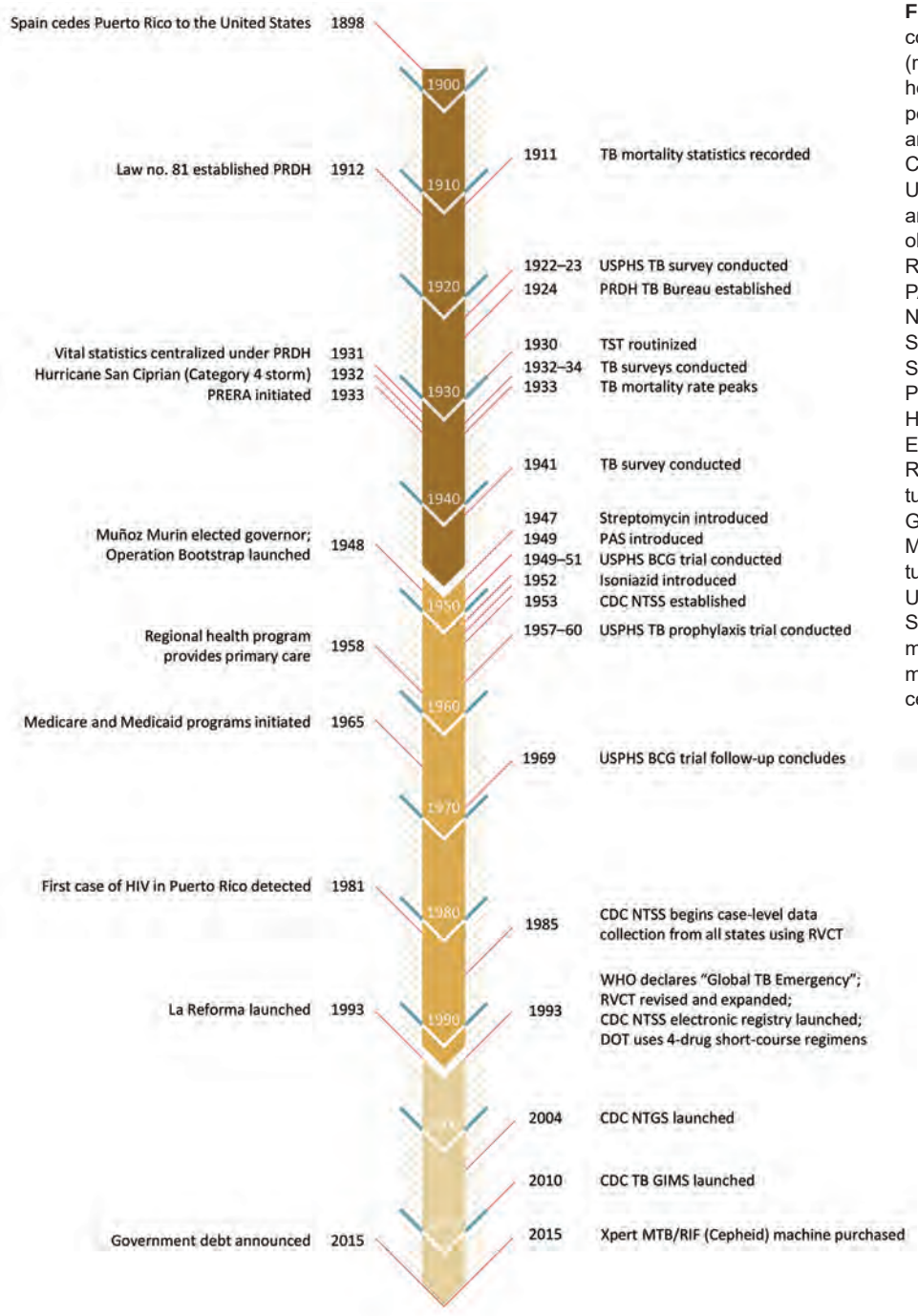

Figure 1. Timeline of key TB control events in Puerto Rico (right) and developments in healthcare and politics (left) over 3 periods: 1898–1946, 1947–1992, and 1993–2015. BCG, bacillus Calmette-Guérin vaccine; CDC, US Centers for Disease Control and Prevention; DOT, directly observed therapy; La Reforma, Reforma de Salud de Puerto Rico; PAS, para-aminosalicylic acid; NTGS, National TB Genotyping Service; NTSS, National TB Surveillance System; PRDH, Puerto Rico Department of Health; PRERA, Puerto Rico Emergency Relief Administration; RVCT, report of verified case of tuberculosis; TB, tuberculosis; TB GIMS, TB Genotyping Information Management System; TST, tuberculin skin testing; USPHS, United States Public Health Service; Xpert MTB/RIF machine, multidrug/rifampin resistance machine (Cepheid, http://www.cepheid.com).

and limited bacteriologic examinations. Several institutions were involved in conducting the surveys, including PRDH, the School of Tropical Medicine of Puerto Rico, Columbia University (New York, NY, USA), and the Rockefeller Foundation and its International Health Board (New York).

The resulting picture of TB on the island was that it primarily affected those 20–40 years of age, nonwhite more than white persons (*16*), urban more than rural areas (*7*), and women more than men (*7*). The most salient underlying factor was poverty, as evidenced by overcrowding, poor living conditions, and malnutrition (*6*,*7*). These findings, combined with the high death rate, led the Puerto Rico Commissioner of Health (Eduardo Garrido Morales) to prioritize TB. Drawing on grants from the Puerto Rico Emergency Relief Administration (established in 1933), government-run TB dispensaries were established and well-ventilated subsidized housing for the poor was

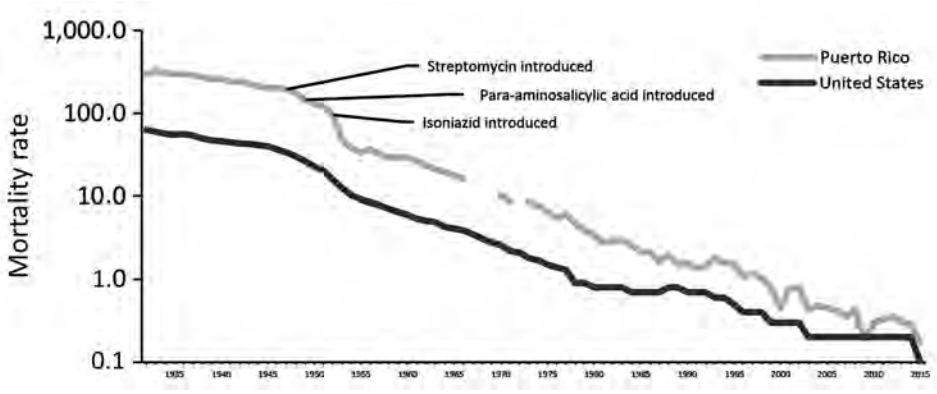

Figure 2. Mortality rate (no. deaths/100,000 population, on logarithmic scale) for reported TB cases in Puerto Rico and the United States, 1932–2015. Mortality data for Puerto Rico were not recorded for 1967–1969 and 1972.

constructed (*8*,*14*). By 1935, of 20 planned TB dispensaries, 4 were operational and outfitted to conduct fluoroscopy, chest radiography, and pneumothorax procedures (treatment by surgically collapsing infected lobes) for patients with active TB; 2 traveling clinics provided services to rural areas (*17*). Culture testing was reserved for special cases (*8*). Patients could be isolated in the TB dispensary for 15 days if they were suspected of having "open," or active, TB (*8*).

Control and Surveillance after the Introduction of TB Chemotherapy (1947–1992)

In 1947, the US Congress allowed Puerto Rico to elect a governor, and the next year, Luis Muñoz Marín was elected. In that same year, to accelerate industrialization and development on the island, the US federal government launched Operation Bootstrap (Operación Manos a la Obra). This operation included projects to improve health; throughout the 1950s, government-subsidized primary healthcare was scaled up and reached islandwide coverage in 1958. In 1965, Medicare and Medicaid programs were initiated on the island.

In 1953, the CDC National Tuberculosis Surveillance System was established. TB remained a major public health challenge in Puerto Rico, accounting for 128 deaths/100,000 population in 1950, a TB mortality rate 5.7 times more than that on the US mainland (Figure 2). TB control, as reflected by the accelerated decline in annual TB mortality rates, was improved by availability of free pharmaceuticals on the island, including streptomycin (introduced in 1947), para-aminosalicylic acid (1949), and isoniazid (1952) (*18*,*19*) (Figure 2). For example, the reported mortality rate fell from 128.4 deaths/100,000 population in 1950 to 10.4 deaths/100,000 population in 1970 (Figure 2). Epidemiologic data revealed that TB continued to affect predominantly those 20–40 years of age but that men were affected more than women. Along with the expanded use of chest radiography for case finding (*18*), TST was used for screening and was required before one could obtain a worker's health certificate. During 1949–1950, PRDH took 234,487 chest radiographs, by which 1,346 (0.6%) cases of active TB were identified. Sputum examination by acid-fast bacilli or culture remained limited or of poor quality (*18*).

Ambulatory treatment was provided, and medications were disbursed in 3-month courses; total treatment duration was ≥2 years (*18*). Subsequent short-course regimens using rifampin were introduced in the 1970s. In-patient care was also managed at an 800-bed TB hospital, which opened in Río Piedras in 1952, and at the US Veterans Administration Hospital in San Juan. In 1958, PRDH TB centers had registered 22,000 patients with active and latent cases; 7,800 were receiving treatment (*18*). An estimated additional 2,600 patients were managed by public and private TB hospitals.

Puerto Rico was the site of 2 major USPHS TB investigations. First, during September 1949–May 1951, the USPHS collaborated with PRDH and the Puerto Rico Department of Education to conduct a controlled trial of bacillus Calmette-Guérin vaccine (*20*–*22*). During this trial, 191,827 children (1–18 years of age) in rural and urban areas were screened for TB; 50,634 (26%) of them received the vaccine, and they were compared against 27,238 (14%) controls (*23*–*25*). Follow-up continued through June 30, 1969. Although the vaccine conferred some benefit to younger persons, it had no longstanding protective effect. Second, during 1957–1960, the USPHS conducted a controlled trial of 1-year treatment with isoniazid prophylaxis (5mg/kg/d), which included 25,000 known TB patient contacts in the United States, Mexico, and Puerto Rico, 12,000 of whom were in Puerto Rico. Contacts were randomized to receive treatment drug or placebo; among those receiving isoniazid, incidence of active TB disease was 60% lower over the course of the trial (*26*,*27*). The Puerto Rico principal study investigators (José Sifontes and Carlos Vicéns) highlighted the value of prophylactic treatment: "A large reservoir of tuberculosis cases for the next generation is already seeded in Puerto

Rico…. The infant with a positive tuberculin in 1962 may be the grandfather who will develop cavitary disease and infect his grandchildren in the year of 2012" (*26*). In tandem, the 2 USPHS TB investigations addressed the international debate about effective TB prevention strategies (bacillus Calmette-Guérin vaccination vs. isoniazid treatment of latent TB), and findings supported isoniazid treatment as being more effective (*25*,*28*).

During the late 1970s and early 1980s, TB funding was reduced nationally, and several PRDH TB clinics were closed (*29*). Studies found incomplete TB reporting (*30*), and decreased support for TB surveillance and control resulted in underestimation of the true population burden of TB (*29*,*31*).

Expanded TB Control and Surveillance (1993–2015)

In 1993, health system reforms were initiated to reduce bureaucracy and increase efficiency. Reforma de Salud de Puerto Rico, locally referred to as La Reforma, was introduced as a government-run healthcare program managed through private insurance companies. Under La Reforma, TB patients could access TB diagnosis and treatment through private healthcare providers, while PRDH continues to provide care at TB clinics (*32*) and retains responsibility for laboratory services, outbreak investigations, determining when cases have had sufficient treatment to be closed, and quarantine.

Since the 1980s, TB care on the island, as elsewhere, has been complicated by 2 factors. First, the emergence of HIV resulted in TB co-infections (*33*). In Puerto Rico, compromised immunity caused by HIV infection was first recognized in 1981, and in 2015, Puerto Rico ranked among the top 10 US jurisdictions in terms of rates of HIV infection diagnoses (*34*). During 1993–2015, a total of 838 (38%) cases of HIV infection were identified among 2,232 TB patients with reported HIV testing results; an additional 930 TB patients did not have reported HIV results. During this same period, of 189,222 TB patients in the United States with reported HIV testing results, 33,227 (18%) were HIV positive.

Second, the proliferation of drug-resistant forms of TB, including multidrug-resistant (MDR) TB (defined as resistance to both isoniazid and rifampin), required new diagnostics to conduct drug-susceptibility testing (DST) and guide appropriate treatment algorithms. In the 1990s, DST was rarely conducted (*32*), although CDC recommended and financially supported PRDH to send isolates for DST to the CDC mycobacteriology laboratory in Atlanta, such as happened during an investigation of nosocomial transmission in an HIV care unit (*35*). More recently, since 2015, detection of TB drug resistance was facilitated by the purchase of an Xpert MTB/RIF machine (Cepheid, http://www.cepheid.com), housed within the PRDH TB laboratory. During 1993–2015, a total of 44 cases of MDR TB were detected, of which 24 (55%) were reported during 1993–1997.

In the early 1990s, the identification of HIV/TB co-infections and drug-resistant TB, along with an overall increase in TB patients in United States, reinvigorated national control and surveillance efforts. In 1993, the National Tuberculosis Surveillance System, which began collecting case-level data from all states in 1985 by using the Report of Verified Case of Tuberculosis (https://www.cdc.gov/tb/programs/rvct/default.htm), expanded to include HIV status, initial drug regimen, DST results, use of directly observed therapy, and completion of therapy, with reporting facilitated by an electronic registry. Furthermore, CDC has steadily introduced molecular surveillance methods (*36*–*39*). In Puerto Rico, TB genotyping has assisted epidemiologic investigations, especially within healthcare facilities (*35*,*40*–*42*). For example, during April 1993–April 1995, genotyping isolates from 113 culture-positive TB patients identified 8 clusters and helped demonstrate transmission within an AIDS care facility (*41*). In 2004, CDC established the National Tuberculosis Genotyping Service to provide genotyping services through molecular evaluation of culture-positive isolates (*43*). In 2010, CDC launched the TB Genotyping Information Management System to manage and analyze molecular epidemiologic data, including rapid detection of genotype clusters appearing across state and territorial boundaries (*44*). Starting in 2018, in the United States and its territories, whole-genome sequencing has been performed on all new isolates of *Mycobacterium tuberculosis* referred for genotyping. Use of whole-genome sequencing will further assist the PRDH TB Control Program with cluster identification and investigation and with surveillance of molecular determinants associated with drug resistance.

Current Epidemiology and Challenges

In 2015, PRDH reported 52 cases of TB, substantially fewer than the 274 cases reported in 1994. During 1993–2015, incidence of reported cases decreased from 7.1 to 1.5 cases/100,000 population; since the late 1980s, this rate has been lower than that of the United States (Figure 3). Of 266 TB cases reported during 2011–2015 in Puerto Rico, 188 (71%) were in male patients and 44 (17%) were in foreign-born patients; 225 (85%) had positive culture results, 14 (5%) met the clinical definition, and 26 (10%) were diagnosed by providers (https://www.cdc.gov/tb/programs/rvct/default.htm) (Table 2). Compared with the 1,272 case-patients reported during 1993–1997, case-patients reported during 2011–2015 were older ($p<0.001$), and a larger proportion received directly observed therapy only (134/266 [50%] during 2011–2015 vs. 252/1,272 [20%]

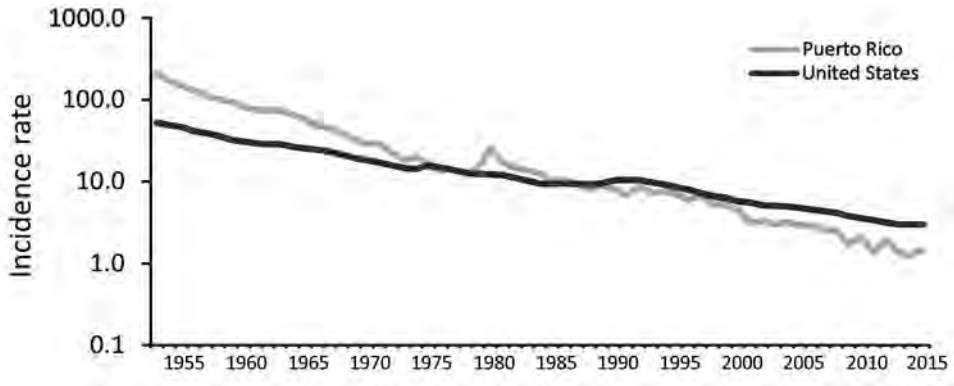

Figure 3. Incidence (no. cases/100,000 population, on logarithmic scale) of reported TB cases in Puerto Rico and the United States, 1953–2015. Puerto Rico data for 1953–1992 sourced from US Centers for Disease Control and Prevention annual TB reports; data not available for 1977; data for 1993–2015 sourced from the Centers for Disease Control and Prevention Online Tuberculosis Information System (https://wonder.cdc.gov/tb.html).

during 1993–1997) and completed therapy within 1 year (140/266 [53%] during 2011–2015 vs. 604/1,272 [47%] during 1993–1997) (Table 2). Of the 266 TB case-patients reported during 2011–2015, culture results were positive for 225. Of those, 209 (93%) isolates underwent genotyping; half (105/209 [50%]) had unique genotypes. Of patients with positive cultures, 217 (96%) isolates underwent initial DST, and 6 (3%) cases of MDR TB were identified. Of the 245 TB patients with known HIV status, 46 (19%) were HIV positive. As of June 2018, of the 266 patients, 187 (70%) had completed treatment, 6 (2%) refused treatment, 48 (18%) died during treatment, 6 (2%) moved out of the country, 5 (2%) did not complete follow-up monitoring, and 14 (5%) had unknown outcomes.

Compared with other US jurisdictions, the low incidence of reported cases in Puerto Rico may result in part from delayed diagnosis and underreporting, as suggested by comparing cases reported during 2011–2015 in Puerto Rico and the United States. The lower proportion of TB cases meeting the clinical case definition in Puerto Rico (5%, 14/266) than in the United States (16%, 7,786/48,955) may denote insufficient clinical awareness of TB symptoms; the higher proportion of culture-positive cases (Puerto Rico 85%, 225/266, vs. United States 77%, 37,723/48,955) may indicate delayed diagnosis because the likelihood of detecting TB through culture increases as disease advances over time. The proportion of TB cases identified after death was also higher in Puerto Rico (5%, 14/266) than in the United States (2%, 1,065/48,955). Puerto Rico has a smaller proportion of foreign-born TB patients (17%, 44/266) than does the United States (65%, 31,764/48,955). Foreign-born patients also differed by country of origin: during 2011–2015, a total of 75% (33/44) of foreign-born Puerto Rico patients reported were from the Dominican Republic; in the United States in 2015, the top 5 countries of origin were Mexico, the Philippines, India, Vietnam, and China (*45*). Given the estimated TB incidence of 45 cases/100,000 population in the Dominican Republic in 2017 (*1*) and the high rates of documented and undocumented immigration to Puerto Rico, the extent of TB among residents originally from the Dominican Republic is poorly understood.

Socioeconomic factors and determinants of TB may also affect progress toward TB elimination in Puerto Rico (*46*). TB surveillance and control may be further complicated by the effect of the government economic debt, which was estimated at $72 billion in June 2015, and of Hurricane Maria, which made landfall on September 20, 2017, as a Category 4 hurricane. Many health professionals have left in the wake of these crises (*47*). More broadly, Puerto Rico has high poverty rates: in 2017, as much as 43.5% of Puerto Rico's population was living under the poverty line, compared with 12.7% in the United States (*48*). Although TB is a disease associated with poverty (*49*), how socioeconomic factors affect TB epidemiology in Puerto Rico is uncertain, including transmission patterns, access to services, and treatment outcomes.

Discussion

Sustained surveillance and control efforts have documented decreased rates of mortality and incidence of reported TB in Puerto Rico. In 2015, incidence of reported TB cases was 1.5 cases/100,000 population, compared with the US incidence of 3.0/100,000 population. A significant increase in patient age distribution, probably resulting from a larger proportion of activated latent cases, further indicates progress. WHO has recognized Puerto Rico as 1 of 33 low-incidence jurisdictions for which TB elimination is possible by 2035 (*3*).

Three major lessons emerge from the experience of TB surveillance and control on the island. First, data collected from sustained TB surveillance have directed control efforts. Second, use of newly developed strategies and techniques has produced evidence-based practices to improve surveillance, control, and patient care. These practices include ambulatory care, use of chemoprophylaxis for latent TB, and molecular surveillance. Third, public health commitment has been crucial to

Table 2. Patient demographics, verification criteria, multidrug-resistant tuberculosis results, HIV status, receipt of directly observed therapy, and therapy completion in ≤1 y for reported tuberculosis case cohorts, 1993–1997 and 2011–2015

Variable	1,272 Reported cases, 1993–1997 no. (%) patients	266 Reported cases, 2011–2015 no. (%) patients
Age, y		
0–4	39 (3)	0
5–14	10 (1)	0
15–24	60 (5)	13 (5)
25–44	449 (35)	66 (25)
45–64	347 (27)	112 (42)
≥65	278 (22)	73 (27)
Not available	89 (7)	2 (1)
Sex		
M	869 (68)	188 (71)
F	403 (32)	78 (29)
Origin of birth		
United States	1,210 (95)	222 (83)
Other than United States	58 (5)	44 (17)
Not available	4 (<1)	0 (0)
Verification criteria		
Positive culture	1,090 (86)	225 (85)
Clinical case definition	82 (6)	14 (5)
Provider diagnosis	70 (6)	26 (10)
Positive smear/tissue	30 (2)	0 ()
Not available	0 ()	1 (<1)
Multidrug-resistant tuberculosis		
Yes	24 (2)	6 (2)
No	753 (59)	210 (79)
Not applicable or available	495 (39)	50 (19)
HIV status		
Positive	386 (30)	46 (17)
Negative	292 (23)	199 (75)
Not reported	594 (47)	21 (8)
Directly observed therapy		
Direct only	253 (20)	134 (50)
Self only	807 (63)	63 (24)
Both	25 (2)	5 (2)
Not applicable or available	187 (15)	64 (24)
Therapy completed in ≤1 y		
Yes	604 (47)	140 (53)
No	113 (9)	6 (2)
Not applicable or not available	555 (44)	120 (45)

TB surveillance and control efforts, as seen by dramatic declines in reported cases and mortality rates since the 1940s and 1950s.

Several areas may affect progress toward TB elimination yet remain poorly understood. Delayed diagnosis and underreporting may contribute to the current relatively low incidence of reported cases; these considerations have been historic problems for TB surveillance and control on the island (*29*–*32*). Despite improvements in care and treatment, only 50% of patients received directly observed therapy and 53% completed treatment within 1 year, indicating areas for further improvement. It remains unclear how Hurricane Maria and socioeconomic changes after the economic downturn will affect TB epidemiology, public health services, provision of care, and infrastructure.

As demonstrated by histories of TB worldwide and in Puerto Rico (*49*), TB thrives in contexts marked by poverty. Addressing socioeconomic factors is part of the WHO framework for eliminating TB in low-incidence contexts (*3*). Although the proportion of Puerto Rico's population living under the poverty line is 3.4 times greater than that of the rest of the United States, the effect of socioeconomic factors on TB epidemiology on the island is unclear.

Although the numbers of reported cases are becoming fewer, the ultimate goal is TB elimination in Puerto Rico through sustained public health surveillance and control efforts, including detection, case management, and treatment success. The use of novel tools, such as molecular surveillance, electronically facilitated case consultation, and smartphone technology as an effective, low-cost measure to guide treatment adherence (*50*), has been shown to be effective for timely public health interventions and may help lead Puerto Rico to TB elimination.

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Whole-Genome Sequencing of Drug-Resistant *Mycobacterium tuberculosis* Strains, Tunisia, 2012–2016

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To investigate transmission of drug-resistant strains of *Mycobacterium tuberculosis* in Tunisia, we performed whole-genome sequencing on 46 multidrug-resistant strains isolated during 2012–2016. Core-genome multilocus sequence typing grouped 30 (65.2%) strains into 3 clusters, indicating extensive recent transmission and Haarlem clone predominance. Whole-genome sequencing might help public health services undertake appropriate control actions.

The emergence of drug-resistant strains of *Mycobacterium tuberculosis* is hampering the control of tuberculosis (TB) worldwide. In Tunisia in 2017, the estimated percentage of TB patients with multidrug-resistant (MDR)/rifampin-resistant TB was 1.1% among those with new infection and 13% among those with previously treated infection (*1*).

Effective and rapid tools are needed to characterize and track the transmission chains of MDR/rifampin-resistant TB. Whole-genome sequencing (WGS) has shown higher discriminatory power for epidemiologic investigations than have other conventional genotyping methods (e.g., spoligotyping, *IS*6110 restriction fragment length polymorphism, and mycobacterial interspersed repetitive unit–variable-number tandem repeat). Indeed, WGS has enabled investigators to rule out false transmission events (*2*–*7*). Furthermore, WGS enables simultaneous determination of polymorphisms and insertions/deletions linked to resistance to first-line and second-line drugs (*8*).

In this study, we used WGS to investigate transmission of MDR and extensively drug resistant (XDR) TB strains isolated in Tunisia over a 4-year period by applying the core-genome multilocus sequence typing (cgMLST) scheme and identifying the drug-resistance marker for first-line and second-line drug resistance. This study was approved by the ethics committee of A. Mami Pneumology Hospital, Ariana, Tunisia.

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The Study

We retrospectively studied 46 MDR *M. tuberculosis* isolates collected from 46 HIV-negative patients in Tunisia during June 2012–June 2016, which represented 57 (80.7%) cases of MDR TB. Of the 46 isolates, 6 represented all (100%) XDR TB cases recorded in the country during that period. We performed drug-susceptibility testing for resistance to first-line drugs (except pyrazinamide) by using the proportion method on Lowenstein-Jensen medium. For pyrazinamide and second-line drugs, we performed drug-susceptibility testing on a Bactec MGIT 960 system (Becton, Dickinson and Company, http://www.bd.com). WGS was performed on the MiniSeq platform (Illumina Inc., https://www.illumina.com) targeting a minimum average reads coverage of 50-fold. To analyze the mutations involved in drug resistance and related to lineage determination, we used PhyResSe and TGS TB (*9*,*10*). We performed the cgMLST scheme version 2.1, considering 2,891 core genes, by using Ridom SeqSphere+ version 5.0.0 software (Ridom GmbH, https://www.cgmlst.org) (*7*). To define a strain as a part of a recent transmission chain, we fixed a threshold of <6 allele variants. For statistical analyses, we calculated p values by using OpenEpi version 3 (https://www.openepi.com) and considered $p<0.05$ to be significant.

Patient origins are reported in the Appendix (https://wwwnc.cdc.gov/EID/article/25/3/18-1370-App1.xlsx). Most MDR TB cases (19; 41.3%) were recorded in the Bizerte region in northern Tunisia. WGS revealed that all MDR/XDR strains belonged to the European-American lineage (lineage 4); the Haarlem family was the most frequent (71.8%) (Figure 1). (WGS files from this study have been submitted to the European Nucleotide Archive as fastq files under study accession no. PRJEB30463, https://www.ebi.ac.uk/ena/data/search?query=PRJEB30463).

The cluster analysis (Figure 2) showed that 30 (65.2%) of 46 isolates were grouped within 3 clusters, and most (90.0%) clustered MDR TB cases belonged to the Haarlem family (Figure 1). Of note, a big cluster of 24 MDR strains linked to Haarlem (cluster 1) was detected over

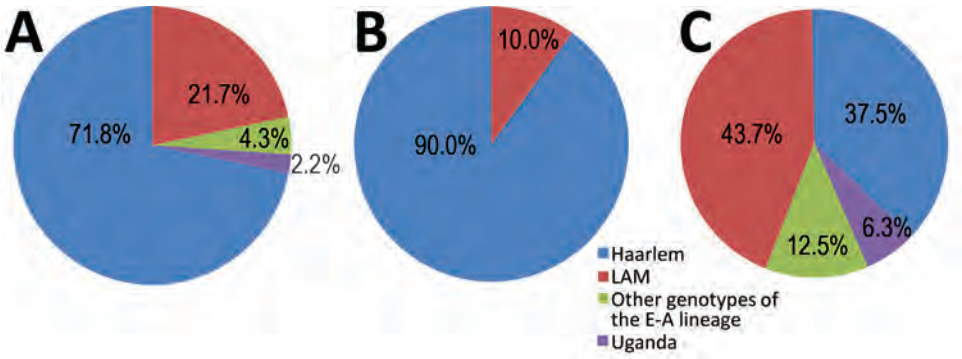

Figure 1. Lineage and family distribution of drug-resistant strains identified during study of drug-resistant *Mycobacterium tuberculosis*, Tunisia, 2012–2016. A) MDR strains. Haarlem was the most frequently represented family (71.8%) among MDR strains. Among other samples, 21.7% belonged to the LAM family, 4.3% presented other genotypes of the EA lineage, and 2.2% the Uganda genotype. B) Clustered MDR strains. Haarlem was also the most frequent family (90.0%); the rest belonged to the LAM family. C) Nonclustered MDR strains. Among nonclustered isolates, LAM was the most frequently represented family (43.7%), followed by Haarlem, which was detected in 37.5% of the strains. EA, European-American lineage; LAM, Latin-American-Mediterranean lineage; MDR, multidrug-resistant.

the entire study period. We found no significant associations between this cluster and patient sex, age (≤35 years, >35 years), and resistance to second-line drugs (XDR TB) (p>0.05); however, we found a significant association with this cluster and pyrazinamide resistance (p = 0.001) and with Bizerte (p = 0.014). Despite the association with the Bizerte region, patients from various regions were part of this cluster (Figure 2). We also found a significant association between cluster 2 (Haarlem) and Beja (p<0.001) and between cluster 3 (Latin-American-Mediterranean) and Ben Arous (p<0.001). Epidemiologic links were confirmed for patients in these 2 transmission chains (Table 1).

All cluster 1 strains shared mutations in drug-resistance genes (e.g., *rpoB*, Ser450Leu, the compensatory

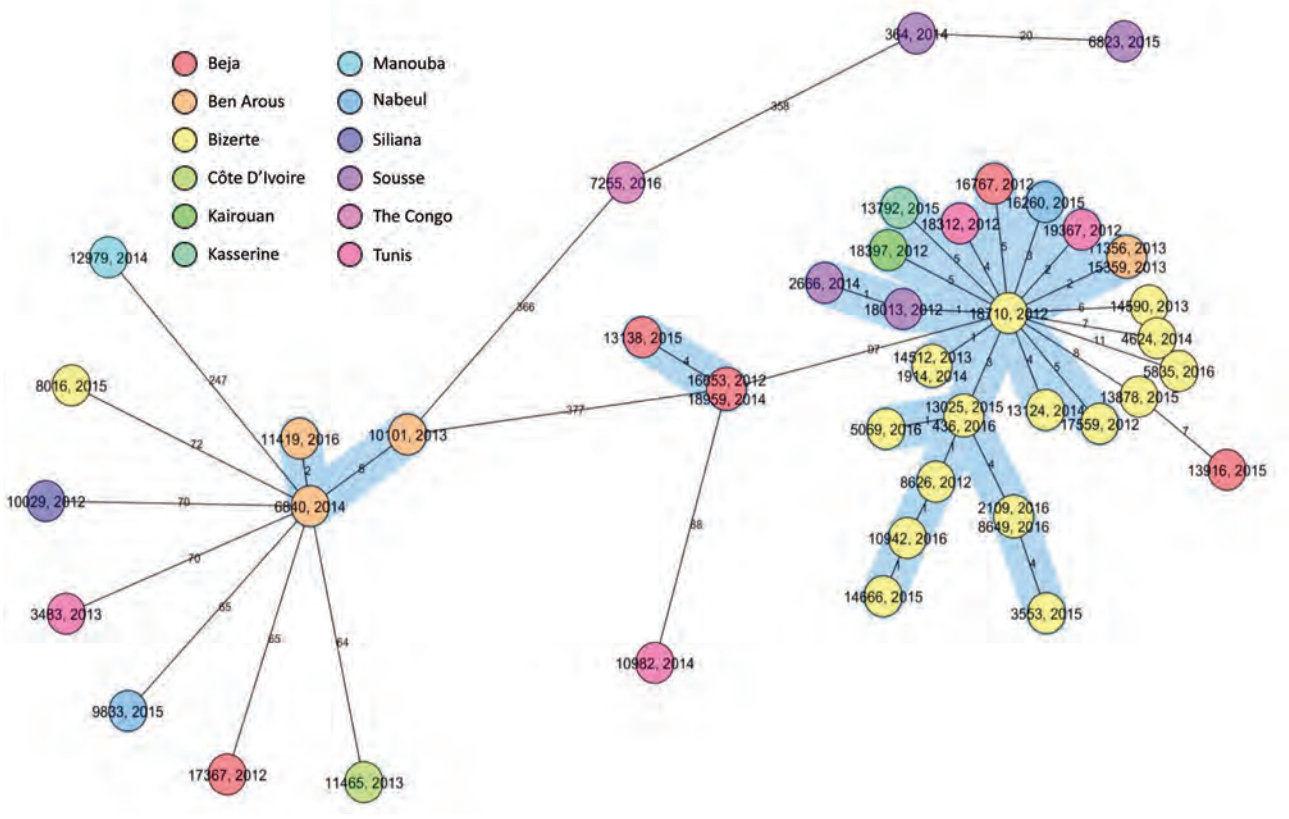

Figure 2. Core-genome multilocus sequence typing–based minimum spanning tree for strains identified during study of drug-resistant *Mycobacterium tuberculosis*, Tunisia, 2012–2016. Ridom SeqSphere+ minimum spanning tree for 46 samples based on 2,891 columns, pairwise ignoring missing values, logarithmic scale. Cluster distance threshold = 5 alleles. Colors indicate regions and countries of patient origin.

Table 1. Epidemiologic information about clustered cases identified during study of drug-resistant *Mycobacterium tuberculosis* strains, Tunisia, 2012–2016*

Cluster, group	*pncA* mutation	No. patients	Epidemiologic links
Cluster 1, Haarlem†			
Group 1	Gln10His	9	Confirmed for patients 10942, 14666 (2 brothers with XDR TB), 8626 (their neighbor with XDR TB) in Bizerte, and patients 13025 and 2109 (2 friends from Bizerte); probable for patients 3553, 436, 5069, 8649 (from Bizerte)
Group 2	Leu4Trp	5	Probable for patients 18013 and 2666 (from Sousse, the central part of Tunisia)
Group 3	A-11C	4	Not available
Group 4	CA deletion (+339, +340)	2	Probable for patients 11356 and 15359 (from Ben Arous, 10 km from the capital Tunis)
Group 5	Cys14Stop	2	Confirmed for patients 1914 and 14512 (brother and sister from Bizerte)
Group 6	Wild type	2	Not available
Cluster 2, Haarlem	Wild type	3	Confirmed for patients 13138, 18959, 16653 (same family: father, son, and uncle; Beja; Northwest of Tunisia)
Cluster 3, LAM	Wild type	3	Confirmed for patients 10101, 11419, 6840 (neighbors from Ben Arous)

*LAM, Latin-American-Mediterranean.
†For the remaining patients of cluster 1, no information was available.

mutation *rpoB*Val534Met (*11*), *katG* Ser315Thr, *embB* Met306Ile, and *gidB*Arg47Trp). However, diversity was noticed in *pncA* mutations conferring resistance to pyrazinamide. On the basis of *pncA* variation, this cluster was split into 6 groups (Table 1).

Four XDR isolates belonged to cluster 1. Three XDR isolates from 2 brothers (patients 10942 and 14666) and their neighbor (patient 8626) shared mutations conferring resistance to second-line drugs and to pyrazinamide (Tables 1, 2), supporting evidence of direct transmission of this XDR strain. The fourth XDR TB case (patient/strain 13792) differed in mutations conferring resistance to pyrazinamide and fluoroquinolones. This case probably shared an ancestral MDR strain with cluster 1, which had evolved differently because of poor adherence of the patient to drug therapy (Table 2; Appendix). Five nonclustered MDR isolates linked to Haarlem (4 patients from Bizerte) were distant by 6–11 alleles from cluster 1 showing the same polymorphisms in *rpoB, katG, embB,* and *gidB* (Figure 2; Appendix). Of note, cluster 2 presented the compensatory mutation *rpoB* Val534Met, but no variation in *embB*, *gidB*, and *pncA* was detected (Appendix). This Haarlem cluster showed limited transmission compared with cluster 1, which was distant by 97 alleles (Figure 2).

In our study, mutation *rpoB*Ser450Leu was mostly associated with rifampin resistance (n = 42, 91.3%), whereas codon 315 of *katG* was most involved with isoniazid resistance (n = 43, 93.4%). Three nonclustered MDR isolates presented a genomic deletion of ≈10.4 kb, which included the entire *katG* gene (strain 3483) and 2 uncommon mutations in *katG*: Gly269Asp and Gly279Asp (patients/strains 9833, 10982) (Appendix). All pyrazinamide-resistant strains detected with the MGIT 960 system (n = 33, 71.7%) had a mutation in the *pncA* gene or its promoter.

Regarding second-line drugs, all 13 fluoroquinolone-resistant strains had a mutation in the quinolone resistance–determining region of *gyrA*, *gyrB*, or both. For second-line injectable drugs, 4 XDR strains had the mutation *rrs* A1401G and 2 had the mutation *eis* C-14T and a frame shift in *tlyA* (Table 2).

Table 2. Mutations detected in genes conferring resistance to fluoroquinolones, aminoglycosides, and capreomycin in study of drug-resistant *Mycobacterium tuberculosis* strains, Tunisia, 2012–2016*

No. strains	Cluster	Resistance to FQ, AG, or CAP on MGIT 960	*gyrA*	*gyrB*	*rrs*	*tlyA*	*eis*–p
3	1	OFX, LVX, KAN, AMK, CAP	Asp94Gly†		A1401G†		
1	1	OFX, LVX, KAN, AMK, CAP		Asp461His/Gly470Cys	A1401G†		
1	1	OFX, LVX	Ala90Val†				
1	1	OFX, LVX	Asp94Gly†				
1	3	OFX, LVX	Ser91Pro†				
1	Not clustered	OFX, LVX, KAN	Asp94Ala†	Asp461Asn			C-14T†
1	Not clustered	OFX, LVX, CAP	Ser91Pro†			INS of 2G†	
1	Not clustered	OFX, LVX	Asp94Ser				
1	Not clustered	OFX, LVX	Ala90Val†				
1	Not clustered	OFX, LVX		Asp461Asn			
1	Not clustered	OFX, LVX	Asp94Tyr†				

*Among second-line drugs, 7 strains were pre-XDR (FQ resistant) and 6 were XDR TB strains on MGIT 960. AG, aminoglycosides; AMK, amikacin; CAP, capreomycin; *eis*-p, *eis* promoter; FQ, fluoroquinolone; INS, insertion; KAN, kanamycin; LVX, levofloxacin ; MGIT 960, Bactec MGIT 960 system (Becton, Dickinson and Company, http://www.bd.com); OFX, ofloxacin; TB, tuberculosis; XDR, extensively drug resistant.
†High-confidence mutations associated with FQ, AG, or CAP resistance according to (*12*).

Conclusions

cgMLST analysis showed that 65.2% of MDR/XDR strains of *M. tuberculosis* were clustered, reflecting extensive transmission in Tunisia, particularly of a Haarlem clone. This Haarlem clone showed polymorphisms *rpoB* Ser-450Leu, Val534Met, *katG* Ser315Thr, *embB* Met306Ile, and *gidB*Arg47Trp, and in *pncA* genes previously identified in a Haarlem MDR TB outbreak in the Bizerte region during 2001–2011 (*13*,*14*). As indicated by statistical association, we conclude that this cluster is still spreading in the Bizerte area. However, diffusion in different regions of the country is alarming and requires intensified efforts to control and diagnose drug-resistant TB.

Only 2 strains, belonging to cluster 1, did not have any mutations in *pncA*. The wild-type *pncA* isolates might represent the genotype of the first strains that emerged in Bizerte and evolved since 2001 by acquiring single-nucleotide polymorphisms in *pncA* and the other genes, including genes involved in resistance to second-line drugs. It has been reported that the mutation rate during a transmission chain or TB latency is not completely stable and is estimated at 0.3–0.5 single-nucleotide polymorphisms/genome/year (*4*,*5*,*15*), which leads to increased numbers of allele variants for some isolates and might explain the results found for 5 MDR strains distant from cluster 1 by 6–11 alleles.

The main limitation of this study is the incomplete number of MDR TB cases (≈19% missing). Epidemiologic information to confirm all clustered cases is lacking.

In summary, cgMLST-based WGS showed extensive transmission of MDR/XDR TB in Tunisia over 4 recent years, thereby indicating that MDR TB is not fully controlled. Use of this molecular approach for surveillance purposes might enable the public health service to undertake appropriate control actions, particularly in specific settings of this country.

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About the Author

Ms. Bouzouita is a PhD student at the National Reference Laboratory for Mycobacteria, Ariana, Tunisia. Her research focuses on TB diagnosis, TB drug resistance, and molecular epidemiology of TB.

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Role of Backyard Flocks in Transmission Dynamics of Highly Pathogenic Avian Influenza A(H5N8) Clade 2.3.4.4, France, 2016–2017

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Highly pathogenic avian influenza A(H5N8) clade 2.3.4.4 spread in France during 2016–2017. We assessed the biosecurity and avian influenza virus infection status of 70 backyard flocks near H5N8-infected commercial farms. One flock was seropositive for clade 2.3.4.4. Backyard flocks linked to commercial farms had elevated risk for H5 infection.

In the past 2 years, major outbreaks of highly pathogenic avian influenza (HPAI) occurred in Europe, resulting in severe socioeconomic effects on the poultry industry (*1*,*2*). During November 28, 2016–March 23, 2017, a total 484 HPAI poultry outbreaks associated with influenza A(H5N8) clade 2.3.4.4 viruses of Eurasia A/goose/Guangdong/1/1996 lineage were reported in France (*2*). Virus introduction into the index farm probably was associated with wild birds; however, other transmission pathways for virus spread between farms have been considered, including trade-related movements and spatial proximity (*2*). Although most outbreaks occurred in commercial flocks (n = 464), outbreaks in ≈20 backyard flocks also were reported (*2*). Backyard flocks are generally assumed to be at risk for avian influenza virus (AIV) introduction from wildlife and from nearby commercial poultry flocks during influenza outbreaks (*3*,*4*). Because little is known about the prevalence of AIV in backyard flocks contiguous to commercial farms, we aimed to quantify the seroprevalence of AIV and H5 subtype and to identify risk factors for infection in backyard flocks near commercial farms affected by HPAI H5N8 during the 2016–2017 epidemic.

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The Study

We conducted our study in Gers Department (1 of the 101 administrative units in France). Gers accounted for 19.8% (96/484) of the HPAI H5N8 outbreaks reported during the epidemic; 55.2% (53/96) of the Gers outbreaks were spatiotemporally clustered during December 11, 2016–January 4, 2017 (*2*). Our study targeted backyard flocks that were located within a 1-km radius from HPAI H5N8 outbreaks reported on commercial farms in Gers (n = 169) (Figure). At the time of our study, no backyard flock in Gers had been reported as HPAI infected.

Using a 28-question form, we conducted face-to-face interviews with each backyard flock owner during March 31–May 10, 2017. The 28 closed or semiclosed questions concerned the species of poultry, biosecurity practices, contacts with other flocks, and health status of the birds. We explained the purpose and methods of the study to all participants, who gave their consent to participate.

We sampled all backyard flocks up to a limit of 10 birds >6 months of age, which ensured that all sampled birds had been exposed to the HPAI outbreaks. Because flock size was as high as 60 birds (median 14 birds), detection thresholds ranged from 20% to 30% with a 95% CI. Not all flock owners consented to or were available for the study; in all, we were able to include 70 of the 169 backyard holdings.

We collected blood samples, tracheal swabs, and cloacal swabs. Blood was stored at 4°C after shipment, then serum was extracted and stored at –20°C. Tracheal and cloacal swabs were stored at –80°C until analysis. We performed serologic testing for AIV by using ELISA (IDVet ID Screen Influenza A Antibody Competition Multi-Species kit, http://www.id-vet.com). We considered a backyard flock as seropositive if ≥1 bird was found to be positive. We then tested AIV-seropositive backyard flocks for H5 antibodies by using the same IDVet ELISA kit, and we used hemagglutination inhibition tests to detect clade 2.3.4.4 H5 or other H5 Eurasian viruses (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1040-App1.pdf). Finally, we individually tested all birds from seropositive backyard flocks for AIV gene M and subtype H5 by using reverse transcription PCR (*5*,*6*). We performed descriptive statistics to assess how seroprevalences

of AIV and H5 subtype were affected by flock owners' practices (Appendix).

Estimated overall flock-level seroprevalence was 25.7% (95% CI 16.9%–37.0%) for AIV and 11.4% (95% CI 5.9%–21.0%) for H5 (Table 1). Estimated overall bird-level seroprevalence was 5.9% (95% CI 4.3%–8.1%) for AIV and 3.3% (95% CI 2.1%–5.0%) for H5. All birds tested were PCR-negative for gene M and H5.

Among H5 ELISA-seropositive birds, only 3 belonging to the same flock showed positive hemagglutination inhibition titers against a clade 2.3.4.4 HPAI H5N8 antigen, and we could not confirm detection of clade 2.3.4.4–specific H5 antibodies with a second clade 2.3.4.4 H5N5 antigen in 1 of these birds. This backyard flock included chickens and ducks and was not adjacent to a commercial farm, and the owner reported working in a poultry meat processing plant.

Other H5 ELISA-positive birds were mainly seropositive for a couple of antigens from other H5 Eurasia lineages instead of clade 2.3.4.4 H5 HPAI virus. We could not distinguish between antibodies targeting low-pathogenicity or HPAI H5Nx viruses that spread in the region during 2015–2016 (*1*) (Appendix Table 1). This finding suggests that backyard flocks might have played a limited role in HPAI H5N8 transmission between farms during the 2016–2017 epidemic. Seroprevalence was higher in ducks than in chickens for AIV (13.1% [95% CI 8.2%–20.2%] vs. 4.1% [95% CI 2.7%–6.3%]) and H5 (9.0% [95% CI 5.1%–15.4%] vs. 1.9% [95% CI 1.0%–3.5%).

Backyard flocks that included ducks were more likely to be AIV-positive (odds ratio [OR] 2.3, 95% CI 1.1–5.1) and H5-positive (OR 5.7, 95% CI 1.6–30.6) than those having only chickens. These results are consistent with several studies emphasizing the role of ducks on AIV shedding and transmission (*1*). Specific attention was paid to flocks having ducks in the sampling design in the field because duck species could be considered as an additional risk factor (*1*). Thus, our study might overestimate the overall seroprevalence at the backyard flock and bird levels. Backyard flocks that had no fencing outdoors or had no covered food distribution area could be considered at higher risk for exposure to wild birds. However, these risk factors were not statistically associated with increased AIV or H5 seroprevalence (Appendix Table 2).

Backyard flocks located on or in close proximity to a commercial poultry farm were significantly more likely to be AIV-positive (OR 6.0, 95% CI 1.5–24.5) and H5-positive (OR 20.5, 95% CI 3.2–215.8). To date, proximity of commercial units to backyard flocks has not been considered as a risk factor, despite airborne transmission being suspected to spread disease (*7*,*8*). On the basis of the influenza A(H7N7) epidemic in the Netherlands, researchers constructed a model that assumed that infected backyard flocks were an example of spillover from commercial farms and that backyard flocks played no part in transmission (*9*). Our results highlight the importance of considering the impact of human activities in both the commercial and backyard flock settings. For commercial flocks, human activities have been described as a main source of secondary spread (*10*), with contacts through persons or shared equipment increasing the risk for AIV transmission (*11*). Consequently, a lack of biosecurity practices for backyard flocks belonging to commercial poultry farmers might have contributed to an increased risk for AIV infection of backyard poultry (Table 2).

Conclusions

We detected high flock- and bird-level seroprevalence of AIV in the backyard flocks we sampled after the 2016–2017 H5N8 epidemic in France. However, we observed very limited circulation of the H5N8 subtype, which indicates the minor role of backyard flocks in the transmission dynamics of H5N8. Backyard flocks belonging to commercial poultry farmers showed a significantly higher risk for infection with other H5 AIVs than backyard flocks having no links with commercial farms. These findings suggest that, from a risk-based perspective, surveillance of AIV circulation in backyard flocks should be focused on those flocks that have ducks and those connected to commercial poultry farms. On that basis, transmission of other more persistent pathogens of interest, such as mycoplasma or herpesviruses, should be further investigated at the backyard–commercial poultry interface (*12*).

Table 1. Results of serologic assays for 70 backyard flocks and 608 birds, by bird species comprising the flock, Gers Department, France, 2016–2017

	Avian influenza virus			Influenza A virus subtype H5		
Species comprising flock	Positive	Total	Seroprevalence, % (95% CI)	Positive	Total	Seroprevalence, % (95% CI)
All backyard holdings	18	70	25.7 (16.9–37.0)	8	70	11.4 (5.9–21.0)
Backyard holdings with only chickens	9	48	18.8 (10.2–31.9)	2	48	4.2 (1.2–14.0)
Backyard flocks with ducks	9	22	40.9 (23.3–61.3)	6	22	27.3 (13.2–48.2)
All birds	36	608	5.9 (4.3–8.1)	20	608	3.3 (2.1–5.0)
Chickens	20	486	4.1 (2.7–6.3)	9	486	1.9 (1.0–3.5)
Ducks	16	122	13.1 (8.2–20.2)	11	122	9.0 (5.1–15.4)

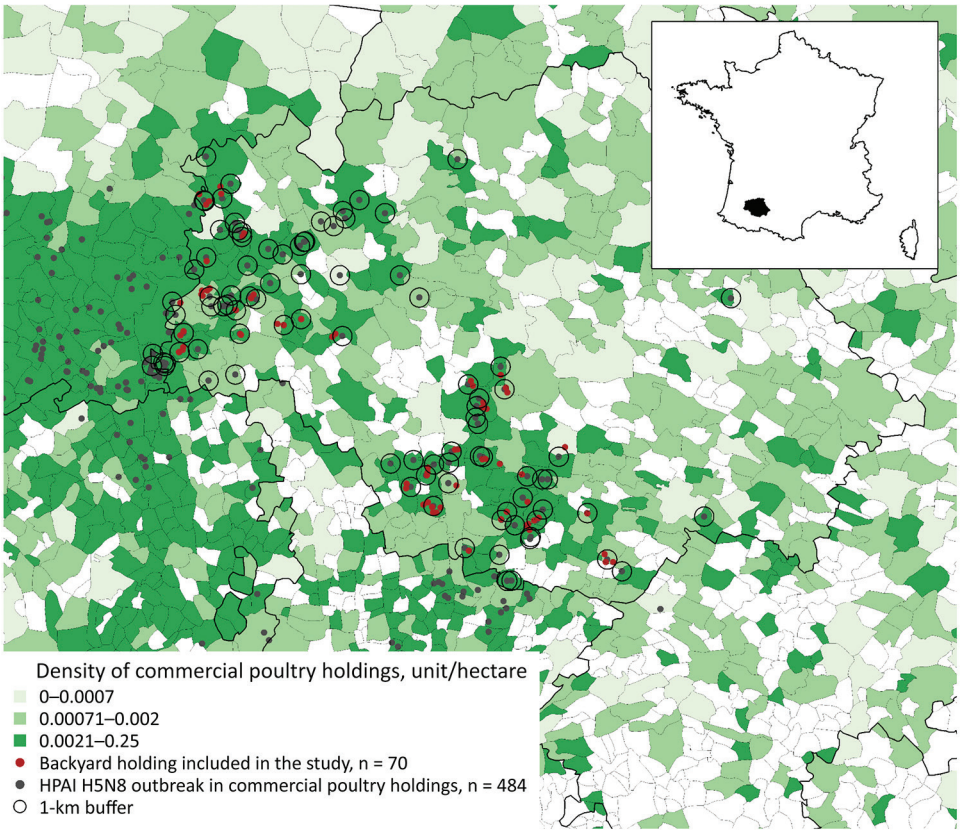

Figure. Locations of 484 commercial poultry holdings with reported outbreaks of HPAI H5N8 and the 70 backyard poultry holdings included in our study, Gers, Department, France, 2016–2017. HPAI H5N8, highly pathogenic avian influenza A virus subtype H5N8.

Table 2. Variables included in the final multivariable logistic regression with avian influenza virus and influenza A virus subtype H5 seroprevalences as outcome variables, Gers Department, France, 2016–2017

Outcome and variable	Odds ratio (95% CI)	p value
Avian influenza virus		
Species included*	2.3 (1.1–5.1)	0.036
Link with poultry industry†	5.8 (1.5–24.5)	0.011
Influenza A virus subtype H5		
Species included*	5.7 (1.6–30.6)	0.019
Link with poultry industry†	20.5 (3.2–215.8)	0.003

*Backyard flocks having ducks (yes vs. no).
†Professional activity of the backyard owner or member of the family home in connection with poultry industry (yes vs. no).

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Longitudinal Outbreak of Multidrug-Resistant Tuberculosis in a Hospital Setting, Serbia

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A retrospective population-based molecular epidemiologic study of multidrug-resistant *Mycobacterium tuberculosis* complex strains in Serbia (2008–2014) revealed an outbreak of TUR genotype strains in a psychiatric hospital starting around 1990. Drug unavailability, poor infection control, and schizophrenia likely fueled acquisition of additional resistance and bacterial fitness–related mutations over 2 decades.

The overall burden of tuberculosis (TB) in Serbia has been greatly reduced in recent years (*1*,*2*). However, a recent study revealed transmission of multidrug-resistant (MDR) *Mycobacterium tuberculosis* complex (MTBC) strains (i.e., MTBC strains resistant to at least rifampin and isoniazid) in Belgrade (*3*). In addition, data retrieved from the national database of MDR TB patients indicate a concentrated burden of MDR TB and extensively drug-resistant (XDR) TB, defined as additional resistance to 1 fluoroquinolone and 1 of the 3 injectable second-line drugs, among psychiatric inpatients in Serbia. To gain more insights into countrywide transmission routes, strain dynamics, and bacterial evolution over time, we retrospectively investigated all (n = 110) patients who received a diagnosis of MDR TB during January 1, 2008–May 31, 2014, in Serbia.

The Study

We subjected 1 MTBC isolate per patient to phenotypic drug susceptibility testing and whole-genome sequencing (WGS) (Appendix 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1220-App1.pdf). We retrieved patients' demographic, epidemiologic, and clinical data from the national database of MDR TB patients, as well as from their medical and laboratory records.

Most patients were male (87/110, 79.1%) and born in Serbia (107/110, 97.3%); mean age was 49.5 years (range 15–83). We observed concurrent conditions for 55 patients; schizophrenia was the most prevalent (26/55, 47.3%). Of the 110 patients, 61 (55.5%) had previously experienced TB. Susceptibility testing results showed that 19/110 (17.3%) MDR MTBC isolates were resistant to all first-line drugs, and 11/110 (10.0%) were classified as XDR (Appendix 1 Table 1). We successfully completed WGS for 103/110 isolates, representing 93.6% of all MDR TB cases recorded over the study period.

We considered 6,512 single-nucleotide polymorphisms (SNPs) differentiating all isolates to analyze their phylogenetic relationships. The MDR MTBC strain population comprised 37/103 (35.9%) isolates classified as lineage 4.2.2.1 (TUR genotype), 20/103 (19.4%) isolates of lineage 4.1.2 (Haarlem genotype), 17/103 (16.5%) isolates of lineage 2.2.1 (Beijing genotype), 15/103 (14.6%) isolates of lineage 4.8 (H37Rv-like strains), 8/103 (7.8%) isolates of lineage 4.4.1.1 (S-type), 2/103 (1.9%) isolates of lineage 4.2.1 (URAL genotype), 1 isolate of lineage 4.1 (Ghana), and 1 nonclassified lineage 4 isolate. Among lineage 2.2.1 Beijing isolates, the previously described Europe/Russia W148 MDR outbreak isolates (*4*) were most prevalent, present in 14/17 (82.4%) of the cases (Figure 1; Appendix 2, https://wwwnc.cdc.gov/EID/article/25/3/18-1220-App2.xls).

Seeking to identify recent chains of transmission, we defined molecular clusters as surrogate markers for epidemiologically linked cases (Appendix 1). Overall, 63/103 (61.2%) isolates could be assigned to 12 different clusters, each including 2–17 patients. The 2 largest clusters, 1 containing 14 and 1 containing 17 cases, comprised isolates of TUR genotype; the next-largest cluster was of 7 Beijing Europe/Russia W148 isolates. For all 63 suggested epidemiologic links, we were able to retrospectively identify 40 (63.5%) epidemiologic links (e.g., household and social contacts) (Appendix 1 Figure 1).

Our main finding was that 35/37 (94.6%) TUR isolates shared identical mutations that confer drug resistance to isoniazid (*katG* S315T), streptomycin (*rpsL* K43R), and ethambutol (*embB* Q497R); we therefore classified them as TUR-outbreak isolates. TUR-outbreak isolates further differentiated into 2 individual transmission chains characterized

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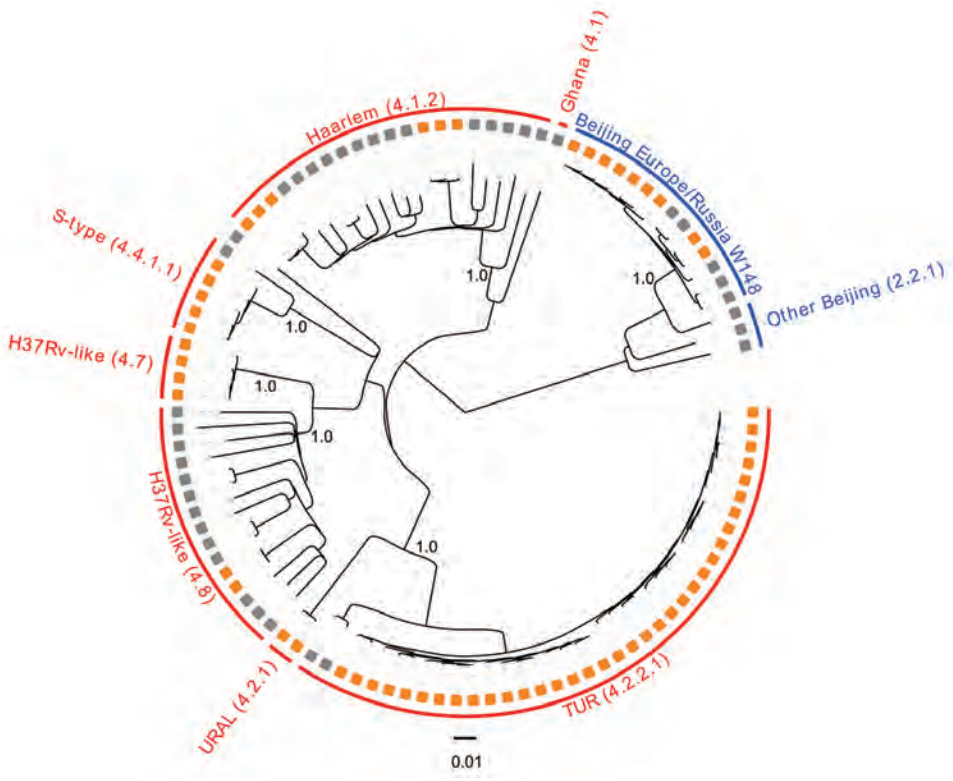

Figure 1. Maximum-likelihood phylogeny, applying a general time-reversible substitution model, of 103 multidrug-resistant (MDR) *Mycobacterium tuberculosis* complex (MTBC) isolates from Serbia sampled during 2008–2014. Orange squares indicate MDR MTBC isolates associated with putative transmission chains (molecular clusters); gray squares indicate other MDR MTBC isolates. All analyzed strains belong to the major MTBC phylogenetic lineage 4 (Euro-American) or lineage 2 (Beijing); red text indicates lineage 4 and blue text, lineage 2. Subgroups are further named according to the single-nucleotide polymorphism barcode nomenclature from Coll et al. (*5*), and to the associated mycobacterial interspersed repetitive unit–variable-number tandem-repeat genotype classification (*6*). Subgroup-defining branches are labeled with bootstrap values based on 1,000 resamples. Scale bar indicates nucleotide substitutions per site.

by 2 distinct rifampin resistance–mediating mutations: *rpoB* S450W in 1998 (95% highest posterior density [HPD] 1993–2001) and *rpoB* S450L in 2003 (95% HPD 2000–2005) (Figure 2, panel A; Appendix 1 Figure 2). Subsequently, both strain populations acquired individual mutations in other RNA polymerase genes (*rpoA* P25R, *rpoC* V431M, and *rpoC* F452L), which have been proposed to enhance the in vitro growth rate of rifampin-resistant strains (*7*). Furthermore, *rpoA* mutations in the entire dataset were more likely to arise in clustered isolates than in unique isolates (20/63 vs. 1/40; $p<0.001$), thus indicating their ability to restore fitness of *rpoB* mutants, increase transmission success, or both.

Of the 35 TUR-outbreak isolates, 26 (74.3%) were from patients hospitalized in Bela Crkva (BC) Hospital, the national center for treatment of all psychiatric patients with concomitant respiratory illnesses. Of note, 22 (84.6%) of these 26 patients had been transferred from 7 different psychiatric hospitals to BC Hospital for pulmonary diagnosis and treatment; 5 were admitted at BC Hospital with either confirmed or suspected TB diagnosis (Appendix 2). Screening for TB at time of admission had not been implemented in BC Hospital during the study period.

To determine the geographic origin of the 3-fold resistant TUR ancestor and to test for the putative independent introduction of 2 different rifampin-resistant cases to the BC Hospital from other hospitals, we extended our Bayesian approach with a discrete trait model introducing the likely place of infection for each patient. We used 2 assumptions: first, a fast disease progression assumed infection and diagnosis of MDR TB within the first 2 years after admission to BC Hospital; and second, a slow disease progression in which patients who received a diagnosis within 2 years after admission were identified as latent MDR TB cases, meaning they had contracted the infection in their hometown or a previous hospital.

The comparison of both models using path sampling clearly favored the fast progression model, suggesting the origin of the TUR outbreak in BC Hospital with a probability of 53% (i.e., node location probability; second likely origin was Belgrade, 12%) (Figure 2, panel A). The 2 unique rifampin-resistance mediating mutations were also more likely to have originated in BC Hospital itself (51% for rpoB S540W node, 95% for rpoB S540L node, and <15% for other location probabilities). Individual transmission events occurred in remote cities but also within Belgrade (Figure 2, panels A, B). In comparison, applying the slow TB progression hypothesis, TUR outbreak strains would have been imported multiple times from different regions throughout the country to BC Hospital, with node location probabilities ≤10% for all locations (Appendix 1 Figure 3). Tracing the time of hospitalization at BC Hospital and MDR TB diagnosis of patients infected with TUR strains backward revealed that the 2 clades (defined by *rpoB* S450L and *rpoB* S450W) indeed coexisted over 2 decades (Appendix 1 Figure 4).

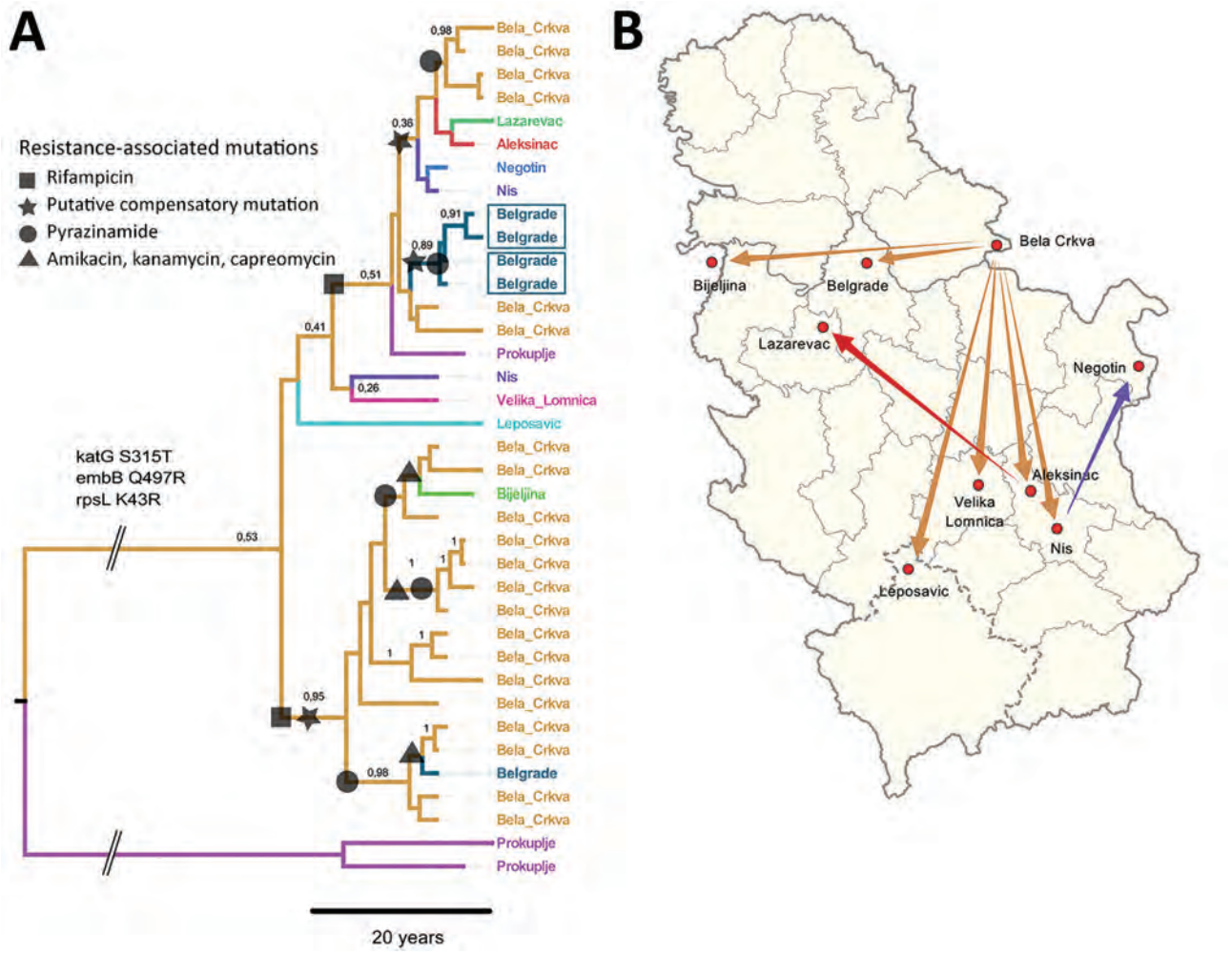

Figure 2. Most likely temporal and spatial origin of *Mycobacterium tuberculosis* complex (MTBC) TUR genotype outbreak strains in Serbia. A) Location annotated time-scaled phylogeny (maximum clade credibility tree) derived from a Bayesian discrete trait phylogeographical analysis of 37 lineage 4.2.2.1 (TUR genotype) multidrug-resistant (MDR) MTBC isolates. Branches are color-coded according to the most likely place of infection, assuming a fast-progression hypothesis (Appendix 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1220-App1.pdf). Branches are annotated with location probabilities; symbols represent acquisition of individual resistance-related mutations shared by all derived strains. B) Regional and countrywide spread of individual TUR genotype outbreak strains originating from Bela Crkva Hospital. Arrows indicate inferred location changes determined from the genealogy shown in panel A.

Conclusions

In a retrospective approach using WGS-based molecular epidemiology, Bayesian statistics, and detailed epidemiologic investigations, we show that MDR TB in Serbia is associated with nosocomial transmission at BC Hospital, likely accompanied by a fast progression to disease within 2 years. Drug unavailability in the 1990s (*8*), schizophrenia as a recognized cause of unsuccessful completion of TB treatment (*9*), and long-term and repeated hospitalizations under extremely adverse living conditions (*10*), together with the absence of a TB infection control program, are believed to be the main drivers of the evolutionary trajectories and success of TUR-outbreak strains in Serbia. The TUR-outbreak strain was considered intrinsically resistant to 3 first-line drugs and probably acquired an MDR genotype in 2 independent events in BC Hospital during the 1990s. Subsequently, putative compensatory mechanisms were selected, the strain acquired individual XDR genotypes, and it spread into other settings in Serbia by family contacts and other modes.

Detection of the extensive transmission network in BC Hospital led to the development and implementation of an appropriate TB infection control program featuring the use of rapid laboratory tests for prompt detection of new cases, completion of appropriate second-line treatment regimens, and markedly expanded contact tracing activities. Since 2015, only 1 new case of MDR TB has been recorded in BC Hospital. However, MDR TB transmission in the general population must continue to be carefully monitored.

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Genomic Analysis of Cardiac Surgery–Associated *Mycobacterium chimaera* Infections, United States

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A surgical heater–cooler unit has been implicated as the source for *Mycobacterium chimaera* infections among cardiac surgery patients in several countries. We isolated *M. chimaera* from heater–cooler units and patient infections in the United States. Whole-genome sequencing corroborated a risk for these units acting as a reservoir for this pathogen.

Mycobacterium chimaera is a species in the *Mycobacterium avium* complex (MAC) (*1*). MAC is the most frequently reported cause of nontuberculous mycobacterium (NTM) infection in the United States, although disseminated *M. chimaera* infections are relatively rare (*2*). In 2012, investigators in Switzerland found that some patients with disseminated *M. chimaera* infections had undergone open-chest cardiac surgeries, during which they were exposed to heater–cooler units (HCUs) (*3*). These devices, Stöckert 3T Heater–Cooler Units (LivaNova PLC, https://www.livanova.com; formerly Sorin Group Deutschland GmbH), manufactured in Germany, were unknowingly contaminated with *M. chimaera* (*4*,*5*). In the same year, a Pennsylvania hospital identified a cluster of invasive *M. chimaera* infections among open-chest cardiac surgery patients exposed to LivaNova 3T HCUs contaminated with *M. chimaera* (*6*), which prompted notification of ≈1,300 patients with exposure to these units (*7*). Additional cases of disseminated *M. chimaera* infection among cardiac surgery patients have emerged worldwide, with evidence implicating bioaerosols produced by contaminated LivaNova 3T HCUs as the source of post–cardiac surgery *M. chimaera* infections (*8*,*9*). We report the relationships among HCU-associated isolates from patients and LivaNova 3T HCUs in the United States and their context among the global outbreak.

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The Study

During 2015–2016, we collected NTM isolates from 3T HCU water (n = 38 isolates) and suspected patient cases (n = 24 isolates) from 8 US locations. We identified isolates and conducted high-throughput whole-genome sequencing using the Illumina Miseq system (https://www.illumina.com). We selected Pennsylvania isolate 2015-2271 (USA_PA_PAT_9) for Pacific Biosciences (https://www.pacb.com) single-molecule real-time sequencing (*10*). We downloaded publicly available *M. chimaera* genomes from isolates collected in Australia, Denmark, Italy, New Zealand, the United Kingdom, and Switzerland from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA). We included Zürich CHE_HCU_1 isolate as a representative of the genotype isolated from HCUs, patients, and manufacturing sites in Europe (*9*). For each isolate, we mapped the sequence reads to the *M. chimaera* strain CDC 2015-22-71 reference genome (GenBank accession no. NZ_CP019221.1) to detect single-nucleotide polymorphisms (SNPs) (Appendix, http://wwwnc.cdc.gov/EID/article/25/3/18-1282-App1.pdf).

We reconstructed phylogenetic relationships among *M. chimaera* isolates collected from post–cardiac surgery patients and HCUs in 8 locations across the United States, as well as HCU-associated strains from Australia, New Zealand, and Europe (Table; Appendix Figure 1). We compared all HCU-associated isolates with 7 *M. chimaera* respiratory isolates obtained from US patients with no history of cardiac surgery. We identified 18,190 SNPs in the 3.82-Mb core genome (62.8% of the reference genome) among 126 *M. chimaera* isolates.

The NeighborNet splitstree (Appendix) of *M. chimaera* showed 3 groups (HCU1, HCU2, and non-HCU; Figure 1). Clade HCU1 (n = 112 isolates; Figure 2) is a discrete cluster composed entirely of HCU-associated isolates from case-patients and HCUs (mean pairwise distance 4 SNPs, range 0–23 SNPs; Appendix Figure 2) from Australia,

Table. *Mycobacteria chimaera* isolated from HCUs, suspected patient case(s), and non–HCU-associated *M. chimaera* isolates in Australia, Europe, New Zealand, and the United States*

Location	No. isolates	No. clinical	No. HCU	Status	Genotypes/location	No. HCU1 genotypes (%)	NCBI BioProject no.	Reference
Iowa 1	9	3	6	HCU	1	9 (100)	PRJNA345021	(*11*); this study
Iowa 2	3	0	3	HCU	1	3 (100)	PRJNA345021	This study
Kentucky	1	0	1	HCU	1	1 (100)	PRJNA345021	This study
Massachusetts 1	4	0	4	HCU	1	4 (100)	PRJNA345021	This study
Michigan	17	8	9	HCU	1	17 (100)	PRJNA345021	This study
Minnesota	1	1	0	HCU	1	1 (100)	PRJNA345021	This study
Pennsylvania 1†	25	10	15	HCU	2	23 (92)	PRJNA344472	(*11*); this study
Virginia	2	2	0	HCU	1	2 (100)	PRJNA345021	This study
Australia 1	6	0	6	HCU	1	6 (100)	PRJEB15375	(*12*)
Australia 2	13	1	12	HCU	3	11 (84.6)	PRJEB15375	(*12*)
Australia 3	7	2	5	HCU	3	5 (71.4)	PRJEB15375	(*12*)
Australia 4	10	2	8	HCU	2	9 (90)	PRJEB15375	(*12*)
Denmark	4	0	4	HCU	1	4 (100)	PRJEB18427	(*13*)
New Zealand 1	2	0	2	HCU	1	2 (100)	PRJEB15375	(*12*)
New Zealand 2	3	0	3	HCU	1	3 (100)	PRJEB15375	(*12*)
New Zealand 3	5	0	5	HCU	1	5 (100)	PRJEB15375	(*12*)
New Zealand 4	2	0	2	HCU	1	2 (100)	PRJEB15375	(*12*)
United Kingdom	3	0	3	HCU	1	3 (100)	PRJNA324238	(*13*)
Zürich	2	0	2	HCU	2	1 (50)	PRJNA313770, PRJNA314007	(*9*)
Italy	1	1	0	Non-HCU	1	0	PRJEB18427	(*9*)
Maryland	1	1	0	Non-HCU	1	0	PRJNA345021	This study
Massachusetts 2	1	1	0	Non-HCU	1	0	PRJNA319839	This study
North Carolina	1	1	0	Non-HCU	1	0	PRJNA345021	This study
Pennsylvania 2	1	1	0	Non-HCU	1	0	PRJNA345021	This study
Tennessee	2	2	0	Non-HCU	1	0	PRJNA319839	This study
Texas	2	2	0	Non-HCU	1	0	PRJNA345021	This study
Total		38	90			112 (95)‡		

*US isolates were collected during 2015–2016. Status refers to HCU-associated isolates (HCU) collected directly from Stöckert 3T Heater–Cooler Units (LivaNova PLC, https://www.livanova.com; formerly Sorin Group Deutschland GmbH) or from patients with suspected HCU-derived *M. chimaera*, and isolates from pulmonary NTM patients without history of HCU exposure (non-HCU). HCU, heater–cooler unit; NCBI, National Center for Biotechnology Information.
†Denotes the location from which the 2 samples (USA_PA_HCU_0, 2015–06–01; and USA_PA_PAT_10, 2015–22–65) did not pass the genomics quality control assessment and were excluded from the analyses.
‡Percentage was derived from the number of HCU1 genotype isolates in the number of isolates collected directly from LivaNova 3T HCU or suspected patient cases.

Denmark, New Zealand, Switzerland, the United Kingdom, and the United States. Clade HCU2 was composed of 3 HCU-associated *M. chimaera* isolates from Switzerland (*2*) and Australia (AUS_HCU_30 and AUS_HCU_31). The mean difference among HCU2 isolates was 21.3 SNPs (range 19–25 SNPs; Appendix). Clade 3 was composed of US non–HCU-associated isolates (non-HCU). Two HCU-associated patient isolates from Australia (mean non–HCU-associated isolate pairwise SNPs 52, range 3–111 SNPs; Appendix) were unclustered. The mean distance between HCU1 and international HCU isolates was 13.58 SNPs (range 0–521 SNPs; Appendix Figure 3); the mean distance between HCU1 and non–HCU-associated isolates was 510.5 SNPs (range 506–610 SNPs; Appendix).

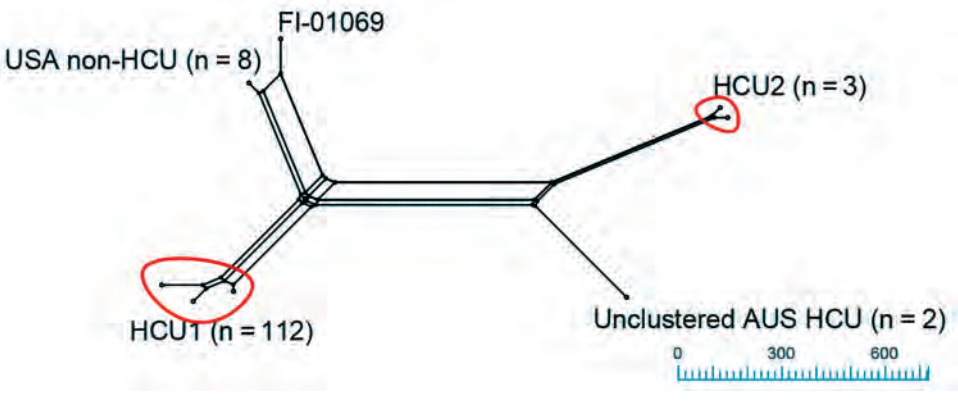

Figure 1. Neighbor Net splitstree of *Mycobacterium chimaera* isolates: relationships between *M. chimaera* isolates (n = 124) mapped against the *M. chimaera* strain CDC 2015–22–71 heater–cooler unit (HCU) reference genome (18,190 single-nucleotide polymorphisms [SNPs] in 3,815,639 core positions). Isolates were grouped with a threshold of ≤500 SNPs to the nearest cluster. Clustered HCU isolates, including the reference strain CDC 2015-22-71, comprise the HCU1 cluster (n = 112) and HCU2 (n = 3). Unclustered isolates include Australian (AUS) HCU isolates (n = 2), USA non-HCU isolates (n=8), and the type strain FI-01069. Scale bar indicates SNPs.

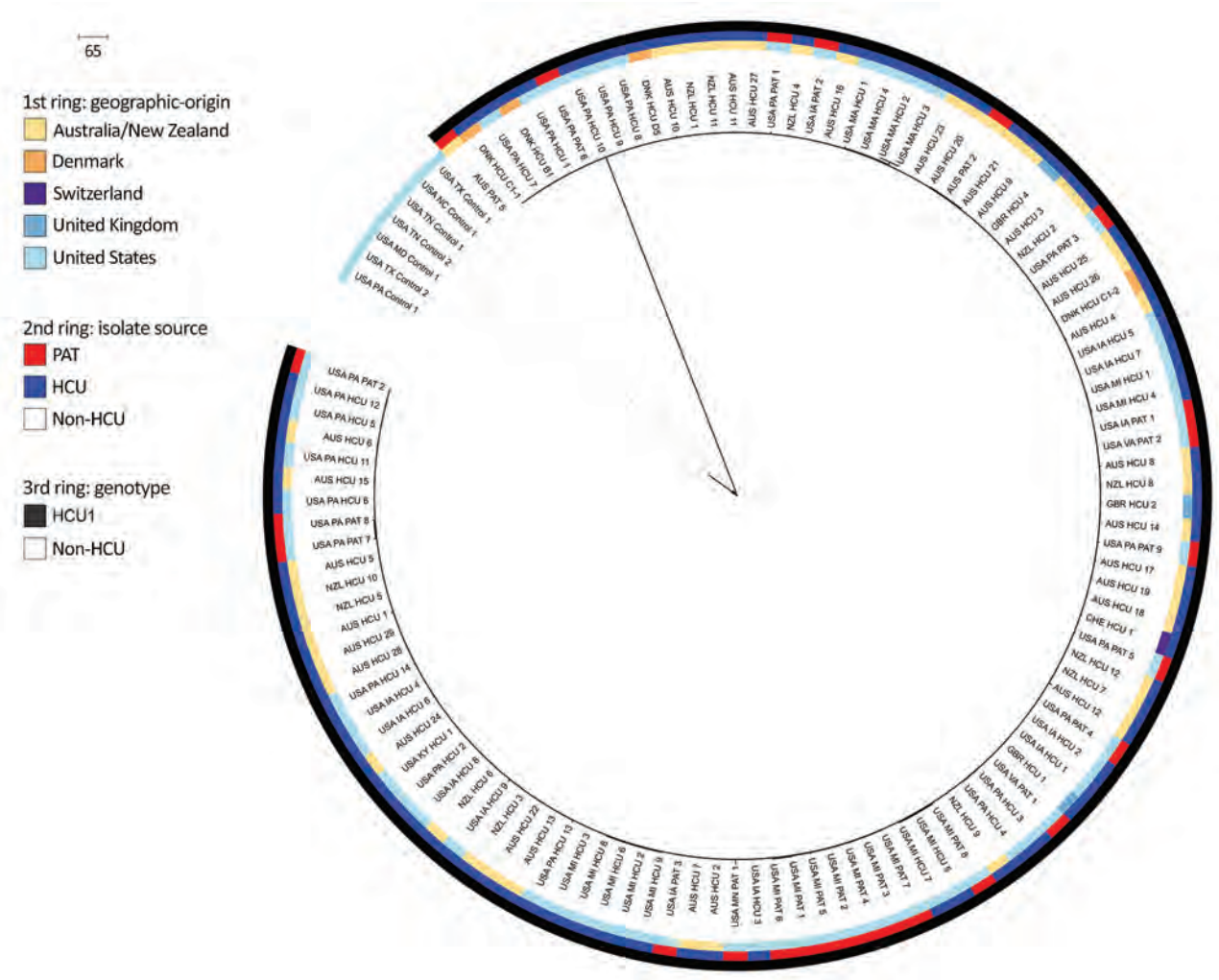

Figure 2. Maximum-likelihood phylogenetic relationships between HCU1 and US non-HCU–associated isolates (651 single-nucleotide polymorphisms [SNPs] in 4,024,718 core positions) as a circular phylogeny. From the center to the perimeter, colored circles indicate the country of origin, isolate source, and HCU genotype(s). Clinical isolate labels use country abbreviation: Australia (AUS), Denmark (DNK), Florence, Italy (FI), New Zealand (NZL), Switzerland (CHE), United Kingdom (GBR), United States (USA); HCU or PAT; isolate number. Non-HCU–associated isolates are from respiratory patients without a history of cardiac surgery. Suspected cases are isolates from blood or tissue samples collected from post–cardiac surgery patients; HCU are isolates collected from hospital HCUs (swabs, water, bioaerosols). Scale bar indicates SNPs. HCU, heater–cooler unit; PAT, suspected case-patient.

In comparison, the mean distance between HCU2 and non-HCU isolates was 17,130.7 SNPs (range 17,057–17,221 SNPs). Of the 117 HCU-associated isolates we analyzed, 112 (95.7%) were HCU1 cluster, 3 (2.6%) were HCU2 cluster, and 2 isolates (1.7%) were not in a major clade.

Whole-genome sequencing of US HCU-associated *M. chimaera* isolates and their comparisons with global HCU-associated isolates provides further evidence for point-source contamination and worldwide dissemination of a *M. chimaera* strain (*3*–*5*). Twenty-two of 24 (92%) US patient isolates associated with HCU exposure during cardiac surgery phylogenetically clustered with international HCU-derived and post–cardiac surgery patient isolates, including those from Australia, Europe, and New Zealand (HCU1). None of the 8 US non–HCU-associated isolates were genetically similar to the HCU1 or HCU2 clusters. Isolates from US post–cardiac surgery patients were genetically more similar to isolates derived from international LivaNova 3T HCUs (mean pairwise distance 4 SNPs) than *M. chimaera* isolates from US patients without a history of cardiac surgery (mean pairwise distance 511 SNPs). This evidence supports the hypothesis that US post–cardiac surgery *M. chimaera* infections were acquired from exposure to factory-contaminated HCUs rather than local populations of waterborne *M. chimaera* in each hospital.

Our analyses revealed that all US *M. chimaera* isolates associated with LivaNova 3T HCU exposure genetically cluster with HCU1 genotype isolates implicated in the global outbreak of post–cardiac surgery *M. chimaera* infections. The HCU2 cluster was not observed in the United States but included 2 isolates from HCUs in Australia, as well as a representative genotype of *M. chimaera* found in HCUs in Europe and at the HCU production site. Consistent with previous findings, this finding suggests the international circulation of a second, less plentiful, strain in the manufacturing site water system (*8*).

These observations support the hypothesis that the LivaNova 3T HCU design provided suitable conditions for both NTM colonization and aerosolization, particularly by *M. chimaera*. Even though production site contamination with *M. chimaera* has been confirmed, the medical community needs to remain alert for HCU-associated NTM infections involving other species (*4*). HCUs are vulnerable to contamination from in-hospital water sources, use of improper water sources, and improper maintenance, each of which may increase the risk of infection by NTM (including *M. abscessus*, *M. chelonae*, and *M. gordonae*, in addition to *M. chimaera*) (*6*). Contaminated HCUs may contain NTM-contaminated biofilms. Furthermore, water from the LivaNova 3T HCUs can become aerosolized during normal function, leading to introduction of potentially infectious particles into the sterile field, onto graft materials, or into the open chest cavity during cardiac surgery. The death rate for HCU-associated *M. chimaera* infections has been reported to be 50%; the latent period to diagnosis can be up to 5 years postsurgery (*4*,*6*,*7*,*9*,*10*), further emphasizing necessary diligence on the part of physicians and cardiac surgery patients to monitor for symptoms of disseminated NTM infection.

Our study has some limitations in methodology. We did not obtain samples from every US hospital that reported LivaNova 3T HCU–associated *M. chimaera* cases; no submitting hospital collected all 3 types of samples (HCUs, non-HCU samples, and suspected case-patients); and HCU samples were not collected by a single person or according to a standardized collection protocol. Despite these limitations, this analysis of US HCU-associated *M. chimaera* isolates clearly shows the clustering of isolates from epidemiologically linked US cases to international LivaNova 3T HCU *M. chimaera* isolates and the HCU1 genotype found within the LivaNova manufacturing site.

In conclusion, the application of WGS has advanced our understanding of *M. chimaera* present in US LivaNova 3T HCUs and patient cases after the initial analysis of suspected cases in Pennsylvania and Iowa. Given the innate drug resistance and the high death rate of HCU-associated *M. chimaera* infections, it remains imperative for hospitals to follow Food and Drug Administration guidelines (*9*) and the manufacturer's instructions to minimize the risk of patient infection. In addition, clinicians should monitor patients who have had cardiac surgery using LivaNova 3T HCUs for signs and symptoms of NTM infection to enable early diagnosis and treatment.

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About the Author

Dr. Hasan is a researcher at the Center for Genes, Environment and Health at National Jewish Health, Denver, Colorado, USA. His main research focuses on nontuberculous *Mycobacteria* comparative genomics to discover genetic markers associated with pathoadaptation and conduct outbreak surveillance.

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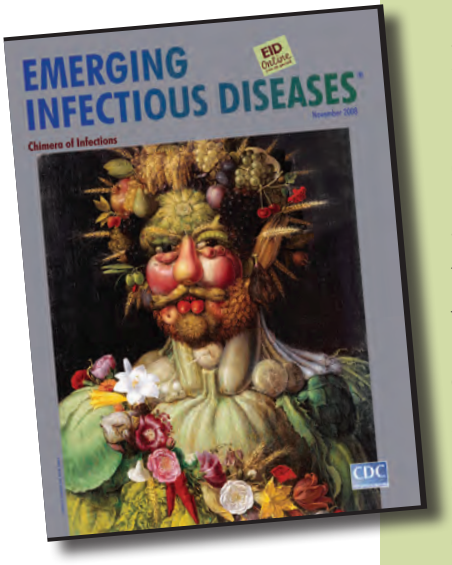

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Chimera

[ki-mir′ə]

From the Greek *Khimaira*, Latin *Chimaera*; she-goat. In Greek mythology: a composite creature with the body and head of a lion, a goat's head rising from its back, and a serpent's tail. In science: an individual organism whose body contains cell populations derived from different zygotes, of the same or different species. Each population of cells keeps its own character, and the resulting animal is a mixture of tissues. Chimera also refers to a substance created from proteins or genes of 2 species, as by genetic engineering. Chimerism is rare in humans; ≈40 cases have been reported.

Source: Dorland's illustrated medical dictionary, 31st edition. Philadelphia: Saunders; 2007; http://www.merriam-webster.com; http://www.medicinenet.com/script/main/hp.asp

https://wwwnc.cdc.gov/eid/article/14/11/e1-1411_article

Drug-Resistant Tuberculosis, Lebanon, 2016–2017

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In a 12-month nationwide study on the prevalence of drug-resistant tuberculosis (TB) in Lebanon, we identified 3 multidrug-resistant cases and 3 extensively drug-resistant TB cases in refugees, migrants, and 1 Lebanon resident. Enhanced diagnostics, particularly in major destinations for refugees, asylum seekers, and migrant workers, can inform treatment decisions and may help prevent the spread of drug-resistant TB.

Populations in crisis-affected areas are particularly vulnerable to tuberculosis (TB) linked to malnutrition, overcrowding, and discontinuity in health services (*1*,*2*). Difficulties accessing diagnosis and starting or completing appropriate treatment can promote the emergence and spread of multidrug-resistant (MDR) TB (resistant to at least rifampin and isoniazid) and extensively drug-resistant (XDR) TB (additionally resistant to ≥1 second-line injectable drug and 1 fluoroquinolone) in the countries of origin or in countries of transit or refuge after migration (*3*).

Lebanon hosts the largest per capita refugee population in the world. In addition to 450,000 refugees from Palestine, ≈1.5 million refugees from Syria are scattered in hundreds of informal sites across the nation (*2*,*4*). Moreover, the country hosts >250,000 migrant domestic workers, mostly originating from regions with high TB incidence rates, such as Ethiopia, Bangladesh, the Philippines, and Sri Lanka (*5*).

The last national survey on the prevalence of drug-resistant TB in Lebanon was performed 15 years ago (*6*), well before the beginning of the Syria crisis in 2011. Even most recent reported MDR TB rates largely relied on estimates rather than on systematic laboratory confirmation (*6*). Second-line drug susceptibility testing (DST) and individualized XDR TB treatments were not available. We report results from a June 2016–May 2017 nationwide study combining extensive phenotypic and molecular testing. This national survey was approved by the ethics committee of the Azm Center for Research in Biotechnology and Its Applications, Lebanese University (document no. CE-EDST-3-2016), authorized by the Lebanese Ministry of Public Health. Informed consent was obtained from the study patients.

The Study

The study included 720 cases of suspected TB, corresponding to all suspected cases reported from June 1, 2016, through May 31, 2017, to the TB centers from the 9 governorates that make up Lebanon's national TB program. After testing of all corresponding microscopy-positive and microscopy-negative samples, 284 were considered confirmed TB cases on the basis of solid (Lowenstein-Jensen [LJ]) or liquid (BBL MGIT Mycobacteria Growth Indicator, BD Diagnostics, http://www.bd.com) culture results or molecular testing results (Xpert MTB/RIF, Cepheid, http://www.cepheid.com). For samples contaminated with blood, Anyplex MTB/NTM Real-time Detection (Seegene, http://www.seegene.com) (Appendix 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1375-App1.pdf) was used. Thirty-four cases could not be subjected to DST because of culture negativity (n = 28), contamination (n = 3), insufficient sample amount for culture (n = 2), or reagent contingencies (n = 1).

Of the 250 remaining patients, 51% (128/250) were men; the mean age was 34 years (Table 1; Appendix 2, https://wwwnc.cdc.gov/EID/article/25/3/18-1375-App2.xlsx). Patients were from Syria (74/250, 29.6%), Lebanon (70/250, 28%), Ethiopia (57/250, 22.8%), Bangladesh (13/250, 5.2%), Palestine (7/250, 2.8%), or other nations (29/250, 11.6%).

Rifampin resistance was detected among 7/250 (2.8%) patients, concordantly with Xpert testing results for all cases (Table 1). We used multivariate logistic regression to test TB history as an independent predictor of rifampin resistance, after adjusting for age, sex, and nationality (Appendix 1). Log-linearity was checked for age. A 2-tailed type I error rate was set at 5%. TB history information was available for 246 (98.4%) patients. The proportion of

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Table 1. Details of 250 TB cases with available phenotypic drug susceptibility profiles, Lebanon, 2016–2017*

Characteristic	No. (%) patients			
	Total, n = 250	New cases, n = 228	Previously treated or relapsed, n = 18	Missing data, n = 4
Sex				
M	128 (51.2)	112 (49.1)	14 (77.8)	2 (50)
F	122 (48.8)	116 (50.9)	4 (22.2)	2 (50)
Country of origin				
Lebanon	70 (28)	64 (28.1)	4 (22.2)	2 (50)
Syria	74 (29.6)	65 (28.5)	9 (50)	0
Ethiopia	57 (22.8)	54 (23.7)	1 (5.6)	2 (50)
Bangladesh	13 (5.2)	13 (5.7)	0	0
Palestine	7 (2.8)	7 (3.1)	0	0
Other	29 (11.6)	25 (11)	4 (22.2)	0
Age, y	34 ± 14	34 ± 14	38 ± 13	23 ± 5
Drug resistance				
RIF	7 (2.8)	3 (1.3)	4 (22.2)	0
Mono	1 (0.4)	1 (0.4)	0	
MDR	3 (1.2)	1† (0.4)	2‡ (11.1)	
XDR	3§ (1.2)	1§ (0.4)	2§ (11.1)	
INH	16 (6.4)	15 (6.6)	1 (5.6)	0
Mono	9 (3.6)	8 (3.5)	1 (5.6)	
INH + SM	7 (2.8)	7 (3.1)	0	
EMB only	1 (0.4)	1 (0.4)	0	0
SM only	23 (9.2)	21 (9.2)	2 (11.1)	0
Susceptible to all first-line drugs	203 (81.2)	188 (82.4)	11 (61.1)	4 (100)

*Age is expressed as mean ±SD; categorical variables are presented as absolute numbers and percentages. EMB, ethambutol; INH, isoniazid; MDR, multidrug resistant; mono, monoresistant; RIF, rifampin; SM, streptomycin; XDR, extensively drug resistant.
†Resistant to RIF and INH.
‡Resistant to RIF, INH, EMB, and SM.
§Resistant to RIF, INH, EMB, SM, amikacin and kanamycin, and levofloxacin (representing all tested drugs for MDR and XDR isolates).

rifampin resistance was 22.2% (4/18) among previously treated patients and patients with relapse and 1.3% (3/228) among patients with new TB cases (adjusted OR 21.4, 95% CI 4.4–105.2; p<0.01). One case in a patient without previous TB history was confirmed by liquid culturing DST as monoresistant to rifampin; 3 other cases, including 1 in a patient without previous TB history, were MDR TB, 2 of which showed resistance to all 4 first-line drugs tested (i.e., ethambutol and streptomycin in addition to rifampin and isoniazid). Moreover, 3 XDR TB cases were detected, including 1 in a patient without previous TB history, showing phenotypic resistance to amikacin, kanamycin, and levofloxacin in addition to all 4 first-line drugs tested. Among all 250 cases, 203 (81.2%) were susceptible to all 4 first-line drugs, 9 (3.6%) were resistant to isoniazid only, 1 (0.4%) to ethambutol only, 23 (9.2%) to streptomycin only, and 7 (2.8%) to isoniazid and streptomycin (Table 1).

To assess their extensive drug-resistance profiles, we subjected isolates from the 3 patients with XDR TB to targeted sequencing by use of a new assay, Deeplex-MycTB (GenoScreen, https://www.genoscreen.fr), which covers 18 drug resistance–associated gene targets (*7*) (Figure; Appendix 1). Two of these cases were confirmed by whole-genome sequencing. In 1 case (patient identification no. 74), no mutation was found to explain phenotypic resistance to amikacin and kanamycin. For the other drugs for this isolate, and for the 2 isolates analyzed by both tests, we detected drug resistance–associated mutations (*8*–*10*) in *rpoB*, *katG* or *inhA*, *gyrA*, *rrs* or *tlyA*, and *embB*, confirming the resistance phenotypes (Table 2). Moreover, we detected different drug resistance–associated deletions in *ethA* in all 3 XDR TB isolates and drug resistance–associated mutations in *pncA* in 2 XDR TB isolates. These mutations predict additional resistance to ethionamide and pyrazinamide, which are not phenotypically tested in Lebanon or in many other countries.

Results of genotypic analysis of the 3 MDR TB isolates by Deeplex-MycTB also were consistent with phenotypic profiling overall, considering that a rare F129S mutation in *katG* was previously described in association with isoniazid resistance (*11*), along with other well-established mutations. An ethambutol resistance–associated M306V mutation in *embB* in 1 isolate was phenotypically undetected, probably reflecting known poor phenotypic reproducibility for this mutation (*10*). Of note, in the same isolate, Deeplex-MycTB testing detected a *gyrA* S91P mutation, which generally confers low levels of levofloxacin resistance (*12*), as a minority population (5.2%). This detection was confirmed by Anyplex results but was not correlated with phenotypic resistance to levofloxacin tested at a standard critical concentration of 1.5 µg/mL. As with the XDR TB isolates, nonsense insertion or deletion mutations additionally detected in *pncA* or *ethA* predicted supplementary pyrazinamide and ethionamide resistance in some isolates.

None of the MDR or XDR TB cases clustered with any other case in the study population tested by standard

Table 2. Genotypic and phenotypic drug susceptibility profiles of drug-resistant TB cases, Lebanon*

			Drug resistance classification					
Category			MDR			XDR		
Patient ID			14	48	125†	74	168	185
TB drug								
RIF	Gene	*rpoB*	S450L	S450L	S450L	S450L	**S450L**	**S450L**
	Phenotypic							
INH	Genes	*katG*	S315T	F129S‡	S315T	S315T		**S315T**
		inhA					**C-15T**	
	Phenotypic							
PZA	Gene	*pncA*		Y103Stop	InserA192	A46P		**H71Q**
	Phenotypic		ND	ND	ND	ND	ND	ND
EMB	Gene	*embB*	Q497R	M306V	M306V	Q497R	**Q497R**	**M306V**
	Phenotypic							
SM	Genes	*rpsL*	K43R		K88R	K43R		**K43R**
		rrs					**A908C**	
	Phenotypic							
AMI/KAN	Gene	*rrs*					**A1401G**	**A1401G**
	Phenotypic							
FQ	Gene	*gyrA*		S91P§		D94A	**S91P**	**D94Y**
	Phenotypic							
CAP	Genes	*tlyA*				InserC313		
		rrs					**A1401G**	**A1401G**
	Phenotypic		ND	ND	ND	ND	ND	ND
ETH	Genes	*ethA*	Deleted¶		141 nt del#	Deleted¶	**DelG632**	**DelG1338**
		inhA					**C-15T**	
	Phenotypic		ND	ND	ND	ND	ND	ND
MIRU-VNTR type**			100-32	19431-157	21404-32	10156-32	21416-15	?-15
M. tuberculosis complex lineage††			2 (Beijing)	4 (Euro-American)	3 (Delhi-CAS)	2 (Beijing)	4 (H37Rv-like)	4 (Haarlem)

*Only genes with detected resistance-associated mutations are shown. No mutation was detected in targets associated with linezolid or bedaquiline and clofazimine resistance. Mutations are shown as amino acid changes with the corresponding codon position, nucleotide changes in promoter regions, or inserted or deleted base (inser or del with position in coding sequence) resulting in a frameshift. Bold text shows mutations concordantly detected by whole-genome sequencing and Deeplex-MycTB (GenoScreen, https://www.genoscreen.fr) in samples subjected to both assays. Other mutations are those detected in samples analyzed by Deeplex-MycTB only. Drug resistance predictions are based on reference data from available scientific literature (*8*–*10*), and for *pncA* also on data from Yadon et al. (*14*). Black represents phenotypic resistance to the different drugs and gray represents phenotypic susceptibility. For phenotypic testing, levofloxacin was the only fluoroquinolone tested. AMI, amikacin; CAP, capreomycin; EMB, ethambutol; ETH, ethionamide; FQ, fluoroquinolones; KAN, kanamycin; INH, isoniazid; ND, not done; PZA, pyrazinamide; RIF, rifampin; SM, streptomycin.
†Deeplex-MycTB result obtained on a primary specimen (sputum). The other results were obtained on indirect samples (primary cultures).
‡Mutation described in association with isoniazid resistance once before by Wang et al. (*11*). This mutation is not detectable by Anyplex testing.
§Detected as a minority variant, at 5.2% in this sample (see text). Percentages of fixation of other mutations within individual samples range from 80.6% to 100%.
¶Putative deletion, as inferred by absence of reads mapped specifically on the corresponding gene target, in contrast to all other, well covered targets.
#Internal deletion, resulting in a frameshift, from gene position 859 to 999.
**According to MIRU-VNTRPlus website (http://www.miru-vntrplus.org) nomenclature (*15*). For patient 185, a question mark in the genotype reflects the absence of a detectable allele in locus 4052.
††According to Deeplex-MycTB (spoligotyping and phylogenetic SNPs) and MIRU-VNTRPlus identification, confirmed by whole-genome sequencing results, when done.

24-locus mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) typing of isolates, showing no support for drug-resistance transmission (Appendix 2). Consistently, 4 of the 6 cases involved were previously treated, and the 2 new cases were in migrant workers, presumably representing imported cases. Two cases were in Syria refugees; 1 patient with MDR TB had repeated failed treatment in Syria, and 1 XDR TB case was a relapse after patient arrival in Lebanon. Of the other previously treated cases, 2 had Beijing strain genotypes; the isolate from an XDR TB case in a patient originating from eastern Europe differed by a single allele from the 100-32 MIRU-VNTR haplotype and the isolate from an MDR TB case in a patient from Lebanon fully matched the 100-32 MIRU-VNTR haplotype (Table 2). This haplotype represents a major, presumably highly transmissible MDR-associated clonal complex epidemically spreading across Eurasia (*13*). Although an XDR TB patient of foreign origin returned to his country after diagnosis because of initial unavailability of proper treatment in Lebanon, the 2 other XDR TB patients received treatment and, as of January 2019, responded positively to ongoing treatments, as were the patients treated for MDR TB.

Conclusions

Although the prevalence of rifampin-resistant TB estimated in Lebanon is relatively low (2.8%), identification of XDR TB and MDR TB cases, including TB strains with strong epidemic potential and complex resistance patterns, calls for sustained diagnosis of MDR TB. We recommend that Lebanon test all TB-positive isolates for resistance to first- and second-line drugs, to inform treatment decisions and prevent the spread of drug resistance. Other major destinations for refugees, asylum seekers, and migrant

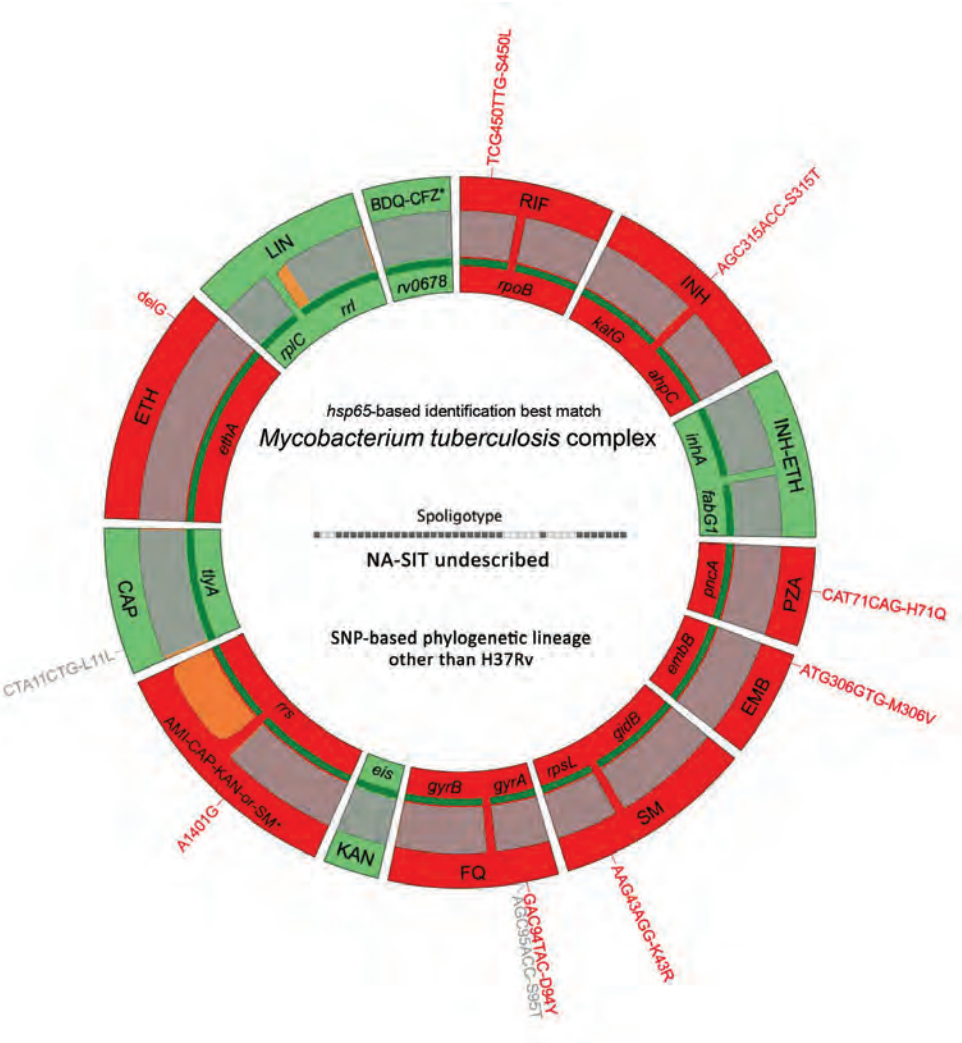

Figure. Deeplex-MycTB (GenoScreen, https://www.genoscreen.fr) results identifying an extensively drug-resistant genotypic profile in an isolate from a tuberculosis (TB) patient in Lebanon. Results correspond to TB patient no. 185 in Table 2. Target gene regions are grouped within sectors in a circular map according to the drug resistance with which they are associated. Red indicates target regions in which drug resistance–associated mutations are detected (red text around the map), whereas green indicates regions where no mutation or only mutations not associated with resistance (gray text around the map) are detected. Dark green lines above gene names represent the reference sequences with coverage breadth above 95%. Limits of detection (LOD) of potential heteroresistance (reflected by subpopulations of reads bearing a mutation), depending on the coverage depths over individual sequence positions, are indicated by gray (LOD 3%) and orange (variable LOD >3%–80%) above the reference sequences. Information on mycobacterial species identification, based on *hsp65* sequence best match, and genotype of *Mycobacterium tuberculosis* complex strain, based on spoligotype and lineage-defining phylogenetic SNP, are shown in the center of the circle. AMI, amikacin; BDQ, bedaquiline; CAP, capreomycin; CFZ, clofazimine; EMB, ethambutol; ETH, ethionamide; FQ, fluoroquinolones; KAN, kanamycin; LIN, linezolid; INH, isoniazid; PZA, pyrazinamide; RIF, rifampin; SM, streptomycin; SNP, single-nucleotide polymorphism.

workers should also consider using enhanced diagnostics to help prevent the spread of drug-resistant TB.

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P.S. is a consultant for Genoscreen; S.D., F.D.M., and C.G. are employees of Genoscreen.

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Epidemiology of Nontuberculous Mycobacterial Infection, South Korea, 2007–2016

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The prevalence and incidence of nontuberculous mycobacterial (NTM) infections increased in South Korea from 2007 to 2016. Annual prevalence of NTM infection increased to 39.6 cases/100,000 population in 2016 and annual incidence to 19.0 cases/100,000 population. Overall prevalence for the study period was higher in the elderly, in females, and in cities.

The prevalence and incidence of nontuberculous mycobacterial (NTM) infection are increasing worldwide (*1*), and it is important to characterize the distribution of NTM infection by demographics or ecologic region to optimize disease control. However, data vary widely by study, and the epidemiology of NTM infection differs among countries. Although the number of patients with NTM infection has increased in South Korea (*2*), representative nationwide population-based epidemiologic data on the extent and distribution of NTM infection are lacking. We evaluated the prevalence, incidence, and spatial distribution of NTM infection in South Korea over a 10-year period, using national health insurance data.

The Study

We based our study on the Health Insurance Review and Assessment Service database, which includes universal health insurance claim data in South Korea. Approximately 97% of the population (≈52 million persons) is included in the system (*3*). We obtained demographic and medical data from the database for 2007–2016. We defined NTM infection as the presence of a diagnostic code associated with NTM infection (International Classification of Diseases, 10th ed., code A31) as either a primary or secondary diagnosis (*4*) and claims data for acid-fast bacilli smears or mycobacterial culture.

We defined annual prevalence as the number of all patients with NTM infection alive during each year and annual incidence as the number of newly diagnosed NTM infections occurring each year after excluding patients who were diagnosed in a previous study period (e.g., patients diagnosed during 2007–2015 were excluded from the estimate of the 2016 incidence rate). We first calculated the crude prevalence rate and incidence rate using each year's total population as the denominator. To adjust for different population structures between years, we calculated the age- and sex-standardized annual prevalence rate and incidence rate of NTM disease using the 2005 population as a standard population, based on the Statistics Korea method (http://kostat.go.kr/portal/eng/index.action). First, we calculated the prevalence and incidence rates for age and sex groups by stratifying all NTM cases into the groups and dividing by the group-specific populations in each year. Then, we multiplied each year's group-specific rates and the group-specific populations in 2005, combined the multiplied values, and divided by the 2005 total population. We calculated standardized overall period prevalence of NTM infection by age, sex, and administrative divisions using the population in the middle of the study period. We conducted statistical analyses using SAS version 9.4 (SAS Institute, http://www.sas.com). Because we used deidentified data in the study, institutional review board approval and patient consent were not required.

A total of 33,974 cases of NTM infection were identified during 2007–2016. The age- and sex-adjusted annual prevalence of NTM infection increased by more than 5-fold, from 6.7 cases/100,000 population in 2007 to 39.6 cases/100,000 population in 2016 (Figure 1). The age- and sex-adjusted annual incidence of NTM infection more than tripled, from 6.0 cases/100,000 population/year in 2008 to 19.0 cases/100,000 population/year in 2016. The overall period prevalence of NTM infection increased with patient age (Figure 2, panel A): prevalence was lowest at ≤19 years of age (1.7 cases/100,000 population), increased dramatically after 50 years of age, and peaked in those ≥70 years of age (223.0 cases/100,000 population). The overall period prevalence of NTM infection was higher among females in all age groups, except for those ≥70 years of age (Figure 2, panel B).

In South Korea, the overall period prevalence of NTM infection tended to be higher in metropolitan cities, which

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[1]These authors contributed equally to this article.

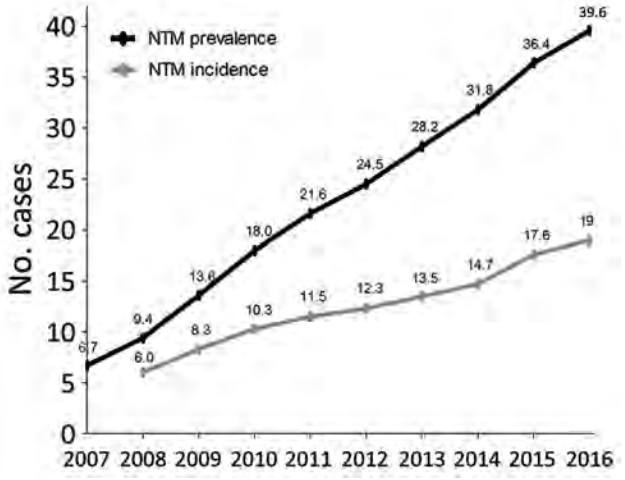

Figure 1. Annual prevalence (total no. cases/100,000 population) and incidence (no. new cases/100,000/year) of nontuberculous mycobacterial infection, adjusted for age and sex, South Korea, 2007–2016.

had greater population densities and higher income levels, than in the provinces (Table 1). The overall period prevalence exceeded 100 cases/100,000 population in most metropolitan cities (5/7, 71.4%) but did not exceed that rate in most provinces (7/9, 77.8%). The prevalence was highest in Gwangju and in Seoul, the capital of South Korea, both of which had >250 cases/100,000 population. Prevalence was lowest in Cheju, the largest and southernmost island (35.8 cases/100,000 population) (Appendix Figure, https://wwwnc.cdc.gov/eid/article/25/3/18-1597-techapp1.pdf).

Analysis of age and underlying conditions indicated that most case-patients (83.4%) were >50 years of age (Table 2). The most frequent underlying respiratory condition was asthma (53.0%), followed by bronchiectasis (43.6%) and chronic obstructive pulmonary disease (COPD) (32.3%). Lung cancer was the most common malignancy (6.3%). Depressive disorder was noted in 23.1% of case-patients.

Conclusions

We identified a remarkable increase in the prevalence and incidence of NTM infection in South Korea from 2007 to 2016. The overall period prevalence increased with patient age and was higher in female patients in most age groups. These trends are consistent with previous studies from Europe (*4*), the United States (*5*,*6*), and Japan (*7*,*8*). A representative US study that analyzed Medicare beneficiaries during 1997–2007 revealed that the prevalence of NTM disease increased from 20 to 47 cases/100,000 population and the prevalence was higher among women (*4*). A study conducted in Germany reported an increasing prevalence of NTM disease, from 2.3 cases/100,000 population in 2009 to 3.3 cases/100,000 population in 2014 (*4*). Studies in Japan, which is geographically close to South Korea, showed similar trends, but the rates were higher than those in the United States or Europe. The estimated annual prevalence of NTM disease in Japan in 2005 was 33–65 cases/100,000 population (*7*), and the incidence in 2015 was 14.7 cases/100,000 population (*8*), which were similar to our data. These results suggest that NTM infection is increasing globally, especially in East Asia, and that epidemiologic distribution can be affected by region or ethnicity. In particular, some studies have indicated that people in East Asia are vulnerable to NTM infection (*9*,*10*).

Another notable finding in our study was the difference in NTM prevalence by administrative division, which indicates that NTM infection is related to population density and the degree of urbanization. Supporting our data, other studies have attributed NTM infection to environmental exposure (*9*), and recent studies in the United States revealed that areas with high risk for NTM infection had higher population densities or higher education and income levels (*9*,*11*). In South Korea, the population density is highest in metropolitan cities. These data suggest the importance of epidemiologic surveillance in understanding NTM infection.

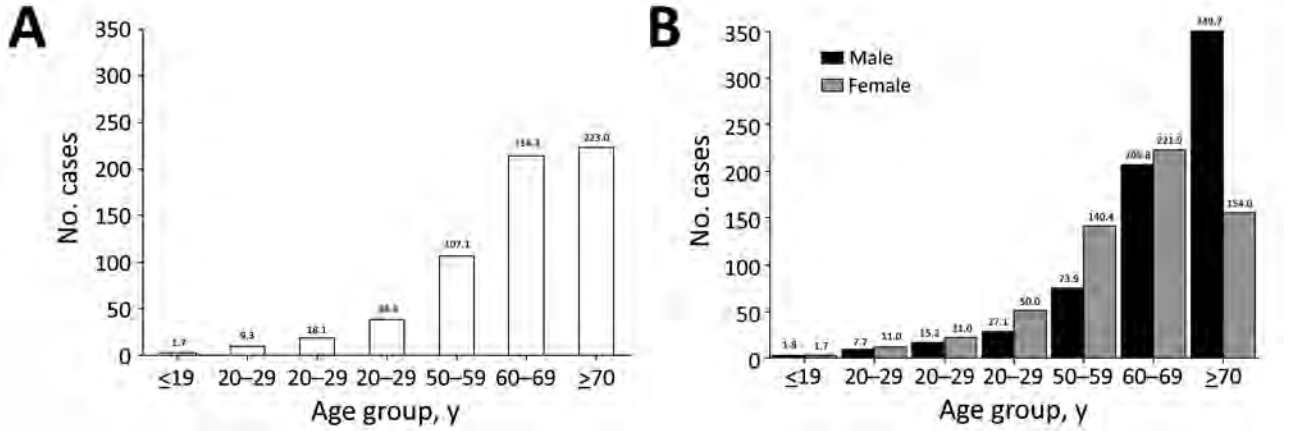

Figure 2. Overall period prevalence (total no. cases/100,000 population) of nontuberculous mycobacterial infection, by age group (A) and by age group and sex (B), South Korea, 2007–2016.

We frequently observed airway diseases in NTM infection in our study. A US study also reported COPD in 41% and bronchiectasis in 37% of NTM patients (*10*), whereas a study in Germany noted COPD or emphysema in 62%–79% of patients (*4*). One study reported mental disorders as concurrent NTM-related conditions (*12*). In our study, depression was also identified in NTM, which might be explained by the burden of COPD (*13*). These data suggest that an assessment of underlying disease is needed in managing NTM infection.

Our study had some limitations. First, because we used diagnosis codes, the distinction between pulmonary and other diseases is unclear, and we could not confirm whether the patients completely met the diagnostic criteria of NTM infection. Second, because we only have data for 2007–2016, we could not exclude patients who had received diagnoses before 2007 when estimating the annual incidence rate. Finally, our definition of NTM infection on the basis of the International Classification of Diseases code may have underestimated the true disease prevalence (*14*,*15*) because prevalence could be affected by loss to follow-up or death during the study period.

In conclusion, we report a substantial increasing trend in the prevalence and incidence of NTM infection in South Korea during 2007–2016 and evaluated regional variations. These data facilitate a better understanding of the epidemiologic trends of NTM infection globally.

Table 1. Overall prevalence of nontuberculous mycobacterial infections by administrative division, adjusted for age and sex, South Korea, 2007–2016

District	No. infections	No. cases/100,000 population
Metropolitan city		
Seoul	13,671	254.5
Busan	2,055	102.6
Daejeon	1,384	194.4
Gwangju	2,121	302.0
Daegu	1,642	119.9
Incheon	1,169	86.3
Ulsan	401	81.5
Province		
Gyeonggi-do	6,195	110.2
Gangwon-do	1,107	108.9
Jeollabuk-do	1,213	98.4
Jeollanam-do	470	35.1
Gyeongsangbuk-do	497	26.8
Gyeongsangnam-do	1,201	66.0
Chungcheongbuk-do	285	30.6
Chungcheongnam-do	444	35.2
Cheju-do (island)	119	35.8

Table 2. Sex, age, and underlying conditions among patients with nontuberculous mycobacterial infection, South Korea, 2007–2016*

Category	No. (%) infections, n = 33,974
Sex	
F	18,555 (54.6)
M	15,419 (45.4)
Age group, y	
≤19	197 (0.6)
20–29	622 (1.8)
30–39	1,479 (4.4)
40–49	3,341 (9.8)
50–59	7,880 (23.2)
60–69	8,961 (26.4)
≥70	11,494 (33.8)
Underlying condition	
Asthma	18,003 (53.0)
Bronchiectasis	14,815 (43.6)
COPD	10,969 (32.3)
Interstitial pulmonary diseases	1,832 (5.4)
Myocardial infarction	1,016 (3.0)
Congestive heart failure	3,581 (10.5)
Cerebrovascular disease	7,789 (22.9)
Cancer	7,808 (23.0)
Lung cancer	2,124 (6.3)
Stomach cancer	1,336 (3.9)
Colon cancer	975 (2.9)
Liver cancer	661 (2.0)
Prostate cancer	606 (1.8)
Breast cancer	577 (1.7)
Uterine cervical cancer	126 (0.4)
Thyroid cancer	673 (2.0)
Liver disease	15,218 (44.8)
Renal disease	1,391 (4.1)
Diabetes mellitus	11,010 (32.4)
Depressive disorder	7,857 (23.1)

*Underlying conditions are listed based on codes from the International Classification of Diseases, 10th edition. COPD, chronic obstructive pulmonary disease.

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Acute Flaccid Myelitis Associated with Enterovirus D68 in Children, Argentina, 2016

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After a 2014 outbreak of severe respiratory illness caused by enterovirus D68 in the United States, sporadic cases of acute flaccid myelitis have been reported worldwide. We describe a cluster of acute flaccid myelitis cases in Argentina in 2016, adding data to the evidence of association between enterovirus D68 and this polio-like illness.

We report a cluster of acute flaccid myelitis (AFM) cases in Buenos Aires, Argentina, in 2016. AFM was defined as acute flaccid paralysis (AFP) with magnetic resonance imaging (MRI) showing lesions predominantly affecting the gray matter of the spinal cord (*1*). We prospectively studied all patients with AFP who were admitted to Hospital de Niños "Ricardo Gutiérrez" in Buenos Aires during April 24–August 24, 2016, under the Argentine National Surveillance Acute Flaccid Paralysis Program for poliovirus as part of the World Health Organization AFP Program in the Americas. We obtained fecal samples or rectal swab specimens, serum samples, nasopharyngeal swab specimens, and cerebrospinal fluid (CSF) samples.

Fecal samples were tested at the National Reference Center for the Argentine National Surveillance Acute Flaccid Paralysis Program for enterovirus, including wild-type and vaccine-derived poliovirus. We screened clinical samples for enterovirus D68 (EV-D68) using a panrhinovirus and enterovirus nested PCR of enterovirus targeting the 5′ untranslated region (*2*). We purified the amplified products and prepared them for Sanger sequencing. We performed BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of GenBank sequences to identify which picornavirus was present. We obtained viral protein 1 partial sequences as previously described (*3*). In addition, we studied a wide panel of viruses (parainfluenza virus 1, 2, and 3; influenza A/B; respiratory syncytial virus; adenovirus; metapneumovirus; rhinovirus; varicella zoster virus; herpes simplex virus; cytomegalovirus) by reverse transcription PCR (RT-PCR) and studied bacteria by culture. We performed MRI and electromyography for all patients.

Fourteen children were admitted with AFP during April–August 2016. Six were confirmed to have AFM by case definition; the other 8 had alternative diagnoses, including Guillain-Barré syndrome (*3*), influenza virus myositis (*2*), encephalitis by echovirus (in 1 child with Down syndrome), acute transient hip synovitis (*1*), and transverse myelitis (*1*). Patients' clinical, demographic, and outcome findings are shown in Table 1, diagnostic findings in Table 2.

In 4 (66.7%) of 6 patients, we confirmed EV-D68 infection by nested RT-PCR. In 1 patient, enterovirus was detected but not typed; in 1 patient, no agent was detected. All patients had distinctive neuroimaging changes. We followed confirmed AFM cases for 6 months to assess clinical improvement.

The median age of patients with AFM was 3.9 (range 1–5) years; 4 (66.7%) of the 6 were female, and 3 (50%) had a history of asthma. All patients had prodromal signs or symptoms before onset of neurologic symptoms: 100% had upper respiratory tract infection (URTI); 4 (66.7%) had fever; and 1 (16.7%) had vomiting and abdominal pain. Neurologic symptoms appeared 1–11 (median 2) days after URTI symptoms.

Results of hematology and chemistry analysis were normal for 5 (83%) patients. Patient 1 had leukocytosis (leukocytes 18,000 cells/mm^3, with 82% neutrophils) and elevated levels of alanine aminotransferase (103 IU/L [reference 10–43 IU/L]), aspartate aminotransferase (97 IU/L [reference 10–35 IU/L]), and creatine kinase (6,591 IU/L [reference 24–170 IU/L]). During follow-up, patient 1 showed an increased creatine kinase level that could not be related to enterovirus infection.

All confirmed AFM case-patients showed T2 gray matter hyperintensity within the spinal cord on MRI. Electromyography showed early signs of denervation and low motor neuron function in all 5 patients in whom the test could be done. Specimen collection was performed 9.5

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Table 1. Demographics, neurologic symptoms, and clinical outcomes for patients with acute flaccid myelitis, Argentina, 2016*

Feature	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age, mo/sex	34/M	15/F	35/M	60/F	12/F	60/F
History of asthma	No	No	No	Yes	Yes	Yes
Preceding illness						
Fever	No	Yes	Yes	Yes	Yes	No
URTI	Yes	Yes	Yes	Yes	Yes	Yes
Gastrointestinal symptoms	No	No	Yes	No	No	No
Neurologic symptoms						
Limb, back, or neck pain	Yes	Yes	Yes	Yes	Yes	Yes
Arm weakness	Yes (bilateral)	Yes (right)	No	Yes (left)	Yes (bilateral)	Yes (bilateral)
Leg weakness	Yes (bilateral)	Yes (progressive, asymmetric, bilateral)	Yes (left progressive to bilateral, asymmetric)	Yes (progressive, asymmetric, bilateral)	Yes (bilateral)	Yes (bilateral)
Neck weakness	Yes	Yes	No	Yes	Yes	Yes
Facial weakness	No	No	No	Yes	No	Yes
Sensitivity involvement	No	No	No	No	No	No
Mental status involvement	No	No	No	No	No	No
Other neurologic deficits	Bulbar weakness	No	No	Left VII cranial nerve palsy	No	Bilateral VII cranial nerve palsy; bulbar weakness; tetraparesis
Severity of disease	ICU care; mechanical ventilation; tracheostomy; feeding support	Weakness	Weakness	ICU care; noninvasive positive pressure ventilation; feeding support	Progressive asymmetric 4-limb weakness	ICU care; mechanical ventilation; tracheostomy; feeding support
Outcome/sequelae	Persistent weakness; feet atrophy; equinus left foot; chronic noninvasive ventilation support	Partial recovery of weakness Atrophy of left foot	Recovery of right leg weakness; equinus left foot	Persistent leg left paralysis; 2 cm atrophy in left quadriceps	Persistent left arm paralysis and left leg weakness	Persistent leg paralysis and arm weakness; noninvasive ventilation support
Duration of hospitalization	6 mo	14 d	10 d	46 d	8 d	4 mo

*ICU, intensive care unit; URTI, upper respiratory tract infection.

(range 3–30) days after URTI symptoms started and 7.5 (range 1–18) days after onset of neurologic symptoms.

We identified enterovirus using nested RT-PCR of nasopharyngeal samples in 5 (83%) of 6 patients; 4 (80%) of 5 were typed as EV-D68, but in 1 patient (20%) the viral load was too low for typing. We identified EV-D68 in 2 (33%) of 6 fecal specimens. We performed molecular characterization of EV-D68 strains based on phylogenetic analyses of a partial VP1 genomic region (Figure).

Results of nested RT-PCR for enterovirus were negative for all CSF samples; results of the respiratory virus panel were negative for all patients. Neither bacteria nor fungus were isolated in blood or CSF samples. Serum PCR to identify herpes simplex virus, varicella zoster virus, and cytomegalovirus also yielded negative results.

Table 2. Diagnostic findings in patients with acute flaccid myelitis, Argentina, 2016

Laboratory tests	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Cerebrospinal fluid findings						
Leukocytes/mm^3 (% mononuclear cells)	195 (85)	4 (100)	23 (84)	130 (96)	40 (70)	16 (54)
Glucose, mg/dL, reference range 40–70	53	58	60	55	57	76
Protein, mg/dL, reference range 15–50	41	70	33	34	41	34
Albuminocytological dissociation	No	Yes	No	No	No	No
Virologic findings						
Enterovirus-D68	Yes	Yes	Yes	Yes	No	No
Nontypable enterovirus	No	No	No	No	No	Yes
Type of positive specimen						
Nasopharyngeal aspirate	Yes	Yes	Yes	Yes	No	Yes
Feces	No	Yes	No	Yes	No	No
Cerebrospinal fluid	No	No	No	No	No	No
Time from prodromal illness to specimen collection	5 d	30 d	13 d	6 d	25 d	3 d

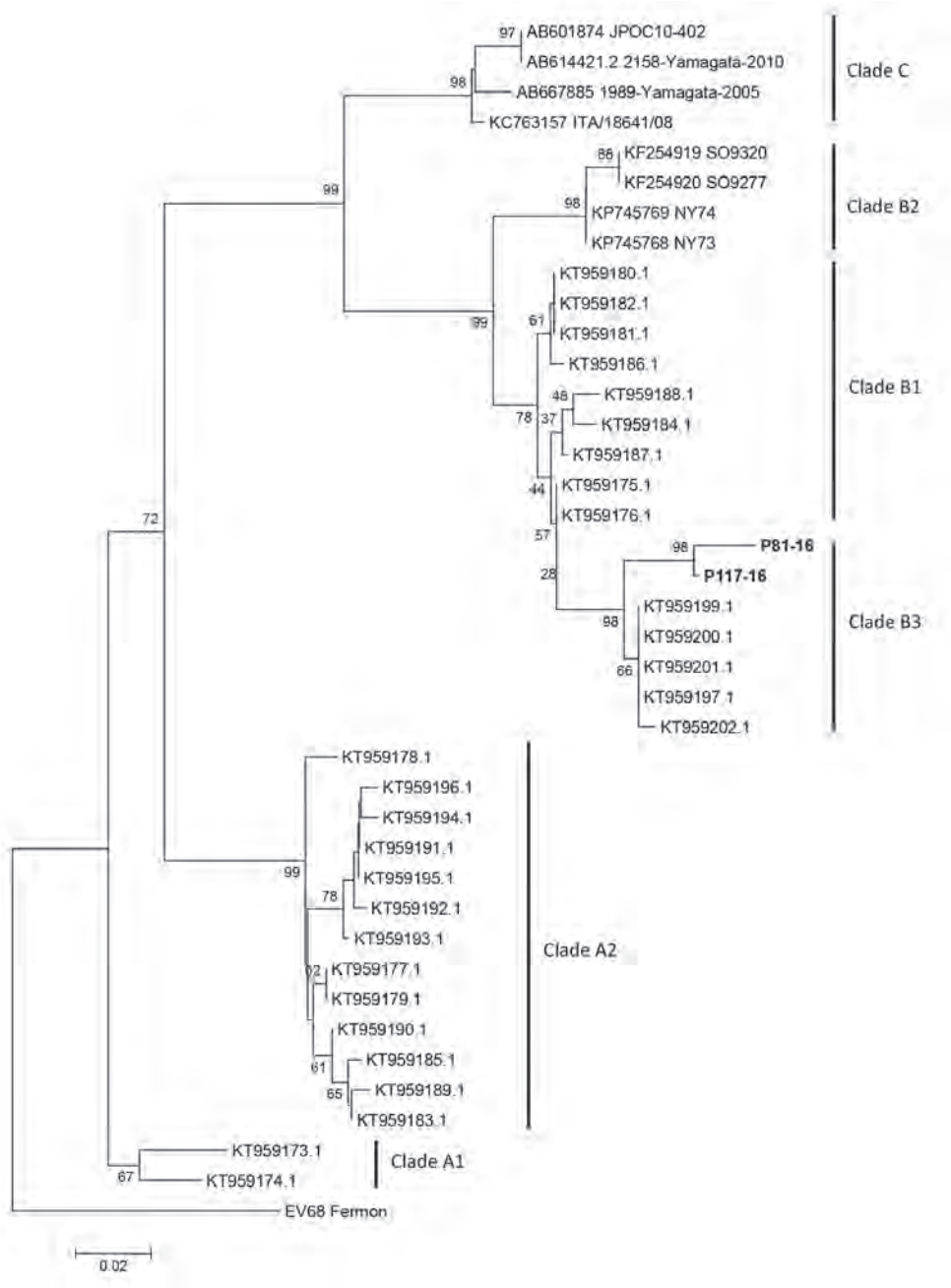

Figure. Molecular characterization of enterovirus D68 strains from Argentina, 2016, compared with reference strains from GenBank. Tree based on phylogenetic analyses of partial viral protein 1 genomic region (nucleotide positions 2554–2799, corresponding to the Fermon strain). Bold indicates strains detected in this study (GenBank accession nos. MF445419–20). We generated trees using the neighbor-joining method, as implemented in MEGA 6 software (http://www.megasoftware.net). Bootstrap values from 1,000 replicates are shown at the nodes. The trees were rooted with the prototype strain Fermon (GenBank accession no. AY426531). Scale bar indicates nucleotide substitutions per site.

Intravenous immunoglobulin was empirically infused in 5 (83%) patients; 2 (33%) received systemic corticosteroids. Three patients required intensive care unit admission. All patients had neurologic sequelae: persisting palsy in ≥1 limbs and atrophy of muscles with a shortening of limbs. Two patients required chronic noninvasive ventilatory support during 6 months of follow-up. No patients died.

Conclusions

AFM has been associated with different etiologic agents (*1*). EV-D68 is a nonpolio enterovirus characterized by affinity for α2–6-linked sialic acids typically found in the upper respiratory tract, making the respiratory tract the preferred target for EV-D68 replication, unlike most enteroviruses, which replicate in the gut (*1*,*4*). Although there is no definitive evidence of causality between EV-D68 and AFM, since the 2014 EV-D68 respiratory outbreak in North America, AFM cases possibly associated with EV-D68 have been reported in the United States, Canada, Australia, Norway, Great Britain, and France (*1*,*5*). We report a cluster of AFM associated with EV-D68 in Argentina; another institution in Argentina (Hospital Garrahan) has also reported a case series of AFM (*6*,*7*).

The cluster in this report occurred over a 3-month period, during the 2016 autumn–winter season, which is the typical enterovirus season in Buenos Aires. Clinical and neurologic findings were similar to those of cases reported in other countries, including URTI preceding the neurologic features (*4*,*8*,*9*). Patients were admitted with asymmetric, acute, and progressive weakness of limbs; areflexia; and muscle pain. These symptoms have been reported as polio-like syndrome; however, testing and MRI should be performed for multiple viruses, including enteroviruses and EV-D68, to detect distinctive spinal cord lesions. No sensory sensitivity involvement was observed. Two patients had cranial nerve dysfunction. Laboratory findings were similar to those previously described, including CSF abnormalities (*1*,*4*,*8*).

Different hypotheses to explain difficulties in isolation of EV-D68 have been reported (*4*). It is possible that most of the nasopharyngeal specimens in previous studies and in our cluster were taken after 7 days of URTI, when the viral load is usually low, as reported by Imamura et al. (*10*). In our case series, enterovirus was identified in respiratory secretions in 5 (83.3%) of 6 patients, even though specimen collection was performed >7 days (mean 9 days) after AFM onset (in 1 patient, viral load was too low for genotyping). The negative nasopharyngeal specimen was collected at 18 days after onset.

Isolation of EV-D68 in fecal samples is uncommon because the virus is both heat and acid labile (*1*). However, in 2 (33.3%) of our 6 patients, EV-D68 was identified in fecal samples.

Reported rates of CSF detection of known neurotropic enteroviruses, such as polioviruses and enterovirus A71, are as low as 0%–5%, although viruses could be detected in brain or spinal cord tissue (*4*,*11*). A recent mouse model of AFM caused by EV-D68 showed that EV-D68 infects anterior horn motor neurons, resulting in motor neuron death (*9*). In our series, CSF samples tested negative for EV-D68 and other pathogens.

No specific treatment for EV-D68 AFM is available; the US Centers for Disease Control and Prevention recommends only support measures (*7*,*12*). Zhang et al. demonstrated that commercial immunoglobulin contained high levels of neutralizing antibodies against EV-D68 strains during the 2014 outbreak in the United States (*13*). No vaccines are available.

EV-D68 belonging to subclade B3 was identified in our cluster by molecular sequencing. This subclade was associated with EV-D68 circulation in the United States and Europe in 2016 (*14*).

We show a cluster of AFM associated with EV-D68 in Argentina. Our findings contribute to global evidence of EV-D68 as a possible cause of localized polio-like illness.

About the Author

Dr. Carballo is a pediatric infectious diseases specialist at the Hospital de Niños "Ricardo Gutierrez" in Buenos Aires. Her research interests are pediatric infectious diseases.

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In Vivo Selection of a Unique Tandem Repeat Mediated Azole Resistance Mechanism (TR_{120}) in *Aspergillus fumigatus cyp51A*, Denmark

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We report a fatal aspergillosis case in which STR*Af* typing and whole-genome sequencing substantiated in vivo emergence of an azole-resistant *Aspergillus fumigatus* with a 120-bp tandem repeat in the promoter region of *cyp51A*. This event, previously restricted to the environment, challenges current understanding of azole resistance development in *A. fumigatus*.

Azole antifungal drug resistance in *Aspergillus fumigatus* is a concern for patients with aspergillosis because of increased risk for disease and death (*1*). Two routes of acquiring azole resistance have been identified: 1) in vivo, as a consequence of long-term azole treatment; and 2) ex vivo, in the environment, resulting from the use of azole fungicides in crop protection. The underlying mechanisms are primarily linked to structural changes or upregulation of the azole target lanosterol 14 α-demethylase encoded by *cyp51A* (*1*). Most environmentally induced resistance mechanisms involve tandem repeats (TRs) in the promoter region of *cyp51A* coupled with nonsynonymous mutations, TR_{34}/L98H and TR_{46}/Y121F/T289A (*1*). However, in vivo resistance development has primarily been associated with nonsynonymous mutations in *cyp51A*-inducing amino acid substitutions of hot spots (e.g., G54, G138, M220, and G448) or non–*cyp51A*-mediated mechanisms, but not a tandem repeat (*1*). We describe a clinical case of infection with azole-resistant *A. fumigatus* that acquired a 120-bp tandem repeat (TR_{120}) resistance mechanism during long-term azole treatment. The finding was substantiated by whole-genome sequencing (WGS).

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The Study

In 2013, a 69-year-old man who was a former smoker with chronic obstructive pulmonary disease (COPD) and severe airflow obstruction sought care at the University Hospital in Århus, Denmark, because of gradually worsening dyspnea, cough, and expectoration. Previously, in 2011, imaging (Figure 1, panel A) and 2 thoracoscopies had been conducted because of suspicion of malignant mesothelioma. Further histopathologic examination and cultures revealed inflammation but no malignancy or mold infection. Subsequently, in 2012, a fistula between pleura and skin led to a persistent air-containing pleural cavity in the right side (Figure 1, panel B). In 2014, a fungus ball in the pleural cavity was found (Figure 1, panel C). *Aspergillus* IgG titer was 1:25,600 (reference range ≤1:200), and azole-susceptible *A. fumigatus* was cultured from sputum (P-1, May 2014). Voriconazole (200 mg 2×/d) was given, alternating with posaconazole (300 mg/d) for 2 years until clinical failure, and 2 azole-resistant *A. fumigatus* isolates were cultured from a new sputum sample (P-2 and P-3, June 2016). Despite amphotericin B inhalations followed by liposomal amphotericin B (3 mg/kg 1×/d), the patient died because of severe hemoptysis 1 year later in 2017.

Three *A. fumigatus* patient isolates (P-1, P-2, and P-3) were available for confirmatory species verification, reference susceptibility testing defined by the European Committee on Antimicrobial Susceptibility Testing using protocol for molds (E.Def 9.3), *cyp51A* Sanger sequencing (using wild-type reference sequence AF338659), and genotyping using the short tandem-repeat *Aspergillus fumigatus* (STR*Af*) assay (*2*,*3*) (Table). We included 4 *A. fumigatus* isolates representing relevant *cyp51A* profiles as control strains (SSI-3614 [wild-type], SSI-7828 [TR_{34}/L98H], SSI-5717 [TR_{46}/Y121F/T289A], and SSI-5197 [F46Y/M172V/E427K]). We detected 3 common Cyp51A variants (F46Y, M172V, and E427K) in the susceptible patient isolate P-1 (GenBank accession no. MG972984). Pan-azole resistance was observed for P-2 and P-3, and both shared *cyp51A* profiles with P-1 but also harbored a TR_{120} mechanism (GenBank accession no. MG972983) in the promoter region (Table). All patient isolates had identical STR*Af* genotypes suggesting that they were isogenic (Table 1) (*4*). Furthermore, the STR*Af* profile was unique among *A. fumigatus* isolates genotyped in Denmark (Appendix Figure, https://wwwnc.cdc.gov/EID/article/25/3/18-0297-App1.pdf).

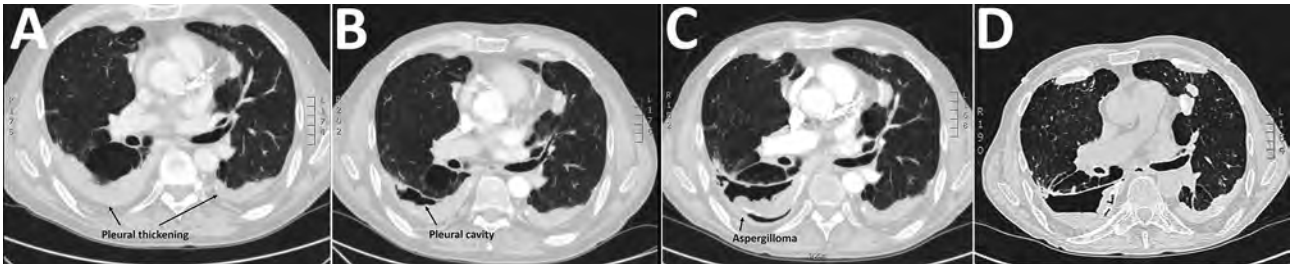

Figure 1. Sequential thoracic computed tomography scan images illustrating the gradual progression from pleural thickening to cavity formation and development of an aspergilloma in a patient with *Aspergillus fumigatus* infection, Denmark, 2013. A) 2011, B) 2012, C) 2014, D) 2016.

We performed WGS for P-1, P-3, and all control strains to investigate relatedness and other potential mechanisms conferring azole resistance. We subjected total DNA (≈10 ng/μL) to WGS (NextSeq 550; Illumina, https://www.illumina.com) by using Nextera DNA library preparation kit (Illumina) and following the manufacturer's instructions. We used NASP (*5*) to detect single-nucleotide polymorphisms (SNPs) after removal of duplicated regions in the *A. fumigatus* strain Af293 chromosomes (http://www.aspergillusgenome.org, genome version s03-m05-r09) using NUCmer (*6*). We inferred relatedness by using FastTree version 2.1.5 (*7*) and a 77.69% core genome (Table; Figure 2). To increase resolution, we conducted a subanalysis for P-1 and P-3 (core genome 79.71%), which identified 41 SNP differences; 6 of the SNPs were nonsynonymous in genes with no previous reported association to azole resistance (Appendix Table 1), and 35 were either synonymous or in noncoding regions (Appendix Table 2).

Conclusions

WGS revealed 41 SNP differences between the susceptible and the resistant patient *A. fumigatus* isolates that evolved during 2 years, similar to a previously described case of in-host microevolution of *A. fumigatus* (*4*). This finding substantiated an isogenic relationship between P-1 and P-3 and demonstrated that the TR_{120} resistance mechanism emerged from P-1, probably during long-term azole therapy. Furthermore, WGS results supported the conclusion that the TR_{120} was the sole mechanism of azole resistance in the azole-resistant patient isolates.

To our knowledge, the TR_{120} is a novel azole-resistance mechanism in *A. fumigatus*, and the in vivo selection of a tandem repeat in the promoter of *cyp51A* is unique. The de novo acquisition of a TR has not previously been shown in vitro or in the environment (i.e., no isolates with L98H or Y121F+T289A combined with wild-type promoters have been reported). However, triplication of an existing TR_{34} on tebuconazole exposure was selected in vitro, and a novel variant, $TR_{46}{}^{3}$, found in clinical and environmental samples, has been derived from sexual mating between TR_{46} parents (*8*,*9*).

Azole resistance involving TRs in the promoter region has been associated exclusively with environmental fungicide selection pressure in *A. fumigatus* and other plant pathogens. Furthermore, although asexual propagation of *A. fumigatus* with TR_{34}/L98H or TR_{46}/Y121F/T289A resistance mechanisms is widespread in the environment, the extent of de novo selection of TR_{34}/L98H and TR_{46}/Y121F/T289A is unclear (*10*). One hypothesis describes both environmental resistance mechanisms as being derived from single events of sexual reproduction (in environmental

Table. *Aspergillus fumigatus* strain characteristics, antimicrobial susceptibility, and molecular data, Denmark, 2013*

Isolate no.	EUCAST-based susceptibility MICs, mg/L VRZ	ITZ	POS	Sanger sequencing: Cyp51A profile§	STR*Af* assay genotyping data:† 2A-2B-2C-3A-3B-3C-4A-4B-4C	WGS data:‡ SNP differences compared with P-1
P-1	1	0.5	0.125	F46Y/M172V/E427K	10–13–10–17–13–8–7–5–6	0
P-2	4	16	0.5	TR_{120}/F46Y/M172V/E427K	10–13–10–17–13–8–7–5–6	NA
P-3	4	>16	0.5	TR_{120}/F46Y/M172V/E427K	10–13–10–17–13–8–7–5–6	41
SSI-5197	1	1	0.125	F46Y/M172V/E427K	10–15–10–28–13–11–7–5–6	4,968
SSI-7413	0.5	0.25	0.125	WT	21–25–19–28–12–6–20–10–8	105,900
Af293 (13)	1	0.5	0.06	F46Y/M172V/N248T/D255E/E427K	26–18–18–46–21–23–11–10–8	102,727
SSI-5946	4	>16	0.5	TR_{34}/L98H	20–21–12–84–10–7–8–9–10	108,901
SSI-5717	>4	0.5	0.25	TR_{46}/Y121F/T289A	26–21–16–32–9–10–8–14–10	108,882

*ITZ, itraconazole; NA, not available; POS, posaconazole; SNP, single-nucleotide polymorphism; STR*Af*, short tandem repeat *Aspergillus fumigatus*; VRZ, voriconazole (isavuconazole MICs were equivalent, data not shown); WGS, whole-genome sequencing; WT, wild-type.
†STR*Af* genotyping was performed as previously described (*3*). Underlined STR*Af* markers are shared with P-1.
‡Reference genome coverage ranged from 88.5% to 90.93%. Sequencing depth based on all assembled contigs >1,000 bp ranged from 57.2× to 80.7×; 71.1× for P-1; and 66.3× for P-3.
§TR_{34}: GAATCACGCGGTCCGGATGTGTGCTGAGCCGAAT, TR46: **AGTTGTCTA**GAATCACGCGGTCCGGATGTGTGCTGAGCCGAAT**GAA**, TR_{120}: TTCTCCTCTAGAAAAAACTCATGAGTGAATAATCGCAGCACCACTCCAG**AGTTGTCTA**GAATCACGCGGTCCGGATGTGTGCTGAGCCGAAT **GAA**AGTTGCCTAATTACTAAGGTGTAGT. GenBank accession numbers are MG972983 with TR_{120} and MG972984 without TR_{120}.

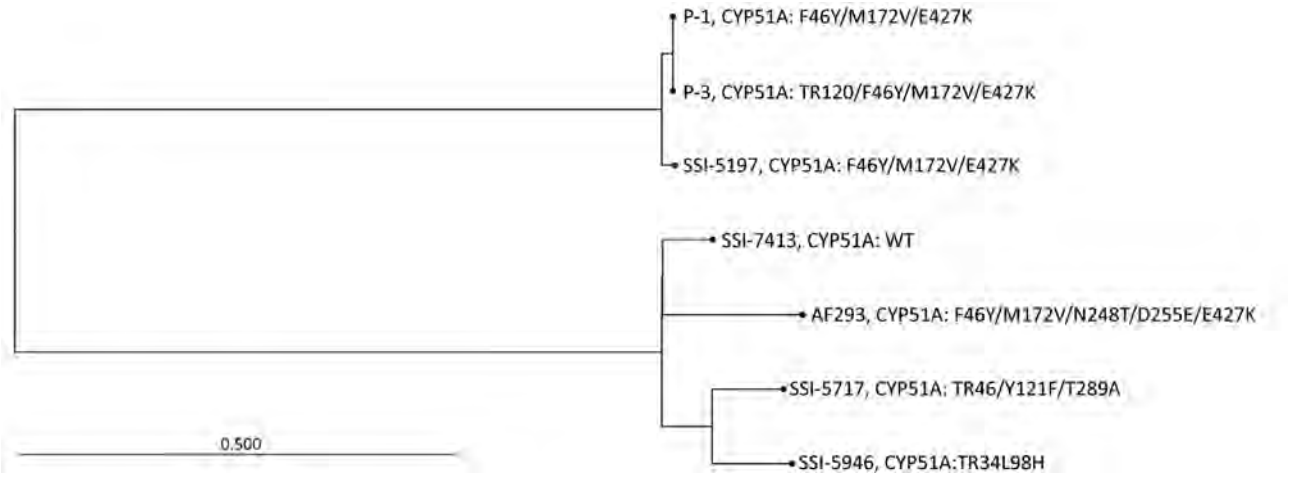

Figure 2. Unrooted phylogenetic tree based on whole-genome sequencing of 2 patient isolates (P-1 and P-3) and 5 reference strains to highlight relatedness between *Aspergillus fumigatus* isolates, Denmark, 2018. We inferred relatedness by using FastTree version 2.1 (*7*) based on a 77.69% core genome. Whole-genome sequencing identified 41 single-nucleotide polymorphism (SNP) differences between P-1 and P-3. We observed subtle differences (<5,000 SNPs) between unrelated patient isolate SSI-5197 and P-1/P-3, whereas >100,000 SNPs differed from P-1/P-3 to the other control strains and Af293. WT, wild-type. Scale bar indicates nucleotide substitutions per site.

habitats) combining the TR with a *cyp51A* mutant. In addition, sexual reproduction might have led to a high genetic diversity among environmental azole-resistant *A. fumigatus*, which otherwise might have indicated multiple origins (*10*). Our finding might challenge the perception that TR azole-resistance mechanisms are exclusive to the environment and might warrant the question of whether TR_{34}/L98H and TR_{46}/Y121F/T289A derive from single events.

Hypothetically, the patient might initially have inhaled isogenic isolates with and without TR_{120}, the resistant one being undetected. However, a patient being co-infected de novo by a susceptible and an isogenic resistant strain has not been previously reported and is considered highly unlikely.

Long-term and subtherapeutic antifungal treatment might facilitate selection of resistance (*11*). Therapeutic drug monitoring was performed once in this patient but without information if the sample was taken according to guidelines as a trough level (lowest level after dosage). Thus, despite a concentration of 4.3 mg/L (within the recommended trough range), potential subtherapeutic levels during the 200 mg 2×/d dosing scheme cannot be ruled out. The F46Y/M172V/E427K substitutions in Cyp51A, found in both susceptible and resistant isolates, have been suggested to play no role or only a minor role in reduced azole susceptibilities (*12*,*13*). TRs in the promoter region of *cyp51A* have previously been linked to increased *cyp51A* gene expression and MICs because of duplicated *srbA* transcription factor binding motifs (SRE1 and SRE2), leading to increased expression of *cyp51A* (*14*,*15*). Taken together, our data suggest that TR_{120} alone is an important driver of pan-azole resistance at a level comparable to that known to be mediated by the TR_{34}/L98H mechanism.

Our WGS results might obviate the desire for in vitro experiments testing the TR_{120} mechanism in laboratory-engineered mutants. Further dissection of the WGS data can help elucidate potential genetic drivers of TR acquisition and add further knowledge as to whether the TR_{34}/L98H and TR_{46}/Y121F/T289A resistance genotypes derived from a single origin. This report adds another piece to the complex picture of emerging azole-resistant *A. fumigatus* and might serve to stimulate further research.

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Listeria monocytogenes Associated with Pasteurized Chocolate Milk, Ontario, Canada

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In an investigation of a listeriosis outbreak in Ontario, Canada, during November 2015–June 2016, pasteurized chocolate milk was identified as the source. Because listeriosis outbreaks associated with pasteurized milk are rare in North America, these findings highlight that dairy products can be contaminated after pasteurization.

Listeria monocytogenes is a formidable pathogen acquired primarily through contaminated food. Invasive listeriosis is a reportable disease in Ontario, Canada; ≈50 case-patients (0.4 cases/100,000 persons) have been reported annually since 2005 (*1*). Recent outbreaks of listeriosis in North America have been associated with delicatessen meats, soft cheeses, raw produce, and unpasteurized dairy products (*2*–*4*). However, listeriosis outbreaks linked to pasteurized fluid milk are rare.

A study in the United States reviewed 83 fluid milk–associated disease outbreaks during 1990–2006; however, only 1 outbreak was attributed to *L. monocytogenes* (*5*). We report an outbreak of listeriosis associated with pasteurized chocolate milk in Ontario, Canada.

The Study

We defined an outbreak case-patient as a person in Ontario with listeriosis symptom onset after November 1, 2015, who had pulsed-field gel electrophoresis (PFGE) pattern combinations LMACI.0015/LMAAI.0024 or LMACI.0015/LMAAI.0069. Thirty-four case-patients met the outbreak definition; only Ontario residents were identified. Eleven case-patients had an onset date during November 14, 2015–February 14, 2016. Onset dates ranged from April 11 to June 20, 2016, for 21 case-patients in the second wave; the remaining 2 case-patients were outliers (Figure 1). Median age was 73 years (range <1–90 years). More than half of the case-patients were female (20/34, 59%). Hospitalizations occurred for 32 (94%) case-patients, and 4 deaths (12%) were reported.

In Ontario, local public health professionals complete the national invasive listeriosis questionnaire and collect food samples. We conducted a case–case analysis by using Ontario case-patients listed in the national listeriosis database as controls. We used a variety of methods to support hypothesis generation, including supplemental questionnaires, centralized interviewing, and reviewing purchase records collected through shoppers' loyalty card programs. A meeting was also held with representatives from a grocery chain that was common for case-patients (retail chain A) for insights into possible sources.

PFGE and whole-genome sequencing were performed at the Public Health Ontario Laboratory, in accordance with PulseNet Canada protocols (Table; Appendix Figure, https://wwwnc.cdc.gov/EID/article/25/3/18-0742-App1.pdf). Food safety investigations, including targeted retail sampling, were conducted by the Canadian Food Inspection Agency and Ontario Ministry of Agriculture, Food and Rural Affairs. Laboratory analyses of food samples were conducted by the Canadian Food Inspection Agency and the Public Health Ontario Laboratory.

Several hypotheses were generated during the course of this outbreak. In the first wave, a concurrent listeriosis outbreak associated with leafy greens was ongoing in the United States and Canada. However, product testing did not establish a relationship between the 2 outbreaks. Cheddar cheese was also suspected, but a food safety investigation, including sampling at the manufacturer, did not support a link to this outbreak (*6*,*7*). Although leafy greens and cheddar cheese were ruled out, 1 commonality remained; shopping at retail chain A was reported frequently by case-patients.

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Table. Characteristics of 23 *Listeria monocytogenes* isolates analyzed by whole-genome sequencing during listeriosis outbreak in pasteurized chocolate milk, Ontario, Canada*

Isolate ID	Isolate source	PFGE pattern, first enzyme/second enzyme	SRA accession no.
ON-1501	Human	LMACI.0015/LMAAI.0069	SAMN09909078
ON-1502	Human	LMACI.0015/LMAAI.0024	SAMN09909079
ON-1503	Human	LMACI.0015/LMAAI.0024	SAMN09909080
ON-1601	Human	LMACI.0015/LMAAI.0069	SAMN09909081
ON-1602	Human	LMACI.0015/LMAAI.0024	SAMN09909082
ON-1603	Human	LMACI.0015/LMAAI.0069	SAMN09909083
ON-1604	Human	LMACI.0015/LMAAI.0024	SAMN09909084
ON-1605	Human	LMACI.0015/LMAAI.0069	SAMN09909085
ON-1606	Human	LMACI.0015/LMAAI.0069	SAMN09909086
ON-1607	Human	LMACI.0015/LMAAI.0069	SAMN09909087
ON-1608	Human	LMACI.0015/LMAAI.0024	SAMN09909088
ON-1609	Human	LMACI.0015/LMAAI.0069	SAMN09909089
ON-1610	Human	LMACI.0015/LMAAI.0024	SAMN09909090
ON-1611	Human	LMACI.0015/LMAAI.0069	SAMN09909091
ON-1612	Human	LMACI.0015/LMAAI.0024	SAMN09909092
ON-1613	Human	LMACI.0015/LMAAI.0069	SAMN09909093
ON-1614	Human	LMACI.0015/LMAAI.0069	SAMN09909094
ON-1615	Human	LMACI.0015/LMAAI.0069	SAMN09909095
ON-1616	Human	LMACI.0015/LMAAI.0069	SAMN09909096
ON-1617	Human	LMACI.0015/LMAAI.0069	SAMN09909097
ON-1618	Human	LMACI.0015/LMAAI.0069	SAMN09909098
ON-1619	Human	LMACI.0015/LMAAI.0069	SAMN09909099
ON-1620	Human	LMACI.0015/LMAAI.0069	SAMN09909100
ON-1621	Human	LMACI.0015/LMAAI.0069	SAMN09909101
ON-1622	Human	LMACI.0015/LMAAI.0069	SAMN09909102
ON-1623	Chocolate milk	LMACI.0015/LMAAI.0069	SAMN09909103

*All strains were from Bioproject PRJNA486837. ID, identification; ON, Ontario; PFGE, pulsed-field gel electrophoresis; SRA, sequence read archive.

A second wave began in April 2016 in which 10 of 17 case-patients reported consuming coleslaw. Six case-patients ate coleslaw from the same manufacturer, which supplied retail chain A and a fast food restaurant chain. However, the food safety investigation, including sampling at the manufacuturer and supplier, did not support this hypothesis.

On May 24, 2016, *L. monocytogenes* isolated from expired bagged chocolate milk collected from the home of 1 case-patient was confirmed to have the outbreak strain PFGE pattern. Fluid milk in Canada is often sold in plastic bags (Figure 2). In this instance, the outer packaging, which is the only area that contains the brand name, was discarded. Thus, the brand name was uncertain, and efforts were undertaken to confirm the source of the chocolate milk. Because the proxy of the case-patient reported purchasing brand B milk, samples of brand B chocolate and white milk were collected from retail for testing. Brand B was the main brand of chocolate milk sold by retail chain A, and it is distributed primarily in Ontario.

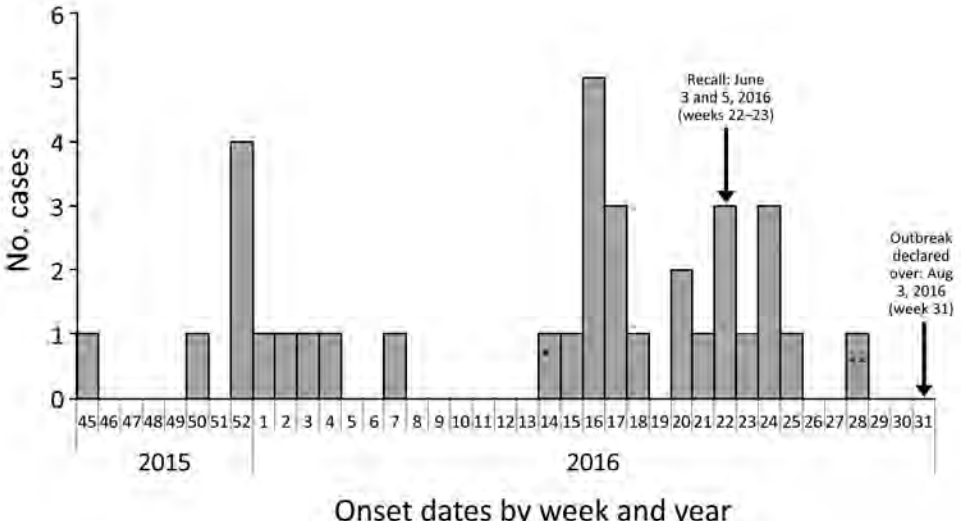

Figure 1. Outbreak cases of listeriosis (n = 34) by onset week and year, Ontario, Canada, November 2015–August 2016. Data were obtained from the Ontario Ministry of Health and Long-Term Care, integrated Public Health Information System database, extracted by Public Health Ontario, August 16, 2016. Weeks are defined according to the Public Health Agency of Canada epidemiologic week calendar. *Neonatal case-patient with symptom onset on April 4, 2016 (week 14), and illness most likely caused by mother-to-child transmission. **Asymptomatic case-patient from whom a specimen was collected on July 13, 2016, and exposure occurred before June 27, 2016 (week 28).

Although the hypothesis-generating questionnaire used stipulated milk, with flavored milk as a prompt, chocolate milk was not specified, and as a result this type of milk might have been underreported. Exposure to pasteurized milk was reported by 60% of case-patients in the first wave compared with 76% of controls. Thus, milk was not originally pursued as a source. However, this new positive isolate led to reinterviewing of case-patients from the second wave and resulted in 9 (75%) of 12 case-patients reporting consuming brand B when asked specifically about chocolate milk.

On June 3, a retail sample of brand B chocolate milk produced at facility C was confirmed positive for *L. monocytogenes*. This finding led to a class I recall of 1 lot of brand B chocolate milk. On June 5, the recall was expanded to all lots of brand B chocolate milk processed at that facility that only distributes in Ontario. Isolates from the original sample and 3 subsequent positive samples of chocolate milk, obtained from extensive retail sampling, matched the outbreak strain by PFGE and whole-genome sequencing. No white milk samples were positive for *L. monocytogenes*.

Environmental sampling at the manufacturer confirmed the presence of the outbreak strain within a postpasteurization pump dedicated to chocolate milk and on nonfood contact surfaces. This postprocess contamination of the chocolate milk line was believed to be the root cause of the outbreak. A harborage site might have been introduced by a specific maintenance event or poor equipment design. The equipment was subsequently replaced, and corrective measures were implemented to prevent recurrence. Chocolate milk production was resumed after rigorous testing for *L. monocytogenes* under regulatory oversight.

Figure 2. Bags of pasteurized chocolate milk as sold in Canada, with outer bag containing brand information removed. A bag of milk similar to these, found at the home of 1 case-patient during investigation of an outbreak of *Listeria monocytogenes* infection associated with pasteurized chocolate milk in Ontario, Canada, was found to be contaminated with the same strain obtained from infected patients.

Conclusions

This outbreak lasted 7 months and resulted in 34 confirmed listeriosis case-patients. Discovering the cause of this listeriosis outbreak was challenging because pasteurized chocolate milk is a commonly consumed product. Although there have been previous outbreaks outside Canada caused by chocolate milk (*8*), pasteurized milk products are generally not expected to be the source. This outbreak highlights that even pasteurized products can be contaminated by and support the proliferation of *L. monocytogenes* when contamination is introduced postpasteurization. The possibility of postprocessing contamination indicates an ongoing need for regulatory oversight and robust quality assurance processes, which include routine sampling of the environment and finished products.

Brand B chocolate milk is a widely distributed product in Ontario, and contamination of this product could have resulted in >34 case-patients. It is possible that a lower number of case-patients were reported because chocolate milk may primarily be consumed by younger, healthier persons, in whom invasive listeriosis is less likely to develop (*9*). Another possible explanation is that the contamination in the milk appeared to be intermittent, with some samples testing positive and others testing negative. As such, careful attention should be given to equipment design and maintenance programs, as harborage sites could result in recurring contamination that goes undetected by routine monitoring. Targeted retail and environmental sampling was instrumental in identifying the root cause in the facility and the breadth of potentially implicated products in the marketplace. Thus, this type of sampling should be considered during outbreak investigations.

Ultimately, the implicated product was determined on the basis of testing of food items obtained from the home of 1 case-patient. This finding highlights the necessity of obtaining a thorough food history and collecting and testing available samples of food that case-patients consumed during the incubation period (*10*). In Canada, where bagged milk is common, labeling of the inner and outer bags with the brand name would facilitate product identification by consumers. This recommendation could extend to other food products in North America (e.g., frozen hamburger patties) that have multiple layers of packaging (*11*).

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Neutralizing Antibody against Enterovirus D68 in Children and Adults before 2014 Outbreak, Kansas City, Missouri, USA[1]

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We evaluated enterovirus D68 seroprevalence in Kansas City, Missouri, USA, from samples obtained during 2012–2013. Neutralizing antibodies against Fermon and the dominant 2014 Missouri isolate were universally detected. Titers increased with age. Widespread circulation of enterovirus D68 occurred before the 2014 outbreak. Research is needed to determine a surrogate of protection.

The first enterovirus D68 (EV-D68) isolate (Fermon) was identified in 1962 (*1*,*2*). Before a 2014 EV-D68 outbreak, US reports of EV-D68 were relatively sparse (<100 sporadic cases and periodic outbreaks in 50 years) (*3*). In autumn 2014, a total of 1,153 confirmed EV-D68 cases occurred in 49 states and the District of Columbia; EV-D68 patients mostly had respiratory symptoms consistent with those in previous EV-D68 outbreaks and cases (*4*). Nationwide, severe disease occurred mostly in school-aged children. Through September 2014, EV-D68 was detected in 338 of 551 children with rhinovirus/enterovirus-positive test results; most (61.3%) were hospitalized at The Children's Mercy Hospital (Kansas City, MO, USA). Hospitalized EV-D68 patients often had asthma or recurrent wheezing. Many of these EV-D68–infected children had unusually severe, refractory bronchospasms, which resulted in 100 intensive care unit stays (*5*).

The Study

We assessed the prevalence of EV-D68 neutralizing antibody in the Kansas City population before the 2014 outbreak using deidentified banked serum samples (stored at The Children's Mercy Hospital) collected in 2012 (n = 155) and 2013 (n = 281) from healthy persons >2 years of age during a poliovirus seroprevalence study (*6*). Age, sex, and race/ethnicity distributions matched 2010 Kansas City census data (Appendix, https://wwwnc.cdc.gov/EID/article/25/3/18-0960-App1.pdf) (*6*).

We performed serology testing at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA). We used an adapted poliovirus microneutralization assay to test samples for neutralizing antibodies (*6*,*7*) against 4 phylogenetically distinct EV-D68 isolates: Fermon (GenBank accession no. NC038308); the 2014 Missouri isolate 14-18949 (clade B1, GenBank accession no. KM851227); and 2 related but non-Missouri 2014 isolates, 14-18952 (clade B2) and 14-18953 (clade A2; GenBank accession nos. KM851230–1; Figure 1; Appendix) (*9*). The proportion of US patients from whom these three 2014 circulating strains were detected was >91% for 14-18949, 7.4% for 14-18952, and <2% for 14-18953 (*10*).

Besides age, sex, and race/ethnicity (Hispanic vs. non-Hispanic), population demographics are descriptive; small subset numbers precluded formal statistical analysis. We performed analyses using Sigmaplot version 12.2 (http://www.sigmaplot.co.uk/index.php; Appendix), and we considered p values <0.05 significant.

Of 436 serum samples, 217 were from male donors; median age was 13 (range 2–81) years. All had neutralizing antibody (i.e., $\geq$3 $\log_2$, $\geq$1:8 titer) against Fermon and 14-18949 (Table); 97% of samples had neutralizing antibody to 14-18953 and 89% to 14-18952. Overall seropositivity for the 4 isolates was not different (p = 0.763).

In total, 50% (24/48) of the 14-18952–seronegative samples and 57% (24/42) of the 14-18953–seronegative samples (p<0.001) came from male donors. Our 2–5-year-old age group made up the largest proportions of these seronegative populations (67% [32/48] of 14-18952 and 36% [15/42] of 14-18953; p = 0.003). The 21 persons seronegative for both 14–18952 and 14–18953 had a median age of 3 (2–61) years. Donor sex and race/ethnicity and season of sample acquisition did not differ between samples seropositive and seronegative for 14-18952 or 14-18953 (data not shown).

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[1]Preliminary results from this study were presented at the Clinical Virology Symposium, May 19–22, 2016, Daytona Beach, Florida, USA.

[2]These authors contributed equally to this article.

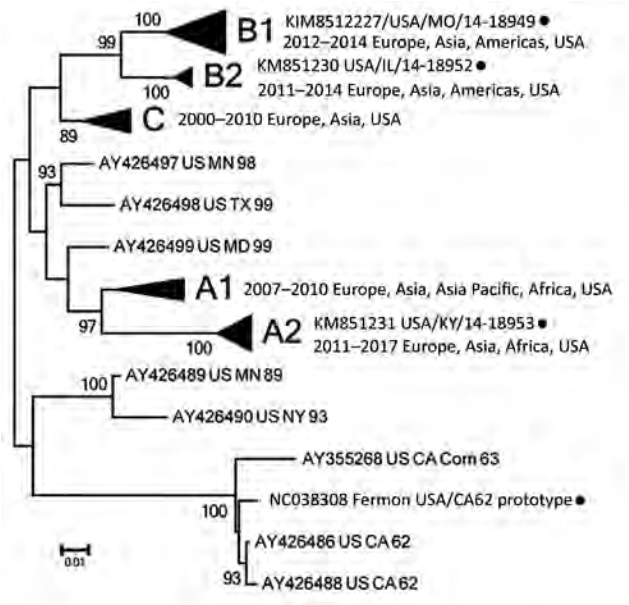

Figure 1. Unrooted neighbor-joining tree depicting phylogenetic relatedness of reference enterovirus D68 isolates (black circles) used in microneutralization assays in study of enterovirus D68 seroprevalence among children and adults in Kansas City, Missouri, USA, 2012–2013. We constructed tree using complete virus protein 1 gene sequences and MEGA6.0 (*8*). Branching within major clades (bolded) is collapsed for clarity.

Neutralizing antibody titers also did not differ by season of sample acquisition, donor race/ethnicity, or donor sex. Mean titers to 14-18949 were lower ($p<0.05$) among self-identifying Hispanics (n = 36; 7.1 $\log_2$, range 3.83–10.5 $\log_2$) than among non-Hispanics (n = 400; 7.83 $\log_2$, range 3.17–10.5 $\log_2$), but this difference might not be clinically significant. Median titers rose with each advancing age group, except against Fermon among 11–15-year-olds ($p<0.001$; Table). The overall median titer was highest for 14-18952 (8.34 $\log_2$, range 2.5–10.5 $\log_2$; $p<0.001$), despite a comparatively lower seropositivity (89%). The overall titer was lowest for 14-18953 (6.83 $\log_2$, range 2.83–10.5 $\log_2$; $p<0.001$), despite a relatively high seropositivity (91%).

All serum samples had neutralizing antibody against the major EV-D68 isolates circulating in the United States in 2014, and most had antibody to the other 2 less frequently detected isolates that year (Figure 2; Appendix Figure). During the 2014 outbreak, 5–10-year-olds (who would have been 3–8-year-olds during the sampling time of our study) had the most severe disease. Severe EV-D68 disease occurred often in children with atopic disease, reactive airway disease, or asthma. In 2014, little EV-D68 disease was noted among adolescents, adults, or the elderly (*4*). The age-associated severe EV-D68 respiratory disease observed in 2014 parallels our finding of lower overall titers in 2–5-year-olds and 6–10-year-olds.

Although introduction of EV-D68 into naive populations could have explained the 2014 outbreak, universal detection of antibody against 14-18949 (dominant 2014 isolate) before 2014 indicates previous widespread exposure to 14-18949 or a related isolate. EV-D68 was also detected in Kansas City as early as 2009 (F. Hassan, University of Missouri at Kansas City, pers. comm., May 2018). Thus, the Kansas City 2014 outbreak did not occur because of population naivete to a 14-18949–like isolate. Indeed, neutralizing antibodies to other EV-D68 isolates were also detected (*4*,*10*). That 2–5-year-olds in our study had lower titers to 14-18949 (Figure 2) suggests that older persons had more experience with 14-18949 or confirms that antibody elicited by non–14-18949 isolates can also neutralize 14-18949. Severe disease during the 2014 outbreak occurred among children who, according to our results, were relatively experienced with this pathogen; they were positive for neutralizing antibodies against 14-18949 but had lower median titers and a reduced reverse cumulative distribution compared with other age groups.

Why this large outbreak was able to occur in a population with a high prevalence of neutralizing antibody against the outbreak isolates remains unclear. One possibility is that respiratory tract mucosal antibody (probably in the form of secretory IgA) is more relevant than serum antibody for protection against respiratory disease (*11*). In this study, we could not address this possibility because only serum samples were available for testing. Also, certain persons could be more susceptible to severe disease because of genetic factors, preexisting atopy or asthma, or differences in other parts of the immune response, including immunopathologic responses. The argument for multiple factors contributing to disease despite the presence of neutralizing antibody is bolstered by the predilection of persons with asthma or atopic disease to have severe disease (*5*); further, asthma patients experience more tight junction injury than persons without asthma during rhinovirus infection (*12*).

The only demographic factor potentially affecting titers was Hispanic race/ethnicity. However, the ≈0.8 $\log_2$ difference might not be clinically significant, considering the median titers in both groups were >5 $\log_2$; thus, whether race/ethnicity is a factor is unclear.

Our data parallel another study with a similar 2011 sampling timeframe conducted in China (*13*). In that study, neutralizing titers against Beijing/2008/01 EV-D68 were low in serum samples collected in 2004 for all age groups, but their 2011 titers resembled our data, despite few reported EV-D68 illnesses in the sampled area during 2007–2011. Their lowest overall titers were also observed in persons of younger age groups.

EV-D68 has been proposed as a cause of acute flaccid myelitis. Increasing reports of this condition underscore the need to better understand EV-D68 seroprevalence and circulation (*14*).

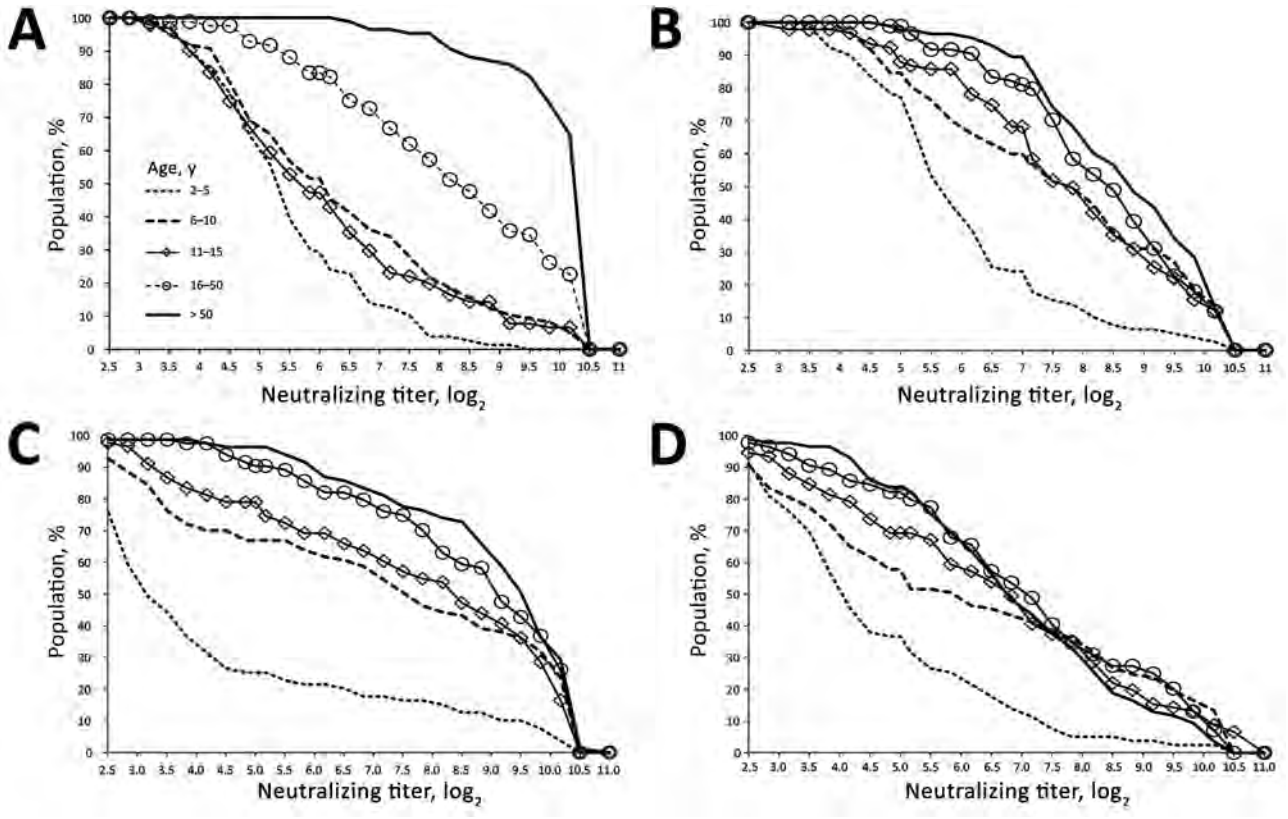

Figure 2. Reverse cumulative distribution curves of neutralizing antibody titers against enterovirus D68 isolates, by isolate and age group, Kansas City, Missouri, USA, 2012–2013. A) Fermon; B) dominant 2014 isolate 14-18949; C) less frequently circulating 2014 isolate 14-18952; D) rare 2014 isolate 14-18953. The reverse cumulative distribution pattern for 14-18949 varied the least by age group.

Table. Neutralizing antibody positivity and titers for each enterovirus D68 isolate, by age group, Kansas City, Missouri, 2012–2013

		% Neutralizing antibody positive; median (range) neutralizing antibody titer*			
Age group, y	No.	Fermon	14-18949	14-18952	14-18953
2–5	79	100; 5.50 (3.17–9.5)	100; 5.83 (3.5–10.5)	60; 3.17 (2.5–10.5)	81; 4.17 (2.5–10.5)
6–10	97	100; 6.17 (3.17–10.5)	100; 7.83 (4.17–10.5)	89; 7.83 (2.5–10.5)	83; 6.17 (2.5–10.5)
11–15	91	100; 5.83 (3.17–10.5)	100; 7.83 (3.17–10.5)	97; 8.50 (2.5–10.5)	93; 6.50 (2.5–10.5)
16–50	84	100; 8.50 (3.83–10.5)	100; 8.50 (4.83–10.5)	98; 9.17 (2.5–10.5)	96; 7.17 (2.5–10.5)
>50	85	100; 10.50 (6.5–10.5)	100; 8.83 (4.83–10.5)	99; 9.50 (2.5–10.5)	98; 6.83 (2.5–10.5)
Total	436	100; 6.83 (2.83–10.5)	100; 7.83 (3.5–10.5)	89; 8.34 (2.5–10.5)	91; 6.50 (2.5–10.5)

*Antibody titers were measured by using the cell viability kit ATPlite (Perkin Elmer, http://www.perkinelmer.com); the titers shown are the $\log_2$ inverse dilution of the lowest antibody concentration with luminescent activity.

Our study had several limitations (Appendix). Because of the retrospective study design, our data and interpretations are limited regionally and temporally. We tested for only 4 select EV-D68 isolates, and antibody reactivity with other isolates might differ.

Conclusions

We detected at least some neutralizing antibody to Fermon and the dominant 2014 isolate (14-18949) in all 436 EV-D68 samples acquired during 2012–2013 in Kansas City. Prospective studies are warranted to define a protective threshold of serum neutralizing antibody (or a surrogate of protection), the distribution of titers in children <2 years of age, and whether antibody levels differ by race/ethnicity.

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Prospective Whole-Genome Sequencing in Tuberculosis Outbreak Investigation, France, 2017–2018

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During June 2017–April 2018, active tuberculosis with Beijing SIT1 isolates was diagnosed in 14 persons living in 4 distant cities in France. Whole-genome sequencing indicated that these patients belonged to a single transmission chain. Whole-genome sequencing–based laboratory investigations enabled prompt tracing of linked cases to improve tuberculosis control.

France is a low-prevalence country for tuberculosis (TB); mean incidence was 7.1 cases/100,000 inhabitants in 2015 (*1*). Auvergne-Rhône-Alpes is the region of France with the second highest prevalence of TB (428 cases notified in 2015). Genotyping is not yet routinely performed for all *Mycobacterium tuberculosis* isolates in France. However, prospective surveys of *M. tuberculosis* strains isolated in the Auvergne-Rhône-Alpes region have been conducted since 2008 by using systematic genotyping to detect TB transmission events and to comprehend complex relationships between certain lineages and the geographic origin of clinical cases (approved by the Comité de Protection des Personnes Sud-Est IV under no. DC-2011–1306) (*2*,*3*). Since November 2016, whole-genome sequencing (WGS) of *M. tuberculosis* isolates was implemented in the Lyon University Hospital laboratory (Lyon, France) on a routine basis to discriminate transmission clusters otherwise undetected by the spoligotyping method. We previously reported on the effect of immigration on imported *M. tuberculosis* cases in the Auvergne-Rhône-Alpes region (*3*); here, we report the rapid spread of a specific *M. tuberculosis* strain and the need for continued vigilant surveillance using advanced molecular techniques.

The Study

During June 2017–April 2018, active TB was diagnosed in 11 persons living in the same city of Auvergne-Rhône-Alpes; all isolates belonged to Beijing SIT1 spoligotype. WGS analysis confirmed that these 11 patients were infected with the same strain, along with 3 other isolates collected in 3 different and distant cities in France, indicating a clonal outbreak.

During June–September 2017, three cases of active TB were diagnosed from the same city in the Auvergne-Rhône-Alpes region. Patients belonged to 2 families without previously known relationships. One of the 3 patients (index case-patient [chronological case-patient 5]) had a 2-year history of confirmed TB; this patient originated from Cape Verde and reported frequent recent travel there. Direct microscopic examination of sputum showed very high titers of acid-fast bacilli, implying a high risk for onward *M. tuberculosis* transmission. In the other 2 case-patients (chronological case-patients 3 and 4), TB was newly diagnosed; they had no history of or suspicion for infection, but both resided in the same household. Isolates from all 3 cases were susceptible to standard first-line anti-TB drugs, and spoligotyping revealed that all belonged to Beijing SIT1 lineage. In parallel with conventional membrane-based spoligotyping, WGS was performed to ascertain whether it was the same strain (Appendix, https://wwwnc.cdc.gov/EID/article/25/3/18-1124-App1.pdf). WGS analysis indicated

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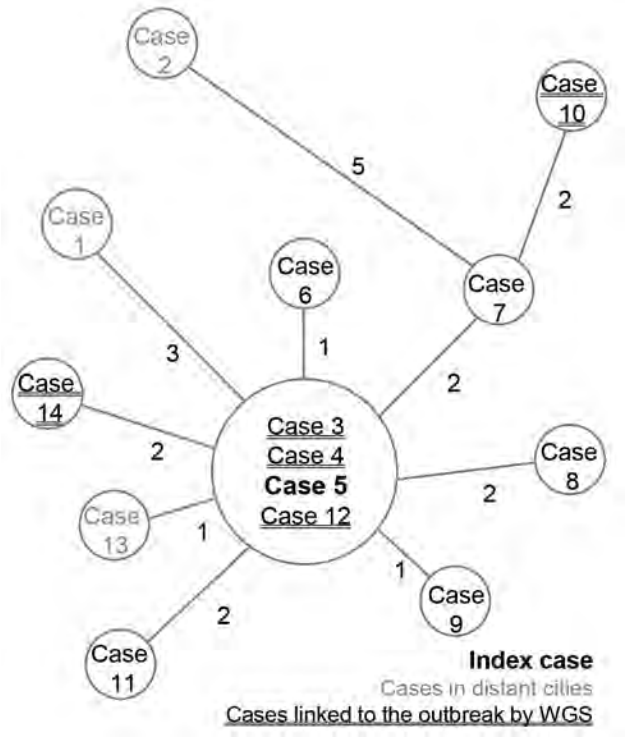

Figure 1. Minimum-spanning tree of single-nucleotide polymorphism differences from 14 *Mycobacterium tuberculosis* outbreak isolates, France, 2017–2018. Cases were numbered according to sampling chronology. Sizes of circles are proportional to the number of isolates with identical genomes; numbers adjacent to lines indicate number of single-nucleotide polymorphism differences between each node. WGS, whole-genome sequencing.

that the strains were identical, meaning 0 single-nucleotide polymorphism (SNP) distance (Figure 1).

Investigations and interviews conducted by the TB control center later revealed that the 2 families did have a connection, through frequent contacts between case-patient 4 and the index case-patient (case-patient 5). These results led the TB control center to widen the circle of investigations beyond household and frequent contacts and to expand the investigation to occasional contacts (including persons from other geographic regions) and persons attending the public places frequented by the index case-patient.

During September 2017–April 2018, extended contact tracing identified 5 additional cases of active TB within the index city and 3 epidemiologically linked cases in distant areas (Île-de-France, Hauts-de-France, and Provence-Alpes-Côte d'Azur). WGS analysis confirmed that all patients belonged to a single transmission chain, supporting a rapid dissemination of this epidemic strain in France. TB had been diagnosed in 1 of the in-contact case-patients as early as 2015; case-patient 1 and the index case-patient lived together in 2015 in Île-de-France, during which time the transmission event was hypothesized to have occurred. These findings led to an extended investigation and communication outreach to general practitioners in the index city. General practitioners were informed of the outbreak and prompted to prioritize performing TB detection in patients with respiratory symptoms suspected to be TB. Consequently, during early 2018, active TB caused by the Beijing SIT1 epidemic strain was diagnosed in 3 previously non–epidemiologically linked patients. Subsequent investigations showed previously missed occasional contacts between the index case-patient and these 3 TB case-patients.

Out of the 14 case-patients identified, 9 (64%) infected by the Beijing strain were household or frequent contacts, and 5 (36%) were occasional contacts (Table). Five case-patients were children. Pulmonary and extrapulmonary disease developed in 4 case-patients; in 2 of those, meningitis, a severe form of TB associated with high rates of death and disability, developed.

Among the 14 cases identified, 5 (36%) were linked to this outbreak through laboratory investigations. WGS analysis enabled us to link strains from 2 initially independent contact tracing investigations and relate 5 cases

Table. Summary of contact tracing and description of cases in a tuberculosis outbreak, France, 2017–2018

Case-patient	Date of sample collection	Contact with index case-patient	Age	Origin	Diagnosis region	TB infection site
1	3rd trimester 2015	Household	Adult	Non–France-born	Île-de-France	Lung
2	1st trimester 2017	Occasional	Adult	Non–France-born	Hauts-de-France	Lung
3	2nd trimester 2017	Household*	Child	France-born	Auvergne-Rhône-Alpes	Lung, meningitis
4	3rd trimester 2017	Frequent	Adult	France-born	Auvergne-Rhône-Alpes	Lung
5†	3rd trimester 2017	Household	Adult	Non–France-born	Auvergne-Rhône-Alpes	Lung
6	3rd trimester 2017	Household	Child	France-born	Auvergne-Rhône-Alpes	Lung, adenitis
7	4th trimester 2017	Household	Child	France-born	Auvergne-Rhône-Alpes	Lung
8	4th trimester 2017	Household	Child	France-born	Auvergne-Rhône-Alpes	Lung
9	4th trimester 2017	Frequent	Adult	Non–France-born	Auvergne-Rhône-Alpes	Lung
10	4th trimester 2017	Occasional	Adult	Non–France-born	Auvergne-Rhône-Alpes	Lung
11	1st trimester 2018	Frequent	Adult	Non–France-born	Auvergne-Rhône-Alpes	Lung
12	1st trimester 2018	Occasional	Adult	Non–France-born	Auvergne-Rhône-Alpes	Lung, adenitis
13	1st trimester 2018	Occasional	Child	France-born	Provence-Alpes-Côte d'Azur	Lung, meningitis
14	1st trimester 2018	Occasional	Adult	France-born	Auvergne-Rhône-Alpes	Lung

*Household from case-patient 4.†Index case-patient.

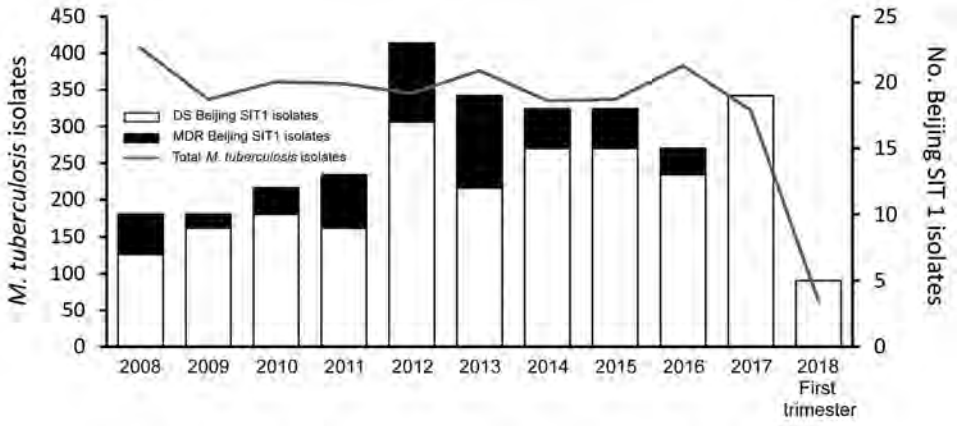

Figure 2. Prevalence of tuberculosis cases and *Mycobacterium tuberculosis* Beijing SIT1 genotype, Auvergne-Rhône-Alpes, France, 2008–2018. DS, drug-sensitive; MDR, multidrug-resistant.

with no previously identified epidemiologic link. Pairwise SNP distance comparison of the isolates revealed a maximum of 7 SNPs separating the index case-patient from the other 13 case-patients and a maximum of 10 SNPs separating other cases (Figure 1). The median SNP difference between cases with a verified epidemiologic link was 2 (range 0–5). This finding is consistent with previous studies in which a 12-SNP threshold excluded transmission (*4*). We compared the cluster we report with other Beijing SIT1 strains isolated in 2017 in our region: pairwise SNP comparison ranged from 140- to 330-SNP distance. This cluster is therefore unrelated to other Beijing SIT1 contemporarily and regionally circulating strains. According to the Shitikov scheme (*5*), those strains belong to the Central Asia group.

Conclusions

The Beijing strain family, regardless of the antimicrobial susceptibility profile, has been involved in many TB outbreaks, including in Europe (*6–10*). Some studies suggest that the epidemic success of the modern Beijing strains might be linked to increased virulence, higher transmission rates, or both, but findings have not been consistent (*11–13*). In the Auvergne-Rhône-Alpes region, Beijing strain accounts for 2.5%–8.1% of all *M. tuberculosis* isolates (average 4.9%) (Figure 2), depending on the year. The increased incidence of Beijing genotype during 2011–2013 (i.e., up to 7.3% in 2012) was related to the migratory influx of patients with multidrug-resistant TB from Eastern Europe and Asia (*14*). The 8.1% increased incidence of Beijing genotype from the second half of 2017 and the first 4 months of 2018 was due to the outbreak we describe.

WGS led to the rapid identification of this transmission event. Real-time feedback between the microbiology laboratory and the TB control center prompted further contact investigations and identification of additional epidemiologically linked cases.

This report highlights how, beyond the traditional contact tracing investigations, molecular *M. tuberculosis* typing enabled detection of otherwise unsuspected transmission and therefore identification of extended clusters. This finding is important because it is not uncommon that TB patients do not cooperate well with interviews (*15*); even in low-prevalence countries, stigma associated with TB diagnosis often hampers adherence of patients to screening. Noncomplying patients do not disclose exhaustive lists of contacts; as shown in this study, case-patients 4 and 5 were found to have frequent contact only through the second contact-tracing interview. In this respect, robust microbiological evidence of transmission may encourage TB control units to perform second interviews and thus increase the chances to document more transmission events. Our observations underscore the added value of molecular epidemiologic tools to prompt TB contact investigations for better detection and disease control.

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Response to Isoniazid-Resistant Tuberculosis in Homeless Shelters, Georgia, USA, 2015–2017

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In 2008, an outbreak of isoniazid-resistant tuberculosis was identified among residents of homeless shelters in Atlanta, Georgia, USA. When initial control efforts involving standard targeted testing failed, a comprehensive approach that involved all providers of services for the homeless successfully interrupted the outbreak.

In 2008, the Tuberculosis Prevention and Control Program at the Georgia Department of Public Health (Atlanta, GA, USA) was notified of 7 cases of active tuberculosis (TB) in Fulton County, Georgia, for which isolate genotypes matched. All cases were linked to 1 homeless shelter in Atlanta, and all isolates exhibited low-level isoniazid resistance (resistant at 0.1 μg/mL but susceptible at 0.4 μg/mL according to BACTEC MGIT [Becton, Dickson and Company, https://www.bd.com]) (*1*). Additional cases with the same genotype were subsequently discovered at other locations. As of December 2017, a total of 110 outbreak-related cases had been identified in Fulton County (Figure 1), an additional 17 cases in other Georgia counties, and 47 cases in 15 other states. Of the 110 Fulton County cases, 41 (37%) patients were coinfected with HIV. A previous report of the outbreak through December 2015 identified several challenges associated with confronting the outbreak, notably lack of accurate rosters for overnight residents in most shelters, lack of voluntary participation in the episodic large-scale screenings (diagnostic testing with a tuberculin skin test [TST] or interferon-γ release assay, or a symptom screening for persons known to have a prior positive diagnostic test result) conducted at the shelters, and lack of consistent TB screening procedures at any of the facilities (*1*). An additional complication was ongoing litigation and efforts by nearby businesses and agencies of the Atlanta city government to close the index shelter (*2*). As a result of these issues, implementation of true targeted testing was initially limited, but in response to the large increase in the number of persons with a diagnosis of active TB caused by the outbreak strain in 2014 (Figure 1), the health department intensified its efforts. We report the results of the outbreak response in Fulton County, conducted from January 2015 through December 2017, illustrating several challenges caused by disjointed healthcare received by persons experiencing homelessness.

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The Study

In June 2014, the Fulton County Board of Health joined with Mercy Care, a local federally qualified health center, to create the Metro Atlanta TB Task Force, including representatives from public health, homeless shelters, and other community service providers to provide a coordinated response in which testing targeted the entire population of persons experiencing homelessness instead of individual facilities. Before the task force interventions, none of the county's 6 homeless shelters had adopted effective TB control guidelines (*1*). Furthermore, a formal survey of shelter employees revealed that their knowledge of TB disease and its transmission was low, a situation further exacerbated by rapid staff turnover. To address these issues, the task force began a series of meetings, led by the health department and Mercy Care, to develop and implement a set of comprehensive TB control guidelines for homeless shelters.

A key feature of these interventions was mandatory TB screening of shelter residents within 7 days of shelter entry and every 6 months thereafter. In addition, the guidelines included the following principles: appointment of a TB liaison at each of the 6 shelters, isolation or separation of residents with cough, maintenance of a bed log that includes every resident's name and sleeping location within the facility, provision of a TB clearance card at the time each person completed screening to allow them access to the 6 shelters, and random inspections of the shelters by the health department to ensure adherence to the guidelines and to provide ongoing staff education. Because publication of the guidelines did not lead to their immediate uptake at all 6 shelters, educational initiatives were undertaken to increase TB knowledge and awareness of guideline content among shelter staff and the homeless community (*3*).

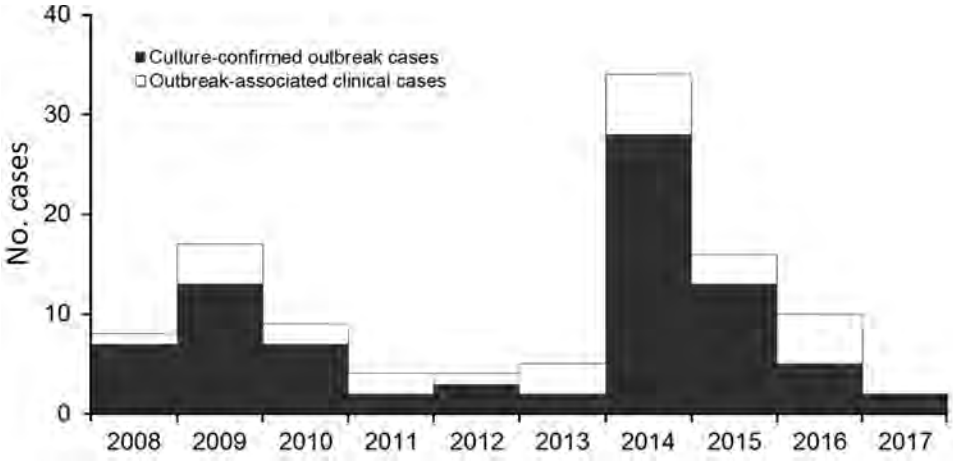

Figure 1. Outbreak-associated tuberculosis cases, Fulton County, Georgia, USA, 2008–2017. Culture-positive patients had ≥1 positive sputum culture with an isolate that had the outbreak-related genotype. Patients with clinical disease did not have a positive culture result but were epidemiologically linked to stays in homeless shelters before the diagnosis of tuberculosis was made.

During the outbreak, persons with a diagnosis of latent TB infection were assumed to be infected with the isoniazid-resistant strain and were therefore offered daily treatment with rifampin for 4 months (*4*). Directly observed therapy was provided for most shelter residents starting in 2008 and for all residents after 2015; incentives ($5 coupons for local grocery stores) were offered weekly for successful completion of all doses.

Data from testing performed before 2015 showed that ≈30% of persons tested by TST did not return for reading of the results. To overcome this problem, starting in 2015, the TST was replaced as the primary test by a single-step blood test, an interferon γ-release assay (QuantiFERON Gold In-Tube; QIAGEN, https://www.qiagen.com).

From January 2015 through December 2017, a total of 14,496 persons were screened for latent TB infection, compared with 2,451 from January 2008 through December 2014 (Figure 2). During 2015–2017, a total of 3 cases of active TB were identified through screening, resulting in disease incidence of 20.7 cases/100,000 persons screened. From January 2, 2015, through December 31, 2017, a total of 430 persons received a diagnosis of untreated latent TB and 391 (91%) started treatment; of those starting treatment, 207 (53%) completed a full course. In 2017, only 2 cases of outbreak-associated TB were reported (Figure 1).

Conclusions

Despite an ongoing downward trend of TB in the United States (*5*), outbreaks among persons with a history of homelessness continue to occur regularly (*6*–*12*). As previously reported, the mobility of persons experiencing homelessness rendered standard contact tracing activities in this investigation difficult (*13*). Greater success was achieved with a systematic approach targeting all shelter residents for testing rather than just individual facilities in which cases had been identified. In particular, the number of persons tested and

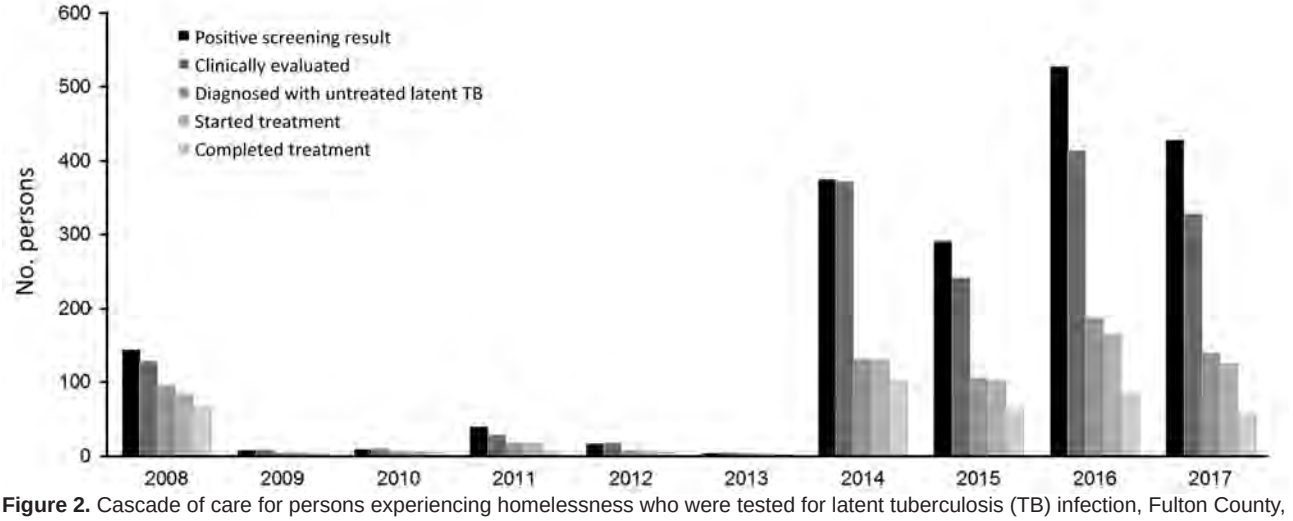

Figure 2. Cascade of care for persons experiencing homelessness who were tested for latent tuberculosis (TB) infection, Fulton County, Georgia, USA, 2008–2017. Positive screening result indicates number with either a new or prior positive test result at screening; clinically evaluated indicates number evaluated by a clinician; diagnosis of untreated latent TB indicates number with a diagnosis of latent TB who required treatment; started treatment indicates number who started treatment for latent TB; and completed treatment indicates number who completed treatment.

provided treatment for latent TB markedly increased after introduction of comprehensive standard screening guidelines and implementation at all shelters.

Since 2015, testing for and diagnosis of latent TB increased despite a decrease in outbreak-associated active TB cases. Acceptance of latent TB treatment was >90%, which in our estimation was attributable to the universal support of the shelter providers. Regular inspections of facilities by public health staff provided frequent opportunities for ongoing education about TB and the need to maintain vigilance despite the downward trend in case numbers.

The primary challenge that remains is finding the substantial numbers of persons with untreated latent TB who are at risk for active TB but no longer in the shelter system. Because of this population, we anticipate that episodic cases of active TB with the outbreak genotype will occur. However, if the comprehensive guidelines now in place are properly implemented, reemergence of TB with the outbreak strain among shelter residents, as was seen in 2014, can be prevented. In addition, efforts are under way to increase the identification of persons with HIV (either not diagnosed or in persons not receiving care) and facilitate the rapid initiation of antiretroviral therapy along with the supportive measures required to maintain treatment adherence.

The presence of isoniazid resistance in the outbreak strain complicated treatment of active disease and latent infection (*4*,*14*). Unfortunately, 1 person with isoniazid-resistant TB diagnosed in 2008 later died of multidrug-resistant TB (*15*).

Homelessness presents several challenges to caring for persons with active TB and those who have been exposed to TB (https://www.cdc.gov/tb/topic/populations/homelessness/default.htm). Congregate living in shelters can provide an efficient means of transmission. Once tested, persons frequently move into, out of, and within shelter systems, making follow-up difficult. Also, shelter residents frequently lack a permanent place to store medications, which necessitates the use of directly observed therapy. In the outbreak we report, these challenges were overcome with a systematic operational approach focused on the entire population of persons experiencing homelessness, illustrating that such approaches can contribute to effective control of TB outbreaks within these populations.

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Exportation of MDR TB to Europe from Setting with Actively Transmitted Persistent Strains in Peru

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We performed a cross-border molecular epidemiology analysis of multidrug-resistant tuberculosis in Peru, Spain, and Italy. This analysis revealed frequent transmission in Peru and exportation of a strain that recreated similar levels of transmission in Europe during 2007–2017. Transnational efforts are needed to control transmission of multidrug-resistant tuberculosis globally.

International migratory movements have created a need for cross-border surveillance of tuberculosis (TB). Monitoring the transmission of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains deserves further analysis (*1*). Through migration, MDR strains can become more widely dispersed; they can be exported from the 30 countries with 89.7% of the incident MDR cases (*2*) to lower prevalence settings.

We performed a transnational molecular epidemiology analysis of MDR TB cases covering a setting with one of the highest resistance rates in Latin America (Lima, Peru) (*2*) and 2 settings in Europe hosting immigrants from Peru (Florence, Italy; and Madrid, Spain) to identify incidents of cross-border transmission. We selected 60 consecutive MDR TB cases (20% of the total MDR cases in Lima) diagnosed during 2014–2015 in one of the poorest districts of Lima (San Juan de Lurigancho), which has the highest incidence of TB (193 cases/100,000 population) in Peru (*3*). MIRU-VNTR (mycobacterial interspersed repetitive unit–variable-number tandem-repeat) analysis (Appendix 1, https://wwwnc.cdc.gov/EID/article/25/3/18-0574-App1.pdf) suggested a high percentage of recent transmission that included 36 (60%) of 60 isolates in 9 clusters (Appendix 2, https://wwwnc.cdc.gov/EID/article/25/3/18-0574-App2.xlsx). A comparison of these isolates with 228 genotyped isolates from the same district 4 years earlier (*3*) revealed that 6 of the 9 strains actively transmitted during 2014–2015 were present in 2011 (Appendix 1 Table 1).

We then investigated whether some of these persistent MDR TB strains actively transmitted in Lima could have been exported to Europe. We used a dataset of 87 MIRU-VNTR genotypes of isolates in Florence obtained from TB cases in Peru during 2001–2010 (*4*) and >300 MDR genotyped isolates obtained nationwide from Italy (*5*). We found that 1 genotype matched between the Lima and Italy MDR datasets; this genotype corresponded to a strain (C8-LPMDR) that infected 11 persons in Florence and 2 in Milan during 2007–2017 (Appendix 1 Table 2). MDR TB strains from Lima were also found in Spain during 2003–2009. Three MDR isolates, matching 3 of the 9 MDR TB strains from Lima, were found in migrants from Peru residing in Madrid (Appendix 1 Table 1). One of these isolates corresponded to the active MDR strain circulating in Italy (C8-LPMDR).

We performed whole-genome sequencing (*6*) with 12 of the 17 isolates of the cross-border MDR TB cluster C8-LPMDR (7 from Florence, 2 from Milan, 2 from Lima, and 1 from Madrid). In a median-joining network analysis, these isolates were distributed along 2 branches (Figure). One branch included all the isolates from Florence. Although we lacked precise data from contact tracing to verify details regarding transmission in Florence, we were able to determine that all the Peru migrants involved came from Lima. In Florence, there is a large community of persons from Peru, which offers opportunities for interacting, such as shared residence and social gatherings. The few differences (0–2 single-nucleotide polymorphisms [SNPs]) found among these isolates strongly suggests these isolates were recently transmitted in Florence. An isolate from Lima (6068) was only 3 SNPs different from a Florence isolate, demonstrating a close genetic

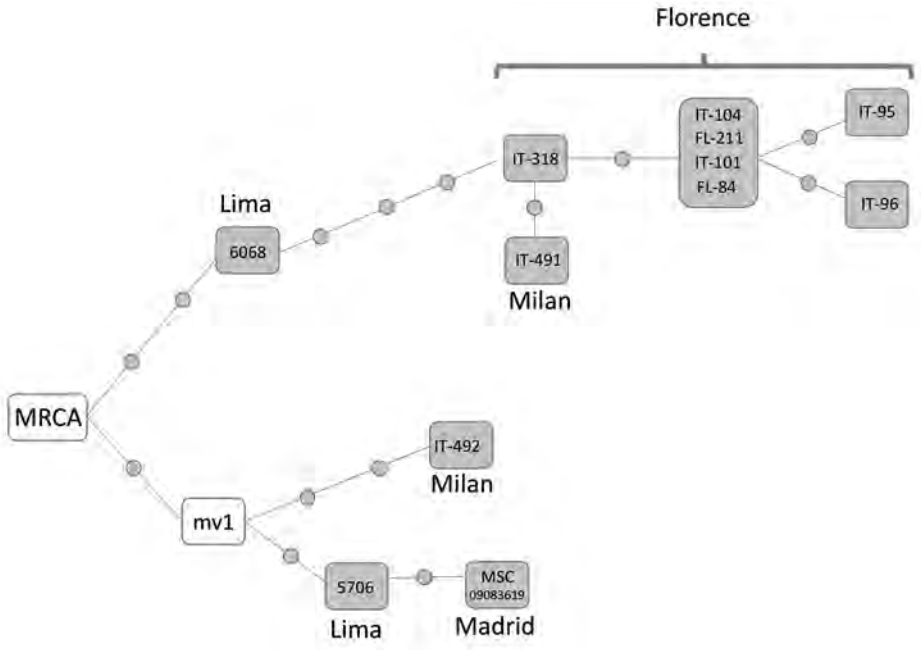

Figure. Median-joining network of whole-genome sequenced isolates of strain C8-LPMDR found in Italy, Peru, and Spain, 2007–2017. Network 4.6.1.6 (http://www.fluxus-engineering.com) was used to perform network analysis. Each dot along the lines linking isolates corresponds to a single-nucleotide polymorphism difference. Isolates within the same box share identical sequences. mv1 corresponds to an unsampled case inferred from the network topology. Sequences were deposited in the European Bioinformatics Institute database (http://www.ebi.ac.uk, accession no. PRJEB25765). FL, Florence; IT, Italy; MRCA, most recent common ancestor.

relationship between the Florence and Lima isolates. This close relationship also suggests that the starting point of this branch was an exportation event of an isolate from Lima. The second branch in the network includes 2 isolates identified in Europe (1 Madrid [city of origin unknown, data not available] and 1 Milan [origin Lima]) and 1 isolate identified in Lima. Because the most recent common ancestor is positioned between the 2 branches and the 2 isolates from Lima are in different branches, these branches probably represent 2 independent exportations of 2 variants of a strain prevalent in Lima that diversified after a prolonged period.

These data reveal that high-risk strains are being exported from Lima to 2 countries of Europe (Italy and Spain). Not only were these strains exported from Lima, but 1 strain caused a prolonged and ongoing transmission event in Italy. The transmission of this strain has caused at least 3 cases in Lima, 11 in Florence, 2 in Milan, and 1 in Madrid.

In another report, the international distribution of an MDR TB strain that caused 10 cases across 3 countries of Europe (Romania, Austria, and Germany) was investigated (*7*). The exportation event discussed in our report is geographically wider (intercontinental, from South America to Europe), involved more cases (17 total, with a transmission cluster of 12 cases in Italy), and occurred over a more extended period (secondary cases spanned 11 years).

Only integrative transnational efforts can provide a clearer picture of transmission of MDR TB, which has become more complex because of international migration. In this cooperative analysis involving Peru, Italy, and Spain, we detected a serious problem of active MDR TB transmission in Lima. This situation led to a pool of persistent strains that were responsible for similar transmission events after exportation to Europe via migration.

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Rectal Lymphogranuloma Venereum, Buenos Aires, Argentina

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Among 34 men with proctitis in Buenos Aires, Argentina, 16 (47%) had *Chlamydia trachomatis* infection, 11 (68.8%) of which were biovar lymphogranuloma venereum. The outbreak was probably local, as in Europe. In Argentina, lymphogranuloma venereum should be a suspected cause of proctitis in HIV-infected men who have had unprotected anal sex with men.

Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by *Chlamydia trachomatis* serovars L1, L2, or L3 and their variants. LGV has been considered endemic to Asia, Africa, and the tropical region of South America. Over the past 2 decades, case reports of LGV in Argentina have been sporadic and regarding only patients who acquired the infection abroad.

In the Netherlands in 2003, an outbreak of rectal LGV among men who have sex with men (MSM), mainly HIV infected, was reported (*1*). This report was followed by many other reports from other developed countries (*2*,*3*).

LGV has been traditionally described as causing inflammation and swelling of the inguinal lymph nodes and also involving the rectum, causing acute proctitis, particularly among HIV-infected MSM (*4*). Since 2015, some clinicians in Argentina have suspected LGV in certain patients with proctitis (regardless of association with inflammatory tumors) in which *C. trachomatis* has been detected but not genotyped. Thus, we conducted a prospective study to assess the *C. trachomatis* genotypes as the causative agent of infectious proctitis in Buenos Aires, Argentina. Our study was conducted in a private practice and a public hospital, under a protocol previously approved by the hospital's ethics committee (no. 201723).

From September 1, 2017, through February 1, 2018, we included in our study every man who visited either the private or public study site and who had rectal signs or symptoms of proctitis and had not taken antimicrobial drugs in the previous month. None of the included patients was referred by a previously included patient. Each participant signed an informed consent form.

Over the first 5 months, we obtained a rectal swab sample from 34 men on their first visit. To detect *C. trachomatis*, we extracted DNA from the samples by using real-time PCR targeting a cryptic plasmid fragment (Alert PCR; ELITech Molecular Diagnostics, https://www.elitechgroup.com). Positive samples were genotyped by *ompA*-based PCR restricted fragment length polymorphism (*5*).

Of the 34 samples analyzed, 16 were positive for *C. trachomatis*; 11 were identified as genotype L2 and 5 as genotypes D, F, or J. All participants reported having engaged in unprotected receptive anal sex in Argentina, except for 1 who had had receptive anal sex while in Mexico. None declared having traveled to an LGV-endemic area. Mean age was 31.63 years (range 22–43 years). All

but 1 were HIV positive, and 5 had another sexually transmitted infection (1 had gonorrhea, 1 had syphilis and gonorrhea, 1 had syphilis only, and 2 had viral condylomas). The signs and symptoms of proctitis included rectal pain; tenesmus; and a discharge that was mucous, crystalline, hematic, or purulent. Endoscopic appearances of proctitis were variable, from mild to severe. Three patients had perianal lesions, 1 had a fistula, and 2 had ulcers. One patient had severe proctitis with rectal stenosis and was suspected of having had inflammatory bowel disease (IBD) for 1 year at the time of the first interview.

All patients were prescribed doxycycline for 3 weeks as recommended (*4*), resulting in complete resolution of proctitis and perianal ulcers. Two patients with chronic complications required additional treatments (surgical resolution of fistula and endoscopic dilatation of stenosis), resulting in complete resolution.

We observed a high prevalence (47%) of chlamydial infection within the studied group (16/34); the most frequent biovar was LGV (68.8% of the *Chlamydia*-infected patients). The cases included in this series were detected within a short period, which suggests a local outbreak. As in the series in Europe, the patients in Argentina were MSM, almost all HIV infected, who reported having had unprotected anal sex (*6*,*7*).

Signs and symptoms from most patients did not suggest LGV: 7 of the 11 with genotype L2 had mild or moderate proctitis similar to non-LGV infection. In 1 patient, severe proctitis mimicked an inflammatory tumor; in another, IBD. Proctitis is a nonspecific manifestation with diverse origins, for which clinical or endoscopic findings are insufficient to determine etiology, as observed in this case series. Also, because in about one quarter of patients with LGV infections the symptoms are mild or absent, diagnosis can be missed or late (*8*,*9*). Thus, given this evidence and the similarities between LGV and IBD, gastroenterologists should consider LGV as a differential diagnosis for patients with proctitis, especially HIV-infected MSM. Because the clinical assessment is not specific and quite inconclusive on which to base a diagnosis with certainty, genotyping is needed to indicate the proper patient care, management, and treatment. Including *C. trachomatis* typing as a routine diagnostic step also helps avoid chronic complications, stop the transmission chain (*3*,*4*,*10*), and provide useful information for epidemiologic surveillance. We conclude that in Buenos Aires, Argentina, as well as in countries in Europe, rectal LGV should be suspected as a cause of proctitis in HIV-infected MSM with a history of unprotected anal sex.

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Confirmed Case of Buruli Ulcer, Senegal, 2018

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Buruli ulcer is a necrotizing skin disease caused by *Mycobacterium ulcerans* and is usually associated with tropical climates and exposure to slow-moving or stagnant water. We report a case of Buruli ulcer that may have originated in an urban semiarid area of Senegal.

In January 2018, a 14-year-old boy came to an urban clinic in Dakar, the capital of Senegal, with a 2-week history of skin lesions. He had a 1 × 1 cm ulcerous erosion over a 6 × 16 cm painful edematous lesion on his right calf; he was febrile, with a temperature of 38.5°C. He was initially treated for cellulitis with amoxicillin and clavulanate acid, along with wound care. Two days later, the lesion had evolved. Debridement revealed considerable necrotic subcutaneous tissue extending 1–3 cm under the epidermal edge. The most proximal of the 3 ulcers had a diameter of 1 cm, the next measured 5 × 6 cm, and the last was an L-shaped lesion measuring 6 × 28 cm, running from midcalf to toes. Infection with *Mycobacterium ulcerans* was suspected because of rapid tissue necrosis, classic undermining edges, patient age, location of the lesions, and failure of standard care (Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/25/3/18-0707-App1.pdf).

The patient was admitted to the hospital and treated with parenteral gentamicin, oral metronidazole, and wound care. The wound bed was swabbed; culture revealed *Acinetobacter* and *Pseudomonas.* Antimicrobial drug therapy was changed to parenteral gentamicin and oral ciprofloxacin. Four swab specimens were obtained from the wound, and quantitative real-time PCR assay targeting the IS2404 putative transposase gene and the mycolactone polyketide synthase gene confirmed the presence of *M. ulcerans*. Targeting IS2404 is considered the diagnostic standard for Buruli ulcer (*1*). Targeting IS2404 PCR analysis for *M. tuberculosis* and negative controls were both negative (Appendix). A skin graft was performed, and the patient was discharged and given rifampin/isoniazid, ciprofloxacin, and wound care.

The patient had been born in rural Guinea-Conakry and moved to Senegal 3 years before his illness. His mother reported that he had been fully vaccinated, although no records remain. He moved to Senegal in 2015 and lived in Dakar for 18 months, then moved east to the semiarid area of Diourbel to attend Koranic school for another 18 months. He denied engaging in any agricultural or mining activities or bathing, washing, or swimming in bodies of fresh water during his 3 years in Senegal. He also denied returning to Guinea-Conakry or other travel since his arrival in Senegal. In Guinea-Conakry, he had been involved in agricultural activities, including rice farming. The family does not use mosquito nets, and he reported occasional insect bites.

Worldwide, Buruli ulcer is the third most common mycobacterial infection, inflicting debilitating cost and social stigma on patients and their families (*2*,*3*). The highest incidence of Buruli ulcer is found in tropical or subtropical sub-Saharan Africa, but 2 cases have been reported in Mali, a semiarid country not usually associated with Buruli ulcer (*3*–*5*). The only other known case of Buruli ulcer in Senegal was in a traveler from Europe who had been building canoes in fresh water along the tropical Senegal–Guinea border (*6*).

The mode of transmission of *M. ulcerans* is poorly understood and may vary by region. The bacterium has been found in aquatic environments, animals, and insects. Animal reservoirs and insect vectors have been proposed, but no definitive vector has been identified (*7*). A systematic review found that poor wound care, living or working near aquatic environments, and failure to wear protective clothing (long pants and long-sleeved shirts) were risk factors associated with *M. ulcerans* infection. Results among other researchers searching for risk factors have been contradictory (*8*). The reported incubation period ranges between 34 and 264 days, with a mean of 4.5 months (*9*). A multicenter study in West Africa demonstrated no significant evidence of protection from *M. ulcerans* infection after bacillus Calmette-Guérrin vaccination (*10*).

This case of Buruli ulcer is noteworthy because it is a confirmed case originating in a semiarid region of West Africa, suggesting that the endemic area of this disease is poorly defined or changing. The patient appears to have contracted the disease in Senegal without the usual water-related risk factors, although he was exposed to insect bites. It is possible but unlikely that he contracted the disease in Guinea-Conakry 3 years earlier, which would mean that he had an incubation period 2 years longer than any previously reported cases. There is no evidence to suggest his possible bacillus Calmette-Guérrin vaccination delayed wound development.

This case illustrates the need to better define the geographic extent and modes of transmission of this debilitating disease so that primary control measures can be identified. In addition, health workers must be provided with the training and tools to diagnose and treat *M. ulcerans*. Research into a point-of-care diagnostic test is needed so that timely treatment can minimize disability and costs to the family.

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Management of Patients with *Candida auris* Fungemia at Community Hospital, Brooklyn, New York, USA, 2016–2018[1]

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Candida auris is an emerging fungus that can cause invasive infections. It is associated with high mortality rates and resistance to multiple classes of antifungal drugs and is difficult to identify with standard laboratory methods. We describe the management and outcomes of 9 patients with *C. auris* fungemia in Brooklyn, New York, USA.

Candida auris is an emerging fungus that can cause invasive infections associated with high mortality rates and is often resistant to multiple classes of antifungal drugs. Risk factors for infection include nursing home exposure; invasive devices, such as tracheostomy tubes or percutaneous endoscopic gastrostomy tubes; immunocompromised status; and use of broad-spectrum antimicrobial drugs (*1*). On the basis of limited data available, echinocandins are recommended as initial therapy for *C. auris* infection (*2*). We review the management of 9 case-patients who had *C. auris* fungemia at a 300-bed community hospital, attached to a 450-bed nursing home, in Brooklyn, NY, USA. There have been 9 occurrences of C. *auris* fungemia at this institution since 2016.

Our case series demonstrates the complex patient population at risk for invasive infection with *C. auris*. Patients infected were generally >70 years of age and had multiple chronic concurrent conditions (Appendix Table, https://wwwnc.cdc.gov/EID/article/25/3/18-0927-App1.pdf). Most patients came from nursing homes, and more than half had invasive devices, such as tracheostomies or

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[1]Preliminary results from this study were presented as a poster presentation at the American Society of Health-System Pharmacists Midyear Meeting, December 3–7, 2017, Orlando, Florida, USA.

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percutaneous endoscopic gastrostomy tubes, placing them at high risk for infection at baseline.

In addition, each patient had a recent history of broad-spectrum antimicrobial drug use; many had concomitant resistant organisms isolated and received concomitant antimicrobial drug therapy during their *C. auris* treatment course. The most common antimicrobial drugs used were meropenem, polymyxin B, and vancomycin. Common bacteria isolated were *Acinetobacter* sp., *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae.* Four patients were admitted to the medical intensive care unit; 2 had prolonged stays in the medical intensive care unit before development of candidemia.

The time from hospitalization to initial infection with *C. auris* varied among the patients. Approximately 60% of the patients came to the hospital with positive blood cultures on day 1, and fungemia developed in the remaining patients after prolonged hospitalization. Most patients had documented clearance of their blood cultures within 3–5 days of initial isolation. However, 1 patient had a second episode of *C. auris* fungemia several weeks after he was initially given treatment and documented to have clearance of blood cultures.

All patients were given micafungin as first-line therapy for an average duration of 22 days. The most common dose used was 100 mg/day of intravenous micafungin. Two of the 9 patients required liposomal amphotericin B after failing to respond to micafungin therapy. Both of these patients remained persistently febrile while receiving micafungin monotherapy; 1 was the patient with 2 episodes of *C. auris* fungemia. The average duration of amphotericin B was 19 days. The in-hospital mortality rate was 22%. Of the 7 patients who were discharged, 43% were discharged to a palliative care service. The average duration of hospitalization for these patients was 65 days.

Limited information is available on interpretation of MIC data for *C. auris* because the Clinical Laboratory Standards Institute (https://clsi.org) does not have breakpoints specific for *C. auris*. Antifungal susceptibility data were determined for each of the patients included in this case series (Appendix Figure). All antifungal susceptibility tests were performed by the Wadsworth Laboratory, the New York State Department of Health Reference Laboratory (https://www.wadsworth.org). Most susceptibility information was not available throughout the course of treatment. Given difficult identification of the organism by using standard laboratory techniques, timely identification and antimicrobial susceptibility information continues to be a challenge when managing patients with invasive *C. auris* (*3*).

All isolates were markedly resistant to fluconazole, and ≈40% were resistant to liposomal amphotericin B (Appendix Figure). This second finding is of particular concern because liposomal amphotericin B is the recommended second-line agent for management of *C. auris* in the setting of micafungin failure. On the basis of the resistance patterns at this hospital, patients who failed monotherapy with micafungin had lisposomal amphoterin B added rather than switching therapy completely.

We encourage clinicians treating *C. auris* infections to consider combination therapy with micafungin plus liposomal amphotericin B in patients who fail monotherapy with micafungin. Laboratory limitations mean that timely identification and susceptibility testing of *C. auris* might not always be possible, and clinicians might often have to defer to local or national epidemiology trends to make the most up-to-date decisions. It is essential to notify the department of health of new cases of infection with *C. auris* as soon as possible and to educate healthcare personnel to help minimize spread. Clinicians should focus on identifying and minimizing risk factors for acquisition of *C. auris* and prevention of spread through enhanced infection control procedures.

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Helicobacter cinaedi Hepatic Cyst Infection with Bacteremia

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Helicobacter cinaedi is an enterohepatic bacillus that causes infections of various manifestations. We report a novel case of hepatic cyst infection with bacteremia caused by *H. cinaedi* in an immunocompetent woman in Japan. Further research is warranted to identify the epidemiologic and clinical features of *H. cinaedi* infection.

Helicobacter cinaedi is a gram-negative, spiral-shaped enterohepatic bacillus found in the digestive tracts of humans and other animals (*1*). Many reports have described that *H. cinaedi* can cause infections in immunocompromised patients (*2*). In recent decades, however, several cases of immunocompetent patients with *H. cinaedi* infection have been reported (*3*). Although various manifestations of *H. cinaedi* infection have been described, to our knowledge, no cases of hepatic cyst infection have been reported. We report a case illustrating an *H. cinaedi* hepatic cyst infection with bacteremia in an immunocompetent patient in Japan.

In July 2017, a 73-year-old woman was referred to the National Center for Global Health and Medicine (Shinjuku, Japan) because of a 2-day history of abdominal pain, vomiting, and fever. She had schizophrenia and had been hospitalized in a psychiatric hospital for >20 years. At admission, her body temperature was 37.5°C, and other vital signs were stable. Physical examination revealed a slight tenderness on her abdomen with mild rebound tenderness. Her laboratory findings showed elevated leukocytes (10,170 cells/mL, reference range 3,300–8,600 cells/μL), neutrophils (87%, reference 40%–71%), and C-reactive protein (11.7 mg/dL, reference 0–0.14 mg/dL). Total bilirubin (0.8 mg/dL), aspartate aminotransferase (13 U/L), alanine aminotransferase (10 U/L), alkaline phosphatase (264 U/L), and gamma-glutamyl transferase (21 U/L) levels were within reference ranges. Enhanced computed tomography (CT) showed slight ascites, but no apparent signs of bowel obstruction. We found a large hepatic cyst (63 mm in diameter) on the left liver lobe (Figure); the cyst had been 44 mm in diameter a month earlier, when she was hospitalized because of a small intestinal obstruction.

On the day of hospital admission, we obtained 2 blood culture samples and started the patient on a regimen of piperacillin/tazobactam. Her fever persisted. On day 5, blood culture results were positive for spiral-shaped gram-negative rod bacteria in both sets of aerobic bottles (after 80.6 h and 104.5 h) (BACTEC, Becton, Dickinson and Company, https://www.bd.com). Enhanced CT on day 5 revealed enlargement of the hepatic cyst from 63 mm to 69 mm in diameter. The patient's tenderness was most pronounced in the upper abdomen. We suspected hepatic cyst infection and placed an ultrasound-guided drainage tube, by which we obtained ≈100 mL of purulent liquid. The patient's fever subsided rapidly, within 1 day after drainage.

We identified the isolates from the blood cultures as *H. cinaedi* by using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (score 2.07). Subsequent PCR testing of pus obtained by drainage also gave a positive result for *H. cinaedi*.

We collected >100 mL of pus in the first 2 drainage days (hospital days 5–6). The patient showed defervescence on day 6. We changed her antibiotic to ampicillin/sulbactam on day 10. A nonenhanced CT scan indicated that the hepatic cyst had collapsed from drainage on day 17. We removed the drainage tube on day 18 and stopped the antibiotic regimen on day 22. The patient's condition was stable after drainage, and she was discharged to the previous psychiatric hospital on day 24.

We report a novel case of hepatic cyst infection caused by *H. cinaedi*. Uwamino et al. reported a retrospective observational study of community-acquired *H. cinaedi* bacteremia (*3*), showing that cellulitis was the most common manifestation in community-acquired cases, whereas bacteremia without any specific focus was the leading type of infection in healthcare-associated and nosocomial settings. In the case we describe, the patient was in a nosocomial setting at the onset. Thus, primary occult bacteremia is the

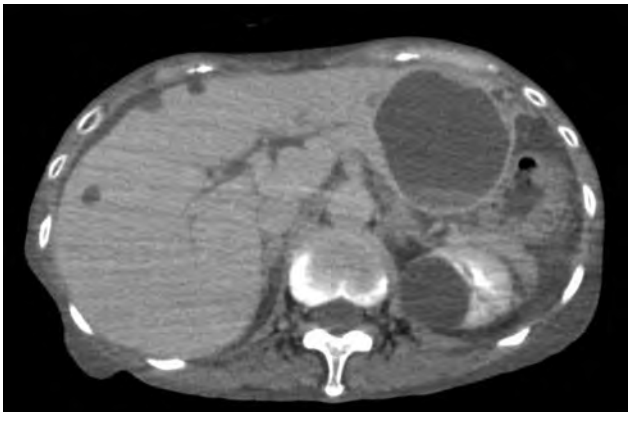

Figure. Enhanced computed tomography image showing a large hepatic cyst (63 mm in diameter) on the left liver lobe in a patient with *Helicobacter cinaedi* infection on the day of hospital admission, Tokyo, Japan, July 2017.

leading hypothesis. However, *H. cinaedi* is an enterohepatic bacterium, and the patient had undergone surgery for an adhesive small intestinal obstruction 1 month before. Her medical and surgical history might have increased the intraintestinal pressure and induced the hepatic cyst infection through biliary reflux.

The patient had schizophrenia but was not immunocompromised beyond her surgical history. Many cases of *H. cinaedi* infection have been reported in immunocompromised hosts (*2*), but reports of *H. cinaedi* infections in immunocompetent patients have been increasing (*3*). Matsumoto et al. showed that *H. cinaedi* bacteremia was found in only 0.06% of total blood samples (*4*); none of the patients in their study were HIV-positive, but many were immunocompromised by other conditions. Kiehlbauch et al. also conducted a retrospective study of *H. cinaedi* bacteremia and found that 45% of patients were HIV-positive (*5*). *H. cinaedi* infection can occur regardless of a patient's immunologic or environmental status.

H. cinaedi infections are often reported in Japan. Miyake et al. reported that the *H. cinaedi* detection rate has increased after introduction of the BACTEC system (*6*). We also used BACTEC bottles. The widespread use of this type of blood culture bottle throughout Japan might contribute to the positivity rate of *H. cinaedi*.

We report a case of *H. cinaedi* hepatic cyst infection with bacteremia. *H. cinaedi* infection can occur in both nosocomial and community-acquired situations and in both immunocompromised and immunocompetent patients; its manifestations vary quite widely. Although the positivity rate of *H. cinaedi* is very low, it might still be overlooked. Further research is warranted to identify the epidemiologic and clinical features of *H. cinaedi* infection.

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Mycobacterium tuberculosis RD-Rio Strain in Kazakhstan

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Mycobacterium tuberculosis RD-Rio strains are still rare in the former Soviet Union countries and Asia. We describe a strain in Kazakhstan that belongs to the RD-Rio secondary branch, which is endemic to northwest Russia and eastern Europe. Although RD-Rio strains are frequently multidrug resistant, this heterogeneous branch included only drug-susceptible isolates.

The RD-Rio strain of *Mycobacterium tuberculosis* was initially described in Rio de Janeiro, Brazil, and was demonstrated to be spread beyond South America (*1*). However, RD-Rio isolates are rare in northern Eurasia (i.e., in Baltic and former Soviet Union countries) (*2*). Phylogenetically, RD-Rio is part of the Latin-American-Mediterranean (LAM) genetic family and is marked by 2 large genomic deletions, RD174 and RD-Rio (*3*); RD-Rio is speculatively associated with particular pathogenic properties (*1*).

We describe a strain from Kazakhstan with confirmed RD-Rio deletion. Molecular analysis and comparison with the global LAM dataset showed that it belongs to the particular secondary branch described in the north of European Russia and Eastern Europe. Although RD-Rio isolates have been associated with multidrug resistance (MDR) (*4*), this branch on a dendrogram (Figure, panel A) included only drug-susceptible isolates.

We conducted this study as part of an ongoing molecular epidemiologic surveillance study of *M. tuberculosis* in Kazakhstan implemented in collaboration with the Centers for Disease Control and Prevention office in Kazakhstan (CDC-Central Asia Region Office). *M. tuberculosis* strain #127 was isolated in Almaty, Kazakhstan, in 2015 from a 52-year-old man with pulmonary tuberculosis (TB). He received anti-TB treatment at the TB hospital of the Interdistrict TB Dispensary in Almaty for 2 months and was discharged. We tested the strain for drug susceptibility to the first- and second-line drugs (streptomycin, isoniazid, rifampin, ethambutol, prothionamide, ofloxacin, kanamycin, capreomycin, and cycloserine). We tested DNA for drug-resistance mutations (in *rpoB*, *katG*, *inhA*, *ahpC*, *embB*, *gyrA*, *gyrB*, *rrs*, *eis*); spoligotyping; 24-locus variable-number tandem-repeat (VNTR) typing; detection of genome deletions RD174, RD-Rio, and RD115; and LAM family–specific single-nucleotide polymorphisms (SNPs) in *Rv0129c* (Appendix, https://wwwnc.cdc.gov/EID/article/25/3/18-1179-App.pdf). The strain was susceptible to all tested drugs and did not bear drug resistance mutations in the tested gene targets. It was assigned to the RD-Rio sublineage, spoligotype SIT20 (according to SITVIT2 database, http://www.pasteur-guadeloupe.fr:8081/SITVIT2/), and Mlva15-9 code #16113-830 (according to https://www.MIRU-VNTRplus.org). We conducted phylogenetic analysis on the 24-MIRU-VNTR profile of this strain along with 357 isolates of the global LAM dataset (*5*) (Figure; Appendix Figure 1, 2).

A recent global LAM study demonstrated that SIT20 is one of the major RD-Rio spoligotypes and is subdivided into 2 branches on the basis of the ETRB locus alleles (*5*). In this study, we showed that, on the global LAM tree, the strain from Kazakhstan clustered within the branch that included only drug-susceptible isolates from northwestern Russia and Latvia with SIT20 and derived SIT1321 spoligoprofiles (Figure, panel B; Appendix Figure 1).

The case-patient's medical record contained no information about his contacts and travel before hospitalization. As a man of working age, he could have traveled to Russia as a migrant worker. It has been estimated that ≈1.9 million Kazakhstan citizens lived in Russia during 1989–2007 (http://focus-migration.hwwi.de/Russian-Federation.6337.0.html?&L=1). In 2015, a total of 2,560,000 persons, including seasonal labor migrants (62% of all outgoing migrants), were known to have migrated from Kazakhstan to Russia (https://www.iom.int/world-migration). Russian law requires that migrants with TB be deported, which may explain why this case-patient preferred to disappear or remain unavailable. Thus, a hypothesis about Russian origin of this strain is based solely on the fact that Kazakhstan is the most common country

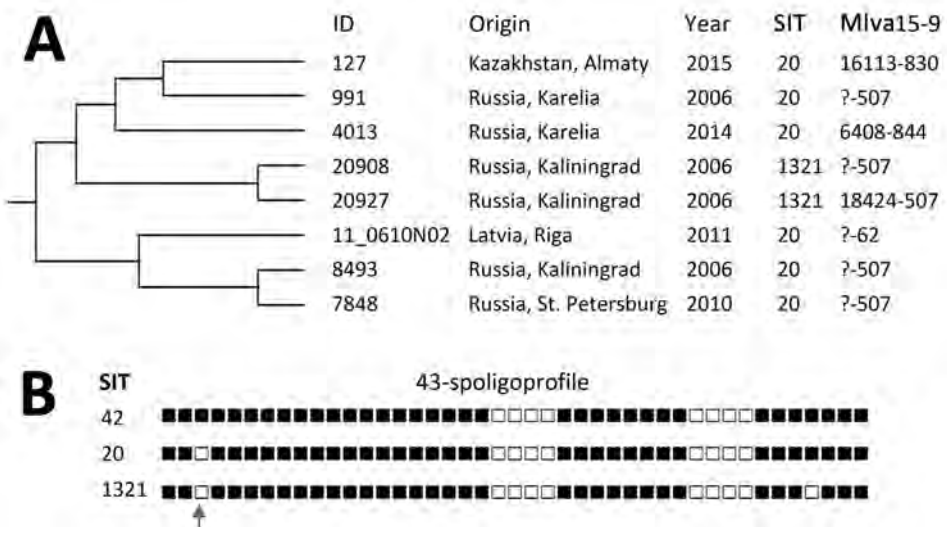

Figure. *Mycobacterium tuberculosis* RD-Rio strain in Kazakhstan. A) Section of the variable-number tandem-repeat–based dendrogram of the Latin-American-Mediterranean family of *Mycobacterium tuberculosis* RD-Rio strain with enlarged branch including SIT20 strain from Kazakhstan. All isolates were drug susceptible. The complete dendrogram with VNTR profiles is provided in Appendix Figures 1, 2 (https://wwwnc.cdc.gov/EID/article/25/3/18-1179-App.pdf). B) Binary spoligoprofiles of the studied strains.

of origin of immigrants to Russia. However, phylogenetic analysis based on high-resolution VNTR loci placed this strain within the branch exclusively made of the isolates from different and neighboring regions in northwestern Russia and Latvia (Figure). We consider this clustering to be evidence that this strain is related to the *M. tuberculosis* population in the European part of Russia. Another example of cross-country *M. tuberculosis* transmission is the "successful Russian strain" Beijing B0/W148-cluster; its overall prevalence in Kazakhstan is low, at 3%, and its isolates were identified in the northern part of the country that is close to Russia (*6*).

Previously, strains of the LAM RD-Rio or SIT20 spoligotype were not described in several countrywide studies in Kazakhstan during 1997–2014 (*2*,*6*–*8*). In neighboring Kyrgyzstan, SIT20 was not described either in the civilian or penitentiary settings (*9*,*10*). In view of the rarity of RD-Rio isolates in northern Eurasia and their previous absence in Kazakhstan, the isolation of such a strain in Kazakhstan, especially in the most distant southern region, deserves attention. That no other isolates have been found through our ongoing surveillance strongly suggests the strain was imported and not acquired in Kazakhstan. The isolates in this SIT20 Russian branch were sufficiently heterogeneous in terms of VNTR locus diversity; they were isolated in different years, and all isolates were drug susceptible (Figure). RD-Rio is known to be associated with MDR, and even by chance, some of these isolates in former Soviet Union countries could have acquired drug resistance under the current adverse conditions of TB control in this region. Nevertheless, these strains have remained drug susceptible. Further surveillance will be needed to determine if additional strains appear and, if so, whether they remain drug susceptible or acquire drug resistance.

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Detection of Influenza C Virus Infection among Hospitalized Patients, Cameroon

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We report 3 cases of influenza C virus in children hospitalized with severe acute respiratory infection in Cameroon. Two of these case-patients had grave clinical manifestations, but all 3 recovered. The lack of specific antiviral drugs for influenza C virus highlights the need to identify and describe cases involving this virus.

Four types of influenza viruses are known: A, B, C, and D (*1*). Unlike influenza viruses types A and B, influenza C viruses generally cause a mild respiratory illness (*2*). However, some cases of lower respiratory infections have been described in children (*1*–*3*). In recent years, severe illness due to influenza C virus has been reported from different geographic regions, but few data have come from Africa (*3*–*6*). Previous detection of influenza C virus in Cameroon reported 2 cases among 561 patients with influenza-like illness (*7*). We identified 3 cases of influenza C virus infection among hospitalized patients with severe acute respiratory infection (SARI) in Cameroon.

Respiratory samples were collected as part of the influenza surveillance system in Centre Hospitalier d'Essos, a SARI site located in the central region of Cameroon that has been involved in surveillance activity since the onset of the program in 2007. At this site, hospitalized SARI patients are screened for other respiratory pathogens, including bacteria and viruses, within the framework of an internal project running concomitantly with influenza surveillance activity since January 2017. The Cameroon National Ethics Committee gave ethics clearance for this study (document no. 2017/03/876/CE/CNERSH/SP). Each child's parent or guardian also provided written consent before sample collection. While awaiting laboratory analyses, the children received presumptive treatments, including antipyretic, antimicrobial, and antimalarial drugs. No antiviral drugs are available to treat patients with influenza C virus infection.

Nasopharyngeal swab specimens were collected in universal transport medium and transported to Centre Pasteur du Cameroon (Yaounde, Cameroon), where we performed analyses. We extracted RNA from the samples using a QIAamp Viral RNA Mini Kit (QIAGEN, http://www.qiagen.com) according to the manufacturer's instructions. We then analyzed samples for the presence of 33 respiratory pathogens using a real-time reverse transcription PCR (Fast-Track Respiratory Pathogens 33 PCR kit; Fast-Track Diagnostics Ltd., https://www.fast-trackdiagnostics.com), obtained through the International Reagent Resource Program (https://www.internationalreagentresource.org). We ran the assay in an Applied Biosystems Prism 7500 thermocycler (Thermo Fisher Scientific, Inc., https://www.thermofisher.com) and considered all reactions with cycle thresholds <37 positive for the tested pathogens. We confirmed influenza C virus in samples from 3 children, 11 months, 3 years, and 4 years of age. The age distribution of the patients is compatible with reports showing that most humans acquire antibodies to influenza C virus early in life (*3*).

Other studies have shown that influenza C infection in infants can be severe enough to require hospitalization, compared with infection in adults (*3*,*8*). All 3 patients had fever, cough, rhinorrhea, asthenia, and conjunctivitis, but 2 had additional grave clinical manifestations. One patient had exacerbated symptoms, including dyspnea, vomiting, diarrhea, and stage II coma; another had an altered general state. All 3 patients recovered.

Unlike similar studies in which influenza C was identified mostly in patients with co-infections of other respiratory pathogens (*4*,*5*), we did not detect co-infection in these 3 patients. Further, absence of underlying or preexisting medical conditions in the children indicates that their illnesses were solely caused by influenza C virus infection. Because all 3 cases were detected in the course of a project focusing on hospitalized patients in a single center, additional cases of influenza C infection among outpatients and hospitalized patients are likely.

We used influenza C sequences available in the GISAID EpiFlu database (https://www.gisaid.org) as of April 18, 2018, to design primers for whole-genome sequencing by next-generation sequencing and Sanger sequencing of the hemagglutinin-esterase (HE) gene. Based on the HE gene, 6 distinct clades of influenza C virus have been identified: C/Taylor/1233/47, C/Mississippi/80, C/Aichi/1/81, C/Yamagata/26/81, C/Kanagawa/1/76, and C/Sao Paulo/378/82 (*5*). Two of the patients' specimens yielded good sequence data, and maximum-likelihood HE gene phylogenetic analysis showed the Cameroon viruses cluster in the C/Sao Paolo/378/82 lineage (Figure), a dominant clade that has been detected in several other countries (*5*,*9*,*10*).

Studies have shown variability in the circulation of influenza C virus, with peaks in winter and spring seasons

(*3*,*4*). These 3 cases all occurred in December 2017, possibly indicating seasonality of influenza C infection in Cameroon, but identification of more cases is required to confirm this hypothesis.

The recent identification of severe illnesses related to influenza C virus infection in Cameroon and the lack of influenza C–specific antiviral drugs highlight the importance of integrating molecular testing for this virus into existing inpatient and outpatient sentinel surveillance systems and for in-depth studies of the epidemiology of influenza C viruses. This process could lead to predominant circulating strains of influenza C virus being included in seasonal influenza vaccines to protect vulnerable populations.

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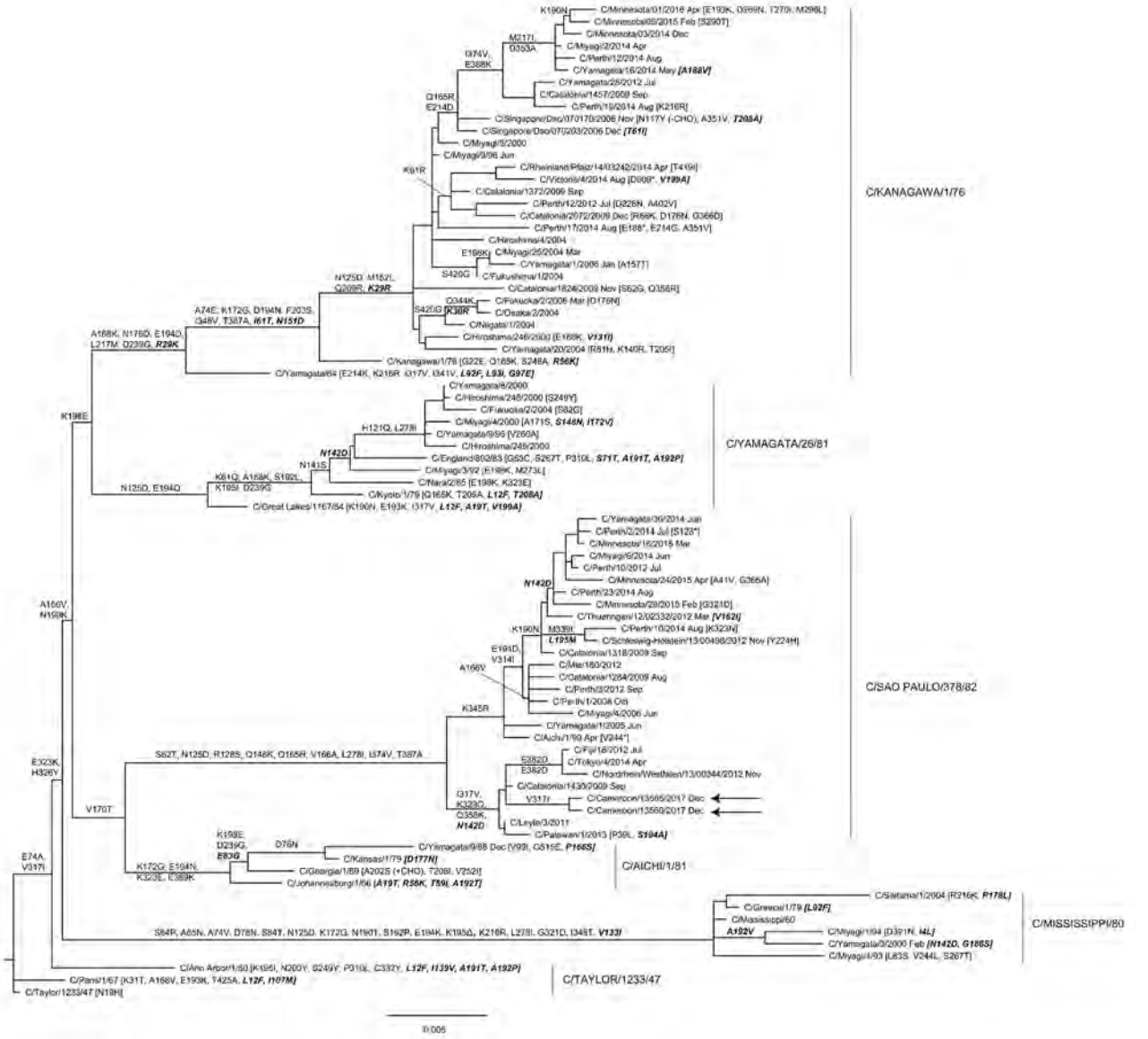

Figure. Hemagglutinin-esterase gene phylogeny for influenza C viruses detected in Cameroon compared with reference viruses. The phylogeny is based on 80 full-length open-reading frames downloaded from the GISAID EpiFlu database (https://www.gisaid.org), with signal peptide coding regions and stop codons removed, yielding products of 1,923 nt. The phylogeny was estimated by using RaxML version 8.2.X (https://sco.h-its.org/exelixis/software.html) with a general time-reversible plus gamma substitution model and then annotated with amino acid substitutions defining nodes and individual virus gene products by using treesub (https://github.com/tamuri/treesub/blob/master/README.md). The phylogeny was visualized by using FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree). Sequences for the 2 viruses from Cameroon (arrows) have been deposited in the EpiFlu database under accession nos. EPI1259829 (C/Cameroon/13560/2017) and EPI1259835 (C/Cameroon/13565/2017).Scale bar indicates nucleotide substitutions per site.

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Healthcare Provider Discrimination toward Pregnant Women with Rifampin-Resistant Tuberculosis

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Little is known about the treatment experiences of pregnant women with rifampin-resistant tuberculosis. We conducted qualitative interviews with 10 women who had this condition; 9 reported facing discrimination from healthcare providers. Our findings underscore an urgent need to ensure a human-rights–based, patient-centered approach for women with rifampin-resistant tuberculosis who are pregnant.

We report discrimination by healthcare providers toward pregnant women with rifampin-resistant tuberculosis (TB) in KwaZulu-Natal Province, South Africa. Pregnant women are at increased risk for all forms of tuberculosis (*1*). Experience in caring for pregnant women with rifampin-resistant TB is limited (*2–4*), and little is known about their experiences during treatment (*5*).

In 2016, a total of 3,280 patients at the King Dinuzulu Hospital Complex in KwaZulu-Natal Province were started on treatment for rifampin-resistant TB; 47% of these patients were women, many in their childbearing years (*6*). During 2016–2017, we conducted qualitative interviews with 10 women as part of an observational study on bedaquiline use during pregnancy, with the goal of describing treatment challenges.

All 10 of the women were postpartum and ranged in age from 22 to 37 years. The mean number of pregnancies was 3 (range 2–5). Two women were on rifampin-resistant TB treatment when they became pregnant, 7 women had rifampin-resistant TB diagnosed during pregnancy, and 1 had rifampin-resistant TB diagnosed and found out she was pregnant at the same time. Nine of the 10 women reported experiencing discrimination while accessing healthcare, either from the antenatal care providers (9 women), the TB care providers (4 women), or both (4 women). (Rifampin-resistant TB and antenatal care services are not integrated in this setting.)

The reported discrimination faced by the women while accessing antenatal care and during delivery was largely

based on a fear of resistant TB transmission. Even when the women were no longer infectious, they were forced to wear masks, the nurses caring for them began wearing gloves, and they were isolated during the delivery and in the postpartum period. Three women reported that they were not evaluated after delivery and that they were left alone without food for themselves or milk for their newborns. Women also reported that they felt pressured into considering pregnancy termination by their antenatal care providers, who sometimes referred to their unborn children as "that thing"; however, none of the women interviewed terminated their pregnancy.

Most of the women interviewed also reported discrimination from rifampin-resistant TB care providers. Some providers told the women they were "foolish" for becoming pregnant during treatment. Several of the women also reported that some care providers ridiculed them for continuing with their pregnancies, stating "We don't know what [you] will give birth to." Some women were offered incomplete treatment regimens (e.g., regimens that were missing ≥1 drugs for part of the treatment period, including ethionamide, injectable agents, or bedaquiline). Some women experienced a delay in the start of rifampin-resistant TB treatment or were started on first-line therapy even though they had a confirmed diagnosis of resistant TB.

Although the number of women in this study is small and their experiences might not be representative of all pregnant women, the common experience of reported discrimination is cause for concern. Proper infection control practices are needed to reduce nosocomial and community transmission of rifampin-resistant TB, but most of these women reported that the discrimination occurred even after they were unlikely to be infectious (*7*).

Some of the discriminatory acts might have placed these women and their newborns at increased risk for poor health outcomes, colored their perceptions of the healthcare systems, and adversely affected future healthcare-seeking behavior for themselves and their children. Additional exploration of this phenomenon is needed along with practical solutions to ensure pregnant women with rifampin-resistant TB receive the best possible care during this challenging period of their lives.

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The study protocol was approved by the Ethics Committee of the South African Medical Research Council (reference no. EC17-6/2016), KwaZulu-Natal Department of Health and King Dinuzulu Hospital, Durban. All participants provided written informed consent and were reimbursed for their time and travel.

All authors had full access to all the data in the study. M.L., S.H., and J.F. conceived and designed the study; M.L. and S.H. conducted the study; M.L., S.H., and J.F. analyzed the data; and M.L., S.H., and J.F. wrote the paper. M.L., as corresponding author, had final responsibility for the decision to submit for publication. The final version of the manuscript has been read and approved by all the authors, and the requirements for authorship have been met. Each author believes that the manuscript represents honest work.

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Mycobacterium chimaera Pulmonary Disease in Cystic Fibrosis Patients, France, 2010–2017

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We report *Mycobacterium chimaera* pulmonary disease in 4 patients given a diagnosis of cystic fibrosis in a university hospital in Montpellier, France. All patients had *M. chimaera*–positive expectorated sputum specimens, clinical symptoms of pulmonary exacerbation, or a decrease in spirometry test results that improved after specific treatment.

Mycobacterium chimaera is a member of the *Mycobacterium avium* complex, which was elevated to species rank in 2004. *M. chimaera* was reported by Tortoli et al. (*1*) as a cause of human lung disease but has been widely known as the bacteria responsible for an outbreak of endocarditis and disseminated infection after cardiac surgery in 2013 (*2*).

Although virulence and pathogenicity of *M. chimaera* in lung disease are currently debated, several cases of *M. chimaera* lung infections have been reported in settings of chronic obstructive pulmonary disease, malignancy, or immunosuppression (*3*–*5*). We found 1 case of *M. chimaera* infection in a patient with cystic fibrosis (*6*). Other nontuberculous mycobacteria (NTM), especially *M. abscessus* and *M. intracellulare*, are well-known pathogens in such a setting (*7*). We report *M. chimaera* pulmonary disease in 4 patients with cystic fibrosis.

After reviewing data for 248 patients who were examined at the Cystic Fibrosis Center of Montpellier, France, during 2010–2017, we observed that 24 (9.7%) of 248 patients had ≥1 respiratory smear sample positive for NTM; for 4 (16.7%) of 24, the sample was positive for *M. chimaera*. The 4 case-patients were Caucasian, age 8–21 years, and who had a newborn diagnosis of cystic fibrosis, preexisting respiratory impairment, and digestive malabsorption. The association of an increased cough and sputum production, breathlessness, and fatigue with a reduction in forced expiratory volume in 1 s (FEV1) or forced vital capacity (FVC) was diagnosed as pulmonary exacerbation (*7*) for all patients (Figure). Diagnosis was confirmed by computed tomography by the presence of bronchiectasis and nodules (tree-in-bud pattern) for case-patients 1 and 3.

Respiratory specimens collected every 3–6 months for 1 year were digested and decontaminated by using the sodium dodecyl sulfate–NaOH method and then centrifuged using fluorescence microscopy. Sputum samples from all patients were negative for acid-fast bacilli. Samples were cultured on solid and liquid media (BACTEC MGIT 960 System; Becton Dickinson Diagnostic Systems, https://www.bd.com) which identified, after 11–41 days, mycobacteria from ≥2 separate sputum samples.

We performed species identification by using a commercial kit (GenoType NTM-DR assay; Hain Lifescience, https://www.hain-lifescience.de) and identified isolates as *M. chimaera*. We also performed molecular identification of isolates as *M. chimaera* as described (*1*,*8*). All isolates were susceptible to macrolides and aminoglycosides. We also isolated several other microorganisms: *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* from case-patient 1; *Haemophilus influenzae* from case-patient 2; *M. avium* and *Stenotrophomonas maltophila* from case-patient 3; and *M. abscessus*, methicillin-resistant *S. epidermidis*, and *P. aeruginosa* from case-patient 4.

Azithromycin, rifampin, and ethambutol (in combination) and ceftazidime, tobramycin, and inhaled colistin were given to case-patient 1. No antimicrobial drugs were given to case-patient 2. Azithromycin and rifampin (in combination) and inhaled colistin were given to case-patient 3. Clarithromycin and linezolid were given to case-patient 4. All 4 case-patients required physiotherapy.

All case-patients were followed up for ≥1 year after the first positive smears for *M. chimaera* were obtained. We found a substantial reduction in symptoms of pulmonary exacerbations and sterilization of sputum specimens for patients given macrolides and rifampin with or without aminoglycosides after 3 months, as well as improvement in FEV1 and FVC after 6 months. In contrast, patients not given treatment (case-patient 2) or given only partial treatment with an anti-NTM antimicrobial drug regimen (case-patient 4) showed a decrease in FEV1 and FVC after 6 months (Figure) and slight recovery or no change after 1 year.

Therefore, we hypothesized that *M. chimaera* showed virulence and pathogenicity in our patients because of their clinical picture and evolution. We are aware that the diagnosis of NTM diseases according to American Thoracic Society criteria (*9*) might not be made with complete certainty because definitive exclusion of other diagnoses was often

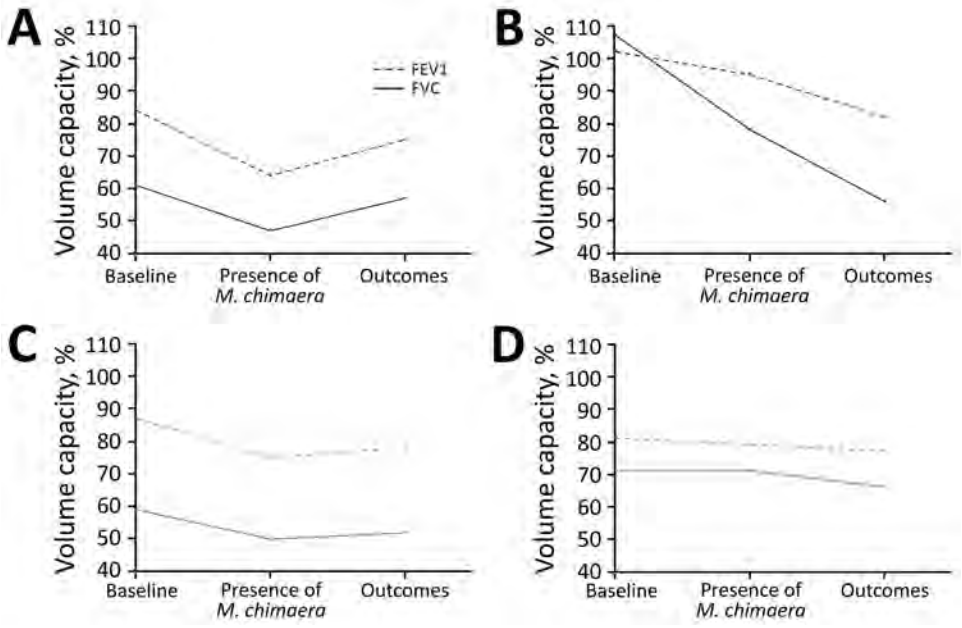

Figure. Evolution of lung function for 4 cystic fibrosis patients with *Mycobacterium chimaera* pulmonary disease, France, 2010–2017. A) Case-patient 1, B) case-patient 2, C) case-patient 3, D) case-patient 4. Case-patients 1 and 3 were given specific treatment for *M. chimaera* disease for 3 months; case-patient 2 was not given specific treatment; case-patient 4 was given only partial treatment. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

difficult for cystic fibrosis patients. Several confounding factors, such as co-infection with conventional pathogens, were observed, which could explain the observed favorable outcome. However, specific treatment against NTM improved outcome, which strengthens our presumption of the potential pathogenic role of *M. chimaera* in lung disease of patients with cystic fibrosis.

Cystic fibrosis transmembrane conductance regulator disorder results in mucus retention and bronchiectasis that favor repeated respiratory tract infection, including NTM diseases (*7*). In these circumstances, cystic fibrosis might promote *M. chimaera* infection in a similar manner to that in patients with chronic obstructive pulmonary disease. The lack of improvement of respiratory function for case-patient 4, who had been given treatment for infection with other pathogens, but only partially for *M. chimaera*, supports our hypothesis. However, whether *M. chimaera* is only a surrogate of respiratory impairment without any virulence or a real pathogenic microorganism remains unknown. In conclusion, *M. chimaera* lung disease should prompt physicians to consider this bacteria as an emergent pathogen in cystic fibrosis patients.

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Biomphalaria pfeifferi Snails and Intestinal Schistosomiasis, Lake Malawi, Africa, 2017–2018

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Two surveys conducted in 2017 and 2018 demonstrated *Biomphalaria pfeifferi* snails in Lake Malawi in Africa. Epidemiologic examination of 175 local children at 3 primary schools confirmed emergence of intestinal schistosomiasis. These findings highlight autochthonous transmission of *Schistosoma mansoni* flukes in Lake Malawi and the need to revise international travel advice.

Throughout sub-Saharan Africa, *Biomphalaria pfeifferi* snails are freshwater intermediate hosts for *Schistosoma mansoni* blood flukes, which cause intestinal schistosomiasis (*1*). Geographic distribution of *B. pfeifferi* snails delineates actual or potentially active zones of *S. mansoni* fluke transmission (*2*). Other than a report of a single *Biomphalaria* shell at Karonga in the far northern portion of Lake Malawi (*3*), considered to be from a marginal swamp (*4*), *B. pfeifferi* snails have not previously been found in Lake Malawi (*5*). However, in November 2017, during malacologic surveillance for intermediate hosts of schistosomiasis in the Mangochi District, Malawi, along the southernmost tip of Lake Malawi, 2 discrete populations of *Biomphalaria* snails were unexpectedly encountered in submerged beds of *Vallisneria* spp. plants (Figure, panel A). DNA sequence analysis of the mitochondrial cytochrome oxidase subunit 1 (*cox*1) (*6*) indicated that the *cox*1 sequences (1,006 bp) of those snails differed from sequences of *B. pfeifferi* snails from Chiweshe, Zimbabwe (GenBank accession nos. DQ084829 [HCO/LCO region] and DQ084872 [Asmit1/2 region]) by only 3 synonymous single-nucleotide polymorphisms.

In May 2018, to confirm *B. pfeifferi* colonization within the lake and suspected risk for intestinal schistosomiasis, we undertook a conjoint malacologic and parasitologic survey with ethics approvals from the Liverpool School of Tropical Medicine, UK (application 17-018) and the Ministry of Health and Population, Malawi (application 1805). Reinspection of all prior malacologic sampling locations and another 43 sites found further populations of *B. pfeifferi* snails (Figure, panel A); large numbers (>50), along with innumerable dead shells, were again found at site 9. All snails were inspected for shedding cercariae, and although cercariae from snails at site 5 were seen, identification by microscopy (×100) was unsuccessful. Supplementary analysis indicated that *cox*1 sequences from 9 snails from sites 2, 5, 7, 10, and 11 were identical.

We conducted an epidemiologic survey of 175 schoolchildren, 5–15 years of age, equal numbers of boys and girls, from 3 primary schools closest to site 9 (Figure, panel B). Mean prevalence of intestinal schistosomiasis, calculated by detection of *S. mansoni* circulating cathodic antigen (CCA) on urine dipstick testing, was 34.3% (95% CI 27.9–41.3); prevalence rates by school were Samama, 46.7% (95% CI 36.7–56.7); Mchoka, 25.0% (95% CI 15.0–36.7); and Palm Beach, 9.1% (95% CI 0.0–22.7). We requested fecal samples from 60 *S. mansoni*–positive children and received samples from 46. Duplicate Kato-Katz examinations confirmed *S. mansoni* ova in 7 children; infection intensities were graded as light (<100 eggs/g feces). All urine samples were inspected for *S. haematobium* ova by syringe filtration (10 mL); general prevalence was 14.9% (95% CI 9.8–20.1); 52% of these samples were also positive by CCA urine dipstick, indicative of *S. mansoni* co-infection. To further determine autochthonous transmission of *S. mansoni* flukes, 2 egg-positive children from Samama and Mchoka took us, on foot, to the shoreline where they regularly swam, which corresponded to snail

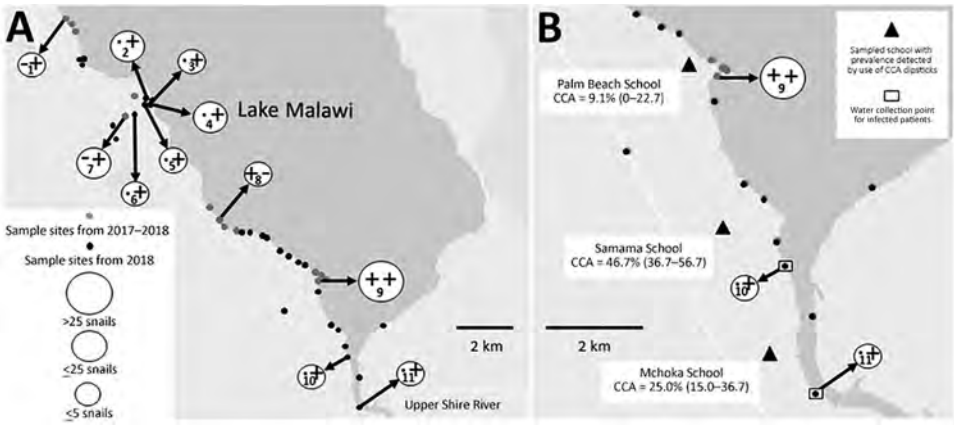

Figure. Locations sampled for *Biomphalaria pfeifferi* snails and of 3 primary schools where children were tested for intestinal schistosomiasis in the region of Lake Malawi, Africa. A) Locations sampled for *B. pfeifferi* snails in November 2017 (gray dots) and May 2018 (black dots), Lake Malawi, Africa. + indicates snails present, – indicates snails absent, and ● indicates site not sampled; symbol position indicates year of sampling (left, 2017; right, 2018). Numbers within circles indicate site numbers. Collected snail numbers are indicated by circle size. In 2017, snails were collected at 2 sites and not collected at 12 sites; in 2018, snails were collected at 10 sites and not collected at 47 sites. On each sampling occasion, >50 *B. pfeifferi* snails were collected at site 9. Coordinates of *B. pfeifferi*–positive sites: site 1, 14.27752°S, 35.10419°E; site 2, 14.31371°S, 35.14174°E; site 3, 14.31424°S, 35.14383°E; site 4, 14.31354°S, 35.14424°E; site 5, 14.31568°S, 35.14030°E; site 6, 14.32033°S, 35.13613°E; site 7, 14.32100°S, 35.13072°E; site 8, 14.36919°S, 35.17629°E; site 9, 14.39363°S, 35.22104°E; site 10, 14.42708°S, 35.23349°E; and site 11, 14.44928°S, 35.23890°E. B) Location of the 3 sampled primary schools (Palm Beach, 14.391346°S, 35.215137°E; Samama 14.417465°S, 35.217580°E; Mchoka 14.439481°S, 35.220644°E) showing local prevalence (% [95% CI]) of intestinal schistosomiasis indicated by *Schistosoma mansoni* circulating cathodic antigen (CCA) detected by urine dipstick. Water collection sites pinpointed by 2 *Schistosoma* egg–positive children from Samama and Mchoka Schools are indicated.

collection sites 10 and 11 (Figure, panel B). Children who were positive for either *S. mansoni* CCA or *S. haematobium* eggs received praziquantel (40 mg/kg).

Colonization of *B. pfeifferi* snails in Lake Malawi and surrounding water is of concern, especially because active *S. mansoni* infections were found in local children. This finding highlights emergence of intestinal schistosomiasis, not previously documented here (*5*,*7*,*8*) or detected in this region by the most recent national survey (F. Fleming, Schistosomiasis Control Initiative, Imperial College London; 2017 Dec 20; pers. comm).

Intestinal schistosomiasis has been detected in children ≈150 km away, along the shoreline of the Lower Shire River (*9*). Finding snails and infected children in Mangochi District suggests recent ecologic and epidemiologic change. In May 2018, the lake was ≈75–80 cm higher than in November 2017, which perhaps favored detection of *B. pfeifferi* snails in the previously more accessible *Vallisneria* plant beds. Seasonal dynamics, such as lake level fluctuations, are well known, along with longer duration perturbations of the lake biota, either induced by climate change or mediated by anthropogenic activities. These changes have altered transmission of urogenital schistosomiasis (*10*); overfishing, particularly of the molluscivorous fish *Trematocranus placodon,* is changing the distribution of many freshwater snails (*5*).

Local aquaculture of fish (e.g., *Oreochromis* spp., called chambo) through use of water pumped inland from the lake has created novel, permanent water bodies colonized by *B. pfeifferi* snails (e.g., sites 2–7), which may now (re)seed snails into the lake for further establishment. Absence of *cox*1 genetic diversity in the *B. pfeifferi* snails we sampled implies a limited number or even a single founder event, but as conditions for autochthonous transmission became favorable, after introduction of *S. mansoni* flukes, intestinal schistosomiasis in local schoolchildren has emerged. This finding is of substantial public health concern in light of current control efforts, which consist only of annual praziquantel distribution in schools (*7*,*8*). We recommend increased surveillance of snails and characterization of schistosomes, along with intensified control interventions to arrest further spread of intestinal schistosomiasis. We also recommend revising and updating health and travel advice given to shoreline community residents and tourists who use the lake.

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Mr. Alharbi is a PhD student under the supervision of L.J. and J.R.S. He has specific interests in medical malacology and molecular epidemiology of schistosomiasis in Africa and the Kingdom of Saudi Arabia.

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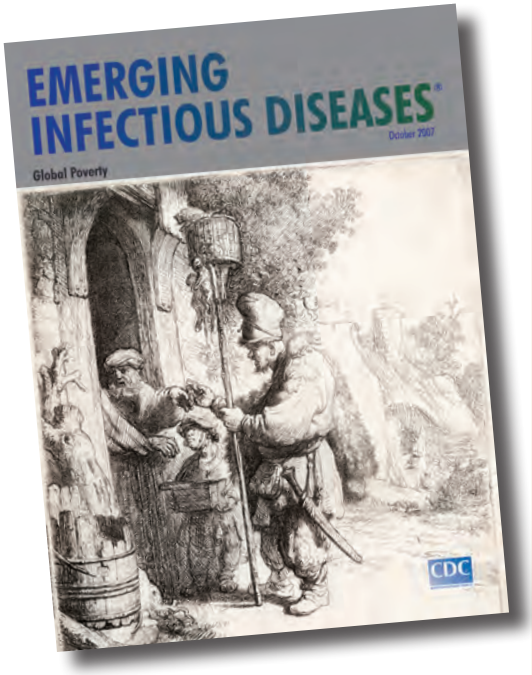

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etymologia revisited

schistosomiasis

[shis”-, skis” to-so-mi’ə-sis],
from the Greek—*skhistos* (split) and *soma* (body)

Infection of the blood with a parasite of the genus *Schistosoma*. Originally thought a single organism with a split body, the parasite was eventually recognized as having male and female forms. Three main species cause human infection: *S. haematobium*, *S. mansoni*, and *S. japonicum*. Each species has its own range of host snails. The parasite releases eggs containing larvae through feces or urine; if the eggs reach water, the larvae are released and may penetrate a snail. A very large number of larvae are then produced inside the snail and released back into the water. Infection is acquired through skin contact with contaminated water.

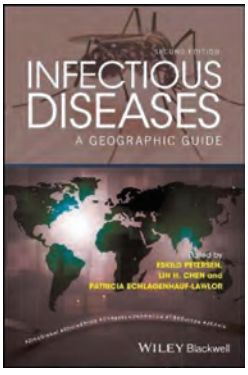
Schistosomiasis, which leads to chronic hepatic and intestinal fibrosis of the urinary tract, was first identified in Egypt in 1851 by German pathologist Theodor Bilharz and is also called bilharzia. Approximately 160 million persons throughout the world are infected, particularly in Africa, the Middle East, South America, and Southeast Asia.

Source: Institute of Tropical Medicine of Antwerp: www.itg.be

https://wwwnc.cdc.gov/eid/article/13/10/e1-1310_article

Infectious Diseases: A Geographic Guide, Second Edition

Eskild Petersen, Lin H. Chen, Patricia Schlagenhauf-Lawlor, editors; Wiley Blackwell, Oxford, UK, 2017; ISBN-13: 978-1119085720; ISBN-10: 1119085721; Pages: 520; Price: US $109.95



In these modern times of frequent overseas travel, a succinct reference describing infectious hazards for each potential destination can prompt travelers to seek preventive medical advice before they travel, rather than treating infections after they return. This second edition of Infectious Diseases: A Geographic Guide makes a useful attempt at providing a resource for travelers and clinicians alike.

The volume is divided into 3 sections. The first introduces concepts related to travel-associated infections. The second, main section includes geographic regions and descriptions of infectious agents endemic to each. The closing chapters provide additional information, such as the impact of migration and climate change on the distribution of infectious agents.

The introductory chapters vary in style, depth, and length; cover some of the history and politics of infectious diseases; and offer insight into the resources and technology required to detect and combat pathogens. Chapter 6, in particular, gives a fairly detailed view of diagnostic tests and lays out the anatomic approach the authors use to describe disease symptoms in the later geographic chapters. Written by the lead editor, Eskild Petersen, this chapter not only explains and compares the types and limitations (e.g., sensitivity and specificity) of diagnostic tests required for detecting the various pathogens but also the accompanying characteristics of other tests (e.g., biochemistry, cytology, radiological imaging) routinely requested when investigating these infections.

The chapters in the geographic section are the focus of the text. Each follows a formula that starts with a boxed summary, then progresses through a head-to-toe anatomic listing of pathogens and associated symptoms in each organ, mostly in the form of tables indicating frequency of occurrence. The authors added a nice touch by further separating some tables into infections with symptoms lasting >4 weeks or <4 weeks, which can help clinicians narrow the differential diagnosis. Each chapter also includes sections describing antibiotic-resistant and vaccine-preventable infections.

Chapters in the geographic section necessarily vary in length and depth depending on the amount of data available, but there are a few unexpected omissions. For instance, plague (*Yersinia pestis*) is absent from the chapters on East Africa and North America, despite being endemic in Madagascar and the United States for more than a century. In addition, severe fever with thrombocytopenia syndrome, an emerging infectious disease identified in China, is absent from the chapter on East Asia. Surprisingly, the index is relatively inconsistent, making it important to read both the accompanying text and the tables. For instance, the East Asia chapter only includes avian influenza H5N1 in the index, not avian influenza H7N9, but the authors discuss only the latter briefly in the text. One table in the North America chapter lists influenza as a rare microorganism under cardiopulmonary infections, which is clearly inaccurate. An additional column devoted to seasonal pathogens might remedy similar inaccuracies in some tables.

This text mostly achieves what the authors propose, although the content of some chapters needs updating. In addition, a more detailed and comprehensive index, ideally separate for the tables and main text, would make it even more helpful for busy clinicians. Overall, this is a useful companion for both the physician and traveler alike.

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Romanticism, *Mycobacterium*, and the Myth of the Muse

Dennis Mahoney and Terence Chorba

At the transition of the 18th into the 19th century, large numbers of deaths in Europe, especially those in urban areas, were associated with tuberculosis. During those two centuries, many celebrated artists, musicians, and literary giants were lost to the disease. Romanticism—Europe's dominant artistic, musical, and intellectual movement that began in the late 18th century and waned after 1850—emphasized individualism and emotion. Characteristic themes included the goodness of people, from which urban life detracted, and the simplicities of childhood and all things natural. A popular myth arose that this movement was favored by tuberculosis, which putatively augmented one's creative faculties. Classicists viewed this belief as consistent with what ancient Greek physicians had called the *spes phthisica*—an earnest hope of recovery from tuberculosis that drove heightened sensitivity and great creativity despite overwhelming illness. Portrayals of this view appear in Alexander Dumas's *La Dame aux Camélias*, Victor Hugo's *Les Misérables*, Giuseppe Verdi's *La traviata*, and Giacomo Puccini's *La bohème*.

Among German writers of the Romantic era who had tuberculosis were Johann Wolfgang von Goethe (1749–1832; best known to English speakers for his poetic drama *Faust*), Friedrich Schiller (1759–1805; trained as a physician and author of "An die Freude"—the Ode to Joy in the final movement of Beethoven's Ninth Symphony), and Georg Philipp Friedrich von Hardenberg (1772–1801; principal poet-theoretician of Early German Romanticism). Goethe received his tuberculosis diagnosis when in his early 20s and recovered fully after several years of convalescence. In contrast, Schiller died of pulmonary tuberculosis at age 46 after a period of increasing lethargy.

The undated and unsigned portrait on this month's cover is that of Hardenberg, better known under his pen name Novalis (i.e., "the clearer of new ground," a poetic goal derived from the surname of his medieval ancestors). Beginning in 1790, he studied at the University of Jena under Schiller and later completed his doctorate in law at the University of Wittenberg, both in what is modern-day Germany. After losing his first fiancée, Sophie von Kühn, to tuberculosis in 1797, he studied mining in Freiberg and fell in love with Julie von Charpentier, daughter of one of his professors. In August 1800, they intended to marry, but he began coughing up blood. His worsening health led him to return to Jena for a consultation with Johann Christian Stark, the physician who had provided treatment for Schiller and Sophie von Kühn. After unsuccessful treatment in Dresden, in January 1801 Hardenberg returned to the family residence of Weissenfels in Saxony, where he died on March 25. At his deathbed was Friedrich Schlegel (1772–1829), who had already published *Hymnen an die Nacht* (Hymns to the Night), a unique combination of rhythmic prose and strophic verse that helped establish Hardenberg's reputation as a poet. Schlegel also published a collection of Hardenberg's poetic writings after Hardenberg died, co-edited by Ludwig Tieck (1773–1853), another leading figure in German Romanticism. This collection included *Heinrich von Ofterdingen*, an eponymous piece of historical fiction,

Artist uncertain, Georg Philipp Friedrich von Hardenberg (Novalis), c. 1772–1801. Oil on canvas, 22.5 in x 20.5 in/57 cm x 52.1 cm. Collection of the *Forschungsstätte für Frühromantik und Novalis-Museum* (Research Center for Early Romanticism and Novalis Museum), *Schloss Oberwiederstedt* (Oberwiederstedt Castle), Wiederstedt, Germany. With publication permission of those institutions, the *Ostdeutsche Sparkassenstiftung* (East German Savings Bank Foundation), and *Sparkasse Mansfeld Südharz* (Mansfield Southern Harz Savings Bank).

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one of the best-known and most uncompromising depictions of the transformative power of art and the metaphysical quest for the unattainable.

Until recently, this cover portrait was widely attributed to Franz Gareis (1775–1803). Gareis was a student of the noted portrait painter Anton Graff, who had painted a portrait of Schlegel in 1798. Later, Schlegel asked Hardenberg to be the subject of a Gareis portrait, but there is no indication that Gareis and Hardenberg ever met. A more likely creator of this portrait was Tieck's sister-in-law, Maria Agatha Alberti (1767–1810), a private student of Graff and Gareis in Dresden at a time when women were not admitted for study at the Dresden Art Academy. Alberti was also part of the circle of friends who ministered to Hardenberg in his final winter. In a letter in June 1801, Karl von Hardenberg, the poet's brother, asked of Tieck that Alberti paint the poet posthumously. Recently published letters show that in 1805, Alberti was in Weissenfels, where she made portraits of Karl, his mother, and his nephew Erasmus. If the Novalis portrait is by Alberti, it is a retrospective depiction, perhaps based on an earlier sketch of the poet, conveying both his frailty and immortal spirit. Tieck attested to the authenticity of this portrait and asked the Hardenberg family whether he might keep it in his possession until his death in 1853.

Memorial at the grave of Novalis in Weissenfels with a bust of the poet created by Fritz Schaper, a renowned sculptor and member of the Prussian Academy of the Arts, in 1901. Photograph by Doris Antony, Berlin, Germany.

Several years later, Jean-Antoine Villemin demonstrated the transmissibility of tuberculosis by infecting laboratory rats with material extracted from human cadavers. In 1882, Robert Koch identified *Mycobacterium tuberculosis* by staining the sputum of patients with pulmonary tuberculosis with alkaline methylene blue; on March 24 of that same year, a generation after Romanticism began to wane, he demonstrated conclusively that *M. tuberculosis* was the causative organism of the disease. A societal change of view then followed; the Romanticist position that consumption was a tragic gift inspiring creativity diminished. By the end of the 19th century, the dominant view was that environmental controls and social distancing benefited the community by providing protection from this contagious illness. The result was dramatically decreased numbers of cases of illness and death from tuberculosis, several decades before effective antimicrobials became available.

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Upcoming Issue

- Resurgence of Vaccine-Preventable Diseases in Venezuela as a Regional Public Health Threat in the Americas
- Mucosal Leishmaniasis in Travelers with *L. braziliensis* Complex Returning to Israel
- Lobomycosis in Soldiers, Colombia
- Tickborne Relapsing Fever in the White Mountains, Arizona, 2013–2018
- Differences in Neuropathogenesis of California Serogroup Viruses
- *Staphylococcus aureus* Bacteremia in Children of Rural Gambia, 2008–2015
- MERS-CoV Infection Dynamics and Antibody Responses among Clinically Diverse Patients, Saudi Arabia
- Pneumonia-Specific *Escherichia coli* Isolates with Distinct Phylogenetic and Virulence Profiles
- Co-infections in Persons with Erythema Migrans, New York, USA
- *Francisella tularensis* Transmission by Solid Organ Transplantation
- Sandfly-Associated Phlebovirus with Evidence of Neutralizing Antibodies in Humans, Kenya
- Seroprevalence of *Borrelia burgdorferi*, *Borrelia miyamotoi*, and Powassan Virus in Residents Bitten by *Ixodes* Ticks, Maine, USA
- Distribution, Host-Seeking Phenology, and Host and Habitat Associations of *Haemaphysalis longicornis* Ticks, Staten Island, New York, USA
- Pneumonic Plague in Dog with Widespread Potential Human Exposure in Veterinary Teaching Hospital
- Prolonged Shedding of Zika Virus RNA in Vaginal Secretions, Nicaragua
- Enterovirus A71 Phenotypes Causing Hand, Foot and Mouth Disease, Vietnam
- Aerosol Transmission of *Aspergillus fumigatus* in Cystic Fibrosis Patients, the Netherlands
- Self-Flagellation as Possible Route of Human T-Cell Lymphotropic Virus Transmission
- Seroprevalence of Zika and Dengue Virus Antibodies among Migrant Workers, Taiwan, 2017

Complete list of articles in the February issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

April 3–5, 2019
National Foundation for Infectious Diseases
2019 Annual Conference on Vaccinology Research
Baltimore, MD, USA
https://www.eventscribe.com/2019/NFIDACVR/

April 13–16, 2019
European Congress of Clinical Microbiology and Infectious Diseases
29th Annual Congress
Amsterdam, Netherlands
http://www.eccmid.org/

April 16–18, 2019
International Conference on One Health Antimicrobial Resistance
Amsterdam, Netherlands
https://www.escmid.org/ICOHAR2019/

May 5–9, 2019
ASM Clinical Virology Symposium
Savannah, GA, USA
https://10times.com/clinical-virology-symposium

June 20–24, 2019
ASM Microbe 2019
San Francisco, CA, USA
https://www.asm.org/index.php/asm-microbe-2018

June 23–28, 2019
Biology of Vector-borne Diseases
Six-Day Training Course
Moscow, ID, USA
https://www.uidaho.edu/cals/center-for-health-in-the-human-ecosystem/education/vector-borne-diseases

July 14–17, 2019
STI and HIV 2019 World Congress
Vancouver, Canada
https://stihiv2019vancouver.com

July 20–24, 2019
American Society for Virology
Minneapolis, MN, USA
www.asv.org

Announcements

Email announcements to EIDEditor (eideditor@cdc.gov). Include the event's date, location, sponsoring organization, and a website. Some events may appear only on EID's website, depending on their dates.

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 75% passing score) and earn continuing medical education (CME) credit, please go to http://www.medscape.org/journal/eid. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers.

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Article Title

Treatment Outcomes in Global Systematic Review and Patient Meta-Analysis of Children with Extensively Drug-Resistant Tuberculosis

CME Questions

1. Your patient is a 10-year-old boy with extensively drug-resistant tuberculosis (XDR TB). On the basis of the global systematic review and individual patient meta-analysis by Osman and colleagues, which one of the following statements about the presentation of children from 11 countries who were managed between 1999 and 2013 for bacteriologically confirmed XDR TB is correct?

A. About half of children had pulmonary TB only
B. Among those with a recorded HIV status, one tenth were HIV-infected
C. The small sample identified over the course of 15 years highlights the serious underdiagnosis and underreporting of XDR TB in children
D. About half of children had had isolates that were resistant to at least 1 of the second-line injectable drugs (kanamycin, amikacin, or capreomycin)

2. According to the global systematic review and individual patient meta-analysis by Osman and colleagues, which one of the following statements about the treatment of children from 11 countries who were managed between 1999 and 2013 for bacteriologically confirmed XDR TB is correct?

A. Mean treatment duration was 4 months for the intensive and 8 months for the continuation phase
B. Most children received bedaquiline or delamanid
C. The most commonly used drugs were an injectable, a fluoroquinolone, cycloserine/terizidone, ethionamide/prothionamide, and para-aminosalicylic acid
D. Kanamycin was the most commonly used injectable

3. According to the global systematic review and individual patient meta-analysis by Osman and colleagues, which one of the following statements about the outcome of children from 11 countries who were managed between 1999 and 2013 for bacteriologically confirmed XDR TB is correct?

A. About half of children had favorable treatment outcomes
B. Four children died, 1 failed treatment, and 2 were lost to follow-up during treatment
C. Mortality was considerably higher than in adults
D. Specific drugs used during treatment predicted unfavorable outcomes

Earning CME Credit

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Article Title

Bacillus Calmette-Guérin Cases Reported to the National Tuberculosis Surveillance System, United States, 2004–2015

CME Questions

1. Your patient is an 81-year-old white man with suspected bacillus Calmette-Guérin (BCG). On the basis of the surveillance study by Wansaula and colleagues, which one of the following statements about surveillance data, demographics, and epidemiology of BCG cases and tuberculosis (TB) cases reported to National TB Surveillance System (NTSS) during 2004 to 2015 is correct?

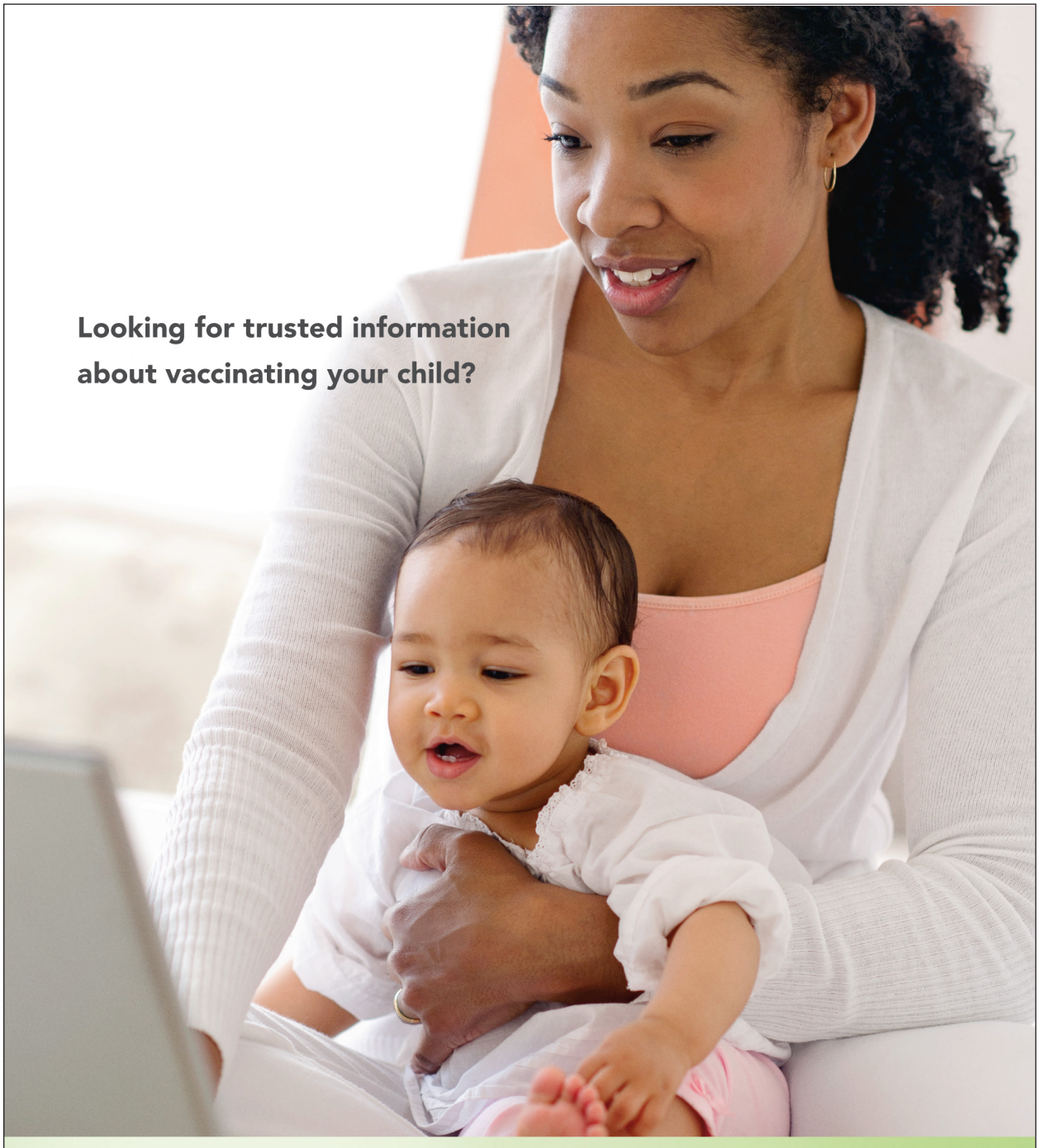
A. During 2004 to 2015, 26 US states reported 118 BCG cases, whereas all 50 US states and the District of Columbia reported 91,065 TB cases
B. Most patients with BCG were not born in the United States
C. BCG cases decreased from 2004–2007 to 2008–2015
D. Patients with BCG were less likely than those with TB to have diabetes and to live in a long-term care facility at the time of diagnosis

2. According to the surveillance study by Wansaula and colleagues, which one of the following statements about clinical characteristics and management of BCG cases and TB cases reported to the NTSS during 2004 to 2015 is correct?

A. All children with BCG had exclusively pulmonary sites of disease
B. Less than half of BCG cases had an extrapulmonary disease site
C. Only 17% of the BCG cases had any pulmonary involvement, including 4 with positive sputum smear findings and 3 with cavitary lesions on chest X-ray
D. Asymptomatic BCG cases should always be treated with anti-TB drugs

3. According to the surveillance study by Wansaula and colleagues, which one of the following statements about clinical implications of these findings regarding BCG cases and TB cases reported to NTSS during 2004 to 2015 would be correct?

A. Clinical history is unlikely to facilitate diagnosis or management of BCG vs TB cases
B. Public health agencies can use these findings to quickly identify likely BCG cases and to avoid inappropriate reporting of BCG cases to the NTSS, as well as costs of unnecessary public health interventions
C. Cases with pulmonary involvement caused by BCG strains should be reported to the NTSS as TB cases
D. Drug susceptibility results are unlikely to help differentiate BCG from TB



Visit www.cdc.gov/vaccines/parents. Learn about the vaccines your baby needs from a reliable source. The CDC's website explains the 14 diseases vaccines prevent, CDC's recommended schedule, possible side effects, how to comfort your baby during vaccine visits and more. Talk to your child's doctor, and visit our website to get the facts about vaccines.

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The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles, see below and visit http://wwwnc.cdc.gov/eid/pages/author-resource-center.htm.

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Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .tif or .jpg files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

Videos. Submit as AVI, MOV, MPG, MPEG, or WMV. Videos should not exceed 5 minutes and should include an audio description and complete captioning. If audio is not available, provide a description of the action in the video as a separate Word file. Published or copyrighted material (e.g., music) is discouraged and must be accompanied by written release. If video is part of a manuscript, files must be uploaded with manuscript submission. When uploading, choose "Video" file. Include a brief video legend in the manuscript file.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words in the main body of the text or include more than 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (not to exceed 150 words), a 1-line summary of the conclusions, and a brief biographical sketch of first author or of both authors if only 2 authors. This section comprises case series papers and concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Research Letters Reporting Cases, Outbreaks, or Original Research. EID publishes letters that report cases, outbreaks, or original research as Research Letters. Authors should provide a short abstract (50-word maximum), references (not to exceed 10), and a short biographical sketch. These letters should not exceed 800 words in the main body of the text and may include either 1 figure or 1 table. Do not divide Research Letters into sections.

Letters Commenting on Articles. Letters commenting on articles should contain a maximum of 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references (not to exceed 15) but no abstract, figures, or tables. Include biographical sketch.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.

In This Issue

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