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Centers for Disease Control and Prevention
Rocky Mountain spotted fever (RMSF) is an emerging public health concern near the US–Mexico border, where it has resulted in thousands of cases and hundreds of deaths in the past decade. We identified 4 patients who had acquired RMSF in northern Mexico and subsequently died at US healthcare facilities. Two patients sought care in Mexico before being admitted to US-based hospitals. All patients initially had several nonspecific signs and symptoms, including fever, headache, nausea, vomiting, or myalgia, but deteriorated rapidly without receipt of a tetracycline-class antimicrobial drug. Each patient experienced respiratory failure.
late in illness. Although transborder cases are not common, early recognition and prompt initiation of appropriate treatment are vital for averting severe illness and death. Clinicians on both sides of the US–Mexico border should consider a diagnosis of RMSF for patients with rapidly progressing febrile illness and recent exposure in northern Mexico.

**R**ocky Mountain spotted fever (RMSF), a life-threatening and rapidly progressing tickborne disease, is caused by infection with the bacterium *Rickettsia rickettsii*. Onset of infection is characterized by nonspecific signs and symptoms that include fever, headache, and muscle pain. Progressing damage to the vascular endothelium can result in organ failure, cutaneous necrosis, and death. RMSF is frequently fatal for persons who do not receive appropriate therapy with a tetracycline-class drug during the first 5 days of illness; half of all deaths occur within the first 8 days (1).

In the United States, RMSF is characteristically a rare and sporadically distributed disease: most cases are reported from mid-Atlantic states (2). Recently, however, epidemic levels of RMSF have been described for areas of eastern and southern Arizona and northern Mexico (3–6). Transmission in these areas is perpetuated by large numbers of brown dog ticks (*Rhipicephalus sanguineus* sensu lato), which are responsible for unusually high incidence of disease in this region (3,5,7). *Rhipicephalus* tick–transmitted RMSF was initially recognized in Mexico during the 1940s, yet during the past 12 years the disease has rapidly reemerged in parts of Baja California and Sonora, Mexico (3,4,6,8,9). We describe 4 patients who acquired RMSF in Mexico and subsequently sought care in the United States. These cases highlight the need for increased healthcare provider awareness of this rapidly progressing disease in communities on both sides of the border.

**Methods**

During 2013–2016, the Arizona Department of Health Services, the California Department of Public Health (CDPH), and the US Centers for Disease Control and Prevention (CDC) identified 4 cases of RMSF in persons who acquired the illness in Mexico and later died in the United States. The cases were identified during the course of routine surveillance and diagnostic testing for this disease at the respective state public health laboratories or CDC. To better characterize the epidemiologic risk factors, clinical progression, and treatment course associated with each of these deaths, we performed a retrospective review of clinical and epidemiologic data and, when available, medical charts (Table). Because the CDC Human Subjects Review Committee determined that this evaluation was not research, this case series was exempt from institutional review board and human subjects review.

**Case Reports**

**Case 1**

In December 2013, fever, chills, and thrombocytopenia developed in a 22-year-old man while he was attending school in Hermosillo, Mexico. While visiting family, he sought care at a hospital in Nogales, Mexico, where he received treatment with penicillin and was released. He subsequently sought care at an emergency department in Nogales, Arizona, USA, where he was found to have fever; hypotension; hepatomegaly; splenomegaly; thrombocytopenia; and elevated levels of creatinine, hepatic transaminases, and bilirubin. He was given intravenous vancomycin and piperacillin/tazobactam and transferred to a tertiary care facility in Tucson, Arizona. Admitting documents at the tertiary care facility noted acute kidney and liver failure. He later became acidoic; his altered mental status and respiratory failure progressed, and he was intubated. Subsequently, the patient experienced a dusky and violaceous rash, bilateral necrosis of his hands and feet, followed by gangrene and severe edema. He remained in an intensive care unit for 2 weeks before experiencing cardiac arrest; he died ≤3 weeks after symptom onset.

Testing of serum obtained on day 19 of the patient’s illness revealed reciprocal IgM and IgG titers reactive with

<table>
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*RMSF, Rocky Mountain spotted fever; *, present; blank cells, absent.

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*R. rickettsii* of 1,024 each, according to an indirect immunofluorescence antibody assay performed at a commercial laboratory. A skin punch biopsy specimen obtained from a rash lesion on the lower abdomen ≈2 weeks after illness onset revealed spotted fever group *Rickettsia* (SFGR) antigens in the endothelial cells of inflamed small blood vessels in the dermis. The sample was tested by an immunohistochemical stain for SFGR at CDC (10) (Figure, panel A).

**Case 2**

In May 2014, a 52-year-old woman from Calexico, California, USA, sought care at an emergency department in El Centro, California, after 3 days of fever, diarrhea, nausea, and vomiting. For the previous 4 days, the patient had self-medicated with ampicillin obtained in Mexico for a toothache. She was hospitalized and received intravenous levofloxacin and cefepime for presumed urosepsis; on day 3 of hospitalization (day 6 of illness), she was transferred to a tertiary care facility in San Diego, California. At arrival, scattered petechiae were visible on her upper and lower extremities; the medical chart reported disseminated intravascular coagulation (DIC) with pancytopenia. Laboratory results showed increased clotting time (elevated international normalized ratio) and elevated levels of hepatic transaminases, but no D-dimers or fibrinogen levels were reported. The patient was intubated and subsequently experienced encephalopathy, cardiomyopathy, and acute renal failure requiring hemodialysis. Intravenous vancomycin and metronidazole were started. The patient never received a tetracycline-class antimicrobial drug before dying of complications of DIC on day 28.

An autopsy revealed bilateral pyelonephritis, acute pancreatitis, pneumonia, ascites, extensive cutaneous necrosis, and widespread ischemic damage. A serum specimen obtained on day 7 of illness revealed a reciprocal IgG titer of <64 and a reciprocal IgM titer of 160, reactive with *R. rickettsii* when tested by an immunofluorescence antibody assay at CDPH. A serum sample obtained on day 24 showed reciprocal IgG and IgM titers of >1,024 and >160, respectively. A skin biopsy specimen of the rash lesion obtained after death and tested by PCR at CDPH was positive for DNA of SFGR species.

The patient had not reported travel for the 1 month preceding illness onset, but relatives frequently visited family in Mexicali, Mexico, and brought their pet dogs across the border with them. An ecologic assessment of the patient’s home in Calexico revealed an extensive brown dog tick infestation of the dogs and the yard. A total of 37 ticks were collected from the domestic and peridomestic setting and tested by PCR at CDPH. One of the 37 ticks was positive for DNA of a *Rickettsia* species. Subsequent testing of this specimen at CDC by a genotyping assay for this agent led to identification of *R. rickettsii* (11).

**Case 3**

On 2 occasions in September 2014, a 39-year-old man sought care at a healthcare facility in Riverside County, California, for fever, cough, dyspnea, diarrhea, nausea, vomiting, and abdominal pain. Both times he was sent home with a suspected diagnosis of viral syndrome. His condition worsened, and he sought care at a third facility on day 3 of his illness, at which time leukopenia and thrombocytopenia

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**Figure.** Histologic slides of autopsy tissue from patients who acquired Rocky Mountain spotted fever in northern Mexico and died at hospitals in the United States, 2013–2016. A) Immunohistochemical stain of *Rickettsia rickettsii* antigens (red) in inflamed blood vessel adjacent to eccrine gland in a skin biopsy specimen from case-patient 1. Immunoalkaline phosphatase with naphthol-fast red and hematoxylin counterstain; original magnification ×50. B) Diffuse pulmonary capillaritis in case-patient 4. Hematoxylin and eosin stain; original magnification ×50. C) Vasculitis involving a small blood vessel in a peripheral nerve of case-patient 4. Hematoxylin and eosin stain; original magnification ×100.
were reported, and a chest radiograph showed pulmonary infiltrates suggestive of pneumonia; a mottled rash also appeared on his extremities. He was hospitalized and given vancomycin, imipenem, azithromycin, and metronidazole. Subsequently, he experienced respiratory failure, requiring ventilator assistance. On day 7, he was given doxycycline, and admitted to an intensive care unit. On day 16, he died.

A plasma specimen obtained on day 7 revealed DNA of an SFGR species when tested by a real-time PCR at CDC (12). No autopsy was performed. The patient had frequently traveled to Mexicali; his most recent trip was 1 week before illness onset.

### Case 4

On 3 occasions in March 2016, an 18-year-old woman sought care in Nogales, Mexico, for fever, headache, myalgia, fatigue, and arthralgia. After each visit she was sent home with palliative treatment for fever, and after 1 of the visits, cephalexin was prescribed for an unspecified illness. On day 7 of illness, she sought care at an emergency department in Nogales, Mexico, for abdominal pain, rash, headache, and extreme fatigue. Laboratory testing detected leukocytosis, thrombocytopenia, and elevated levels of pancreatic enzymes and hepatic transaminases. The patient was transported across the border to Nogales, Arizona, for further medical care, but she died of cardiac arrest at arrival.

An autopsy revealed a widespread petechial rash; perivascular inflammation of the heart, lungs, and liver; and petechial hemorrhages in the epicardium and lung pleura. Postmortem specimens of whole blood, urine, and vitreous humor were positive for DNA of *R. rickettsii* when tested by PCR at CDC; immunohistochemical assay, also performed at CDC, demonstrated abundant intravascular antigens of SFGR in sections of lung, liver, heart, spleen, and central nervous system tissue (Figure, panels B and C).

### Discussion

During 2013–2016, passive surveillance identified 4 cases of fatal RMSF in persons who had traveled to or resided in areas of northern Mexico and who died in the United States. Epidemic RMSF is an emerging public health concern in portions of northern Mexico (3,4,13). During 2009–2016, a total of 967 cases of RMSF, including 132 deaths, were reported in Mexicali (3). Similarly, during 2004–2015, a total of 1,129 cases and 188 deaths from RMSF were reported from Sonora, Mexico, prompting the Secretary of Health in Mexico to declare an epidemiologic emergency (13). Cases of RMSF have also been increasingly reported from the Mexico states of Coahuila and Chihuahua. During 2015, approximately 181,300,000 persons crossed into the United States from a Mexico land border (23,800,000 into Arizona, 72,400,000 into California, 2,400,000 into New Mexico, and 82,700,000 into Texas), making these border crossings some of the busiest in the world (14). Although transborder cases of RMSF could be infrequent, they underline the need for improved clinical awareness regarding the diagnosis and treatment of this disease on both sides of the border, and they highlight the value of ongoing communication between health authorities in the United States and Mexico.

Each of the 4 patients we report sought care at a healthcare facility for fever and other nonspecific signs and symptoms including headache, nausea, vomiting, or myalgia; thrombocytopenia was reported during early illness (within the first 3–4 days of illness) for at least 2 patients. Rash was observed for all patients during the course of their illness but was not noted in the original clinical description for any. Two patients sought care in Mexico before being admitted to US-based facilities, and all patients reported having made multiple visits to healthcare facilities before admission. All patients sought care within the first 3 days of illness and were admitted to the hospital within 7 days of illness onset, reflecting the rapidly progressing nature of RMSF. Respiratory failure and cutaneous necrosis of the extremities were common end-stage manifestations of this severe disease. DIC was a reported end-stage manifestation for 1 patient but was not validated by specific laboratory assays. True DIC is rarely documented in cases of RMSF (15). Among the 4 patients, death occurred 7–28 days after illness onset, and several patients received life support before death. Each case met ≥1 laboratory criteria for a confirmed spotted fever rickettsiosis (16).

Early suspicion of RMSF and prompt initiation of tetracycline-class antimicrobial drug therapy are critical for averting severe sequelae and death from RMSF (17,18). In this case series, all patients received a non–tetracycline-class antimicrobial drug within the first week of illness, and only 1 patient received doxycycline at any point during illness. Doxycycline should be initiated immediately whenever a rickettsial disease, including RMSF, is suspected and should never be delayed while awaiting the appearance of a rash or confirmatory laboratory result. Clinicians along the US–Mexico border should be cognizant of the occurrence of RMSF in this region and should consider this diagnosis for patients with otherwise unexplained febrile or septic syndromes. RMSF can be difficult to distinguish from various other life-threatening infectious and noninfectious conditions, including measles, leptospirosis, thrombotic thrombocytopenic purpura, and meningococcemia, particularly during the early stages of disease (19). Differentiation becomes even more challenging in light of recent epidemics of arboviral infections such as those caused by Zika, dengue, and chikungunya viruses, which can have similar signs and symptoms early.
in illness, including fever, malaise, arthralgia, and rash (20,21). Results of blood chemistries, including complete blood counts and hepatic function panels, may help distinguish between these infections. For example, platelet levels <100,000 cells/mm<sup>3</sup> are rarely found in patients with Zika or chikungunya virus infection but are often found in patients with advanced RMSF and dengue, particularly those with dengue hemorrhagic fever (19–21). Lymphopenia is often found in patients infected with chikungunya virus but less commonly found in patients infected with Zika and dengue viruses (20,21). Leukocyte counts are typically within reference range or slightly elevated in patients with RMSF, although patients with more advanced stages of disease may have lymphocytosis with a predominant left shift (4,7,19).

Questions about epidemiologic risk factors are helpful for clinical evaluations. Of the 4 patients reported here, 3 had spent time in regions of northern Mexico recognized for high rates of RMSF (3). At least 1 patient reported having been bitten by a tick in the week before illness onset, and 2 reported having had contact with dogs.

Reduction of duplication and delayed access to lifesaving care for patients with RMSF can be facilitated by increased clinical awareness, in-depth clinical and social histories, and improved binational communication. Increased clinical education along the US–Mexico border can help clinicians correctly recognize and promptly treat suspected cases of RMSF.

Acknowledgments

We thank the clinical laboratory experts at CDC and the CDPH Viral and Rickettsial Disease Laboratory who provided the vital work to confirm these cases, including Atis Muehlenbachs and Wun-Ju Shieh, Sandor Karpathy, Shezeen Gillani, Ida Chung, Cecilia Kato, Natasha Espinosa, Alex Espinosa, Oliver Oyler, and Larry Penning. We also thank the healthcare providers who participated in the clinical care of these patients and the local public health investigators who completed home investigations and interviews presented herein.

Ms. Drexler is an epidemiologist with the Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC. Her research interests include the prevention and control of tickborne rickettsial diseases.

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https://wwwnc.cdc.gov/eid/content/21/8/contents.htm
Limited data are available describing extrapulmonary nontuberculous mycobacteria (NTM) infections in the general population. We describe results from statewide population-based laboratory surveillance in Oregon, USA, during 2007–2012. We defined a case of extrapulmonary NTM infection as ≥1 isolate from skin/soft tissue, disseminated sites, lymph node, joint, or other sites. The annual incidence of extrapulmonary NTM infection (other than *Mycobacterium gordonae*) was stable, averaging 1.5 cases/100,000 population. Median age of the 334 patients was 51 years, and 53% of patients were female. Half of cases were caused by *M. avium* complex, but rapid-growing NTM species accounted for one third of cases. Most extrapulmonary NTM infections are skin/soft tissue. Compared with pulmonary NTM infection, more extrapulmonary infections are caused by rapid-growing NTM species. The designation of NTM as a reportable disease in Oregon in 2014 will result in better detection of changes in the incidence and patterns of disease in the future.

Non-tuberculous mycobacteria (NTM) are ubiquitous in water and soil and are a cause of opportunistic pulmonary and extrapulmonary infections. Extrapulmonary manifestations include disseminated, skin, joint, and lymph node infections. Extrapulmonary NTM infections are typically sporadic but may be associated with nosocomial outbreaks (1), clinical procedures (2), or nail salon pedicures (3). Treatment is species dependent, typically consisting of 3–6 months of multidrug antimicrobial therapy (4). Although numerous case series are found in the literature, few data exist to describe the population-based epidemiology of extrapulmonary NTM infections. Recent studies have shown an increase in the prevalence and incidence of pulmonary infections (5,6). We describe the results of a statewide laboratory surveillance study in Oregon, USA, that identified all patients from whom NTM was isolated from extrapulmonary sites during 2007–2012. We report characteristics of patients with extrapulmonary NTM infection by species and site and calculate the annual incidence over the study period.

**Methods**

Methods have been described elsewhere (5). In brief, we requested positive NTM culture results from January 1, 2007, through December 31, 2012, from all 17 laboratories that perform acid-fast bacillus culture in Oregon or are used as reference laboratories. We excluded *M. tuberculosis* and *M. bovis*. Additional data for each positive culture included patient name, age at collection or date of birth, address or county and ZIP code of residence, species isolated, date of collection, and body source of isolate. One laboratory in central Oregon was unable to provide enough information to assign a state of residence. Given a low likelihood of these patients coming from out of state (this lab is >120 miles from Washington, Idaho, or California), however, we considered all these patients to be Oregon residents. After we identified the patients, we linked patients with extrapulmonary isolates to the state HIV database to identify any who were infected with HIV.

**Case Definition**

We defined an extrapulmonary NTM infection case as having ≥1 isolates from skin/soft tissue (wound, abscess, tissue, or exit catheter); disseminated sites (blood, bone marrow, cerebrospinal fluid, pericardial fluid, or peritoneal fluid); lymph node (lymph node or neck abscess); joint (synovial or joint fluid); or other (urine, eye, sinus, or nasopharyngeal). We excluded isolates from an unknown source or from feces, saliva, or gastric sites. *M. gordonae* was reported but is considered nonpathogenic, so we excluded it from estimates of disease incidence. Rapid-growing mycobacteria (RGM) species include *M. chelonae/abscessus* complex, *M. fortuitum*, and *M. chelonae* (4); we included these in the analysis.

**Statistical Analysis**

We described patients by age, sex, and species isolated overall and by source of specimen. We considered extrapulmonary cases to be incident at the time of isolation, given that treatment for extrapulmonary infections is typically in the range of weeks to several months and the cure rate is high (4). We calculated the annual incidence as the number
of new cases in a given year divided by the midyear population using population data from the Portland State University Population Research Center (7) and report the average annual incidence and standard 95% CIs for 2007–2012. We used Poisson models using a log link to estimate the overall incidence rate trend over the study period. We imported all data into SAS version 9.3 (SAS Institute Inc., Cary, NC) for analysis. The study was considered to be public health practice (nonresearch) by the Oregon Health Authority and was conducted under Oregon Administrative Rule 333–019–0005 (Conduct of Special Studies).

Results

Patient Characteristics and Species Isolated
We identified 334 patients with extrapulmonary isolates, including 1 patient with 2 distinct infections. Overall, 176 (53%) patients were female, and the median age was 50 years (range 0.8–92 years) (Table). Half (n = 167, 50%) of patients had M. avium/intracellulare complex (MAC) infection, 129 (38.6%) RGM infection, and 21 (6.3%) M. marinum infection.

Results by Site of Infection
Among the 334 extrapulmonary NTM infections, 197 (59.0%) were skin/soft tissue, 57 (17.1%) were disseminated, 28 (8.4%) were lymph node, 14 (4.2%) were joint, and 38 (11.4%) were other (Table). The overall species distribution was 50% MAC, 22.8% M. chelonae/abscessus complex or M. chelonae, 9.6% M. fortuitum, and 6.3% M. marinum. We identified an additional 13 patients with M. gordonae isolates. Patients with skin/soft tissue infections were more commonly female (58%), whereas disseminated infections occurred predominantly in male patients (67%); of these, 79% of patients had MAC infection. The median age of patients with disseminated infections was 41 years, and 60% of these infections occurred in HIV-positive patients. Lymph node infections were 82.1% MAC; 18% of these patients were HIV positive. Among 23 HIV-negative patients with lymph node infections, 10 (44%) were <5 years of age. Only 14 joint infections were reported; these patients had a median age of 70 years, and 64.3% of infections were caused by MAC. Of other infections, 52.6% were caused by MAC and 31.6% by M. chelonae/abscessus complex or M. chelonae.

Estimates of Annual Incidence
The average annual incidence of extrapulmonary NTM infection during 2007–2012 was 1.5 (95% CI 1.1–1.8) cases/100,000 population. The incidence was 1.4 (CI 1.0–1.8) cases/100,000 population in 2007, peaked at 1.7 (CI 1.3–2.1)/100,000 in 2009, and decreased to 1.3 (CI 1.0–1.7)/100,000 in 2012 (Figure). The Poisson estimate of change in annual incidence was not significant, at −1.8% (95% CI −0.8 to 0.04; p = 0.6). The incidence by site of isolation was 0.9 cases/100,000 population for skin/soft tissue, 0.2/100,000 for disseminated, 0.2/100,000 for lymph, 0.1/100,000 for joint, and 0.2/100,000 other. The average annual incidence was 0.7 cases/100,000 population for MAC, 0.2/100,000 for

---

<table>
<thead>
<tr>
<th>Table. Characteristics of 334 nontuberculous mycobacterium infections, Oregon, USA, 2007–2012*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Annual incidence/100,000 population</td>
</tr>
<tr>
<td>Patient demographics</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>Median age, y (range)</td>
</tr>
<tr>
<td>HIV positive</td>
</tr>
<tr>
<td>Mycobacterium species</td>
</tr>
<tr>
<td>Rapid-growing species</td>
</tr>
<tr>
<td>M. chelonae/abscessus complex</td>
</tr>
<tr>
<td>M. fortuitum complex</td>
</tr>
<tr>
<td>M. chelonae</td>
</tr>
<tr>
<td>Slow-growing species</td>
</tr>
<tr>
<td>M. avium/intracellulare complex</td>
</tr>
<tr>
<td>M. marinum</td>
</tr>
<tr>
<td>M. goodii</td>
</tr>
<tr>
<td>M. aubagnense</td>
</tr>
<tr>
<td>M. xenopi</td>
</tr>
<tr>
<td>M. alvei</td>
</tr>
<tr>
<td>M. neoaurum</td>
</tr>
<tr>
<td>Other species†</td>
</tr>
</tbody>
</table>

*Values are no. (%) patients except as indicated. Total excludes 13 M. gordonae isolates: 6 skin/soft tissue, 1 disseminated, 1 lymph node, 5 other.
†Skin/soft tissue: wound, abscess, tissue, or exit catheter; disseminated: blood, bone marrow, cerebrospinal fluid, pericardial fluid, or peritoneal fluid; lymph node: lymph node or neck abscess; joint: synovial or joint fluid; other: urine, eye, sinus, or nasopharyngeal.
‡Other species were 8 unspeciated and 1 each of M. asiaticum, M. brandtii, M. brisbanense, M. heckeshomense, M. immunogenum, M. interjectum, M. kansasi, M. lentiflavum, M. linterense, M. mucogenicum/phocaicum, M. obuense, M. simiae, M. thermoresistibile, and M. vaccae.
Extrapulmonary NTM Infections, Oregon

Figure. Observed incidence (cases/100,000 population) of extrapulmonary nontuberculous mycobacterium infection (excluding Mycobacterium gordonae), Oregon, USA, 2007–2012.

M. abscessus/chelonae complex, and 0.1/100,000 each for M. fortuitum, M. chelonae, and M. marinum.

Discussion

We describe Oregon’s population-based experience with extrapulmonary NTM infections before these infections were made reportable in 2014. The annual incidence remained stable over the study period, averaging 1.5 cases/100,000 population. In contrast with pulmonary NTM disease, which occurs predominantly in female patients, half of patients with extrapulmonary infections and two thirds of patients with disseminated NTM are male (8,9). A smaller proportion of extrapulmonary infections is caused by MAC than by pulmonary disease, and RGM cause 43.1% of skin/soft tissue infections. Overall, 15% of extrapulmonary infections occurred in HIV-positive patients.

Our observed overall average incidence was comparable to the annualized prevalence (1.6 cases/100,000 population) originally reported in Oregon during 2005–2006 (10). The skin/soft tissue prevalence/incidence in each time period was also identical at 0.9 cases/100,000 population, accounting for 58.9% of all extrapulmonary infections in our study. More recently, Smith et al. reported a higher prevalence of extrapulmonary NTM infection of 2.8 cases/100,000 population in 3 counties in North Carolina during 2006–2010 (11). Of 184 North Carolina patients with non–M. gordonae extrapulmonary NTM isolates, 51 (28%) were from a sterile site (equivalent to joint/disseminated/lymph node by our definition), 15 (8%) were dermal, 7 (4%) were catheter/implant related, and 111 (60%) were categorized as other. Because of their different classifications, it is difficult to compare results by site directly. However, in North Carolina, a similar proportion of extrapulmonary infections overall was caused by rapidly growing NTM (37%, compared with 31% in our study).

RGM are most commonly associated with skin/soft tissue infections. We observed a similar proportion of skin/soft tissue infections caused by RGM in Oregon compared with our prior study (43% vs. 51% in 2005–2006). Although describing small subsets of our skin/soft tissue infection category, a similarly or slightly higher proportion of dermal (10/15, 67%) and catheter/implant-related (2/7, 43%) extrapulmonary infections in North Carolina were caused by RGM (11). In our data, >80% of RGM M. fortuitum and slow-growing M. goodii and M. marinum infections were associated with skin/soft tissue infections.

Other categories of extrapulmonary NTM infections were less common. Disseminated infection, representing 17% of extrapulmonary infections, typically occurs in severely immunocompromised patients with AIDS (CD4+ counts <50), hematologic malignancies, or transplants (12,13). Positive HIV status was a notable contributor to infection in our study, associated with 60% of disseminated NTM. As reported previously, median annual incidence of disseminated NTM in Oregon during 2007–2012 in HIV-positive patients was high, at 110 cases/100,000 HIV person-years (13). Given the lower proportion of lymph, skin/soft tissue, or other infections with HIV, it is possible that some of these infections also represent disseminated infection in HIV patients.

In our data, only 43% of patients with pediatric lymphadenitis were <5 years of age, even after excluding those with HIV. Pediatric lymphadenitis occurs primarily in immunocompetent children <4 years of age, so this is an unusual pattern (14). Lymph node infections represented <10% of extrapulmonary infections. In the North Carolina study, only 3% of cases were isolated from the lymph node, although some may have been misclassified as neck infections (11). Of the 12 neck isolations in North Carolina, 8 were in children <3 years of age. In contrast, patients in our study with NTM infections of the joint (4% of total extrapulmonary infections), with a median age of 70, likely represent surgical site infections. Oregon previously investigated a cluster of 9 NTM infections involving joint prostheses occurring in 2013–2014 (15).

The strengths of this study include complete capture of extrapulmonary cases statewide over a 7-year period, allowing population-based analyses and analysis of trends. The disease incidence should be considered a minimal estimate, requiring the physician to order the appropriate diagnostic test (acid-fast bacillus culture). We were also able to link to the state HIV database and identify HIV-positive patients. Study limitations included a lack of clinical information to identify other underlying conditions and risk factors for infection. We were also unable to distinguish M. chelonae from M. abscessus in the M. chelonae/abscessus complex cases.
More detailed clinical data and exposure history for NTM infections in Oregon will be available in the future from state surveillance efforts, aiding in the identification of outbreaks that require public health intervention. However, it is likely that relatively few cases are associated with outbreaks. During the first 2 years of reportability in Oregon, only 11 (11%) of 98 extrapulmonary NTM isolates were linked epidemiologically (16). Reporting and follow-up of all patients with extrapulmonary isolates may be useful for detecting previously unidentified environmental sources of NTM, such as specific watersheds. Subspeciation and molecular typing of isolates may be necessary to identify clusters of more common species. Detailed follow-up on patients isolating M. gordonae to confirm whether it is the most likely cause of disease will inform whether or not to include it as a reportable infection along with other NTM species.

In conclusion, unlike pulmonary infections, which are increasing, extrapulmonary NTM incidence in Oregon is stable. Similar to pulmonary NTM, MAC causes most disseminated and lymph node infections. In contrast, RGM species are much more common causes of skin/soft tissue and other infections. Although the literature highlights clusters and outbreaks, most extrapulmonary NTM infections are likely isolated cases. Now that extrapulmonary NTM infections have been made reportable in Oregon (2014) and other states, we anticipate additional population-based estimates to be made available in the future.

This study was supported by the University of Rochester Respiratory Pathogens Research Center/National Institute of Allergy and Infectious Disease (HHSN272201200005C).

E.H. contributed to the acquisition, analysis, and interpretation of data, and developed the manuscript; S.S., K.H., and K.L.W. contributed to the concept and study design, interpretation of data, and reviewing the manuscript critically for intellectual content.

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References


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Whole-genome sequencing is rapidly replacing current molecular typing methods for surveillance purposes. Our study evaluates core-genome single-nucleotide polymorphism analysis for outbreak detection and linking of sources of *Salmonella enterica* serovar Typhimurium and its monophasic variants during a 7-month surveillance period in Denmark. We reanalyzed and defined 8 previously characterized outbreaks from the phylogenetic relatedness of the isolates, epidemiologic data, and food traceback investigations. All outbreaks were identified, and we were able to exclude unrelated and include additional related human cases. We were furthermore able to link possible food and veterinary sources to the outbreaks. Isolates clustered according to sequence types (STs) 19, 34, and 36. Our study shows that core-genome single-nucleotide polymorphism analysis is suitable for surveillance and outbreak investigation for *Salmonella* Typhimurium (ST19 and ST36), but whole genome–wide analysis may be required for the tight genetic clone of monophasic variants (ST34).

The foodborne pathogen *Salmonella* is responsible for tens of millions of human infections worldwide each year (1). It constitutes a substantial health and economic burden, especially in developing countries (1). Fast, accurate, and highly discriminatory typing methods are crucial for detecting outbreaks, identifying sources of the outbreaks, and preventing further spread of the bacteria as part of effective surveillance. In Denmark, 1,122 cases of human *Salmonella* infections were registered in 2014. *Salmonella enterica* serovar Typhimurium accounted for 17.6% of cases, and its monophasic variants accounted for 20.5%. Cases are often associated with consumption of swine and poultry products (2).

In Denmark, as in many countries worldwide, the monophasic *Salmonella* Typhimurium variants have emerged in the past decades (3–8). The monophasic variants are circulating in multiple clonal lineages, and owing to the relatively rapid emergence of the clones that often also exhibit multidrug resistance, these types of monophasic variants are considered an important epidemic health risk (3,9–11).

Whole-genome sequencing (WGS) is a widely used technique for molecular subtyping of bacteria, and it is replacing the more laborious current molecular typing methods. The vast amount of data provided by this method not only enables high-resolution typing for surveillance but also provides valuable additional data regarding further characterization of emerging clones based on genetic differences and evolutionary studies. Several studies have proven WGS-based typing to have an enhanced discriminatory power in comparison to current molecular typing methods used for *Salmonella* (12–19), although few studies have evaluated WGS analysis in real-time surveillance. A retrospective study of *Salmonella* Enteritidis showed that single-nucleotide polymorphism (SNP)–based WGS analysis was suitable for surveillance purposes (15). However, the authors also emphasized the importance of evaluation and interpretation of the SNP-based analysis within serovars or even lineages before applying the method in real-time surveillance.

In Denmark, surveillance of *Salmonella* Typhimurium and its monophasic variants is conducted at Statens Serum Institut (human clinical isolates) and the National Food Institute (food and veterinary isolates). Surveillance is based on serotyping, drug-susceptibility testing, and multilocus variable-number tandem-repeat analysis (MLVA). The aim of our study was to evaluate WGS as a typing method for routine surveillance of *Salmonella* Typhimurium and its monophasic variants. To do so, we selected an already typed collection of strains from 2013 and 2014 and reanalyzed them to redefine outbreaks and detect outbreak sources based on core-genome SNP analysis.
**Material and Methods**

We selected 372 isolates of *Salmonella Typhimurium* and its monophasic variants for this study (online Technical Appendix 1, [https://wwwnc.cdc.gov/EID/article/23/10/16-1248-Techapp1.xlsx](https://wwwnc.cdc.gov/EID/article/23/10/16-1248-Techapp1.xlsx)). The collection included 292 human clinical isolates from the national surveillance system in Denmark (Statens Serum Institut, Copenhagen) collected during January 2013–April 2013 (previously sequenced isolates) and June 2014–October 2014 (isolates sequenced during this study). During the 7 months of surveillance, 8 outbreaks were previously defined based on epidemiologic data, serotyping, drug-susceptibility testing, and MLVA (Table 1). Outbreak investigations during that period were initiated when 5 isolates with an indistinguishable MLVA profile were collected within a 4-week period. The investigations included patient interviews, typing of food and veterinary isolates, and examination of isolates with closely related MLVA (1 locus difference) and resistance profiles.

In addition, we selected 80 food and veterinary isolates linked or possibly linked to the outbreaks from the National Food Institute collection in Denmark (DTU Food, Technical University of Denmark, Lyngby, Denmark). The food and veterinary isolates were isolated from swine, poultry, cattle, and feed in 2010, 2013, and 2014.

**WGS and Sequence Analysis**

We analyzed all human isolates by using WGS at Statens Serum Institut’s Department of Microbiology and Infection Control and sequenced food and veterinary isolates at the Technical University of Denmark’s National Food Institute. We sequenced isolates by using an Illumina Miseq (Illumina, San Diego, CA, USA). All sequences were de novo assembled and sequence type (ST) determined. We identified core-genome SNPs by using an in-house SNP pipeline, and we then analyzed a selected subgroup of isolates by using the SNP pipelines NASP (20) and CSI Phylogeny 1.2 (27). A description of sequencing procedures and sequence analysis is provided (online Technical Appendix 2, [https://wwwnc.cdc.gov/EID/article/23/10/16-1248-Techapp2.pdf](https://wwwnc.cdc.gov/EID/article/23/10/16-1248-Techapp2.pdf)). We assessed quality of the sequences and excluded 6 isolates from the study because of poor quality. Additional information on sequences also is provided (online Technical Appendix 1).

Sequence reads were deposited in the European Nucleotide Archive (study accession no. PRJEB14853).

**Results**

We detected core-genome SNPs in the entire isolate collection by using the complete genome of *Salmonella Typhimurium* 14028S (ST19) as the reference genome. The SNP analysis resulted in 14,326 SNPs. We constructed a maximum-parsimony tree from the core-genome SNPs and observed 3 ST-specific groups (Figure 1); 1 group mainly consisted of ST19 strains, 1 solely consisted of ST34 stains, and 1 solely consisted of ST36 strains. A long branch separated all 11 Typhimurium ST36 isolates from the remaining isolates with 3,707 SNPs. Furthermore, we observed a distinct cluster of 242 isolates of ST34. The close genetic cluster included isolates of both serovar Typhimurium and monophasic variants, with the monophasic variants being most prevalent. The remaining 113 isolates clustering together were ST19, ST376, ST568, and ST2212, all identified as serovar Typhimurium.

**Outbreak Investigation**

We analyzed the 3 observed ST groups separately. Core-genome SNPs were detected by using an internal de novo assembled reference genome for each ST group. We examined the 8 previously defined outbreaks (outbreaks A–H; Table 1) on the basis of the genetic relatedness of the isolates, the epidemiologic data, and the food traceback investigations. We identified and redefined all 8 outbreaks on the basis of the SNP analysis (Table 2). We also plotted the distribution of the outbreaks over time (online Technical Appendix 2, Figure).

**The ST36 Group**

We analyzed 11 ST36 isolates and detected 3,146 core-genome SNPs with SNP distances between isolates ranging from 0 to 1,694. The previous definition of outbreak A included 6 human cases and no suspected food and veterinary isolates (Table 1). The SNP analysis clustered all 6 isolates with a SNP distance between the isolates of 0 to 7 SNPs. Nearest neighbor isolate was separated from the cluster with 143 SNPs, clearly differentiating the outbreak cluster from the remaining isolates (Figure 2; Table 2).

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**Table 1.** Previously defined outbreaks included in the data collection in an outbreak investigation of *Salmonella enterica* serovar Typhimurium and its monophasic variants, Denmark*

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>No. sequenced strains</th>
<th>Year</th>
<th>Outbreak characteristics</th>
<th>Serovar variant</th>
<th>Food/veterinary isolates linked to outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>2014</td>
<td>Same MLVA and resistance profile</td>
<td>Typhimurium</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>35</td>
<td>2013</td>
<td>Same MLVA, various resistance profiles</td>
<td>Typhimurium</td>
<td>Yes</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>2013</td>
<td>Same MLVA and resistance profile</td>
<td>Typhimurium</td>
<td>No</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>2013</td>
<td>Same MLVA, various resistance profiles</td>
<td>Typhimurium</td>
<td>No</td>
</tr>
<tr>
<td>E</td>
<td>24</td>
<td>2014</td>
<td>Same MLVA, various resistance profiles</td>
<td>Monophasic</td>
<td>Yes</td>
</tr>
<tr>
<td>F</td>
<td>19</td>
<td>2014</td>
<td>Various MLVA, same resistance profile</td>
<td>Monophasic</td>
<td>Yes</td>
</tr>
<tr>
<td>G</td>
<td>14</td>
<td>2014</td>
<td>Same MLVA, various resistance profiles</td>
<td>Monophasic</td>
<td>No</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>2014</td>
<td>Same MLVA and resistance profile</td>
<td>Monophasic</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*MLVA, multilocus variable-number tandem-repeat analysis.
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 23, No. 10, October 2017

The ST19 Group

The ST19 group comprised 98 human isolates and 5 food and veterinary isolates. Within the ST19 group, we detected 6,549 SNPs with distances between isolates ranging from 0 to 982 SNPs (Figure 3). Two outbreaks (B and C) were detected in 2013; B comprised 35 human isolates, and C comprised 7 human isolates (Table 1).

A tight genetic cluster with 0 to 4 SNP differences between the isolates comprised all 35 isolates previously defined in outbreak B. Two additional human isolates from 2013 with closely related MLVA profiles were located in this cluster and regarded as part of the outbreak, as defined by the SNP analysis. Likewise, 1 isolate from 2014 clustered with the outbreak cases but was not included in the new outbreak definition because of the difference in time. Patient interviews pointed to consumption of pork as the likely contamination source. Two suspected food isolates from pork with the same MLVA profile were, at the time, collected from 2 different meat-distributing companies. However, we could not confirm a clear connection between the food isolates and the human cases. Our SNP analysis showed that the 2 suspected isolates were located in the outbreak cluster and therefore provided additional evidence that pork was the likely source of the outbreak. The cluster was separated from the nearest neighbor isolate with 34 SNPs (Figure 3; Table 2).

All 7 isolates previously defined in outbreak C had identical core-genome SNPs. No food or veterinary isolates were linked to the cases, and the outbreak cluster was separated from the nearest neighbor with 64 SNPs (Figure 3; Table 2).

The ST34 Group

Most of the isolates observed in this study were ST34, and this ST group was dominated by the monophasic variants. The ST34 group included 169 human isolates and 73 food and veterinary isolates. Within the ST34 group, we detected 1,673 SNPs with distances between isolates ranging from 0 to 327 SNPs (Figure 4). Three outbreaks (D–F) were detected in 2014; D comprised 7 human isolates, E comprised 22 human isolates, and F comprised 9 human isolates (Table 1).

A tight genetic cluster with 0 to 3 SNP differences between the isolates comprised all 22 isolates previously defined in outbreak E. Two additional human isolates from 2014 with closely related MLVA profiles were located in this cluster and regarded as part of the outbreak, as defined by the SNP analysis. Likewise, 2 isolates from 2015 clustered with the outbreak cases but were not included in the new outbreak definition because of the difference in time. Patient interviews pointed to consumption of pork as the likely contamination source. Two suspected food isolates from pork with the same MLVA profile were, at the time, collected from 2 different meat-distributing companies. However, we could not confirm a clear connection between the food isolates and the human cases. Our SNP analysis showed that the 2 suspected isolates were located in the outbreak cluster and therefore provided additional evidence that pork was the likely source of the outbreak. The cluster was separated from the nearest neighbor isolate with 327 SNPs (Figure 4; Table 2).

All 7 isolates previously defined in outbreak F had identical core-genome SNPs. No food or veterinary isolates were linked to the cases, and the outbreak cluster was separated from the nearest neighbor with 20 SNPs (Figure 4; Table 2).

Table 2. New definitions of previously defined outbreaks based on core-genome SNP analysis in an outbreak investigation of *Salmonella enterica* serovar Typhimurium and its monophasic variants, Denmark.*

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>No. human cases</th>
<th>Included/excluded compared with previously defined</th>
<th>Food/veterinary isolates</th>
<th>Sources</th>
<th>No. SNPs</th>
<th>Maximum SNP distance</th>
<th>SNP distance from nearest neighbor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>–</td>
<td>0</td>
<td>Swine</td>
<td>8</td>
<td>7</td>
<td>143</td>
</tr>
<tr>
<td>B</td>
<td>37</td>
<td>+2</td>
<td>2</td>
<td>Swine</td>
<td>11</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>+1/–2</td>
<td>4</td>
<td>Swine</td>
<td>2 (6)</td>
<td>2 (6)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>E</td>
<td>20</td>
<td>+1/–5</td>
<td>13</td>
<td>Swine/cattle</td>
<td>6 (7)</td>
<td>3 (4)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>F</td>
<td>22</td>
<td>+3</td>
<td>2</td>
<td>Cattle</td>
<td>4</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>G</td>
<td>9</td>
<td>–</td>
<td>0</td>
<td>Cattle</td>
<td>2</td>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>–</td>
<td>1</td>
<td>Swine</td>
<td>0</td>
<td>0</td>
<td>4 (7)</td>
</tr>
</tbody>
</table>

*Previous outbreaks shown in Table 1. SNPs in parentheses are derived from reanalysis of closely related clusters with an internal de novo assembled reference genome. SNP, single-nucleotide polymorphism.
possibly linked food and veterinary isolates, and 5 outbreaks were detected (outbreaks D–H; Table 1).

The SNP analysis of the 242 isolates resulted in 1,488 core-genome SNPs. SNP distances ranged from 0 to 95. Based on core-genome SNPs, the genetic relation between isolates was distinctly more close in comparison to ST36 and ST19. For some clusters, few SNPs separated the isolates, so defining outbreaks based on the analysis was complicated. We recalculated 2 close clusters, which included outbreaks D, E, and H, separately with an internal de novo assembled reference genome to obtain a higher resolution (Figure 4; Table 2). The recalculation added a few extra SNPs; however, conclusions were still not clearcut. Analyzing statistics from our SNP pipeline revealed that ≈20% of the reference genome was discarded when all sequences were analyzed using the closed reference genome (Table 3). Likewise, in some cases, 10% of the reference genome was not used when analyzing an apparently closely related cluster separately. To rule out whether the disregarded data were attributable to the SNP pipeline used, we additionally analyzed the cluster including outbreaks E and H by using the 2 alternative core-genome SNP pipelines NASP (20) and CSI Phylogeny 1.2 (21). From our in-house pipeline, we identified 374 core-genome SNPs within this cluster. The NASP pipeline identified 404 SNPs, and CSI Phylogeny identified 361 SNPs. No further obvious changes were observed in the overall phylogeny in this cluster or for the resolution within the outbreaks, supporting the robustness of our pipeline.

Outbreak D, detected in 2013, previously comprised 8 human isolates. From our analysis, we could include 1 additional human case and exclude 2, on the basis of the genetic relatedness of the isolates (Figure 4). Patient interviews revealed a likely source being consumption of pork from a specific butcher. No relevant food samples from the butcher, patient households, or the companies distributing meat to the butcher were available. Isolates with the same MLVA profile were, at the time, isolated from different slaughterhouses. The SNP analysis linked 3 swine isolates collected from 3 different slaughterhouses with 2–4 SNP differences with the human cases. Another food isolate from pork separated with 6 SNPs was also likely connected to the outbreak. Four food and veterinary isolates, also separated with few SNPs to the outbreak cases, were collected in 2014 and therefore not considered as part of the outbreak. The SNP analysis provided additional evidence for the connection with consumption of pork, further indicating multiple possible sources and the presence of the strain in the food production in 2014.

A large outbreak (outbreak E) was detected in 2014; our collection included 24 isolates from that outbreak. Of the 24 isolates, 23 clustered with few SNP differences (Figure 4). Because the outbreak isolates were located in a closely related cluster in the ST34 group, a clear outbreak definition based on SNP analysis was difficult to make. One human isolate was clearly separated from the cluster and could be excluded as an outbreak case. One additional isolate with a different MLVA profile clustered with 0 SNPs to cases and was included. The probable source of the outbreak was consumption of pork. Samples from swine with the same MLVA profile were collected from a slaughterhouse and suspected as the primary source. Traceback investigations pointed to a specific swine herd with high Salmonella carriage rate supplying swine to the slaughterhouse during the same period. We collected additional samples from a company cutting and distributing meat from the slaughterhouse and from products from companies receiving meat from the primary sources. Isolates from the suspected primary and secondary sources clustered with 0 SNPs to outbreak cases, confirming the connection. Further analysis of the accessory genome identified the presence of 1 region unique for 20 of the human outbreak isolates and the 13 confirmed...
food and veterinary isolates. The identified region had an approximate size of 3,600 bp and contained a ColRNA1-like (92% sequence identity) compatibility gene related to plasmids, 2 genes associated with plasmid regulation (copG and rop), and 1 hypothetical protein (99% protein similarity with predicted plasmid protein identified in an enteropathogenic *Escherichia coli* 0119:H6 strain [GenBank accession no. AP014807.1]). The region was located on an entire single contig with a higher average read coverage and ≈10% lower GC-content than the average of the genome. The nearest neighbor isolates considered for inclusion in the outbreak did not harbor the plasmid-related region and were not included in the final definition (Table 2).

Our results showed that all 19 isolates defined in 2014 as part of outbreak F clustered with 0 to 3 SNPs differences. Three additional human isolates (all with different MLVA profiles) clustered with 0 SNPs to cases and were included in the new cluster definition. The nearest neighbor isolate was located 20 SNPs away, and the outbreak could clearly be defined. Outbreak and food traceback investigations identified beef as the most likely source. We confirmed 2 samples from companies with 0 to 3 SNPs differences with human cases (Figure 4; Table 2).

Likewise, outbreak G was clearly defined by the SNP analysis. Nine human isolates clustered with 0 to 2 SNPs and separated from nearest neighbor with 31 SNPs. Five isolates previously included were distantly related to the cluster and not included in the new outbreak definition (Figure 4; Table 2). No linked food and veterinary isolates were available; however, we did observe a geographic connection to northern Denmark. Three of 5 excluded patients were interviewed, and no travel to northern Denmark was reported, further indicating the exclusion of the patients from the outbreak.

Last, all 5 isolates previously defined in outbreak H clustered with 0 SNPs. Consumption of a specific swine product was reported by 2 case-patients, and a food sample from the specific product clustered with 0 SNPs (Figure 4; Table 2).
Influence of Reference Genome

We examined the influence of the choice of reference genome used in the SNP analysis on the cluster formation. As mentioned previously, we analyzed each ST group and each outbreak separately by using an internal de novo assembled reference genome for each ST group and outbreak. We evaluated the size of core-genome used, percentage of reference genome used, and number of called SNPs (Table 3). Our results showed that using an internal reference genome for ST36 yielded an extra 259 SNPs compared with using the complete ST19 genome 14028S. Using an internal reference for ST19 resulted in 47 fewer SNPs and using an internal reference for ST34 resulted in 2 fewer SNPs compared with using the 14028S genome. No extra SNPs were called within the outbreaks for ST19 and ST36, regardless of reference used or group of isolates analyzed. A few extra SNPs were added when analyzing the outbreaks in ST34 separately with an internal reference. Extra SNP resolution was mostly added on the longer branches and not within tight clusters. We also evaluated including poor-quality genomes in the analysis; inclusion resulted in a considerable loss of data (Table 3).

Discussion

In this retrospective study, we showed that core-genome SNP analysis could be applied for surveillance of "Salmonella" Typhimurium and its monophasic variants. We were able to recover the 8 previously defined outbreaks based on the SNP analysis, epidemiologic data, and food traceback investigations. With the analysis, we could exclude unrelated human isolates and include related isolates not previously defined in the outbreaks based on MLVA. Furthermore, we were able to link possible sources to the outbreaks and reject previously suspected food and veterinary sources. In 4 out of the 8 outbreaks, we could identify the likely source of the outbreak as related to swine. In 1 outbreak, consumption of beef product was confirmed. The remaining 3 outbreaks were not linked to any known sources. In Denmark, "Salmonella" Typhimurium and its monophasic variants are commonly isolated from swine or pork (2) and pork meat is considered the main source of infection as observed and reported in many other countries (9,22–24).

The overall phylogeny of all isolates showed 3 groups of isolates corresponding to the ST. The most commonly isolated ST19 and ST34 isolates clustered together, with ST34 isolates defined in a distinct tight cluster. ST36 isolates were separated from ST19 and ST34 isolates with a long branch indicating a distant relation to ST19 and ST34. The distant relation is further confirmed by ST19 and ST34 belonging to e-BurstGroup 1 (eBG1), whereas
ST36 is located in eBG138, having 3 out of 7 alleles identical with eBG1 (25).

For isolates with ST19 and ST36, the outbreak clusters were well-delimited and separated, as was the case for 2 outbreaks in the ST34 group. For 3 outbreaks with ST34 isolates, the low diversity of the core-genome complicated clear conclusions based on SNP differences between isolates. With further analysis of accessory genes and information from the outbreak investigation, we could more clearly define the outbreaks. Results from the SNP analysis showed that 20% of the reference genome was discarded when analyzing the entire ST34 group, indicating a large amount of accessory data not being used in the analysis. The close core-genome correlates well with the fact that the ST34 is considered a newly expanding clone (3,10,11,24). Additionally, the large variation detected in the accessory genome corresponds well with the findings of Petrovska et al. (26), which also revealed a high amount of microevolution within a clonal expansion of ST34 in the United Kingdom.

Our results show that the SNP analysis is a suitable typing method in relation to surveillance of Salmonella Typhimurium, with the possible exception of some lineages of the monophasic variants. Before applying the method in real time, parameters like isolates analyzed (e.g., ST and clusters), choice of reference genome, and sequence quality need to be addressed and taken into account when setting up a workflow. The reference genome used and the group of isolates analyzed had some, although mostly minor, effect on the SNPs called. However, in general, the choice of reference genome and the selection of isolates analyzed did not change our outbreak definitions. For ST19 and ST34, an overall reference genome, either ST19 or ST34, could be applied. A few extra SNPs were added within outbreaks when analyzing smaller clusters of isolates or outbreaks with an internal reference genome. However, within the tight ST34 group, the few extra SNPs within outbreaks might help in defining some outbreaks more clearly. We observed the largest differences when analyzing the ST36 isolates with a close ST36 reference genome compared with results using an ST19 reference genome. This analysis identified an extra 259 SNPs, and because the ST36 group is distantly related to ST19 and ST34, we recommend using an ST36 reference genome for this group of isolates.

The parameter that did affect the outcome considerably was the quality of the genomes used. We excluded 6 genomes out of 372 isolates because of poor quality. Including these in the analysis resulted in ≈29% fewer SNPs; therefore, we recommend quality assessment of the genomes before analysis. Last, SNP analysis does not give a static value easily communicated between institutions. Adding new isolates to the analysis results in new calculations and a new core-genome. Potential output differences might occur when different pipelines are used; however, the 3 SNP pipelines used in this study did not result in any major differences in phylogeny and had no influence on the outbreak investigations. A clear consensus of the workflow, quality criteria, and the bioinformatics tools used would resolve practical issues regarding the method. Alternatively, the widely used gene-by-gene approach is faster in real-time surveillance and more easily comparable between laboratories provided identical schemes are used. However, this approach requires expensive software (unless data are uploaded to public repositories) and agreement on schemes and curation of allele databases.

Studies on other Salmonella serovars using SNP analysis for outbreak investigation and detection revealed a variable number of SNP cutoff values for defining outbreak clusters, ranging from 0 to 30 SNPs (14,15,17–19,27,28). Likewise, our study shows that SNP cutoffs for outbreaks vary, even within a single serovar, lineage, or clone, and evaluation from outbreak to outbreak is needed. The nature of the outbreak must be taken into account when defining a single outbreak, given that many parameters are possibly affecting the results (e.g., the genetic makeup of the serovar, routes of infection, source types, and time). The

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Table 3. Statistical data from core-genome SNP analysis of different subgroups with complete genome of Salmonella enterica Typhimurium 14028S or an internal de novo assembled genome as reference in an outbreak investigation of Salmonella Typhimurium and its monophasic variants, Denmark

<table>
<thead>
<tr>
<th>Isolates within</th>
<th>Selection of isolates</th>
<th>Size of core-genome used, bp</th>
<th>% Reference genome used</th>
<th>Called SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14028S</td>
<td>Internal de novo</td>
<td>14028S</td>
</tr>
<tr>
<td>All</td>
<td>All</td>
<td>3806685</td>
<td>–</td>
<td>78.16</td>
</tr>
<tr>
<td>ST36</td>
<td>All</td>
<td>4527849</td>
<td>4494995</td>
<td>92.97</td>
</tr>
<tr>
<td></td>
<td>Outbreak A</td>
<td>4604894</td>
<td>4611321</td>
<td>94.55</td>
</tr>
<tr>
<td>ST19</td>
<td>All</td>
<td>4314989</td>
<td>4304908</td>
<td>88.80</td>
</tr>
<tr>
<td></td>
<td>Outbreak B</td>
<td>4648245</td>
<td>4662681</td>
<td>95.44</td>
</tr>
<tr>
<td></td>
<td>Outbreak C</td>
<td>4711565</td>
<td>4720046</td>
<td>96.74</td>
</tr>
<tr>
<td>ST34</td>
<td>All</td>
<td>4055543</td>
<td>4034423</td>
<td>83.27</td>
</tr>
<tr>
<td></td>
<td>Outbreak D</td>
<td>4665201</td>
<td>4771546</td>
<td>95.79</td>
</tr>
<tr>
<td></td>
<td>Outbreak E</td>
<td>4706765</td>
<td>4871858</td>
<td>96.64</td>
</tr>
<tr>
<td></td>
<td>Outbreak F</td>
<td>4705075</td>
<td>4836637</td>
<td>96.61</td>
</tr>
<tr>
<td></td>
<td>Outbreak G</td>
<td>4694550</td>
<td>4826771</td>
<td>96.39</td>
</tr>
<tr>
<td></td>
<td>Outbreak H</td>
<td>4734518</td>
<td>4886190</td>
<td>97.21</td>
</tr>
</tbody>
</table>

*SNPs in parentheses are called SNPs with inclusion of poor-quality sequences. SNP, single-nucleotide polymorphism; ST, sequence type.
SNP analysis and other WGS methods for typing provide a higher discrimination of isolates in comparison with other conventional typing methods (12–19) and gives the advantage of many additional analyses. However, new typing approaches also lead to many new questions on how to interpret data and, in a surveillance context, how to define outbreak cases and sources. Despite the high resolution of the new typing methods, detailed and extended information on epidemiology and food traceback are still crucial elements in effective surveillance. This study has not only provided valuable information on the core-genome SNP analysis for surveillance and outbreak source detection but also has given insight into the phylogenetic relationships between isolates of *Salmonella* Typhimurium and its monophasic variants in Denmark.

**Acknowledgments**

We thank Kristoffer Kiil and Kim Ng for assistance with bioinformatics analyses and Christian Vråby Pedersen for technical assistance.

The Danish Council for Independent Research (Ministry of Higher Education and Science, Denmark) funded this study (grant DFF-4090-00138).

Ms. Gymoese is a PhD student at the University of Copenhagen, Denmark, and is employed at Statens Serum Institut’s Department of Microbiology and Infection Control. Her main research interests are genomics using whole-genome sequence analysis to investigate and optimize surveillance of foodborne bacterial infections.

**References**


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### March 2014: Mycobacterial Infections

- Invasive Fungal Infections after Natural Disasters
- Monitoring Water Sources for Environmental Reservoirs of Toxigenic Vibrio cholerae O1, Haiti
- High-Level Relatedness among Mycobacterium abscessus subsp. massiliense Strains from Widely Separated Outbreaks
- Hendra Virus Vaccine, a One Health Approach to Protecting Horse, Human, and Environmental Health
- Possible Role of Songbirds and Parakeets in Transmission of Influenza A(H7N9) Virus to Humans
- Hantavirus Infections among Overnight Visitors to Yosemite National Park, California, USA, 2012
- Minimal Diversity of Drug-Resistant Mycobacterium tuberculosis Strains, South Africa
- Surveillance for Antimicrobial Drug Resistance in Under-Resourced Countries
- Drought and Epidemic Typhus, Central Mexico, 1655–1918
- Nontoxigenic tox-Bearing Corynebacterium ulcerans Infection among Game Animals, Germany
- Postmortem Diagnosis of Invasive Meningococcal Disease
- Urban Epidemic of Dengue Virus Serotype 3 Infection, Senegal, 2009
- IgG against Dengue Virus in Healthy Blood Donors, Zanzibar, Tanzania
- Mimivirus Circulation among Wild and Domestic Mammals, Amazon Region, Brazil
- Infective Endocarditis in Northeastern Thailand
- Crimean-Congo Hemorrhagic Fever among Health Care Workers, Turkey
- Influenza A(H1N1)pdm09 Virus Infection among Giant Pandas, China
- Mixed Scrub Typhus Genotype, Shandong, China, 2011
- Neisseria meningitidis Serogroup W, Burkina Faso, 2012
- Role of Placental Infection in Miscarriage

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**Emerging Infectious Diseases**

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 23, No. 10, October 2017 • 1639
Enteric Infections Circulating during Hajj Seasons, 2011–2013

Moataz Abd El Ghany,1,2,3 Mona Alsomali,1 Malak Almasri,1 Eriko Padron Regalado, Raecee Naeem, AbdulHafeez Tukestani, Abdullah Asiri, Grant A. Hill-Cawthorne, Arnab Pain,2,3 Ziad A. Memish2,3

In support of improving patient care, this activity has been planned and implemented by Medscape, LLC and Emerging Infectious Diseases. Medscape, LLC is jointly accredited by the Accreditation Council for Continuing Medical Education (ACCME), the Accreditation Council for Pharmacy Education (ACPE), and the American Nurses Credentialing Center (ANCC), to provide continuing education for the healthcare team.

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Learning Objectives

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

• Assess common countries of origin of cases of infectious diarrhea in the current study of Hajj.
• Evaluate the clinical presentation of patients with gastroenteritis at Hajj.
• Differentiate the microbiology of gastroenteritis at Hajj.
• Distinguish the most common bacterial pathogen isolated from cases in the current study.

CME Editor

Jude Rutledge, BA, Technical Writer/Editor, Emerging Infectious Diseases. Disclosure: Jude Rutledge has disclosed no relevant financial relationships.

CME Author

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Authors

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Hajj, the annual Muslim pilgrimage to Mecca, Saudi Arabia, is a unique mass gathering event that raises public health concerns in the host country and globally. Although gastroenteritis and diarrhea are common among Hajj pilgrims, the microbial etiologies of these infections are unknown. We collected 544 fecal samples from pilgrims with medically attended diarrheal illness from 40 countries during the 2011–2013 Hajj seasons and screened the samples for 16 pathogens commonly associated with diarrheal infections. Bacteria were the main agents detected, in 82.9% of the 228 positive samples, followed by viral (6.1%) and parasitic (5.3%) agents. Salmonella spp., Shigella enteroinvasive Escherichia coli, and enterotoxigenic E. coli were the main pathogens.

1These first authors contributed equally to this article.
2These senior authors contributed equally to this article.
3These authors were co-principal investigators.
associated with severe symptoms. We identified genes associated with resistance to third-generation cephalosporins ≈40% of *Salmonella* and *E. coli*-positive samples. Hajj-associated foodborne infections pose a major public health risk through the emergence and transmission of antimicrobial drug–resistant bacteria.

Hajj, the annual pilgrimage by Muslims to Mecca, Saudi Arabia, is a unique mass gathering event in terms of scale (i.e., the number of pilgrims), diversity of the pilgrims, nature of the activities performed, and regularity. Approximately 2 million pilgrims from 185 countries, in addition to hundreds of thousands of residents of Saudi Arabia, travel to holy sites in Mecca each year (1). This enormously diverse population (in terms of ethnic origin, socioeconomic status, sex, age, and health status) comes together to perform the same activities within a relatively short period over a limited area of land (2), which allows for the mixing of infectious agents and susceptible populations (3). Mass gatherings such as Hajj therefore increase the potential for the emergence and dissemination of infections and raises public health concerns in Saudi Arabia and globally (4). Hajj-associated communicable public health hazards mainly involve the transmission of respiratory infections, foodborne diseases, bloodborne diseases, and zoonotic infections (4).

Globally, diarrheal infections remain the leading cause of mortality in children <5 years of age and contribute to ≈10% of child deaths each year (5–7). In addition, travelers’ diarrhea is still the most common illness observed in travelers returning from regions where diarrheal diseases are endemic (8,9). The main etiologic agents detected are consistently bacteria (*Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp.); viruses (rotavirus, norovirus, and adenovirus); and parasites (*Cryptosporidium* spp., *Giardia lamblia*, and *Entamoeba histolytica*) (10,11).

Despite substantial advances in food and water hygiene in many countries, mass gathering events still represent the perfect environments for the transmission of enteric infections (12,13). Diarrheal infections and foodborne diseases are commonly associated with the Hajj pilgrimage (14). Although diarrheal infections and other enteric infections are some of the most common complaints among pilgrims, little information is available regarding incidence, etiologic agents, and the abundance of antimicrobial drug–resistant strains. Published reports have mainly been based on analyses of hospital admission data that lack full characterization of the nature of the infections (15–17). Moreover, estimates of the incidence of Hajj-associated gastrointestinal disease based on hospital admission data can vary considerably (14). Recently, a few studies have shown an increase in the carriage rates of enteric pathogens that include *Tropheryma whippelii* (18), multidrug-resistant nontyphoidal *Salmonella* (19), and carbapenemase-producing *E. coli* (20) among pilgrims from France returning from Hajj. These findings, coupled with the growing threat of drug-resistant microorganisms (21), increase the risks associated with the Hajj pilgrimage and fuels the emergence and dissemination of drug-resistant enteric pathogens.

We conducted a large-scale study to catalog the circulating enteric pathogen population in Hajj pilgrims with diarrheal symptoms. We report on the use of molecular and antigenic approaches to characterize the etiologic agents associated with enteric infections in pilgrims who sought medical treatment while performing Hajj during the 2011–2013 seasons.

**Materials and Methods**

**Ethics Statement**
The samples were originally collected for diagnostic purposes; therefore, collection was not experimental in nature. The Ministry of Health of Saudi Arabia anonymized all identifiable information, and only deidentified records and samples were available to the researchers. The King Fahad Medical City institutional review board approved the study protocol (approval no. 11–157, dated October 4, 2011). The Institutional Biosafety and Ethics Committee of King Abdullah University of Science and Technology also approved the study in 2013.

**Study Design**
We conducted the study for 3 successive Hajj seasons, starting in 2011. Fecal samples from pilgrims having medically attended diarrhea while performing Hajj were collected. Healthcare facilities distributed along the Hajj sites were enrolled in the study.

We included patients with symptoms who were seeking medical care for diarrhea or who were admitted to hospitals or primary care centers established in the holy sites during the 7–10-day Hajj period. We defined diarrhea as the occurrence of ≥3 unformed stools in a 24-hour period or passing stool more frequently than normal for the patient, accompanied by ≥1 other gastrointestinal symptom (abdominal pain/cramps, vomiting, or bloody or mucoid stools). Patients who had unformed stool with visible blood were defined as having cases of dysentery. Patients with increased body temperature were categorized as having either mild (>37.5°C and <39°C) or severe (>39°C) fever.

We categorized the patients into 2 groups according to degree of symptom severity. We defined severe diarrhea as ≥6 unformed stools per day; diarrhea requiring hospitalization; or diarrhea accompanied by fever, dehydration, or bloody or mucoid stools. We classified patients with diarrhea not fulfilling the criteria for severe symptoms as having...
mild cases. We screened all the samples molecularly, antigenically, or both for a panel of 16 infectious agents commonly associated with diarrheal infection.

**Antigenic Detection of Viral and Parasitic Pathogens**

We used qualitative enzyme immunoassays for the initial detection of viral agents in the fecal samples according to manufacturers’ instructions. We used the IDEIA Norovirus test (Oxoid, Basingstoke, UK) to detect norovirus genogroups 1 and 2 and ProSpecT tests (Oxoid) to detect group A rotaviruses, adenoviruses, and astroviruses. For parasitic agents, we used the Giardia/Cryptosporidium Quik Chek test (TechLab, Blacksburg VA, USA) for the detection and differentiation of Cryptosporidium oocyst antigen and Giardia cyst antigen.

**Isolation of DNA Using QIAsymphony Platform**

We used the QIAsymphony SP (QIAGEN, Hilden, Germany), an automated high-throughput platform, for the isolation and purification of total DNA from the collected fecal samples. We used the QIAsymphony DNA 800 complex kit (QIAGEN) to extract DNA from 800 μL of pretreated diluted samples according to the manufacturer’s instructions.

**Molecular Characterization of Bacterial Species**

We used 3 previously established multiplex PCR assays (M1, 2, and 3) in parallel to detect the bacterial pathogens commonly associated with diarrheal infections (22). The M1 multiplex PCR used primers targeting genes eae and bfpA (enteropathogenic E. coli), aggR (enteroaggregative E. coli), and Vero cytotoxin (enterohemorrhagic E. coli). The M2 multiplex PCR used primers targeting the genes elt and st (enterotoxigenic E. coli [ETEC]), daaE (diffusely adherent E. coli), and virF and ipaH (Shigella spp./enteroinvasive E. coli [EIPEC]). The M3 multiplex PCR used primers targeting the hipO gene (Campylobacter jejuni), internal transcribed spacer region (Salmonella spp.), Yersinia stable toxin gene (Yersinia enterocolitica), and rtxA gene (Vibrio cholerae). Primer details and the expected PCR fragment sizes are provided (online Technical Appendix Table 1). We evaluated the differences between the sets of the categorical data by using the Pearson χ² test. We defined statistical significance as p<0.05.

**Results**

Durin 3 consecutive Hajj seasons (2011–2013), we collected 544 fecal samples from pilgrims who had diarrhea while performing Hajj and who sought treatment at healthcare facilities (Tables 1, 2). These patients originated from 40 countries on 5 continents (online Technical Appendix Table 4). Most patients (434, 79.8%) originated from 7 countries: Saudi Arabia (24.82%, n = 135), Nigeria (15.07%, n = 82), Egypt (12.87%, n = 70), Bangladesh (8.09%, n = 44), Pakistan (6.43%, n = 35), Yemen (6.25%, n = 34), and India (6.25%, n = 34). Median (+quartile deviation) patient age was 40.17 (±13.17) years. By Hajj season, median age was 40 (±12.25) years in 2011, 40 (±13.25) in 2012, and 40.5 (±14) years in 2013 (Table
represented 27.12% of patients in 2011, 28.28% in 2012, and most patients were men (72.98%, n = 397); women represented 27.12% of patients in 2011, 28.28% in 2012, and 23.26% in 2013 (Table 1).

We summarized the distribution of the clinical features among the patients during the 3 Hajj seasons (Table 2). Most patients were seen as outpatients (86.95%,

### Table 1. Demographic characteristics of persons who acquired enteric infections during their travel for Hajj, 2011–2013

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Year</th>
<th>Statistical analyses*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2011</td>
<td>2012</td>
</tr>
<tr>
<td>No. patients</td>
<td>118</td>
<td>297</td>
</tr>
<tr>
<td>Median (±quartile deviation)</td>
<td>40 (±12.25)</td>
<td>40 (±13.25)</td>
</tr>
<tr>
<td>No. countries of origin</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

| Sex, no. (%)                    |            |                       |                       |            |     |         |
|---------------------------------|------------|-----------------------|-----------------------|------------|     |         |
| F                               | 32 (27.12) | 84 (28.28)            | 30 (23.26)            | 146 (26.84) | 1.07 | 0.59    |
| M                               | 86 (72.12) | 213 (71.72)           | 98 (75.97)            | 397 (72.98) |     |         |

*Comparison between Hajj seasons.

### Table 2. Clinical characteristics of persons who acquired enteric infections during their travel for Hajj, 2011–2013

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Year</th>
<th>Statistical analyses*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2011</td>
<td>2012</td>
</tr>
<tr>
<td>Hospitalization, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outpatient</td>
<td>94 (79.66)</td>
<td>263 (88.55)</td>
</tr>
<tr>
<td>Inpatient</td>
<td>24 (20.34)</td>
<td>33 (11.11)</td>
</tr>
<tr>
<td>Not defined</td>
<td>0</td>
<td>1 (0.34)</td>
</tr>
<tr>
<td>Stool consistency, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unformed†</td>
<td>57 (48.31)</td>
<td>187 (62.96)</td>
</tr>
<tr>
<td>Watery‡</td>
<td>60 (50.85)</td>
<td>101 (34.01)</td>
</tr>
<tr>
<td>Not defined</td>
<td>1 (0.85)</td>
<td>9 (3.03)</td>
</tr>
<tr>
<td>Abdominal pain/cramps, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>106 (89.83)</td>
<td>282 (94.95)</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not defined</td>
<td>12 (10.17)</td>
<td>15 (5.05)</td>
</tr>
<tr>
<td>Bowel movements/d, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>9 (7.63)</td>
<td>5 (1.68)</td>
</tr>
<tr>
<td>3–5</td>
<td>78 (66.1)</td>
<td>212 (71.38)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>19 (16.1)</td>
<td>65 (21.89)</td>
</tr>
<tr>
<td>Not defined</td>
<td>12 (10.17)</td>
<td>15 (5.05)</td>
</tr>
<tr>
<td>Duration of diarrhea, d, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>61 (51.69)</td>
<td>140 (47.14)</td>
</tr>
<tr>
<td>3–5</td>
<td>42 (35.59)</td>
<td>119 (40.07)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>12 (10.17)</td>
<td>24 (8.08)</td>
</tr>
<tr>
<td>Not defined</td>
<td>3 (2.54)</td>
<td>14 (4.71)</td>
</tr>
<tr>
<td>Presence of mucus, no. (%)</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>38 (32.2)</td>
<td>165 (55.56)</td>
</tr>
<tr>
<td>No</td>
<td>80 (67.8)</td>
<td>126 (42.42)</td>
</tr>
<tr>
<td>Not defined</td>
<td>0</td>
<td>6 (2.02)</td>
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<tr>
<td>Presence of blood, no. (%)</td>
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<td></td>
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<td>Yes</td>
<td>14 (11.86)</td>
<td>26 (8.75)</td>
</tr>
<tr>
<td>No</td>
<td>104 (88.14)</td>
<td>265 (89.23)</td>
</tr>
<tr>
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<td>0</td>
<td>6 (2.02)</td>
</tr>
<tr>
<td>Vomiting, no. (%)</td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>33 (27.97)</td>
<td>80 (26.94)</td>
</tr>
<tr>
<td>No</td>
<td>85 (72.03)</td>
<td>211 (71.04)</td>
</tr>
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<td>0</td>
<td>6 (2.02)</td>
</tr>
<tr>
<td>Fever, no. (%)§§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No fever</td>
<td>87 (73.73)</td>
<td>167 (56.73)</td>
</tr>
<tr>
<td>Moderate</td>
<td>23 (19.49)</td>
<td>72 (24.24)</td>
</tr>
<tr>
<td>Severe</td>
<td>3 (2.54)</td>
<td>32 (10.77)</td>
</tr>
<tr>
<td>Not defined</td>
<td>5 (4.24)</td>
<td>26 (8.75)</td>
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<tr>
<td>Dehydration, no. (%)¶¶</td>
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</tr>
<tr>
<td>Yes</td>
<td>42 (35.59)</td>
<td>53 (17.85)</td>
</tr>
<tr>
<td>No</td>
<td>76 (64.41)</td>
<td>238 (80.13)</td>
</tr>
<tr>
<td>Not defined</td>
<td>0</td>
<td>6 (2.02)</td>
</tr>
</tbody>
</table>

*Comparison between Hajj seasons.
†Bristol 6.
‡Bristol 7.
§Moderate fever defined as >37.5°C and <39.0°C; severe fever defined as ≥39.0°C.
¶Dehydration defined as ≥2 of the following signs or symptoms: thirst, dry mouth, weakness/lightheadedness, and darkening of the urine/decrease in urination.
n = 473), and the most frequently reported symptoms were abdominal pain/cramps (90.26%, n = 491), presence of mucus in the stool (44.85%, n = 244), watery diarrhea (38.42%, n = 209), dehydration (24.63%, n = 134), vomiting (22.98%, n = 125), and moderate fever (22.06%, n = 120). Less common symptoms were bloody stool (9.38%, n = 51) and severe fever (6.62%, n = 36). We observed significant differences in the frequencies of these symptoms across the 3 Hajj seasons (Table 2).

Characterization of Bacterial Pathogens
We screened the 544 fecal samples collected from the patients during the 2011–2013 Hajj seasons for 16 infectious agents, including bacteria, viruses, and parasites commonly associated with diarrheal infections. We calculated the number of the samples tested and the number and percentage of the positive samples from each season (Table 3). We detected >1 of the pathogens screened for in 41.91% (n = 228) of the samples. We observed no significant difference between the numbers of positive samples during the 3 seasons ($\chi^2 = 0.63; p = 0.73$). The percentages of positive samples detected were 43.22% (n = 51) for 2011, 40.40% (n = 120) for 2012, and 44.19% (n = 164) for 2013. Bacterial pathogens were the predominant infectious agents detected for the 3 Hajj seasons and the agents identified in 34.74% (n = 189) of the total samples, followed by viral (2.57%, n = 14) and parasitic (2.21%, n = 12) agents. Thirteen patients (representing 2.39% of the total samples) had samples testing positive for >1 pathogen. We observed no significant difference in the distribution of infectious agents across the 3 seasons ($\chi^2 = 8.84; p = 0.18$).

We calculated the distribution of patients by age group and the enteric pathogens identified (Figure, panels A, B). The highest proportion of patients having diarrhea of known etiology, compared with unknown, was the <20-year-old age group (odds ratio [OR] 2.46; p = 0.0002). Conversely, the highest proportion of patients having diarrhea of unknown etiology compared with known was the 40–60 years age group (OR 0.52; p = 0.0004). For most of the age groups, bacteria were the main cause of diarrhea in patients, with no significant difference detected across the 3 Hajj seasons ($\chi^2 = 8.59; p = 0.2$).

We also calculated the distribution of the bacterial agents associated with the diarrheal patients during 2011–2013 Hajj seasons by age group (Figure, panel C). E. coli was the most frequent species present, detected in 43.39% (n = 82) of the bacteria-positive samples. Of the serovars tested, ETEC was the most common, detected in 25.4% (n = 48) of the positive samples, followed by enteropathogenic E. coli (8.47%,...
Enteric Infections during Hajj Seasons, 2011–2013

n = 16), enteroaggregative E. coli (3.7%, n = 7), diffusely adherent E. coli (3.7%, n = 7), and enterohemorrhagic E. coli (2.12%, n = 4). We detected Salmonella spp. in 32.80% (n = 62) and Shigella/EIEC in 21.69% (n = 41) of the bacteria-positive samples. We observed significant differences in the distribution of bacterial pathogens across the 3 Hajj seasons ($\chi^2 = 12.89; p = 0.01$) and among the different age groups ($\chi^2 = 21.62; p = 0.01$).

Characterization of Viral and Parasitic Pathogens

We calculated the distribution of the viral and parasitic agents associated with diarrheal infections of pilgrims during the 2011–2013 Hajj seasons (Table 3). Screening for adenoviruses, astroviruses, noroviruses, and rotaviruses showed rotaviruses were most common, detected in 42.86% (n = 6) of the samples positive for the screened viruses. Astroviruses were detected in 21.43% (n = 3), noroviruses in 28.57% (n = 4), and adenoviruses in 7.14% (n = 1) of the virus-positive samples. We used reverse transcription PCR and Sanger sequencing to determine the genotypes of the astroviruses, noroviruses, and rotaviruses detected (online Technical Appendix Table 5). All norovirus genotypes identified were recovered from pilgrims from inside Saudi Arabia. Also, 80% of the identified astrovirus genotypes were recovered only from pilgrims from inside Saudi Arabia (astrovirus 2 or 5), whereas the single astrovirus 1 genotype was recovered from a pilgrim from Morocco (online Technical Appendix Table 5).

Giardia spp. were the most common parasitic agent, identified in 83.33% (n = 10) of the parasite-positive samples, followed by Cryptosporidium spp. in 16.66% (n = 2) of the samples. We isolated Giardia spp. from patients originating from 10 countries: 4 from Pakistan, 3 from Nigeria, 2 from Bangladesh, and 1 each from Ethiopia, Somalia, Egypt, Jordan, Niger, India, and Afghanistan. We identified Cryptosporidium spp. in 2 children (<5 years of age) from Saudi Arabia and 1 older pilgrim (65 years of age) from Chad (online Technical Appendix Table 6).

Relationship between Severity of Diarrheal Disease and Etiologic Agent

We calculated the distribution of the etiologic agents by severity of disease (Table 4). The percentage of samples with identified etiologic agents was significantly higher in patients with severe cases compared with those with mild cases (OR 1.69; p = 0.01). Similarly, the percentage of bacterial agents was significantly higher in patients with severe cases compared with those with mild cases (OR 1.58; p = 0.04). The main bacterial contributors to the severe disease of Hajj-associated diarrheal illness were Salmonella, Shigella/EIEC, and ETEC.

Antimicrobial Drug Resistance

We calculated the distribution of $\beta$-lactamase genes among the identified bacterial samples (Table 5). $\text{bla}_{\text{CTX-M-15}}$ and $\text{bla}_{\text{qom}}$ were the most common antimicrobial resistance genes, associated predominantly with Salmonella (n = 25/62) and ETEC (n = 16/48). This finding suggests that 40.32% of Salmonella infections and 33.33% of ETEC infections associated with the Hajj might be resistant to at least some third-generation cephalosporins, and this number might be growing with successive seasons.
Discussion

Enteric infections are commonly associated with mass gathering events, including the annual Hajj pilgrimage to Mecca, Saudi Arabia. The host country and the country of origin of many of the pilgrims are endemic for enteric pathogens and increasingly high levels of antimicrobial resistance. In addition, the lack of effective vaccines against major bacterial infections is challenging (30). These circumstances raise serious public health challenges for Saudi Arabia, with potential intercontinental and global implications. A key challenge is the paucity of information available on the structure of the circulating enteric pathogens encountered and thus are more highly susceptible to infection when traveling overseas (32).

In this study, we used integrated antigenic and molecular approaches to screen 544 fecal samples from pilgrims with medically attended diarrheal illness for 16 pathogens to identify the etiologic agents responsible for patients seeking care at healthcare facilities during 3 consecutive Hajj seasons. Bacterial pathogens were the most common causes of Hajj-associated diarrheal disease, followed by viruses and parasites, and this pattern was maintained during all 3 seasons.

Our data demonstrate that Hajj-associated diarrheal disease is usually caused by 1 bacterial agent, with ETEC, Salmonella spp., and Shigella/EIEC being the most common. This association is distinct to the pattern of travelers’ diarrhea observed in travelers from Finland, where multiple bacterial pathogens have been identified in 53% of patients with ongoing diarrhea and 25% of those without symptoms (37). However, this observation is not surprising; Hajj-associated diarrheal disease is likely to be different from travelers’ diarrhea because of the different populations involved. Most of Hajj pilgrims originate from intermediate- and high-risk regions for enteric pathogens. In contrast, many travelers’ diarrhea patients are nonimmune persons from developed countries who are naive to many of the enteric pathogens encountered and thus are more highly susceptible to infection when traveling overseas (32).

Viruses ranked second and parasites third as the most commonly detected pathogens in patients with Hajj-associated diarrhea. Of note, all of the identified noroviruses and most astroviruses and rotaviruses were recovered from pilgrims from inside Saudi Arabia. The emergent norovirus genotype GII.4 that was first identified in Sydney, Australia, in 2012 and subsequently resulted in global outbreaks had already begun circulating among pilgrims from Saudi Arabia during the Hajj seasons. Bacterial pathogens were the most common causes of Hajj-associated diarrheal disease, followed by viruses and parasites, and this pattern was maintained during all 3 seasons.

Table 4. Relationship between severity of diarrheal disease and identified etiologic agents among persons who acquired enteric infections during their travel for Hajj, 2011–2013*

<table>
<thead>
<tr>
<th>Category</th>
<th>Severity of diarrheal disease</th>
<th>Statistical analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe</td>
<td>Mild</td>
</tr>
<tr>
<td>Total no. cases</td>
<td>412</td>
<td>132</td>
</tr>
<tr>
<td>Positive for etiologic agent, no. (%)</td>
<td>185 (44.9)</td>
<td>43 (32.58)</td>
</tr>
<tr>
<td>Bacterial agents, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>153 (37.14)</td>
<td>36 (27.27)</td>
</tr>
<tr>
<td><em>Shigella/EIEC</em></td>
<td>35 (8.5)</td>
<td>6 (4.55)</td>
</tr>
<tr>
<td>ETEC</td>
<td>43 (10.44)</td>
<td>5 (3.79)</td>
</tr>
<tr>
<td>EPEC</td>
<td>13 (3.16)</td>
<td>3 (2.27)</td>
</tr>
<tr>
<td>EHEC</td>
<td>3 (0.73)</td>
<td>1 (0.76)</td>
</tr>
<tr>
<td>DAEC</td>
<td>7 (1.7)</td>
<td>0</td>
</tr>
<tr>
<td><em>EAEC</em></td>
<td>5 (1.21)</td>
<td>2 (1.52)</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>2 (0.49)</td>
<td>2 (1.52)</td>
</tr>
<tr>
<td>Viral agents, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrovirus</td>
<td>13 (3.16)</td>
<td>1 (0.76)</td>
</tr>
<tr>
<td>Norovirus</td>
<td>2 (0.49)</td>
<td>1 (0.76)</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>4 (0.97)</td>
<td>0</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>6 (1.46)</td>
<td>0</td>
</tr>
<tr>
<td>Parasitic agents, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td>10 (2.43)</td>
<td>2 (1.52)</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>8 (1.94)</td>
<td>2 (1.52)</td>
</tr>
<tr>
<td>Mixed infectious agents, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacteria and virus</em></td>
<td>9 (2.18)</td>
<td>4 (3.03)</td>
</tr>
<tr>
<td><em>Bacteria and parasite</em></td>
<td>4 (0.97)</td>
<td>2 (1.52)</td>
</tr>
<tr>
<td><em>Bacteria, virus, and parasite</em></td>
<td>5 (1.21)</td>
<td>1 (0.76)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1 (0.76)</td>
</tr>
</tbody>
</table>

*EAEIC, enterohemorrhagic Escherichia coli; EHEC, enteroinvasive E. coli; EPEC, enteropathogenic E. coli; ETEC, enterotoxigenic E. coli; DAEC, diffusely adherent E. coli; OR, odds ratio.
†Compared with total number of cases. OR 1.69, p=0.01.
‡Compared with total number of cases. OR 1.89, p=0.04.
§Compared with total number of positive bacteria. OR 0.47, p=0.04.
¶Compared with total number of cases. OR 2.96, p=0.02.
Arabia in late October and early November of the 2012 Hajj season. Major causes of diarrhea among children living in Saudi Arabia include rotaviruses (accounting for 6.0% incidence), noroviruses (3.5%), astroviruses (1.9%), and adenoviruses (1.4%) (33).

The 3 most commonly identified bacteria in our study (Salmonella spp., Shigella spp., and E. coli) have all been identified by the World Health Organization as being among the top 9 bacteria likely to have a serious impact on global public health (21). Of particular concern were the presence of extended-spectrum β-lactamase (ESBL) (primarily bla_{CTX-M-15}) and carbapenemase (e.g., bla_{NDM}) genes in ≥40% of Salmonella spp. and E. coli–positive samples collected.

Recently, travelers’ diarrhea has been shown to be an independent risk factor for contracting ESBL-producing Enterobacteriaceae (ESBL-PE) but not carbapenemase-producing Enterobacteriaceae (CPE), with the rate of acquisition varying by destination (34). Saudi Arabia and the countries of origin for many of the pilgrims are countries at high risk for the acquisition of diarrheal (9,32) and ESBL-PE infections (34). Recent surveillance studies have also reported increasing prevalence of CPE and ESBL-PE isolates in the Gulf Cooperation Council countries (35), with some research institutes in Saudi Arabia finding that up to 65% of E. coli isolates are ESBL producers (36). Recently, the rates of bla_{CTX-M-15} infection in Hajj pilgrims have been found to be 31% in 2013 and 34.83% in 2014 (37).

Collectively, these results suggest that further epidemiologic investigations need to be carried out during pilgrimages to identify potential food sources of pilgrim infections. In addition, antimicrobial drug susceptibility testing is needed to inform treatment.

This study used a retrospective approach and a anonymized specimen from each patient enrolled in the study. One advantage of this approach is that the study population is more representative of the highly diverse Hajj population, with samples collected from patients originating from 40 different countries. However, a prospective approach with pre- and post-Hajj samples collected from each patient would have provided information on the role of the pilgrimage in contracting the pathogens identified.

In addition, even though integrated molecular and antigenic approaches were used, >50% of the tested samples had no identifiable etiologic agent. These samples require further examination using more comprehensive high-throughput sequencing and metagenomic approaches. High-throughput shotgun sequencing has been used successfully to study population structures and define the epidemiologic links of many enteric pathogens (38–42). Moreover, metagenomic approaches have been used successfully to identify viral (43,44) and bacterial (45) agents associated with enteric infections. This approach could enable estimation of the ratio of pathogenic to commensal bacteria in pilgrims’ guts, thereby characterizing the acquisition of potential pathogens and their dynamics before and during infections.

Finally, in this study, the assessment of antimicrobial drug susceptibility was only performed by detecting resistance-related genes. The presence of such genes does not necessarily mean the pathogen identified is carrying them.

<table>
<thead>
<tr>
<th>Year/bacteria</th>
<th>bla_{CTX-M-15}</th>
<th>bla_{NDM}</th>
<th>bla_{KPC}</th>
<th>bla_{IMP}</th>
<th>bla_{OXA-48}</th>
<th>bla_{VIM}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011 Salmonella</td>
<td>3+2†</td>
<td>2†</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>0</td>
</tr>
<tr>
<td>Shigella/EIEC</td>
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<td>1†</td>
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<td>0</td>
<td>0</td>
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<tr>
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<td>4†</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EHEC</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2012 Salmonella</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shigella/EIEC</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EPEC</td>
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<td>EHEC</td>
<td>2</td>
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</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>2+1†</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2013 Salmonella</td>
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<td>0</td>
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<tr>
<td>Shigella/EIEC</td>
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</tr>
<tr>
<td>ETEC</td>
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<td>1†</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EPEC</td>
<td>6+1†</td>
<td>1†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EAEC</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

†Multidrug-resistant; both bla_{CTX-M-15} and bla_{NDM} were detected.

Table 5. Distribution of β-lactamase genes among the identified bacterial agents among persons who acquired enteric infections during their travel for Hajj, 2011–2013†
and these genes might be associated with other commensal carriage. We focused on those resistance genes that are posing the most risk to global health and can be easily shared among the Enterobacteriaceae, rather than the genes that can confer resistance to the antibiotics widely used for treating enteric infections.

The data we have collected are alarming and highlight the need for further studies to explore the impact of Hajj on public health in Saudi Arabia and globally. Longitudinal studies are required to monitor the changes in colonization patterns of pilgrims during the Hajj, identify the key factors that control these changes, detect the emergence of novel variants (particularly those associated with drug resistance), and understand the dynamics of disease transmission. In addition, active surveillance for enteric diseases is needed to define the potential impact of Hajj on the baseline status of enteric infections in residents of Saudi Arabia and to investigate foodborne outbreaks of disease in a timely manner.

Acknowledgments
We thank Elizabeth Barnes, Mahmoud Diaf, and Moustafa El Badry for their advice regarding the statistical analysis.

This work was supported by the Saudi Ministry of Health (Z.M.) and King Abdullah University of Science and Technology (faculty baseline funding [BAS/1/1020-01-01] to A.P.), and Marie Bashir Institute and Sydney Medical School Foundation (M.A. and G.H.).

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References


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In 2007, a waterborne outbreak of Cryptosporidium hominis infection occurred in western Ireland, resulting in 242 laboratory-confirmed cases and an uncertain number of unconfirmed cases. A boil water notice was in place for 158 days that affected 120,432 persons residing in the area, businesses, visitors, and commuters. This outbreak represented the largest outbreak of cryptosporidiosis in Ireland. The purpose of this study was to evaluate the cost of this outbreak. We adopted a societal perspective in estimating costs associated with the outbreak. Economic cost estimated was based on totaling direct and indirect costs incurred by public and private agencies. The cost of the outbreak was estimated based on 2007 figures. We estimate that the cost of the outbreak was \( >€19 \) million (\( ≈€120,000/\text{day of the outbreak} \)). The US dollar equivalent based on today’s exchange rates would be $22.44 million (\( ≈$142,000/\text{day of the outbreak} \)). This study highlights the economic need for a safe drinking water supply.

Cryptosporidium spp. are protozoan parasites that might be present in inadequately treated water. Human infection can result in watery diarrhea, stomach cramps, bloating, vomiting, and fever (1–3). Although usually a self-limiting illness in otherwise healthy persons, cryptosporidiosis might be associated with chronic gastrointestinal sequelae in some persons and might be fatal for persons with impaired immune function (4). In Ireland, animal contact is the main source of transmission (5). Endemic disease usually occurs in the spring, is predominantly rural, and is generally associated with C. parvum (4,6). C. hominis is primarily a parasite of primates, including humans, and is less common in Ireland.

Several large waterborne outbreaks of cryptosporidiosis have been reported (5,7,8). In 1993, contamination of the municipal water supply affected an estimated 403,000 persons in Milwaukee, Wisconsin, USA (7). In March 2001 in North Battleford, Saskatchewan, Canada, an estimated 7,000 persons became ill from contaminated water (9).

Many studies have estimated the economic costs of microbial contamination of drinking water supplies (7,10–13). However, there is no standard method for performing such analysis. Halonen et al. estimated the cost of the lost workdays to be €1.8–2.1 million (11). Corso et al. included medical costs and loss of productivity related to cryptosporidiosis in Milwaukee and estimated the costs to be $96.2 million (7). A similar approach was used in a study in Canada by the Safe Drinking Water Foundation (Saskatoon, Saskatchewan, Canada) in 2015, which conducted a full cost-benefit analysis of microbial contamination of the water supply in Walkerton, Ontario (13). Regardless of the approach used by researchers, there is agreement that costs of outbreaks are considerable and that benefits of preventive measures need to be investigated (11–13).

Outbreaks of cryptosporidiosis are common in Ireland. During 2011–2014, a total of 100 outbreaks (84 of which were outbreaks in families) were reported that included 305 cases (5). In March 2007, the largest outbreak of cryptosporidiosis in Ireland since surveillance began was identified and was associated with contamination of the public water supply serving an urban area (Galway, Ireland) and surrounding areas. The outbreak was distinguished from the usual spring peak not only by the number of cases but also by the predominantly urban distribution and the infecting species (C. hominis). The outbreak lasted for 5 months, by which time there were 242 confirmed cases of cryptosporidiosis (Health Service Executive [HSE], Galway, Ireland, unpub. data), although it was likely that the actual number affected was higher (14). A boil water notice was put in place for the duration of the outbreak (158 days); the order affected \( ≈120,432 \) persons. The outbreak ended in August 2007 after major investments by local authorities in water treatment infrastructure and major disruption to residents and local businesses.
Water quality incidents, such as the outbreak of cryptosporidiosis in Galway in 2007, can have major economic effects on the entire community (residential and commercial) (7,15). The challenge associated with managing waterborne infection with Cryptosporidium spp. is that infection is not inactivated by chlorination of water, which is the primary method of water treatment in many areas (6). Several technologies are available to remove or inactivate Cryptosporidium spp. during water treatment. These technologies include filtration and ultraviolet light treatment systems.

We assessed economic costs associated with the waterborne outbreak of cryptosporidiosis in Galway, Ireland, during 2007 from the societal perspective. Assessment of costs included not only costs incurred by the 242 reported cases but costs incurred by persons who were ill but did not seek healthcare, as well as by the wider public, local businesses, the healthcare system, local authorities, national agencies, and tourism. We also examined the ratio between the investment needed to mitigate risk for contamination with Cryptosporidium spp. and costs averted by such an investment. The aim of such analysis was to aid decision makers with public investment decisions and to inform other stakeholders about economic consequences associated with outbreaks of this type.

Materials and Methods

Costs

A societal perspective was adopted in estimating costs associated with this outbreak. The costs associated with the waterborne outbreak of cryptosporidiosis in Galway in 2007 have a multilevel structure (Figure). Consistent with previous research and traditional health economic frameworks, we included direct and indirect costs in calculations (15). Direct costs were medical and healthcare costs, cost of provision of alternative water, and response costs. Indirect costs were loss of income, loss of business, and loss of productivity (15). We describe the economic effect of the outbreak on those directly affected, and in the wider community, local businesses, and government agencies. The economic cost estimated in our analysis is based on totaling direct and indirect costs incurred by public and private economic agencies (Figure) associated with the outbreak.

Costs included are consistent with those included in previous economic assessments of waterborne outbreaks of infectious diseases (7,15,16). In our analysis, most costs are reported to have occurred during the outbreak in 2007. Thus, use of a discount rate was not considered necessary. Although the final update was completed in 2009, we could not differentiate what proportion of this update occurred in what period. Thus, the value for the update that was provided to us was not discounted.

Data Sources and Assumptions

Data, sources, and assumptions (if any) made in estimating costs are provided (Table 1). To estimate household costs, we used data for the Simulation Model of the Irish Local Economy (SMILE) (17) to determine the number of households and their socioeconomic characteristics if located in the boil water notice area. SMILE (17) is a synthetic data-set that is spatially representative of households and farms.

Figure. Multilevel structure of costs associated with waterborne outbreak of cryptosporidiosis, Galway, Ireland, 2007. EPA, Environmental Protection Agency of Ireland; GP, general practitioner; HSE, Health Service Executive.
Table 1. Description of costs included in economic assessment of waterborne outbreak of cryptosporidiosis, Galway, Ireland, 2007*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value and assumption</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Private sector</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. households</td>
<td>45,160</td>
<td>(17) HSE, 2007; estimated bottled water retail price</td>
</tr>
<tr>
<td>Cost of extra bottled water</td>
<td>48% reported buying bottled water; 80% increased bottled water use from 3.2 L to 16 L/wk; 20% increased use from 3.2 L to 20 L/wk; €0.50/L</td>
<td></td>
</tr>
<tr>
<td>Cost of boiling water</td>
<td>Use of boiled water/household: drinking &gt;12%, 2.1L/adult/d, 1 L/child/d; cooking &gt;30%, 2 L/household/d; dishwashing &gt;43%, 10 L/household/d; hygiene &gt;14% 250 mL/person/d; €0.01/L</td>
<td>(15)</td>
</tr>
<tr>
<td><strong>Sick persons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. reported</td>
<td>242</td>
<td>HSE, 2007</td>
</tr>
<tr>
<td>No. not reported</td>
<td>498, 71% not reported</td>
<td>HSE, 2007</td>
</tr>
<tr>
<td>GP visits</td>
<td>€50/visit, 1 GP visit/confirmed case; all reported patients consulted GP; GMS cost €50.46 (assumed to be €50)</td>
<td>HSE primary care reimbursement service, 2008</td>
</tr>
<tr>
<td>Self-medications</td>
<td>€9.26 for antidiarrheal medication; €6.99 for ORS (1 pack); 30% self-medication for reported; 17.6% self-medication for not reported</td>
<td>Pharmacy prices; (7)</td>
</tr>
<tr>
<td>Loss of income</td>
<td>€122.85/d; 5 d of work missed (all nonhospitalized reported patients and 17.4% of not reported patients); 10 d of work missed for hospitalized patients</td>
<td>Central Statistics Office, 2007; HSE, 2007</td>
</tr>
<tr>
<td>Loss of income for caregivers</td>
<td>€122.85/day; children &lt;15 and persons &gt;65 years of age (n = 195); 1 caregiver took 5 d off from work; average 10 d off from work for hospitalized patients</td>
<td>Central Statistics Office, 2007; (4); HSE, 2007</td>
</tr>
<tr>
<td><strong>Cost of missing college/school</strong></td>
<td>Reported: €69.20/student/d; 5 d missed (10 d for hospitalized patients); not reported: 19% took time off from school/college; average 5 d missed, €69.20/student/d</td>
<td>Central Statistics Office, 2007; (16); HSE, 2007</td>
</tr>
<tr>
<td><strong>Cost to businesses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Productivity loss</td>
<td>€134/person/d; average 5 d; 10 d for hospitalized persons and caregivers</td>
<td>Health and Safety Times, 2011</td>
</tr>
<tr>
<td><strong>Hotels, B and Bs, hostels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra water</td>
<td>4.2 L/room/d; 2.1 L/person/day</td>
<td>(18)</td>
</tr>
<tr>
<td>Cancellations</td>
<td>13% cancelation rate; 57% occupancy rate</td>
<td>IPSOS Mori survey</td>
</tr>
<tr>
<td>Other assumptions</td>
<td>No. businesses: 70 hotels, 134 B and B, and 18 hostels; no. rooms: 79/hotel, 5/B and B, and 134/hostel; price: €66 hotels, €65 B and B, and €17 hostels</td>
<td></td>
</tr>
<tr>
<td><strong>Care industry†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottled water</td>
<td>2.1 L/person/d</td>
<td>(18)</td>
</tr>
<tr>
<td>Boiled water</td>
<td>1.5 L/person/d</td>
<td></td>
</tr>
<tr>
<td>Other factors</td>
<td>18 nursing homes in boil water–alert area; average no. residents 41, total no. residents for analysis 742; 168 nurseries/day care centers and 19 child minders; assume 3 children/child minder; average 29 children/nursery or day care center; no. children in nurseries or day care centers 4,929</td>
<td></td>
</tr>
<tr>
<td><strong>Public sector costs, €</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galway City Council</td>
<td>3,388,840.33</td>
<td>Galway City Council</td>
</tr>
<tr>
<td>Galway County Council</td>
<td>2,472,837</td>
<td>Galway County Council</td>
</tr>
<tr>
<td>EPA</td>
<td>20,000</td>
<td>EPA</td>
</tr>
<tr>
<td>HSE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency department cost</td>
<td>100; 1.3% of reported patients visited ED</td>
<td>HSE, (14)</td>
</tr>
<tr>
<td>5,810 extra species detection tests</td>
<td>46.06</td>
<td>University Hospital Galway (no. laboratory tests); commercial service provider (cost)</td>
</tr>
<tr>
<td>3,000 extra fecal culture tests</td>
<td>59.58</td>
<td>University Hospital Galway (no. laboratory tests); commercial service provider (cost)</td>
</tr>
<tr>
<td><strong>Hospital admissions</strong></td>
<td>753/person/d; 35% admitted to hospital for 10 d</td>
<td>HSE, 2007</td>
</tr>
</tbody>
</table>

**Response team (representatives from all public sector categories)**

Opportunity cost of labor | 356/consultant/meeting; 16 persons; 28 meetings | IPS |

*B and Bs, bed and breakfast; ED, emergency department; EPA, Environmental Protection Agency of Ireland; GMS, general medical scheme; GP, general practitioner; HSE, Health Service Executive; IPS, Institute of Public Administration; ORS, oral rehydration solution.
†Nursing homes, nurseries, and day care centers.
at an electoral district level and includes several datasets (e.g., the Living in Ireland Survey, Small Population Statistics, and the Geo-Directory) (16).

In the context of this analysis, we used SMILE data for 2006. On the basis of these data, there were 45,160 households in the area containing 120,432 persons, which included 5,034 children <5 years of age, 13,471 children >5–<18 years of age, 87,970 adults <65 years of age, and 13 persons ≥65 years of age.

Not Reported Case-Patients
Many studies have reported variation in the number of not reported cases of gastroenteritis in outbreak settings (7,14–16). Fitzgerald et al. reported that 71% of persons with gastroenteritis whose health was affected were not reported as case-patients (14). Corso et al. estimated that during the outbreak of cryptosporidiosis in Milwaukee in 1993, a total of 25% of the population in the area were affected, but 88% of them were not reported (7). If the estimate of 25% of the population is applied in this study, the estimated number of cases not reported would have been 25,291.

For our economic assessment, we adopted a more conservative approach and assumed that 71% of persons with signs or symptoms were not reported as case-patients. In addition, we assumed that cases not reported would be in less vulnerable persons 5–64 years of age (=101,441 persons or 84% of total population in the area estimated by using SMILE data). Therefore, on the basis of the number of reported cases, we estimated that 498 persons who were ill did not seek healthcare or were not reported. The costs we estimated are based on the most conservative figure, but we acknowledge that there is substantial uncertainty regarding the number of persons infected, and the actual number infected may be >498 persons.

We assumed that all confirmed case-patients visited a general practitioner at least once (estimated cost/visit €50). The same cost was assigned to all private patients, public patients, or general medical services patients because general medical services general practitioners claim for out-of-house services is €50.64 (HSE primary care reimbursement service data for 2008). We assumed that 30% of reported case-patients and 17.6% of not reported case-patients self-medicated (7) with an anti-diarrheal agent and an oral rehydration solution. We also assumed that each self-medicated case-patient purchased 1 packet of anti-diarrheal medication and 1 packet of oral rehydration solution (Table 1).

Shortly after the outbreak, the HSE Western Area (Limerick, Ireland) commissioned Ipsos MORI (London, UK), a private marketing research company, to conduct a postoutbreak survey to determine the effect of the outbreak on residents of the area affected by the boil water notice and for persons visiting the area for work or recreational activities (commuters and tourists). Results of this survey were available, and we used these results in our economic assessment. The survey found that all nonhospitalized reported cases-patients and 17.4% of not reported case-patients missed on average 5 days of work, and those who were hospitalized (35% of reported case-patients) were absent from work for an average of 10 days. Because of uncertainty about employment status and sector of persons under consideration, we assumed that all persons 22–65 years of age were employed, received an average industrial wage, and were not paid for days of work missed because of illness (HSE, 2007, unpub. data).

We assumed that dependents (189 symptomatic children <15 years of age and 6 elderly persons ≥65 years of age) would require a full-time caregiver for the duration of their illness (5 days for nonhospitalized case-patients and 10 days for hospitalized case-patients). Loss of income (for reported and not reported case-patients) was estimated as the average industrial wage rate in Ireland in 2007 (€122.85/d for 2007; Central Statistics Office, Cork, Ireland). Moreover, persons <21 years of age (reported and not reported case-patients) were assumed to either attend college/school or were unemployed. Consistent with previous research by Safefood (16), we assigned the opportunity cost to the time they were ill at the minimum wage rate in 2007 (>€69.20/d for 2007; Central Statistics Office).

On the basis of the Ipsos MORI survey results, we assumed that 48% of households bought extra bottled water, 80% increased bottled water consumption from 3.2 L/wk to 16.1 L/wk, and the remaining 20% increased consumption from 3.2 L/wk to 20 L/wk, at a cost of €0.50/L of bottled water purchased. On the basis of the study of Ailes et al. (15), we assumed that 12% of households boiled water for drinking, 30% for cooking, 43% for dishwashing, and 14% for hygiene use, at a cost of €0.01/L. This cost was based on National Electricity Supply Board (Dublin, Ireland) tariffs reported in 2007 and energy required. Further assumptions about the number of liters boiled for each purpose are provided (Table 1).

Public Sector Costs
We made assumptions related to costs incurred by the public sector. These assumptions include cost of emergency department attendance, which is assumed to apply to 1.3% of reported case-patients (14) (€100/visit, which is usually charged to private patients in Ireland (HSE data); cost of a hospital stay (€753/d reported by the HSE in 2015); costs incurred by local authorities (Galway City Council €3,388,840.33 and Galway County Council €2,472,837); cost of the response team (28 meetings attended by 16 senior representatives of HSE West, Galway County Council, and Galway City Council representing an estimated opportunity cost of €356/person/meeting); and costs incurred by...
the Environmental Protection Agency (Dublin, Ireland), which were estimated to be €20,000.

**Private Sector Costs**

Costs to the private business sector in the area proved to be more challenging to estimate because of lack of available data. It proved particularly difficult to obtain reliable data on costs incurred by restaurants. Therefore, we excluded these costs from calculations. The remaining business costs (service sector [lodging businesses] and care sector [nurseries, child care, nursing homes]), sources, and assumptions are provided (Table 1).

**Results**

We estimated that the overall cost of the waterborne outbreak of cryptosporidiosis in Galway in 2007 was €19 million or €120,000/day of the outbreak (Table 2). The US dollar equivalent based on today’s exchange rates would be $22.44 million (≈$142,000/day of the outbreak). The estimated cost to households in the affected area was ≈€3.9 million. This estimate translates into an average cost of alternative water of ≈€87/household in the boil water notice zone (or €0.55/household/day of the outbreak). The overall loss of income to households with symptomatic persons was estimated to be €287,957. This cost includes the loss of income of ill persons and their caregivers. The cost for not reported case-patients was estimated to be €74,002. If the average household income in the boil water zone was estimated to be €27,251/year (as estimated by using SMILE data) or €11,796 in 158 days, the loss translates into 0.8% of household income in the affected area during the outbreak.

We estimated that cost for lodging and care businesses was €8 million (€50,000 lost by local businesses/day of the outbreak). We also estimated that businesses in the lodging sector lost €5.4 million because of cancellations and an additional €1.7 million required for provision of alternative water to customers. Care businesses provided alternative water to persons in their care at an estimated cost of €525,929.

<table>
<thead>
<tr>
<th>Table 2. Overall estimated cost of waterborne outbreak of cryptosporidiosis, Galway, Ireland, 2007*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category</strong></td>
</tr>
<tr>
<td><strong>Private sector costs</strong></td>
</tr>
<tr>
<td>Household costs</td>
</tr>
<tr>
<td>Bottled water</td>
</tr>
<tr>
<td>Boiled water</td>
</tr>
<tr>
<td>Sick (reported and not reported) costs</td>
</tr>
<tr>
<td>Sick reported</td>
</tr>
<tr>
<td>Sick reported wage loss</td>
</tr>
<tr>
<td>School days lost</td>
</tr>
<tr>
<td>General practitioner</td>
</tr>
<tr>
<td>Self-medication (reported)</td>
</tr>
<tr>
<td>Caregiver income loss (reported)</td>
</tr>
<tr>
<td>Sick not reported</td>
</tr>
<tr>
<td>Sick not reported wage loss</td>
</tr>
<tr>
<td>School days lost (not reported)</td>
</tr>
<tr>
<td>Self-medication (not reported)</td>
</tr>
<tr>
<td>Caregiver income loss (not reported)</td>
</tr>
<tr>
<td><strong>Business costs</strong></td>
</tr>
<tr>
<td>Hotel cancelations</td>
</tr>
<tr>
<td>Hotel bottled water bill</td>
</tr>
<tr>
<td>Nurseries, day care centers, and nursing homes water bills</td>
</tr>
<tr>
<td>Caregiver productivity loss</td>
</tr>
<tr>
<td>Sick reported</td>
</tr>
<tr>
<td>Sick not reported</td>
</tr>
<tr>
<td>Sick productivity loss</td>
</tr>
<tr>
<td>Reported</td>
</tr>
<tr>
<td>Not reported</td>
</tr>
<tr>
<td><strong>Public sector costs</strong></td>
</tr>
<tr>
<td>Local authorities</td>
</tr>
<tr>
<td>Galway City Council</td>
</tr>
<tr>
<td>Galway County Council</td>
</tr>
<tr>
<td>EPA</td>
</tr>
<tr>
<td>HSE</td>
</tr>
<tr>
<td>Accident and emergency</td>
</tr>
<tr>
<td>Hospital</td>
</tr>
<tr>
<td>Extra laboratory tests</td>
</tr>
<tr>
<td>Response team†</td>
</tr>
<tr>
<td><strong>Total cost of outbreak</strong></td>
</tr>
</tbody>
</table>

*EPA, Environmental Protection Agency of Ireland; HSE, Health Service Executive.†Local authorities, EPA, and HSE.
Discussion
The waterborne outbreak of cryptosporidiosis that occurred in Galway in 2007 resulted in 242 reported cases of illness and a conservative estimate of 498 additional cases that were not reported. This outbreak also generated a considerable cost to residents, visitors, public bodies, and local businesses. This study highlights the economic need for a safe drinking water supply by reporting public expenditure on mitigating results of the outbreak and private costs to households and businesses in the area. The outbreak was believed to have occurred because the lake that serves as the source of drinking water for the city became contaminated with *C. hominis*, and the treatment process in place was not sufficient to eliminate or inactivate the parasite before water was distributed in the municipal supply.

Our results indicated that there are economic benefits of investing in safe drinking water supplies and water treatment enhancement (e.g., treatment with UV light, which effectively inactivates *Cryptosporidium* oocysts) (19,20). Hutton et al. reported a return of $5–$46 per $1 investment in water and sanitation improvements; all water improvement interventions examined in their study were cost-beneficial (12).

We recognize a major limitation in our approach to assessing relative costs. There is uncertainty associated with number of reported and not reported cases. The outbreak was the largest reported in Ireland, but other studies support the conclusion that when public water supply is contaminated with *C. hominis*, large proportions of populations are affected (7,21). There is also no basis on which to estimate the frequency with which a source water contamination event likely to result in a comparable outbreak occurs. If such a contamination event occurs frequently (e.g., annually), the cost of implementation greatly outweighs the associated costs of infection. If such an event occurs every 100 years, then the situation might be reversed. In the context of a municipal supply based on a large surface water body in which source protection is challenging, we believe contamination is likely to occur relatively frequently. Methods to define the annual probability of a major contamination event for a particular water supply more precisely would be of value.

Costs assessed for this evaluation related to the period of an outbreak. However, there is reason to believe that some economic impacts continued for years afterward related to the undermining of public trust in the water supply and affected Galway area and local businesses because of reduced numbers of visitors. As many as 13% of respondents to the Ipsos MORI survey indicated that they were less likely to return to the Galway area because of the outbreak. Thus, the economic effect might be greater.

Limited data is one of the obstacles that resulted in the number of assumptions made in this study. Thus, our results should be interpreted carefully, and we advise careful examination of assumptions before drawing conclusions. The lack of data related to businesses in the area and the effect of the outbreak on business operations prevented us from accounting for these effects in our calculations. Moreover, there is an uncertainty about the number of persons who were ill as a result of the outbreak, but did not seek help.

We estimated that the overall cost of this outbreak was €19 million (€120,000/day of the outbreak). These findings strongly support the value of a sustainable economic model to ensure that water infrastructure upgrades anticipate and prevent outbreaks. This study identified that availability of appropriate data are a limiting factor in completion of such economic assessments and provided valuable evidence that investment in safe drinking water supplies and water treatment enhancement benefits public health and the wider economy.

Acknowledgments
We thank all relevant representatives of the Galway County Council, Galway City Council, Health Service Executive, and the Environmental Protection Agency of Ireland for providing data. This study was supported by the Environmental Protection Agency of Ireland as part of the Research Programme 2014–2020.

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http://www.hpsc.ie/A-Z/Gastroenteric/Cryptosporidiosis/Publications/EpidemiologyofCryptosporidiosisinIrelandAnnualReports/


Address for correspondence: Dearbháile Morris, Discipline of Bacteriology, National University of Ireland Galway, Galway, Ireland; email: dearbhaile.morris@nuigalway.ie

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We investigated whether outpatient antimicrobial drug prescribing is associated with Neisseria gonorrhoeae antimicrobial drug susceptibility in the United States. Using susceptibility data from the Gonococcal Isolate Surveillance Project during 2005–2013 and QuintilesIMS data on outpatient cephalosporin, macrolide, and fluoroquinolone prescribing, we constructed multivariable linear mixed models for each antimicrobial agent with 1-year lagged annual prescribing per 1,000 persons as the exposure and geometric mean MIC as the outcome of interest. Multivariable models did not demonstrate associations between antimicrobial drug prescribing and N. gonorrhoeae susceptibility for any of the studied antimicrobial drugs during 2005–2013. Elucidation of epidemiologic factors contributing to resistance, including further investigation of the potential role of antimicrobial drug use, is needed.

Neisseria gonorrhoeae, the causative pathogen of gonorrhea, has been designated an urgent antimicrobial drug resistance threat by the Centers for Disease Control and Prevention (CDC) (1). Since the introduction of antimicrobial drugs in the first half of the 20th century, N. gonorrhoeae has successively developed resistance to each antimicrobial agent recommended for gonorrhea treatment (2). In the United States, the prevalence of resistance in N. gonorrhoeae often varies by sex of partner and by geographic region (3,4). Prevalence is often greater in isolates from gay, bisexual, and other men who have sex with men (MSM) than from men who have sex only with women (MSW), and prevalence is often highest in the West and lowest in the South (4). Resistant strains, in particular penicillinase-producing N. gonorrhoeae, fluoroquinolone-resistant N. gonorrhoeae (PPNG), and gonococcal strains with reduced cephalosporin susceptibility, seemed to emerge initially in the West (Hawaii and the West Coast) before spreading eastward across the country (5–9). These geographic patterns seem to support the idea that importation of resistant strains from other regions of the world, such as eastern Asia, is a primary factor of the emergence of resistant gonococci in the United States (5–9). Whereas antimicrobial drug prescribing patterns have been clearly associated with the emergence of resistance in other bacterial pathogens, the degree to which domestic antimicrobial use and subsequent selection pressure contributes to the emergence of gonococcal antimicrobial resistance in the United States is unclear (10–13). Using an ecologic approach, we sought to investigate the potential geographic and temporal association between antimicrobial drug susceptibility among US N. gonorrhoeae isolates and domestic outpatient antimicrobial drug prescribing rates in the United States during 2005–2013.

Methods

Data Sources
We used data from 3 sources: N. gonorrhoeae antimicrobial drug susceptibility data from the Gonococcal Isolate Surveillance Project (GISP), antimicrobial drug consumption data from IMS Health, and US Census data for population denominators. GISP is a CDC-supported sentinel surveillance system that has monitored gonococcal antimicrobial susceptibility in the United States since 1987 (4). GISP includes selected publicly funded sexually transmitted infection (STI) clinics in 25–30 cities and 4–5 regional laboratories each year. Each month, up to 25 N. gonorrhoeae urethral samples are collected consecutively from men with gonococcal urethritis attending participating STI clinics; these samples are submitted to regional laboratories for antimicrobial drug susceptibility testing.
Health statistical and analytic teams (4). Sampling men with gonococcal urethritis is an efficient means to detect gonococcal infections: urethral infections in men are likely to be symptomatic (prompting patients to seek healthcare), and gonococcal urethritis can be rapidly diagnosed by Gram stain (4). In addition, sampling of men allows for monitoring of susceptibility among infections in MSM and heterosexual sexual networks (4).

We abstracted deidentified epidemiologic data from medical records. When analyzing data, we limited the number of isolates to ≤300/city/year to minimize over-representation of individual cities; we chose isolates at random for removal from the analytic dataset when >300 isolates were submitted from a site in a year. Gonococcal isolates collected at each clinic are subcultured at the clinic’s local public health laboratory on supplemented chocolate medium and frozen in trypticase soy broth containing 20% glycerol. Isolates are shipped monthly to 1 of the regional laboratories for β-lactamase production testing and agar dilution antimicrobial drug susceptibility testing. From 2005 through 2013, the testing panel consistently included azithromycin, ceftriaxone, ciprofloxacin, penicillin, spectinomycin, and tetracycline. Cefixime susceptibility testing was conducted from 2005 through 2006, temporarily halted in 2007 due to lack of availability of cefixime in the United States, and resumed in 2009. Cefpodoxime susceptibility testing was conducted from 2009 through 2012. Standardized bacterial suspensions are inoculated on Difco GC Medium Base supplemented with 1% IsoVitaleX Enrichment (Becton, Dickinson and Company Diagnostic Systems, Sparks, MD, USA).

For antimicrobial drug prescribing data, we extracted QuintilesIMS (Danbury, CT, USA) data on systemic oral antimicrobial drug prescriptions dispensed in the United States during 2005–2013. During the study period, QuintilesIMS captured >70% of all outpatient prescriptions in the United States, reconciled them to wholesale deliveries, and projected to 100% coverage of all prescription activity using a patented projection method based on a comprehensive sample of patient-deidentified prescription transactions (collected from pharmacies that report their entire pharmacy business to the company each week) (14). These data represent outpatient prescriptions across all payers and include county of prescriber (used in this analysis); data are from community pharmacies and federal government and nongovernmental mail service pharmacies. The IMS projection method standardizes these data into estimated prescription counts and uses geospatial methods to align the estimated prescriptions for the non-sample pharmacies to prescribers with observed prescribing behaviors for the same product in nearby sample pharmacies. The method is routinely validated at various levels of granularity by IMS Health statistical and analytic teams (15). We obtained population data on age, sex, and county from US Census bridged-race population estimates published by the CDC.

Data Analysis
We restricted the analysis to GISP sites that participated continuously from 2005 through 2013. We focused on azithromycin, cefixime, ceftriaxone, and ciprofloxacin because these agents are currently recommended for gonorrhea treatment (azithromycin and ceftriaxone), are in the same antimicrobial class as a recommended antimicrobial drug (cefixime), or are widely used for outpatient treatment of non-STD infections or are of renewed interest because of potential novel diagnostics for detection of resistance determinants (ciprofloxacin) (16,17). We calculated geometric mean MICs and the percentage of isolates with resistance or reduced susceptibility for each antimicrobial drug by GISP site and year. Per Clinical and Laboratory Standards Institute criteria, we categorized ciprofloxacin resistance as MIC ≥1 µg/mL (18). In the absence of established resistance breakpoints for other antimicrobial drugs of interest, we categorized reduced cefixime susceptibility as ≥0.25 µg/mL, reduced ceftriaxone susceptibility as ≥0.125 µg/mL, and reduced azithromycin susceptibility as ≥2.0 µg/mL.

Using IMS data for each county corresponding to each of the 23 included GISP sites, we summarized antimicrobial drug prescription counts by specific antimicrobial drug (azithromycin, cefixime, ceftriaxone, and ciprofloxacin) and aggregated antimicrobial category (macrolides, cephalosporins, and fluoroquinolones). To reflect the sex and age distribution of men with gonorrhea sampled in GISP, we limited the antimicrobial drug prescription counts to prescriptions to men 10–59 years of age. We calculated prescription rates for the aggregated antimicrobial drug categories (defined as the number of prescriptions per 1,000 men 10–59 years of age) by county and year using US Census data for denominators. Using the prescribing rate for each antimicrobial category by county and year, we calculated the median prescribing rate for each county across years. The degree of overlap between the county and catchment area of the corresponding STD clinic is expected to be somewhat imprecise and vary by geographic site: some clinic catchment areas may include a small section of a large county, whereas other catchment areas may extend beyond the borders of the corresponding county.

We performed linear regression analyses in which the dependent variable was antimicrobial susceptibility (geometric mean MIC) at each GISP site and the independent variable of interest was the prior year prescription rate (i.e., the prescribing rate during the year before the year corresponding to the antimicrobial drug susceptibility results) at each county and year. We considered 2 representations of the prescribing rate variable: the original lag variable and
the centered lag variable (19,20). We used the noncentered lag variable to calculate the results. We performed separate longitudinal models for each drug by geometric mean MIC and the rate of prescribing of the corresponding antimicrobial class (azithromycin susceptibility and macrolide prescribing; cefixime and ceftriaxone susceptibility and cephalosporin prescribing; and ciprofloxacin susceptibility and fluoroquinolone prescribing) (19,20). We performed exploratory analyses to determine if there was a linear relationship between the susceptibility outcome and time. The linear assumption was satisfied, so we did not perform any transformation. To examine the association between susceptibility outcomes and prescribing rate, we then constructed multivariable linear mixed models for repeated measures with intercept and time as random effects. The models included 3 potential confounders based on a priori decisions (as these variables have been found to be associated with antimicrobial drug prescribing and/or gonococcal susceptibility): geographic region; sex of sex partner (defined as the percentage of MSM at each GISP site per year, based on GISP data); and race (defined as the percentage of men with urethral gonorrhea who were black or African American at each GISP site per year, based on GISP data) (3,4,15,21). We conducted all analyses in SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA), using Proc Mixed for restricted maximum-likelihood estimation for small size samples. We calculated CIs at α = 0.05 to determine statistically significant associations.

Results

Antimicrobial Susceptibility

Of 33 GISP sites that participated at some point during 2005 through 2013, 23 participated continuously and were included. From these sites, 44,957 isolates were collected and submitted to GISP (range per site in a given year 49–393) and, after removal of observations if >300 isolates were submitted by a site in a year, we included data from 43,852 (97.5%) isolates in the analysis. The percentage of gonococcal isolates with reduced ceftriaxone susceptibility increased from 0.1% in 2005 to 1.6% in 2011 and decreased to 0.5% by 2013 (Table 1). Overall, geometric mean cefixime MICs increased slightly from 2006 to 2009 and then remained stable. The percentage of isolates with reduced ceftriaxone susceptibility increased slightly from 2005 to 2011 and then decreased; the geometric mean increased slightly from 2006 to 2007 and then remained stable. The percentage of isolates with reduced

<table>
<thead>
<tr>
<th>Results†</th>
<th>Cefixime</th>
<th>Ceftriaxone</th>
<th>Azithromycin</th>
<th>Ciprofloxacin</th>
</tr>
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<tbody>
<tr>
<td>2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>0.1</td>
<td>0.1</td>
<td>0.6</td>
<td>10.1</td>
</tr>
<tr>
<td>2006</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>15.4</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>NT</td>
<td>0.010</td>
<td>0.024</td>
<td>0.027</td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>NT</td>
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<td>0.242</td>
<td>0.024</td>
</tr>
<tr>
<td>2009</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>0.9</td>
<td>0.3</td>
<td>0.3</td>
<td>14.7</td>
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<tr>
<td>2010</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>1.6</td>
<td>0.4</td>
<td>0.6</td>
<td>14.2</td>
</tr>
<tr>
<td>2011</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>1.6</td>
<td>0.4</td>
<td>0.3</td>
<td>14.4</td>
</tr>
<tr>
<td>2012</td>
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<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>1.0</td>
<td>0.3</td>
<td>0.3</td>
<td>16.1</td>
</tr>
<tr>
<td>2013</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Reduced susceptibility, %</td>
<td>0.5</td>
<td>0.1</td>
<td>0.6</td>
<td>17.1</td>
</tr>
</tbody>
</table>

*Results are for 23 sites that participated in GISP for the entire study period. Cefixime MIC testing range was 0.001–0.5 µg/mL during 2005–2006 and 0.015–0.5 µg/mL during 2009–2013; ceftriaxone MIC testing range was 0.001–2.0 µg/mL during 2005–2006 and 0.008–2.0 µg/mL during 2007–2013; azithromycin MIC testing range was 0.008–16 µg/mL during 2005–2006 and 0.03–16 µg/mL during 2007–2013; ciprofloxacin MIC testing range was 0.001–16 µg/mL during 2005–2006 and 0.008–16 µg/mL during 2007–2013. NT, not tested.
†Reduced susceptibility indicates isolate’s resistance or reduced susceptibility to the indicated drug. Reduced cefixime susceptibility was defined as MIC ≥0.25 µg/mL, reduced ceftriaxone susceptibility MIC ≥0.125 µg/mL, reduced azithromycin susceptibility MIC ≥2 µg/mL, and ciprofloxacin resistance defined as MIC ≥1 µg/mL.

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azithromycin susceptibility varied between 0.2% and 0.6%; the geometric mean appeared to peak in 2008 and increased again during 2011–2013. The percentage of isolates with ciprofloxacin resistance increased during 2005–2007 and increased again during 2009–2013; the geometric mean MIC increased during 2005–2013. For each antimicrobial drug, geometric mean MICs varied by site and year (online Technical Appendix Tables 1–4, 8, https://wwwnc.cdc.gov/EID/article/23/10/17-0488-Techapp1.pdf). Sites with the highest median cefixime and ciprofloxacin geometric mean MICs were in the West; those with the lowest were in the South and Midwest (online Technical Appendix Tables 1, 4). Sites with highest median azithromycin geometric means were in the Midwest and West, and those with the lowest were in the South (online Technical Appendix Table 3). We found little variation in median ceftriaxone geometric mean MICs across sites (online Technical Appendix Table 2).

Antimicrobial Drug Use

Counties with the highest cephalosporin, macrolide, and fluoroquinolone prescribing rates, such as Jefferson County, Alabama, and Oklahoma County, Oklahoma, were located in the South (online Technical Appendix Tables 5–7). Counties with the lowest prescribing rates, such as Multnomah County, Oregon, and San Diego and San Francisco, California, were located in the West. Cephalosporin prescribing rates increased in many counties but decreased in sites such as those in Honolulu, Hawaii, and Los Angeles, California (online Technical Appendix Table 5). During 2005–2013, macrolide prescribing increased in all counties (online Technical Appendix Table 6). Fluoroquinolone prescribing increased in most counties, with the largest absolute increases occurring in counties in the South (online Technical Appendix Table 7). The multivariable models did not demonstrate associations between N. gonorrhoeae susceptibility and antimicrobial drug prescribing for any of the studied antimicrobial drugs (Table 2).

Discussion

Using an ecologic approach, we did not find an association between population-level outpatient prescribing rates of clinically relevant antimicrobial drugs and N. gonorrhoeae antimicrobial drug susceptibility among urethral isolates from men in the United States. Prescribing rates were lowest in sites both where ciprofloxacin resistance and reduced cefixime susceptibility initially emerged and where the prevalence of resistance or reduced susceptibility has been highest, such as Honolulu, Hawaii, and West Coast sites (4, 22, 23). Conversely, prescribing rates are highest in the southern United States, the region where the prevalence of gonococcal resistance has tended to be the lowest (4).

Bacterial antimicrobial drug resistance is clearly broadly linked to antimicrobial drug use, but the association is probably complex, interacting through several possible mechanisms and varying by bacteria, mode of transmission, antimicrobial drug, prevalence of resistance, and geographic location (24). For some bacterial pathogens, such as Streptococcus pneumoniae and Escherichia coli, associations between population-level antimicrobial drug prescribing and resistance in the United States and Europe have been described (10–13). We did not find such an association for N. gonorrhoeae.

There are at least 2 possible explanations for the apparent lack of county-level association between domestic antimicrobial drug prescribing and N. gonorrhoeae susceptibility. First, factors other than population-level prescribing rates, such as importation of resistant strains from other countries, might contribute to emergence of gonococcal resistance in the United States. Previously

<p>| Table 2. Adjusted linear regression coefficients for change in antimicrobial geometric mean MIC associated with 10% increase in corresponding antimicrobial prescribing rate for 23 sites, Gonococcal Isolate Surveillance Project, United States, 2005–2013* |</p>
<table>
<thead>
<tr>
<th>Effect</th>
<th>β coefficient</th>
<th>SE</th>
<th>d.f.</th>
<th>95% CI of β coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>-0.0087</td>
<td>0.003</td>
<td>155</td>
<td>-0.0146, -0.0029</td>
</tr>
<tr>
<td>Macrolide prescribing†</td>
<td>-0.0155</td>
<td>0.002</td>
<td>155</td>
<td>-0.0502, 0.0191</td>
</tr>
<tr>
<td>Cefixime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>0.0011</td>
<td>0.0001</td>
<td>109</td>
<td>0.0008, 0.0014</td>
</tr>
<tr>
<td>Ceftriaxine</td>
<td>0.0016</td>
<td>0.0013</td>
<td>109</td>
<td>0.00010, 0.00041</td>
</tr>
<tr>
<td>Cephalosporin-prescribing‡</td>
<td>0.0004</td>
<td>0.001</td>
<td>155</td>
<td>0.00002, 0.0005</td>
</tr>
<tr>
<td>Macrolide-prescribing‡</td>
<td>0.0002</td>
<td>0.0006</td>
<td>155</td>
<td>0.00009, 0.0013</td>
</tr>
<tr>
<td>Ciprofloxacin-prescribing§</td>
<td>0.0004</td>
<td>0.0021</td>
<td>155</td>
<td>-0.0038, 0.0046</td>
</tr>
</tbody>
</table>

*All models were adjusted for percent of MSM at each site (using GISP data), race (percentage of men coded as black versus non-black in GISP data), and geographic region. Time was based on 1-year intervals. Estimate is statistically significant if the 95% CI of β coefficient does not cross 0.

†Per 10% increase in macrolide prescribing during the previous year; includes azithromycin, clarithromycin, and erythromycin.

‡Per 10% increase in ceftriaxone prescribing during the previous year; includes cefaclor, cefadroxil, cefdinir, cefditoren pivoxil, cefixime, cefpodoxime proxetil, cefprozil, cefditoren pivoxil, cefuroxime axetil, cephalexin, cephradine, and loracarbef.

§Per 10% increase in ciprofloxacin prescribing during the previous year; includes ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, norfloxacin, ofloxacin, and trovafloxacin.
Antimicrobial Drug Prescription and N. gonorrhoeae

published epidemiologic data have strongly suggested that resistant strains, such as PPNG and fluoroquinolone-resistant N. gonorrhoeae, and strains with reduced cefixime susceptibility (also investigated with genomic data), emerged initially in other parts of the world, particularly eastern Asia, and subsequently spread to the United States through Hawaii and the West Coast (5–9).

Second, it is possible that our data sources, methodology, or both lacked sufficient sensitivity to detect an association. The time period analyzed might have played a role: antimicrobial drug prescribing could conceivably be of greater or lesser importance during different phases of the emergence or persistence of resistance. As an inherent limitation of an ecologic approach, it is also possible that the prescribing rates we used in our analysis do not necessarily reflect antimicrobial drug use patterns among persons at risk for gonorrhea. For example, men seeking care in publicly funded STI clinics may be underinsured and thus lack access to routine medical care and be less likely to receive antimicrobial drug prescriptions. On the other hand, men diagnosed with gonorrhea in STI clinics may have been exposed to repeated antimicrobial drug courses for repeated STIs. Antimicrobial drug use might differ between MSM and MSW, so the availability of population-level drug prescribing data that include the sex of sex partners would be helpful.

Future investigations of associations between antimicrobial drug use and gonococcal susceptibility may also be strengthened by the availability of the indication for treatment (not available in the QuintilesIMS data), allowing for analyses linking susceptibility to antimicrobial drug use specifically for gonorrhea treatment. Investigators have previously identified links between individual-level antimicrobial drug use and gonococcal resistance in the United States (25,26). Nearly 30 years ago, Zenilman et al. found that persons with gonorrhea in Dade County, Florida, who had medicated themselves with illicit antibacterial drugs were more likely to be infected with PPNG than penicillin-sensitive strains (odds ratio 3.6; 95% CI 1.9–6.8) (25). Of note, our dataset does not include illicit or nonprescribed antimicrobial drugs. Among persons with gonorrhea in California during 2000–2003, antimicrobial drug use in the 3 months before diagnosis with gonorrhea was independently associated with infection with a fluoroquinolone-resistant strain (26). Using multisite GISP data for 2005–2010, we previously found that recent antimicrobial drug use was independently associated with N. gonorrhoeae ciprofloxacin, penicillin, and tetracycline resistance among men with gonococcal urethritis (3). However, the magnitude of the association between resistance and antimicrobial drug use was dwarfed by the magnitude of the association between resistance and geographic region and sex of sex partner, and use of an antimicrobial drug was not associated with reduced susceptibility to azithromycin, cefixime, and ceftriaxone.

The antimicrobial drugs that clinicians choose to treat gonorrhea may influence the susceptibility of N. gonorrhoeae populations. In the early 1970s, an increase in the recommended dosage of penicillin in response to increased resistance was followed by a plateau in penicillin resistance; experts speculated that the updated and highly effective treatment schedule retarded the selection of resistant mutants (27). Recently, cephalosporin susceptibility in the United States appeared to improve following updates in CDC treatment guidelines that recommended routine dual therapy, a preference for injectable ceftriaxone over oral cefixime, and a higher ceftriaxone dose (28). In contrast, some gonorrhea treatment approaches might promote resistance. Treatment with azithromycin alone is not recommended because of concerns about the ease with which N. gonorrhoeae can develop macrolide drug resistance; previously published cases seem to illustrate selection of higher azithromycin MICs following gonorrhea treatment with azithromycin monotherapy (29–31). Spectinomycin resistance was observed to emerge rapidly among US service members stationed in South Korea after spectinomycin was adopted as the primary gonorrhea treatment by the US military (following the emergence of PPNG) (32).

Emergence and persistence of gonococcal-resistant phenotypes is probably influenced by a complex (and not yet fully understood) interplay of bacterial and host factors, such as the ease with which the gonococcal strain can acquire necessary mutation(s); the effect of the mutation(s) on bacterial fitness; the anatomic site of infection (which can influence symptomatology, likelihood of treatment success, and coexistence of N. gonorrhoeae with other bacteria with which DNA may be shared); host mobility (including international travel); host sexual behavior; the nature of the sexual network within which the resistant strain emerges; prevalence of resistance; provider screening practices; and antimicrobial drug exposure (32–37). Furthermore, the relative importance of each factor may differ by resistance phenotype. The framework posited by Lipsitch and Samore may prove useful for considering mechanisms by which antimicrobial drugs might contribute to N. gonorrhoeae resistance, such as emergence of resistance during treatment or clearance of a susceptible majority bacterial population and subsequent transmission of a resistant minority population (24,29). However, much work remains to be done to understand these complex relationships.

Our analysis has other limitations. Ecologic analyses are limited by the potential for unmeasured and uncontrolled confounding. Conclusions of this ecologic analysis are based on counties or geographic site, rather than individual patients. Prescribing data were derived from counties that in some instances do not fully overlap with the STD...
clinic catchment areas from which the susceptibility data were derived. Our analyses were limited to data from men. However, the inclusion of data from women is unlikely to have influenced the results: women may consume more antimicrobial drugs than men, and gonococcal isolates from women tend to be more susceptible to antimicrobial drugs than those from men (similar to isolates from MSW and substantially more susceptible than isolates from MSM) (38,39). An important caveat is that our findings are only applicable to the United States: they should not be extrapolated to other countries and regions. It is possible that rates of population-level antimicrobial drug prescribing or use in other countries may select for resistant gonococcal strains, which in turn may spread across international borders. Further investigation to understand region- or county-specific factors contributing to resistance is urgently needed.

The findings of our analysis suggest that population-wide domestic antimicrobial drug prescribing rates might not play a prominent role in the emergence of gonococcal resistance in the United States. Other means, such as importation from other countries, might play larger roles. Through this lens, enhanced surveillance for and public health capacity to respond to imported resistant strains are important strategies. However, it is possible that the choice of antimicrobial drugs that clinicians prescribe for gonorrhea therapy might influence the persistence or spread of resistant gonococcal strains that emerge in the United States. US-based healthcare providers should treat gonorrhea according to CDC STI treatment guidelines with dual therapy of 250 mg ceftriaxone as a single intramuscular dose plus 1 g azithromycin orally (16). The remarkable ability of *N. gonorrhoeae* to develop resistance to each antimicrobial drug used for treatment (2), combined with the declining number of new drugs (40), highlight the need to develop and apply interventions to slow the emergence and spread of gonococcal resistance.

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References


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Poliovirus Excretion in Children with Primary Immunodeficiency Disorders, India

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Prolonged excretion of poliovirus can occur in immunodeficient patients who receive oral polio vaccine, which may lead to propagation of highly divergent vaccine-derived polioviruses (VDPVs), posing a concern for global polio eradication. This study aimed to estimate the proportion of primary immunodeficient children with enterovirus infection and to identify the long-term polio/nonpolio enterovirus excreters in a tertiary care unit in Mumbai, India. During September 2014–April 2017, 151 patients received diagnoses of primary immunodeficiency (PID). We isolated 8 enteroviruses (3 polioviruses and 5 nonpolio enteroviruses) in cell culture of 105 fecal samples collected from 42 patients. Only 1 patient with severe combined immunodeficiency was identified as a long-term VDPV3 excreter (for 2 years after identification of infection). Our results show that the risk of enterovirus excretion among children in India with PID is low; however, systematic screening is necessary to identify long-term poliovirus excreters until the use of oral polio vaccine is stopped.

Oral polio vaccine (OPV) has been a key factor in global polio eradication and has proven highly effective because of its ease of administration and ability to transmit to secondary contacts (1–4). Primary immunodeficiencies (PIDs) are a heterogeneous group of inherited disorders resulting from developmental defects or dysfunction of the immune system components (5). Patients with PID can potentially be infected by immunizations if they receive live vaccines (6). OPV immunization has been associated with poliovirus infection in patients with primary antibody deficiencies and combined immunodeficiencies, which can lead to paralysis (7,8). In addition, some vaccinated patients with PID may shed vaccine-derived polioviruses (VDPVs) because of a prolonged period of intestinal replication. VDPVs show increased nucleotide divergence in the viral protein 1 (VP1) coding region in all variants of OPV serotypes (PV1, PV2, and PV3); this divergence is associated with increased neuropathogenicity (9). More than 100 immunodeficiency-related VDPV (iVDPV) infections have been reported in PID patients worldwide to date (10,11). As potential reservoirs for neuroviral VDPV strains, patients with PID represent a global risk to nonimmunized contacts and to the Global Polio Eradication Initiative (10,12).

Several studies have investigated iVDPVs in patients with different types of immunodeficiencies, such as combined immunodeficiency, antibody deficiency, and other immunodeficiencies (8,10,13,14). Recognizing the risk that iVDPV poses to global poliovirus eradication, the World Health Organization (WHO) maintains a registry of known iVDPV cases and promotes global surveillance of iVDPVs (15). However, it is difficult to estimate the number of iVDPV excreters worldwide in the absence of systematic screening of immunodeficient patients for enterovirus excretion, especially in the developing world (15).

To establish screening facilities for children with PID in India, we collaborated with the National Institute of Immunohaematology (NIIH, the referral laboratory for PID in India) and Bai Jerbai Wadia Children’s Hospital (a tertiary care unit) in Mumbai, India, which has established facilities for diagnosis of PIDs. The study aimed to identify the proportion of children with PID who have enterovirus infection and also to identify long-term polio/nonpolio enterovirus excreters among them.

Materials and Methods

Study Design
The study is being conducted in collaboration with the clinicians of the PID surveillance group at NIIH and Wadia Children’s Hospital. This article describes the data regarding children with PID who were enrolled in the study.
Poliovirus in Immunodeficient Children, India

during September 2014–April 2017. Blood samples were collected at Wadia Children’s Hospital from patients (≤18 years of age) suspected of having immunodeficiencies, as part of routine surveillance; diagnosis was confirmed by standard procedure at NIIH.

We obtained ethical clearance from the Ethics Committee of NIIH, Wadia Children’s Hospital, and Enterovirus Research Centre (ERC), Mumbai, and obtained informed consent from the patients’ parents. The investigations were guided by the clinical presentation, immunological abnormalities, and molecular diagnosis wherever feasible, according to the phenotypic classification of the International Union of Immunological Societies (affiliated with WHO) (16). Initial investigations involved a complete blood count with a differential count on the leukocytes and mean platelet volume on a Sysmex XS-800i 5-part automated hematological analyzer (Sysmex Co., Kobe, Japan); serum immunoglobulin estimation (IgG, IgA, IgM, IgE) by nephelometry (BNProspec, Siemens); nitroblue tetrazolium blood test by microscopy; and lymphocyte subset analysis by flow cytometry using BD Multitest 6-color TBNK reagent (BD Biosciences). We made specific PID diagnoses on the basis of clinical features and laboratory investigations (16,17).

We collected fecal samples from the children with PID who routinely visit the PID outpatient department of Wadia Children’s Hospital for checkup and intravenous immunoglobulin (IVIG) treatment. We followed up with these patients regularly for monthly fecal samples (1 sample in each month for 2 consecutive months); both Wadia Children’s Hospital and ERC staff reminded patients to return with their samples. ERC performed fecal sample processing (as described later), culture, isolation, and characterization of virus isolates. We followed up by taking monthly samples from the patients whose samples were found positive for enteroviruses until 2 consecutive samples became negative for enteroviruses. We followed up again with those patients for enterovirus detection after a 6-month interval.

Enterovirus Isolation
We performed enterovirus isolation from fecal extracts as described in the WHO laboratory manual (18). We used human rhabdomyosarcoma (RD) and transgenic mouse cell line expressing polio receptor (L20B) for enterovirus culture. We treated fecal extracts (10%, wt/vol) prepared in phosphate-buffered saline (Sigma cat. no. D8662; Sigma-Aldrich, St. Louis, MO, USA) with 10% (vol/vol) chloroform before inoculation in cell cultures. We infected cells in duplicate with 200 μL of stool extract, incubated at 36°C, and observed microscopically for cytopathogenic effect (CPE) daily for 5 days. We freeze-thawed cell cultures showing CPE and collected culture medium for virus identification. We scored samples as negative if 2 consecutive passages in the same cell line did not produce CPE.

Enterovirus Typing
We extracted viral RNA from culture medium of CPE-positive freeze-thawed cells using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. For nonpolio enteroviruses, we used primer pairs 222/224 and 88/89 for partial VP1 sequencing for identification of enterovirus type (19,20). For poliovirus isolate VP1 region amplification (≈900 nt), we performed reverse transcription PCR in a single tube using reverse primer Q8 and forward primer Y7, as described previously (21). We performed the sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. We resolved the sequences on an ABI 3130xl Genetic Analyzer (Applied Biosystems) and edited them using Sequencher version 4.10.1 software (Gene Codes, Ann Arbor, MI, USA). For nonpolio enteroviruses, we subjected the sequences to BLAST search (https://www.ncbi.nlm.nih.gov/BLAST) and defined the virus type using the criteria of ≥75% nucleotide and ≥85% amino acid similarity in the VP1 region (22). For poliovirus isolates, we compared sequences with the Sabin reference strain in the VP1 region.

Results
A total of 1,393 patients with suspected PID were screened at the PID Outpatient Department of Wadia Children’s Hospital during a period of 2 years and 7 months; of these, the NIIH PID screening group confirmed that 151 patients had PID. We were able to follow up with 42 patients with humoral, combined, and other PIDs for fecal sample collection to test for enterovirus excretion. There were 33 male and 9 female participants. We found a striking predominance of male participants in all types of PID except IgG subclass deficiency. The age of the study participants ranged from 4 months to 18 years of age (Table 1). The PID types showed a mixture of antibody deficiencies, combined T and B cell deficiencies, phagocytic defects, and other immunodeficiencies (Table 1).

Of 42 children with PID, 40 (95%) had received OPV 1–96 months before fecal specimen collection; the parents of the remaining 2 (5%) children were not able to recall whether their children had received OPV. All patients with antibody-mediated immunodeficiency were under replacement IVIG prophylaxis. We collected fecal samples before the monthly IVIG therapy was administered. Of the 42 patients enrolled...
for the study, 5 (11.9%) underwent bone marrow transplant and 10 (23.8%) died during the study period.

We received 105 fecal samples from 42 patients with confirmed PID; we tested these for polio and other enteroviruses. Among the fecal samples from these 42 patients, specimens of 8 patients (19%) tested positive for enteroviruses. Of these, we found 1 patient with severe combined immunodeficiency (SCID) who had excreted poliovirus for 2 years and then abruptly stopped excreting it. Fecal specimens of a 4-month-old patient with SCID and 1 patient with familial hemophagocytic lymphohistiocytosis (FLH) tested positive for poliovirus (Sabin type 1) (Table 2). A total of 4 patients with SCID were enrolled in this study; SCID was diagnosed in 2 of them at 2 months of age, and fecal specimens collected for detection of enterovirus excretion were found negative. Both the patients died before the second sample collection. Samples from 3 patients with antibody deficiencies and 2 patients with other immune deficiencies tested positive for nonpolio enterovirus (NPEV), but subsequent samples tested negative (Table 3). None of the samples from patients with common variable immune deficiency (CVID) tested positive for enterovirus excretion.

**Poliovirus Excretors**

A 6-year-old boy with a case of leaky SCID had been excreting poliovirus for 2 years. Genetic sequencing of virus isolates identified type 3 VDPV with up to 41 (4%) nt changes at 4 years of age, which subsequently showed up to 93 (10%) nt divergence in VP1 region (from the parental Sabin strain) at 6 years of age. After a prolonged excretion for 2 years, the child abruptly stopped excreting; subsequent collections were found negative for poliovirus. The child had received routine immunizations, as well as Pulse Polio doses on National Immunization Days, until he was 14 months of age; he was also immunized with IPV at 3.5 years of age. The first and second samples from a 4-month-old child with SCID tested positive for Sabin1 poliovirus (separate samples collected each month until patient becomes negative for enterovirus excretion); as of this writing, the third sample is due for collection. The fecal specimen of another patient with FLH tested positive for poliovirus in the second sample, but 2 subsequent samples were negative (Table 2). This child was being treated for FLH but had peripheral demyelinating neuropathy unrelated to this disease, and died 5 days after the fourth specimen collection.

One patient with X-linked agammaglobulinemia (XLA) was enrolled in our study; this patient had a previous record of VDPV2 excretion detected by acute flaccid paralysis (AFP) surveillance by the National Polio Surveillance Program (NPSP, WHO) being conducted at our center. The child had a paralytic attack 3 months after receiving the last dose of OPV, 2 years before enrollment in our study.

### Table 1. Baseline characteristics of confirmed PID cases in children recruited from Wadia Children’s Hospital, Mumbai, India, September 2014–April 2017

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>PID types</th>
<th>No. cases</th>
<th>M/F ratio</th>
<th>Age range, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hemophagocytic lymphohistiocytosis</td>
<td>11</td>
<td>10/1</td>
<td>4–96</td>
</tr>
<tr>
<td>2</td>
<td>X-linked agammaglobulinemia</td>
<td>7</td>
<td>7/0</td>
<td>27–216</td>
</tr>
<tr>
<td>3</td>
<td>Chronic granulomatous disease</td>
<td>4</td>
<td>3/1</td>
<td>7–80</td>
</tr>
<tr>
<td>4</td>
<td>Severe combined immunodeficiency</td>
<td>4</td>
<td>4/0</td>
<td>4–48</td>
</tr>
<tr>
<td>5</td>
<td>Common variable immunodeficiency</td>
<td>3</td>
<td>2/1</td>
<td>84–120</td>
</tr>
<tr>
<td>6</td>
<td>Chédiak–Higashi syndrome</td>
<td>3</td>
<td>1/2</td>
<td>26–86</td>
</tr>
<tr>
<td>7</td>
<td>Hypogammaglobulinemia</td>
<td>2</td>
<td>1/1</td>
<td>96–108</td>
</tr>
<tr>
<td>8</td>
<td>IgG subclass deficiency</td>
<td>2</td>
<td>0/2</td>
<td>84–114</td>
</tr>
<tr>
<td>9</td>
<td>Hyper-IgM syndrome</td>
<td>2</td>
<td>2/0</td>
<td>17–59</td>
</tr>
<tr>
<td>10</td>
<td>Autoimmune lymphoproliferative syndrome</td>
<td>1</td>
<td>0/1</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>B cell expansion with NF-xB and T cell anergy</td>
<td>1</td>
<td>1/0</td>
<td>18</td>
</tr>
<tr>
<td>12</td>
<td>Hyper-IgE syndrome</td>
<td>1</td>
<td>1/0</td>
<td>42</td>
</tr>
<tr>
<td>13</td>
<td>Interleukin 12 receptor β1 defect</td>
<td>1</td>
<td>1/0</td>
<td>156</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>42</strong></td>
<td><strong>33/9</strong></td>
<td><strong>4–216</strong></td>
</tr>
</tbody>
</table>

*PID, primary immunodeficiency

### Table 2. Demographic and clinical data for patients with PID whose fecal samples tested positive for polioviruses, Wadia Children’s Hospital, Mumbai, India, September 2014–April 2017

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Age, mo/sex</th>
<th>PID type</th>
<th>OPV†</th>
<th>Months last IVIG therapy</th>
<th>BMT</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48/M</td>
<td>SCID</td>
<td>Yes</td>
<td>ND</td>
<td>D1</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>48/M</td>
<td>FLH</td>
<td>No</td>
<td>ND</td>
<td>D1</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>4/M</td>
<td>SCID</td>
<td>No</td>
<td>ND</td>
<td>D1</td>
<td>ND</td>
</tr>
</tbody>
</table>

*BMT, bone marrow transplant; D, day of collection following first collection; FLH, familial lymphohistiocytosis; IVIG, intravenous immunoglobulin; NA, not available; ND, not done; neg, negative; OPV, oral polio vaccine; P1SL, polio1 Sabin-like; P3SL, polio 3 Sabin-like; PID, primary immunodeficiency disease; SCID, severe combined immunodeficiency; VDPV3, type 3 vaccine-derived poliovirus.†Time from last OPV to first fecal sample collection.
history showed detection of VDPV2 through AFP surveillance, with 7 nt changes from the parent virus. The child was routinely receiving replacement IVIG therapy because of suspected PID; subsequent specimens tested for poliovirus excretion were found negative. No virus shedding was detected in the monthly specimens collected after this child was enrolled in our study.

### Nonpoliovirus Excreters

Five (12%) patients tested positive for nonpolio enteroviruses; 1 patient with IgG subclass deficiency excreted enterovirus 75 (EV-75) and 1 patient with XLA excreted EV-76 in only 1 specimen each. These 2 patients were receiving IVIG replacement therapy and being followed up for enterovirus excretion. A patient with the rare immunodeficiency disorder dedicator of cytokinesis 8 deficiency (DOCK8 deficiency, or hyper IgE syndrome) excreted echovirus 13 in the first fecal sample, and another patient with the rare immunodeficiency disorder B cell expansion with NF-κB linked immunodeficiency disease (BENTA) disease excreted echovirus 5 in the first fecal sample. The patient with DOCK8 deficiency was lost to follow-up; repeated monthly samples from the patient with BENTA disease tested negative for enterovirus. The first specimen from a patient having chronic granulomatous disease, a phagocytic disorder, tested positive for echovirus 14, but the patient died before the next specimen collection (Table 3).

### Discussion

Children with PID are at risk for prolonged infection with enteroviruses and may excrete iVDPV after receiving OPV or after being exposed to contacts excreting poliovirus. Such patients are at risk for developing paralytic poliomyelitis and must be identified, as they may pose a risk of reintroduction of the virus into the population after global eradication of poliovirus.

The first objective of this study was to identify chronic or long-term poliovirus/nonpolio enterovirus excreters among children with various PIDs being treated at a tertiary care unit in Mumbai. Although there was no chronic (>5 years) poliovirus excretor among the participants, we identified 1 patient with SCID as a long-term (>6 months) VDPV3 excreter who was excreting VDPV for 2 years. One of the notable highlights of our study was that 6 (75%) of 8 children with PID who were excreting enterovirus had combined or other immunodeficiencies, rather than only antibody-mediated immunodeficiency. Madkaikar et al. reported the distribution pattern of PID in the same tertiary care unit in Mumbai, which varied considerably from those reported by United States, Europe, Africa, and other Asian countries, as follows: diseases of immune dysregulation (29%), phagocytic defects (29%), predominant antibody deficiency (13%), combined T and B cell deficiency (19%), and other well-defined diseases (10%) (17). Our study showed a similar pattern of distribution in PID participants: diseases of immune dysregulation (31%), predominant antibody deficiency (34%), phagocytic defects (17%), and combined T and B cell deficiency (14%). Except for 1 patient (a VDPV2 excreter), none of the participants had paralytic disease at the time of enrollment in the study or developed paralysis during the study period.

The VDPV3 isolates excreted by the patient with SCID showed 4% nucleotide change at 4 years of age, indicating that the source of virus was most probably the OPV given at birth. The patient has been receiving IVIG at regular intervals since he received the diagnosis of SCID. In an outbreak of poliovirus infection in Minnesota, USA, in 2005, a patient with PID continued to shed iVDPVs while receiving immunoglobulin therapy for several months and finally stopped shedding virus after a second bone marrow transplant (23). According to the WHO Update on VDPVs (24,25), 6 patients with SCID, 5 of them with no paralytic manifestations, were excreting VDPV2 for a long time (>4 months to several years), and 1 continued virus excretion even after a bone marrow transplant. Unlike a Sri Lanka study (14) that reported that children with SCID may not pose a threat to the community because of their short life span, our study, along with studies in Israel, Iran, Libya, and Turkey (24,25), showed that some patients with SCID can excrete the virus

Table 3. Demographic and clinical data for patients with PID whose fecal samples tested positive for nonpolio enteroviruses, Wadia Children’s Hospital, Mumbai, India, September 2014–April 2017

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Age, mo/sex</th>
<th>PID type</th>
<th>Months from last OPV†</th>
<th>IVIG therapy</th>
<th>BMT</th>
<th>Results by collection day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>114/F</td>
<td>IgG subclass deficiency</td>
<td>60</td>
<td>Yes</td>
<td>ND</td>
<td>D1, neg; D62, EV75; D214, neg; D250, neg; D419, neg; D476, neg; D685, neg</td>
</tr>
<tr>
<td>2</td>
<td>42/M</td>
<td>Hyper-IgE syndrome</td>
<td>24</td>
<td>No</td>
<td>ND</td>
<td>D1, E13; lost to follow-up</td>
</tr>
<tr>
<td>3</td>
<td>18/M</td>
<td>BENTA disease</td>
<td>NA</td>
<td>No</td>
<td>ND</td>
<td>D1, E5; D185, neg; D273, neg</td>
</tr>
<tr>
<td>4</td>
<td>30/M</td>
<td>CGD</td>
<td>NA</td>
<td>No</td>
<td>ND</td>
<td>D1, E14; D10, died</td>
</tr>
<tr>
<td>5</td>
<td>54/M</td>
<td>XLA</td>
<td>1</td>
<td>Yes</td>
<td>ND</td>
<td>D1, EV76; D107, neg; D136, neg; D260, neg</td>
</tr>
</tbody>
</table>

†Time from last OPV to first fecal sample collection.

*BENTA, B cell expansion with NF-κB and T cell anergy; BMT, bone marrow transplant; CGD, chronic granulomatous disease; D, day of collection; DOCK8 deficiency, or hyper IgE syndrome; E5, echovirus 5; E13, echovirus 13; E14, echovirus 14; EV75, enterovirus 75; EV76, enterovirus 76; IVIG, intravenous immunoglobulin; NA, not available; ND, not done; neg, negative; OPV, oral polio vaccine; PID, primary immunodeficiency disease; XLA, X-linked agammaglobulinemia.
for a prolonged period even after bone marrow transplant, with a high rate of nucleotide changes from the parent Sabin strain. The life expectancy of these children varies depending on the type of SCID/mutation, so it is necessary to analyze the immunological workup of these patients for visualizing whether the child can survive or excrete viruses. Another striking feature common to these SCID poliovirus excreters is that most of them excrete VDPV without exhibiting any paralytic symptoms. Of the 4 participants with SCID in our study, 2 of them received their diagnoses at 2 months of age; their fecal specimens were found negative for enteroviruses, but the infants died before the next sample collection at the age of 4 months. One reason for the survival of 1 child with SCID could be that his condition was diagnosed as leaky SCID (26), whereas the 2 infants who died exhibited classical SCID. Further studies on the patient with leaky SCID are in progress to enumerate the changes in immunological parameters that might have contributed to stopping virus excretion. The information about this child was provided to NPSP for screening of contacts and other precautionary measures.

FLH accounted for the largest number of patients in our study, which enrolled 11 patients with FLH. The reason could be that the FLH treatment protocol requires frequent hospitalization and follow-up, so the patients with FLH were therefore accessible for fecal sample collection. One of the children with FLH excreted Sabin1 poliovirus only once, and subsequent specimens tested negative.

The incidence of agammaglobulinemia has been estimated to be ≈1/100,000 live births in the United States and Europe, but no estimates have been made for developing countries (27). We enrolled 7 patients with XLA (17%), ranging in age from 20 months to 18 years; all of them had received routine OPV and are currently undergoing IVIG replacement therapy. One patient with XLA had a history of early demyelination with Guillain-Barré syndrome and onset of paralysis; VDPV2 was detected in this patient at 10 months of age through the WHO AFP surveillance program. Aside from patients with SCID, patients with B cell–mediated PIDs such as XLA and CVID have been reported to excrete VDPVs (14,25,28). There are controversies regarding replacement IVIG therapy preventing enterovirus infections in B cell deficiency disorders (29,30). In our study, the role of IVIG in stopping VDPV shedding cannot be ruled out for the patient with SCID who excreted VDPV3 and the patient with XLA who excreted VDPV2.

Eight (19%) of 42 participants were excreting enteroviruses with no associated disease, which can be considered normal for most enterovirus infections. The excretion of enteroviruses in our study was not restricted to antibody-mediated immunodeficiency; rather, it included patients with different spectrums of PID, although there was only 1 long-term excreter. Three patients had CVID, but none of them has excreted enteroviruses so far, as reported in other studies (14,31). The reason could be that all 3 participants received their diagnoses after the age of 7 years and would thus have had their diagnoses missed by either AFP or PID surveillance, as all of them are asymptomatic (31,32).

Our data have limitations in that they represent the PID participants in a tertiary care unit in Mumbai, India, restricting the study population to only Mumbai and parts of Maharashtra state. Patient follow-up, sharing of fecal samples, and collection of specimens at regular intervals were challenging because of lack of awareness and financial burden. Although 151 patients with diagnosed PID were recorded during our study period, only 42 could be followed up. Our study does not allow a precise quantification of the risk for prolonged excretion of poliovirus among patients with PID because there was only 1 patient who excreted for >6 months. Because we used the RD and L20B cell lines for isolation of enteroviruses, we could have missed some enteroviruses that are not cultivable in these cell lines. Furthermore, owing to the lack of affordable treatment and expensive clinical management of infants and children with persistent and chronic infections, many young children probably died from PID before diagnosis. Because our study included only children ≤18 years of age, persons with CVID who often do not develop signs or symptoms of immune deficiency until they are young adults would have been missed (33). Some patients with B cell immunodeficiency could not be followed, so this study may not be an accurate reflection of all patients with B cell immunodeficiency disorders.

Our study demonstrated that the proportion of children who have PID and enterovirus infection in the study area is not high, which may indicate that the risk of chronic excretion of poliovirus among patients with PID in India is low. However, owing to sample size limitations, we recommend future studies expanding the scope and intensity of surveillance activities at additional referral hospitals in other areas of the country. A large-scale multicenter study is needed for the determination of the pattern of excretion of poliovirus in children in India who have received diagnoses of PID.

Acknowledgments
We thank Swapnil Varose and C.B. Sawant for their help in fecal sample collection and data entry and the staff of the Isolation Lab of the Enterovirus Research Centre for their technical assistance. We acknowledge the National Polio Surveillance Program, World Health Organization, for the Polio Lab Facility.

This study was supported by intramural funds from the Indian Council of Medical Research, New Delhi, India.

Dr. Mohanty is a research scientist at Enterovirus Research Centre, Indian Council of Medical Research, Mumbai, India. Her primary research interest is innate immune responses to polio and nonpolio enteroviruses.
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• Transmission of Babesia microti Parasites by Solid Organ Transplantation
• Immune Responses to Invasive Group B Streptococcal Disease in Adults
• Ambulatory Pediatric Surveillance of Hand, Foot and Mouth Disease As Signal of an Outbreak of Coxsackievirus A6 Infections, France, 2014–2015
• Increased Hospitalization for Neuropathies as Indicators of Zika Virus Infection, according to Health Information System Data, Brazil
• Global Escherichia coli Sequence Type 131 Clade with blaCTX-M-2, Gene
• Multidrug-Resistant Corynebacterium striatum Associated with Increased Use of Parenteral Antimicrobial Drugs
• Risk Factors for Middle East Respiratory Syndrome Coronavirus Infection among Healthcare Personnel
• Epidemiology of La Crosse Virus Emergence, Appalachian Region, United States
• Reassortant Eurasian Avian-Like Influenza A(H1N1) Virus from a Severely Ill Child, Hunan Province, China, 2015
• Serotype IV Sequence Type 468 Group B Streptococcus Neonatal Invasive Disease, Minnesota, USA
• Capsular Switching and Other Large-Scale Recombination Events in Invasive Sequence Type 1 Group B Streptococcus
• Changing Pattern of Chlamydia trachomatis Strains in Lymphogranuloma Venereum Outbreak, France, 2010–2015

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• Early Growth and Neurologic Outcomes of Infants with Probable Congenital Zika Virus Syndrome
• Severe Fever with Thrombocytopenia Syndrome Complicated by Co-infection with Spotted Fever Group Rickettsiae, China
• Guinea Worm (Dracunculus medinensis) Infection in a Wild-Caught Frog, Chad
• Dog-Mediated Human Rabies Death, Haiti, 2016
• Staphylococcus aureus Colonization and Long-Term Risk for Death, United States
• Group B Streptococcus Serotype III Sequence Type 283 Bacteremia Associated with Consumption of Raw Fish, Singapore
• Group B Streptococcus Serotype Sequence Type 283 Disease Linked to Consumption of Raw Fish, Singapore
• Novel Levofloxacin-Resistant Multidrug-Resistant Streptococcus pneumoniae Serotype 11A Isolate, South Korea

Cross-sectional studies suggest an increasing trend in incidence and relatively low recurrence rates of *Clostridium difficile* infections in Asia than in Europe and North America. The temporal trend of *C. difficile* infection in Asia is not completely understood. We conducted a territory-wide population-based observational study to investigate the burden and clinical outcomes in Hong Kong, China, over a 9-year period. A total of 15,753 cases were identified, including 14,402 (91.4%) healthcare-associated cases and 817 (5.1%) community-associated cases. After adjustment for diagnostic test, we found that incidence increased from 15.41 cases/100,000 persons in 2006 to 36.31 cases/100,000 persons in 2014, an annual increase of 26%. This increase was associated with elderly patients, for whom incidence increased 3-fold over the period. Recurrence at 60 days increased from 5.7% in 2006 to 9.1% in 2014 (p<0.001). Our data suggest the need for further surveillance, especially in Asia, which contains ≈60% of the world’s population.

*Clostridium difficile* infection (CDI) is a major cause of nosocomial illness worldwide. CDI is associated with high rates of mortality and illness (1) and has a case-fatality rate of up to 14% within 30 days after diagnosis (2). Disease recurrence further increases illness rates, reduces quality of life, and might occur in up to 27% of the incident cases (3). These features place a major burden on healthcare systems.

The disease burden of CDI has been well studied in Europe and North America. Although antimicrobial drug stewardship programs have contributed to a decrease in incidence in some countries, such as the United Kingdom and Finland (2,4), CDI is still a major health burden in other countries. In South Korea, a nationwide study showed an increasing trend in incidence of CDI (5). Disease occurrence in the United States has doubled during 2001–2010 (6). A similar trend of increase was also observed in a prospective surveillance study in Australia (7) and a retrospective observational study in Germany (8).

Emergence of community-associated CDI (CA-CDI), which originates in a community without traditional risk factors (9), is also a concern. A major proportion of CDI cases was attributable to a community in the United States (1). In Finland, episodes of CA-CDI have shown a major increase versus an overall decrease in disease incidence (2). Surveillance of CDI cases diagnosed during admission of patients showed that up to 50% of CDI cases were community associated (3,7).

Although Asia contains ≈60% of the world’s population, epidemiologic data of CDI for this continent are sparse (10). The disease burden and secular trend of incidence, especially CA-CDI, has not been reported for this region. Therefore, we conducted a large territory-wide study to investigate the disease burden and clinical outcomes of CDI in Hong Kong, China.

**Methods**

**Study Population and Case Identification**

We identified all patients in Hong Kong given a diagnosis of CDI during January 1, 2006–December 31, 2014, from the Clinical Data Analysis and Reporting System (CDARS), which is a computerized database of patient records managed by the Hong Kong Hospital Authority. The database contains laboratory and clinical information, including patient demographics, disease diagnoses, investigations, procedures, and drug prescription records in the public hospital system. It also contains information regarding residence in homes for elderly persons and medical

1These authors contributed equally to this article.
care provided by the Community Geriatric Assessment Team in the ambulatory care setting. This public hospital system is composed of 41 hospitals within 7 service clusters and provides >90% of inpatient medical services in the territory. There were >1 million inpatient discharges and deaths in this system during 2013–2014. In addition to the ward-based services, the system also provides the outpatient clinics and geriatric ambulatory care in Hong Kong. This electronic database has been used for conducting robust population studies (11,12).

We defined a case of CDI as a positive result on culture, toxin, or molecular assay for a diarrheal stool specimen obtained from an inpatient resident ≥18 years of age. Patients with samples obtained >48 hours after admission or those who were hospitalized in a healthcare facility within the previous 4 weeks were classified as having cases of healthcare-associated CDI (HA-CDI). Patients who had not been hospitalized in a healthcare facility within the previous 12 weeks were classified as having cases of CA-CDI. Patients who had been hospitalized in a healthcare facility (including long-term care facilities, such as homes for elderly persons and palliative care centers) within the previous 4–12 weeks were classified as indeterminate. We defined an incident case as a CDI episode without a positive laboratory test result in the previous 60 days.

Data Extraction
We obtained anonymized clinical information, including patient demographics, disease diagnoses, laboratory results, and clinical outcomes. Patient demographic data included age, sex, and residence in homes for elderly persons. Relevant disease diagnoses were identified by using codes from the International Classification of Diseases, 9th Revision (https://www.cdc.gov/nchs/icd/icd9.htm), including inflammatory bowel disease (555.0–556.9), Crohn’s disease (555.0), ulcerative colitis (556.0), colectomy (45.7 or 45.71–45.79), or surgical intervention during the same admission, and other concurrent conditions.

We retrieved data on medication prescriptions, including antimicrobial drug use within 8 weeks before CDI diagnosis. Medications prescribed under hospital authority–affiliated clinics and long-term care facilities were accessible in the database. Severe CDI was defined by either a maximum leukocyte count ≥15,000 cells/µL or a >50% increase in serum creatinine level, according to Cohen et al. (13). These data were captured from CDARS from 1 day before to 7 days after the CDI diagnosis date. Refractory disease referred to nonresponding disease requiring >14 days of continued treatment. We defined death as dying within 30 days after the diagnosis of CDI and recurrence as a recurrent diarrheal stool specimen with a positive test result for C. difficile within 60 days after completion of CDI treatment.

Statistical Analysis
We reported descriptive statistics as median, interquartile range (IQR), and percentage and calculated the overall crude incidence of CDI as the number of patients given a diagnosis of CDI/100,000 persons ≥18 years of age. The midyear population was obtained from Hong Kong Census and Statistics Department.

We also estimated incidences of health-associated and community-associated cases in the same manner. We used the χ² test for trend to compare differences in incidences, mortality rates, and recurrence rates. Assuming a Poisson distribution, we calculated 95% CIs for the incidence rate. We analyzed potential predictors for 30-day mortality rate and 60-day recurrence rate by using univariate and multivariate forward Wald logistic regression. We used Cox proportional hazard regression to identify factors that decreased the time to recurrence after an episode. We also used SPSS for Windows version 22.0 (IBM Corp., Armonk, NY, USA) to perform statistical analysis.

Ethical Statement
This study was conducted in accordance with the Declaration of Helsinki (2013 version) and approved by the Joint Clinical Research Ethics Committee of the Chinese University of Hong Kong and Hospital Authority New Territory East Cluster. All clinical data were anonymized by the CDARS, and all potential patient identifiers were removed upon return of database searches.

Results

Disease Burden, Incidence, and Clinical Outcomes
We identified 15,753 CDIs during 2006–2014. These infections included 14,402 (91.4%) healthcare-associated and 817 (5.1%) community-associated infections. The remaining 534 infections were indeterminate. The median age of case-patients was 78 (range 64–86) years, and there were more women (51.6%) in the entire cohort. The diagnostic test-adjusted incidence increased significantly from 15.41 cases/100,000 persons in 2006 to 36.31 cases/100,000 persons in 2014 (p<0.01 by χ² test for trend). We observed the trend of increase across all age groups. However, the incidence for elderly persons (>65 years of age) increased by the largest margin from 12.98 cases/100,000 persons in 2006 to 36.31 cases/100,000 persons in 2014 (p<0.01 by χ² test for trend). The overall 30-day mortality rate was 22.5%. The disease recurrence rate was 7.8% at 60 days after completion of initial CDI treatment. A total of 30.2% of the patients
lived in a home for the elderly, and most (79.4%) patients had visited a healthcare facility ≤4 weeks before disease onset. Most (>75.6%) patients had taken an antimicrobial drug known to be associated with medium to high risk for CDI before disease onset (14,15). A total of 47.8% had taken a proton-pump inhibitor, and 47.3% had taken a histamine-2 antagonist (Table 2).

Clinical Characteristics and Outcomes of HA-CDI and CA-CDI

We studied demographic and clinical characteristics in CDI patients of the 2 epidemiologic categories. Charlson comorbidity scores and proportions with severe CDI were similar between the 2 groups. Median ages (IQR) were 79 (65–86) years for patients with HA-CDI and 75 (57–85) years for patients with CA-CDI. For the CA-CDI group, male sex (41.2%), residence in a home for the elderly (24.9%), and exposure to high-risk antimicrobial drugs (36.6%) were less common than for HA-CDI group. The 30-day all-cause mortality rates were 9.8% for the CA-CDI group and 23.2% for the HA-CDI group, and the 60-day recurrence rates were 3.0% for the CA-CDI group and 8.0% for the HA-CDI group. However, a higher proportion of patients with CA-CDI than patients with HA-CDI had inflammatory bowel diseases (4.4% vs. 0.4%) (Table 2).

Risk Factors for Death and Recurrence

Logistic regression modeling suggested that advanced age (adjusted odds ratio [OR] 5.04, 95% CI 3.88–6.55), nonmetastatic tumor (adjusted OR 1.60, 95% CI 1.45–1.75), and healthcare-associated infection (adjusted OR 1.55, 95% CI 1.12–2.09) were the major predictors for death in 30 days. Other risk factors, including residence in a home for the elderly, exposure to high-risk antimicrobial drugs, and having renal diseases, each increased the risk for CDI by 39% to 47% (Table 3).

Table 1. Estimated crude incidence of Clostridium difficile infections, Hong Kong, China, by epidemiologic category, 2006–2014*

<table>
<thead>
<tr>
<th>Year</th>
<th>Adult population†</th>
<th>Overall Crude</th>
<th>HA-CDI Crude</th>
<th>CA-CDI Crude</th>
<th>Overall Adjusted§</th>
<th>HA-CDI Adjusted</th>
<th>CA-CDI Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>5,571,096</td>
<td>16.21</td>
<td>15.01</td>
<td>0.86</td>
<td>14.26</td>
<td>0.82</td>
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</tr>
<tr>
<td>2007</td>
<td>5,553,789</td>
<td>15.29</td>
<td>14.15</td>
<td>0.63</td>
<td>13.29</td>
<td>0.59</td>
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</tr>
<tr>
<td>2008</td>
<td>5,635,881</td>
<td>16.91</td>
<td>15.76</td>
<td>0.73</td>
<td>15.35</td>
<td>0.67</td>
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<tr>
<td>2009</td>
<td>5,711,689</td>
<td>20.24</td>
<td>18.94</td>
<td>0.74</td>
<td>23.11</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>5,788,704</td>
<td>24.00</td>
<td>22.18</td>
<td>1.07</td>
<td>20.77</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>5,865,870</td>
<td>35.44</td>
<td>32.22</td>
<td>1.79</td>
<td>23.40</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>5,943,512</td>
<td>43.34</td>
<td>39.39</td>
<td>2.37</td>
<td>33.32</td>
<td>2.82</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>6,014,771</td>
<td>47.28</td>
<td>43.13</td>
<td>2.71</td>
<td>34.71</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>6,085,892</td>
<td>49.36</td>
<td>44.38</td>
<td>2.96</td>
<td>36.31</td>
<td>2.18</td>
<td></td>
</tr>
<tr>
<td>Overall†‡</td>
<td>NA</td>
<td>29.46</td>
<td>26.94</td>
<td>1.51</td>
<td>24.10</td>
<td>1.22</td>
<td></td>
</tr>
</tbody>
</table>

*CDI, Clostridium difficile infection; CA-CDI, community-associated CDI; HA-CDI, healthcare-associated CDI; NA, not applicable.
†Midyear population ≥18 years of age reported by the Census and Statistics Department of Hong Kong.
‡There were 534 indeterminate cases according to criteria of Cohen et al (13): n = 19 (2006); n = 28 (2007); n = 24 (2008); n = 32 (2009); n = 43 (2010); n = 84 (2011); n = 94 (2012); n = 87 (2013); n = 123 (2014).
§Overall incidence adjusted for diagnostic method use, assuming equal sensitivity across tests. The 2010 cohort was used as the reference population.
¶Values in parentheses in this row are 95% CIs.
#p<0.01 by χ² test for trend.
When we considered the 60-day disease recurrence, Cox regression analysis showed that the use of a toxin detection assay (adjusted hazard ratio 1.79, 95% CI 1.53–2.11) and healthcare-associated infection (adjusted hazard ratio 1.52, 95% CI 1.06–2.20) were the major predictors. Other risk factors included severe CDI and exposure to high-risk antimicrobial drugs (Table 4). When compared with incident cases, we found that the odds for recurrence among the first recurrent cases was 1.57 (95% CI 1.35–1.82). These odds increased to 2.10 (95% CI 1.62–2.74) after 1 recurrent episode, and further increased by ≈10% after 2 recurrent episodes, and further increased by ≈10% to 2.22 (95% CI 1.38–3.57) after the third episode.

### Secular Changes in Mortality and Recurrence Rates

During 2006–2014, the crude 30-day all-cause mortality rate decreased slightly from 25.7% to 21.0% (OR 0.77, 95% CI 0.64–0.92; p = 0.01). Healthcare-associated case-patients in the 2014 cohort had an ≈20% reduced risk for death (OR 0.80, 95% CI 0.67–0.97; p = 0.02). The reduction was more apparent among the community-associated case-patients (OR 0.30, 95% CI 0.17–0.51; p<0.01). Despite the decreasing mortality rate, the recurrence rate increased significantly from 5.7% in 2006 to 9.1% in 2014 (p<0.01 by χ² test for trend) (Figure 2, panel A). This increase represented an ≈70% increase in the recurrence rate (OR 1.66, 95% CI 1.23–2.24). Further analysis suggested that the prevalence of severe disease, change of diagnostic test used, and exposure to proton-pump inhibitor changed over time (Table 5). We observed the same increasing trend for CA-CDI (2.5% vs. 5.7%) and HA-CDI (5.7% vs. 9.5%) during 2006–2014. Most recurrences occurred within 60 days after completion of initial treatment (Figure 2, panel B).

### Table 2. Characteristics of patients with *Clostridium difficile* infections, Hong Kong, China, 2006–2014*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall, n = 15,753†</th>
<th>HA-CDI, n = 14,402</th>
<th>CA-CDI, n = 817</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;44</td>
<td>1,040 (6.6)</td>
<td>893 (6.2)</td>
<td>105 (12.9)</td>
</tr>
<tr>
<td>45–64</td>
<td>2,930 (18.6)</td>
<td>2,621 (18.2)</td>
<td>203 (24.8)</td>
</tr>
<tr>
<td>65–84</td>
<td>7,026 (44.6)</td>
<td>6,495 (45.1)</td>
<td>301 (36.9)</td>
</tr>
<tr>
<td>&gt;85</td>
<td>7,577 (30.2)</td>
<td>4,393 (30.5)</td>
<td>208 (25.4)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>7,624 (48.4)</td>
<td>7,028 (48.8)</td>
<td>337 (41.2)</td>
</tr>
<tr>
<td>F</td>
<td>8,129 (51.6)</td>
<td>7,374 (51.2)</td>
<td>480 (58.8)</td>
</tr>
<tr>
<td>Resident of home for elderly persons</td>
<td>4,757 (30.2)</td>
<td>4,393 (30.5)</td>
<td>203 (24.9)</td>
</tr>
<tr>
<td>Severe disease</td>
<td>6,868 (43.6)</td>
<td>6,294 (43.7)</td>
<td>340 (41.6)</td>
</tr>
<tr>
<td>Antimicrobial drug use‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-risk drug</td>
<td>10,397 (66.0)</td>
<td>9,822 (68.2)</td>
<td>299 (36.6)</td>
</tr>
<tr>
<td>Medium-risk drug</td>
<td>11,909 (75.6)</td>
<td>11,320 (78.6)</td>
<td>318 (38.9)</td>
</tr>
<tr>
<td>Low-risk drug</td>
<td>221 (1.4)</td>
<td>216 (1.5)</td>
<td>74 (1.4)</td>
</tr>
<tr>
<td>Diagnostic test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>4,883 (31.0)</td>
<td>4,421 (30.7)</td>
<td>259 (31.7)</td>
</tr>
<tr>
<td>Toxin detection</td>
<td>5,246 (33.3)</td>
<td>4,940 (34.3)</td>
<td>195 (23.9)</td>
</tr>
<tr>
<td>NAAT</td>
<td>5,624 (35.7)</td>
<td>5,041 (35.0)</td>
<td>363 (44.4)</td>
</tr>
<tr>
<td>Use of proton-pump inhibitor</td>
<td>7,530 (47.8)</td>
<td>7,086 (49.2)</td>
<td>180 (22.0)</td>
</tr>
<tr>
<td>Use of histamine-2 receptor antagonist</td>
<td>7,451 (47.3)</td>
<td>6,927 (48.1)</td>
<td>273 (33.4)</td>
</tr>
<tr>
<td>Concurrent condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1,497 (9.5)</td>
<td>1,411 (9.8)</td>
<td>29 (3.6)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>5,183 (32.9)</td>
<td>4,825 (33.5)</td>
<td>176 (21.6)</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>2,410 (15.3)</td>
<td>2,232 (15.5)</td>
<td>100 (12.2)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2,899 (18.4)</td>
<td>2,693 (18.7)</td>
<td>103 (12.6)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>3,592 (22.8)</td>
<td>3,341 (23.2)</td>
<td>113 (13.8)</td>
</tr>
<tr>
<td>Nonmetastatic tumor</td>
<td>3,970 (25.2)</td>
<td>3,701 (25.7)</td>
<td>134 (16.4)</td>
</tr>
<tr>
<td>AIDS</td>
<td>79 (0.5)</td>
<td>58 (0.4)</td>
<td>13 (1.6)</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>95 (0.6)</td>
<td>58 (0.4)</td>
<td>36 (4.4)</td>
</tr>
<tr>
<td>Deaths</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During hospital stay</td>
<td>3,733 (23.7)</td>
<td>3,528 (24.5)</td>
<td>51 (6.2)</td>
</tr>
<tr>
<td>30-d all-cause</td>
<td>3,544 (22.5)</td>
<td>3,341 (23.2)</td>
<td>80 (9.8)</td>
</tr>
<tr>
<td>60-d all-cause</td>
<td>5,088 (32.3)</td>
<td>4,781 (33.2)</td>
<td>106 (13.0)</td>
</tr>
<tr>
<td>Recurrence, d§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>961 (6.1)</td>
<td>907 (6.3)</td>
<td>17 (2.1)</td>
</tr>
<tr>
<td>60</td>
<td>1,229 (7.8)</td>
<td>1,152 (8.0)</td>
<td>25 (3.0)</td>
</tr>
<tr>
<td>90</td>
<td>1,339 (8.5)</td>
<td>1,267 (8.8)</td>
<td>28 (3.4)</td>
</tr>
<tr>
<td>180</td>
<td>1,481 (9.4)</td>
<td>1,397 (9.7)</td>
<td>33 (4.0)</td>
</tr>
</tbody>
</table>

*CA-CDI, community-associated *Clostridium difficile* infection; CDI; HA-CDI, healthcare-associated *Clostridium difficile* infection; NAAT, nucleic acid amplification test.
†Sum of HA-CDI and CA-CDI cases might not equal number of overall CDI cases because of missing information in the registry.
‡Antimicrobial drug use 6 weeks before diagnosis was stratified into high risk (fluoroquinolones, cephalosporins, and clindamycin); medium risk (penicillins, macrolides, and sulfonamides); and low risk (tetracyclines).
§Defined as reappearance of symptoms after initial resolution and a positive CDI test result.
Discussion
Our main finding was the increasing incidence of CDI during 2006–2014 by an adjusted rate of 21% annually for HA-CDI and CA-CDI in Hong Kong. This increase was less than the 39% annual increase in South Korea (5) but higher than the 24% annual increase in Australia (7). This difference might be partially caused by increasing clinical suspicion, introduction of sensitive diagnostic tests in 2010, an aging population, or endemicity and virulence of the C. difficile bacterium. Prescriptions for antimicrobial drugs in Hong Kong have been closely monitored, and relevant use guidelines are available (16). In the United Kingdom and Finland, introduction of antimicrobial drug stewardship programs has considerably reduced CDI in hospitals (2,4).

In our study, the aging population contributed to the temporal increase. Advanced age is a well-established risk factor for CDI; we observed a 2% increased risk for disease for each additional year of age (17). In our population, the incidence rate for elderly persons >75 years of age was higher than that for other age groups. In Hong Kong, the population is aging rapidly because of increased life expectancy and reduced birthrate. According to the latest United Nations report, the life expectancy at birth in Hong Kong is the longest in the world (18). Given the predicted increase in elderly persons >75 years of age from 7.3% in 2014 to 17.8% in 2041, the incidence of CDI is expected to reach 75.86 cases/100,000 persons (19). In addition, our logistic regression analysis showed that mortality and recurrence rates were much higher in elderly patients. Thus, the incidence of CDI might be expected to further increase, which represents a large burden on the healthcare system. Relevant surveillance should be enhanced and public health measures should be incorporated to reduce the disease burden, especially in places with an aging population.

Stratification of cases into epidemiologic categories showed that healthcare-associated infections contributed >90% of all incident cases detected. This contribution was considerably higher than those in some studies reporting up to 70% cases of healthcare origin (1,2,7). However, our rate was comparable to rates reported in 2 other studies in Asia (10,20). These findings highlight possible discrepant epidemiologic etiologies of the infection at different locations (21). Recent emergence of C. difficile ribotype 002 in the region may also account for the discrepancy (15,22,23).

This common circulating ribotype has a greater propensity to sporulate and produce toxins. These spores and toxin

---

Table 3. Association between 30-day all-cause deaths and potential independent variables for patients with Clostridium difficile infections, Hong Kong, China, 2006–2014*  

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis†</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>OR (95% CI)</td>
<td>p value</td>
<td>Adjusted OR (95% CI)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;44</td>
<td>NA</td>
<td>1.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>45–64</td>
<td>0.38</td>
<td>1.46 (1.34–1.58)</td>
<td>&lt;0.01</td>
<td>0.39</td>
</tr>
<tr>
<td>65–84</td>
<td>1.02</td>
<td>2.77 (2.45–3.14)</td>
<td>&lt;0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;85</td>
<td>1.77</td>
<td>5.87 (4.57–7.56)</td>
<td>&lt;0.01</td>
<td>1.62</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.10</td>
<td>1.11 (1.03–1.19)</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>Resident of home for elderly persons</td>
<td>0.55</td>
<td>1.73 (1.59–1.87)</td>
<td>&lt;0.01</td>
<td>0.33</td>
</tr>
<tr>
<td>Severe disease‡</td>
<td>−0.01</td>
<td>0.99 (0.92–1.08)</td>
<td>0.94</td>
<td>NA</td>
</tr>
<tr>
<td>Antimicrobial drug use§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-risk drug</td>
<td>0.62</td>
<td>1.85 (1.68–2.04)</td>
<td>&lt;0.01</td>
<td>0.34</td>
</tr>
<tr>
<td>Medium-risk drug</td>
<td>0.17</td>
<td>1.16 (1.09–1.28)</td>
<td>&lt;0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>Low-risk drug</td>
<td>0.24</td>
<td>1.27 (0.94–1.72)</td>
<td>0.12</td>
<td>NA</td>
</tr>
<tr>
<td>Diagnostic test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>NA</td>
<td>1.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Toxin detection</td>
<td>−0.33</td>
<td>0.72 (0.65–0.79)</td>
<td>&lt;0.01</td>
<td>−0.25</td>
</tr>
<tr>
<td>NAAT</td>
<td>−0.13</td>
<td>0.88 (0.80–0.97)</td>
<td>&lt;0.01</td>
<td>−0.05</td>
</tr>
<tr>
<td>Use of proton-pump inhibitor</td>
<td>0.37</td>
<td>1.44 (1.34–1.56)</td>
<td>&lt;0.01</td>
<td>0.24</td>
</tr>
<tr>
<td>Use of histamine-2 receptor antagonist</td>
<td>0.15</td>
<td>1.16 (1.08–1.25)</td>
<td>&lt;0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Healthcare-associated disease</td>
<td>0.63</td>
<td>1.88 (1.65–2.13)</td>
<td>&lt;0.01</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*NA, not applicable; NAAT, nucleic acid amplification test.
†p = 0.74 by Hosmer-Lemeshow test.
‡Severe disease was diagnosed according to the according to criteria of Cohen et al (12).
§Antimicrobial drug use 8 weeks before diagnosis was stratified into high risk (fluoroquinolones, cephalosporins, and cindamycin); medium risk (penicillins, macrolides, and sulfonamides); and low risk (tetracyclines).
Table 4. Cox proportional hazard regression analysis of potential independent variables associated with time to recurrence of *Clostridium difficile* infections, Hong Kong, China, 2006–2014*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th></th>
<th>Multivariate analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>Hazard ratio (95% CI)</td>
<td>p value</td>
<td>β</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;44</td>
<td>NA</td>
<td>1.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>45–64</td>
<td>0.14</td>
<td>1.15 (0.86–1.55)</td>
<td>0.34</td>
<td>0.01</td>
</tr>
<tr>
<td>65–84</td>
<td>0.29</td>
<td>1.33 (0.91–1.95)</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>&gt;85</td>
<td>0.41</td>
<td>1.50 (1.14–1.98)</td>
<td>&lt;0.01</td>
<td>0.16</td>
</tr>
<tr>
<td>Male sex</td>
<td>–0.02</td>
<td>0.99 (0.88–1.10)</td>
<td>0.79</td>
<td>NA</td>
</tr>
<tr>
<td>Resident of home for elderly persons</td>
<td>–0.02</td>
<td>0.98 (0.87–1.11)</td>
<td>0.99</td>
<td>NA</td>
</tr>
<tr>
<td>Severe disease†</td>
<td>0.32</td>
<td>1.38 (1.22–1.55)</td>
<td>&lt;0.01</td>
<td>0.35</td>
</tr>
<tr>
<td>Antimicrobial drug use‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-risk drug</td>
<td>0.49</td>
<td>1.55 (1.36–1.77)</td>
<td>&lt;0.01</td>
<td>0.32</td>
</tr>
<tr>
<td>Medium-risk drug</td>
<td>0.41</td>
<td>1.51 (1.33–1.72)</td>
<td>0.01</td>
<td>–0.01</td>
</tr>
<tr>
<td>Low-risk drug</td>
<td>0.11</td>
<td>1.12 (0.69–1.83)</td>
<td>0.66</td>
<td>NA</td>
</tr>
<tr>
<td>Diagnostic test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>NA</td>
<td>1.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Toxin detection</td>
<td>0.69</td>
<td>1.89 (1.62–2.21)</td>
<td>0.01</td>
<td>0.57</td>
</tr>
<tr>
<td>NAAT</td>
<td>0.27</td>
<td>1.31 (1.12–1.52)</td>
<td>0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>Use of proton-pump inhibitor</td>
<td>0.08</td>
<td>1.09 (0.97–1.22)</td>
<td>0.16</td>
<td>NA</td>
</tr>
<tr>
<td>Use of histamine-2 receptor antagonist</td>
<td>0.18</td>
<td>1.19 (1.07–1.34)</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Healthcare-associated disease</td>
<td>0.49</td>
<td>1.65 (1.51–1.83)</td>
<td>0.01</td>
<td>0.42</td>
</tr>
<tr>
<td>Concurrence condition</td>
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</tr>
<tr>
<td>Myocardial infarction</td>
<td>0.09</td>
<td>1.10 (0.92–1.32)</td>
<td>0.32</td>
<td>NA</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>0.49</td>
<td>1.63 (1.46–1.82)</td>
<td>&lt;0.01</td>
<td>–0.15</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>–0.05</td>
<td>0.95 (0.82–1.12)</td>
<td>0.56</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.08</td>
<td>1.08 (0.94–1.24)</td>
<td>0.30</td>
<td>NA</td>
</tr>
<tr>
<td>Renal disease</td>
<td>0.05</td>
<td>1.05 (0.92–1.20)</td>
<td>0.47</td>
<td>NA</td>
</tr>
<tr>
<td>Nonmetastatic tumor</td>
<td>–0.24</td>
<td>0.79 (0.69–0.90)</td>
<td>0.01</td>
<td>–0.15</td>
</tr>
<tr>
<td>AIDS</td>
<td>–0.58</td>
<td>0.56 (0.48–1.74)</td>
<td>0.32</td>
<td>NA</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>0.14</td>
<td>1.15 (0.62–2.14)</td>
<td>0.66</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA: not applicable; NAAT, nucleic acid amplification test.
†Severe disease was diagnosed according to the criteria of Cohen et al (13).
‡Antimicrobial drug use 8 weeks before diagnosis was stratified into high risk (fluoroquinolones, cephalosporins, and clindamycin); medium risk (penicillins, macrolides, and sulfonamides); and low risk (tetracyclines).

Producers might facilitate bacterial dissemination in the hospital environment and increase the number of symptomatic patients. Because data were available for tertiary care settings in our study, community cases might not be recognized unless patients became sufficiently ill for hospital admission. A delay in diagnosis is rather common for CA-CDI (16).

Emergence of CA-CDI has been increasingly recognized. Persons with this type of infection have had no traditional risk factors associated with nosocomial infection. The younger age of the disease population has led to concern over loss of productivity and years of life lost (3,24). Despite the relatively low prevalence, we observed a higher increase of incidence for the community than that for healthcare settings. Risk factors for acquisition of CDI in the community are unclear, but bacterial, host, and environmental factors have been suggested to play a role (25). Contamination of retail meats with *C. difficile* spores might represent a potential reservoir for infection of humans (26,27). Contact with contaminated raw meat has been recognized as a risk factor for nasal colonization with *Staphylococcus aureus* in humans (28). Molecular typing of *C. difficile* isolates from contaminated raw meat and those from exposed personnel might identify a novel reservoir for asymptomatic carriage. Although not yet proven for the community, asymptomatic carriers have been shown to contribute to transmission of *C. difficile* strains in healthcare facilities (29).

The 30-day all-cause mortality rate decreased slightly during the study period. This finding was consistent with other reports in which 20%–30% reductions were detected (5,30). When compared with mortality rates for CA-CDI, mortality rates at different times were consistently higher in the HA-CDI group. This finding is consistent with another report in which a 4-fold higher case-fatality rate for HA-CDI was observed (2). Consistently, logistic regression analysis suggested that healthcare-associated cases, in addition to advanced age, were the major risk factors for 30-day all-cause mortality rates.

The recurrence rate for our population was considered lower than rates reported for Western countries (3,5,8). Although this discrepancy would require further validation, our data might indicate lower disease recurrence rates for Asia. Because various therapies other than antimicrobial drugs, including colectomy and fecal microbiota transplant, have been considered for managing recurrent CDI.
Figure 2. Clostridium difficile infections in adults, Hong Kong, China, 2006–2014. A) Prevalence of 60-day recurrence increased significantly (p<0.001 by \( \chi^2 \) test for trend). B) Recurrence rates, which were higher for healthcare-associated infections.

Cases, this lower recurrence rate might indicate a need to reconsider the risk–benefit balance when deciding which therapies to use. Further studies are warranted to investigate differences in recurrence rates.

We also observed a major increase in recurrence rate over the study period. The factors accounting for this change were multifactorial and might include change of diagnostic method used over time, increasing prevalence of severe disease, and exposure to proton-pump inhibitors. A systematic review of 68 studies concluded that use of antimicrobial drugs after diagnosis, in addition to older age, were major risk factors for recurrence (31). However, in our study, we did not investigate exposure to antimicrobial drugs after diagnosis. Similarly, increased ward-level prescriptions for antimicrobial drugs have been shown to increase CDI in hospitalized patients (32).

Table 5. Temporal change in exposure prevalence for patients with Clostridium difficile infections in association with trend in recurrence, Hong Kong, China, 2006–2014*

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Period, prevalence (standardized Pearson residuals), %</th>
<th>Absolute residual difference, %†</th>
<th>p value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;44</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>45–64</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>65–84</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>≥85</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male sex</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Resident of home for elderly persons</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Severe disease</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Antimicrobial drug use§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-risk drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium-risk drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial culture</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Toxin detection</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NAAT</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Use of proton-pump inhibitor</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Use of histamine-2 receptor antagonist</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Healthcare-associated disease</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Concurrent condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebrovascular disease accident</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Renal disease</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Nonmetastatic tumor</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AIDS</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
</tbody>
</table>

*NAAT, nucleic acid amplification test.
‡By \( \chi^2 \) test for trend.
§Antimicrobial drug use 8 weeks before diagnosis was stratified into high risk (fluoroquinolones, cephalosporins, and clindamycin); medium risk (penicillins, macrolides, and sulfonamides); and low risk (tetracyclines).
Our study was strengthened by use of a large territory-wide population. Because public hospitals provide >90% of the entire inpatient service in Hong Kong, our study was representative of the region. Our study also investigated the temporal trend of burden of CDI. All data, including demographics, laboratory findings, and clinical records, were objectively recorded in a database. Thus, there was no cause for concern regarding recall bias.

Nevertheless, our study had some limitations. First, the study relied on inpatient data for which cases diagnosed in outpatient clinics might have been missed. This limitation might lead to underestimation of disease burden, particularly for community-associated case-patients with milder disease, who have been managed as outpatients without the need for hospitalization. This limitation might have also skewed clinical characteristics and outcomes for this group, although all patients with severe cases requiring hospitalization would have been represented in our data.

Second, because of lack of coding, our database was not able to capture fecal microbiota transplants as a novel therapy for recurrent CDI. Nevertheless, because of operational and logistic difficulties, fecal microbiota transplant has been used sparsely for selected patients (33). This procedure was unlikely to have caused any major changes in overall disease epidemiology.

Third, we did not include repeated exposure to antimicrobial drugs after initial CDI diagnosis in our database. This limitation might potentially serve as a major factor for subsequent disease recurrence.

Fourth, as with other retrospective studies (1,2,7,34,35), the diagnosis of CDI in our study was based on different laboratory methods, including bacteriological culture, toxin detection, and molecular assays. Given the variable sensitivity and specificity of these tests, this limitation could have biased estimation of disease incidence. Cases of pseudomembranous colitis diagnosed only by histologic analysis might have been missed. Thus, the incidence of CDI might be underestimated.

In conclusion, the incidence of CDI is increasing at a rapid rate in Hong Kong. Further surveillance of this infection in this area is urgently needed.

This study was supported by the Food and Health Bureau Health and Medical Research Fund (grant no. 15140132, HK-13-03-02), the General Research Fund (grant no. 24103516), the Shenzhen Science and Technology Program (grant no. JCYJ20150630165236954), and Department of Science and Technology of Guangdong Province, Natural Science Foundation of Guangdong Province (grant no. 2015A030313886). S.H.W. is supported by the Croucher Foundation and the Li Ka Shing Institute of Health Sciences. S.H.W. conceived the study and critically reviewed the paper; J.H. and R.Z.W.D. obtained data, performed data analysis, and prepared the paper; T.N.Y.K., X.W., L.Z., M.I., R.C., P.M.K.H., K.L.Y.L., M.C.S.W., G.T., and W.K.K.W. provided intellectual input and revised the paper; and M.T.V.C., F.L.K.C., J.Y., J.J.Y.S., S.C.N., N.L., and J.C.Y.W. managed and supervised the study.

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References


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Molecular Tracing to Find Source of Protracted Invasive Listeriosis Outbreak, Southern Germany, 2012–2016

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We investigated 543 Listeria monocytogenes isolates from food having a temporal and spatial distribution compatible with that of the invasive listeriosis outbreak occurring 2012–2016 in southern Germany. Using forensic microbiology, we identified several products from 1 manufacturer contaminated with the outbreak genotype. Continuous molecular surveillance of food isolates could prevent such outbreaks.

Listeriosis is a serious, life-threatening infectious disease caused by Listeria monocytogenes that mainly affects elderly and immunocompromised persons, pregnant women, and neonates (1,2). In Germany, listeriosis cases have been predominantly reported among nonpregnant women and men, and the source of most infections was unknown (3). The major risk factors identified for these cases were immunosuppressive therapy, immunocompromising diseases, gastric acid suppression, and frequently consumed ready-to-eat foods (i.e., packed and presliced cheese and boiled sausages) (4). L. monocytogenes is usually transmitted through food prone to contamination during manufacturing or postproduction processing before packing. In 2012, the number of L. monocytogenes cases in Germany started continuously increasing; 707 cases (incidence rate 0.9/100,000 population) and a case-fatality rate of 7% were reported in 2016 (3). Among the 6 most predominant enteric pathogens in Germany, L. monocytogenes has accounted for the highest number of years of potential life lost (5).

The Study
At the end of 2015, an outbreak of invasive listeriosis caused by L. monocytogenes serotype 1/2a was reported in southern Germany (6). The outbreak became apparent because analysis of the L. monocytogenes isolates from patients residing in the federal states Bavaria, Baden-Württemberg, and Hesse revealed the same novel pulsed-field gel electrophoresis (PFGE) pattern: 13a/54. As of July 14, 2017, the National Reference Centre for Salmonella and Other Bacterial Enteric Pathogens and the Binational Consiliary Laboratory for Listeria, a collaborative agreement between the Robert Koch Institute (Wernigerode, Germany) and the Austrian Agency for Health and Food Safety (Wien, Austria), had received 84 human isolates with this PFGE pattern. These isolates could be assigned to 78 surveillance cases reported in the national mandatory notification system. Subsequent whole-genome sequencing (WGS) and core-genome multilocus sequence typing (MLST) (7) resulted in the assignment of 57 human isolates to the unique cluster type 1248 (sequence type 8 according to the Institut Pasteur MLST database; http://bigsdb.pasteur.fr/). A possible case was defined as signs and symptoms of acute invasive listeriosis in a patient with disease onset November 2012–October 2016 from which L. monocytogenes with the PFGE pattern 13a/54 was isolated. Confirmed cases met the aforementioned criteria and included assignment of the L. monocytogenes isolate into the core-genome MLST cluster type 1248 (6). Analysis of the patients’ food consumption habits, which were recorded during explorative interviews, did not identify the causative food item.

We analyzed 543 environmental L. monocytogenes isolates of molecular serotype IIa (comprising the conventional serotypes 1/2a and 3a) that corresponded with the spatial and temporal distribution of cases in southern Germany. Staff of official food control laboratories had acquired these isolates from food matrices and food processing plants in the affected federal states. With these isolates, we performed PFGE analysis (with only Apal for initial...
screening) and WGS at the National Reference Laboratory for *L. monocytogenes* (Berlin, Germany).

Analysis showed that 26 isolates from food had the same *AscI* (13a) and *ApaI* (54) restriction patterns as the human outbreak strain; patterns from 55 isolates showed ≥90% similarity to the PFGE pattern *ApaI* 54 (Figure 1). We sequenced all 13a/54 isolates and 17 of the 55 isolates representative of the cluster with ≥90% similarity. In addition, 148 isolates were directly subjected to WGS. We conducted comparative genomic analyses to find single-nucleotide polymorphisms (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/10/16-1623-Techapp1.pdf) and compared the isolates closely related to the outbreak strain by core-genome MLST (Figure 2) (8).

We defined the threshold for strain affiliation with the outbreak cluster as a difference of ≤10 alleles (7).

In May 2016, we identified an *L. monocytogenes* isolate (BfR-LI-02473) with the 13a/54 PFGE pattern that belonged to the core-genome MLST cluster type 1248. This isolate had been found at a retail store in Bavaria 2 months prior in a smoked pork belly sampled by food inspectors. The meat product had been manufactured by a meat processing plant in Germany that distributed food products in southern Germany. The highly contaminated batch of smoked pork belly (bacterial concentration 1.9 × 10⁹ CFU/g) was recalled from consumers and withdrawn from the market.

Follow-up investigations of this meat processing plant revealed isolates identical to BfR-LI-02473 (BfR-LI-02689, BfR-LI-02690, and BfR-LI-02647) in another batch of smoked pork belly, in vegetarian sausages, and in 2 types of boiled pork sausages. The bacterial concentration in these products was ≤100 CFU/g. At the end of May 2016, all food products from this meat producer were banned from sale, and those already on the market were withdrawn.

Two more core-genome MLST cluster types found in raw meat and sausage were identified in the company’s production chain. However, based on the PFGE data, we could not assign these isolates to any human cases that occurred in southern Germany during 2012–2016. Furthermore, an *L. monocytogenes* isolate obtained from the fecal sample of an employee differed from the outbreak strain. Environmental sampling of the suspected plant led to the identification of a potential contamination hotspot: a conveyor belt on which food products were directly placed before packaging. However, no isolates were available for sequencing to confirm this hypothesis.

Overall, this outbreak investigation revealed 26 isolates from food with the 13a/54 PFGE pattern: 24 isolates
either originating from or associated with food products from the suspected outbreak source assigned to the cluster type 1248 and 2 isolates not epidemiologically linked to the suspected outbreak source that differed from cluster type 1248 by ≥32 alleles. Isolates closely related to the outbreak strain and included in cluster B (online Technical Appendix Figure) differed by ≥22 alleles by core-genome MLST analysis (Figure 2). The human outbreak isolates differed from each other by ≤3 alleles, and among all human isolates, the maximum difference was 8 (6). Compared with the human outbreak isolates, the outbreak isolates from food having the 13a/54 PFGE pattern and belonging to the core-genome MLST cluster type 1248 contained only 2–4 allele differences based on single-nucleotide polymorphisms, indicating a close phylogenetic relationship (Figure 2). On-site investigation in the household of a patient who regularly consumed smoked pork belly distributed by the suspected outbreak source revealed an isolate in cheese belonging to cluster type 1248 and identical to the patient isolate. Comparative analyses of unopened packages of the same batch of cheese were negative for *L. monocytogenes*, suggesting cross-contamination in the patient’s household.

The meat processing plant predominantly supplied grocery stores of a single company. Patients and their relatives often shopped at these grocery stores and frequently ate pork products. Altogether, food consumption

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**Figure 2.** Minimum spanning tree estimating the phylogenetic relationships among outbreak and nonoutbreak *Listeria monocytogenes* isolates from humans and from food products, southern Germany, 2012–2016. We conducted bioinformatics analyses using the Ridom SeqSphere+ software version 3.1.0-2016-01 (Ridom GmbH, Münster, Germany). The core-genome multilocus sequence typing scheme for whole-genome sequencing–based typing of *L. monocytogenes* relies on a set of 1,701 target genes (alleles) that are present in >99% of the known genomes of the species (7). Each circle represents an allelic profile. The numbers on the connecting lines illustrate the number of differing alleles in a pairwise comparison. Closely related genotypes (≤10 allele difference) designated a cluster type and are indicated with bold red numbers. CT, cluster type; PFGE, pulsed-field gel electrophoresis.
histories of patients were compatible with our molecular typing results, but we could not prove the producer was the source of the infections. After the production plant was shut down, the outbreak strain was isolated from only 3 more persons who either might have consumed or consumed pork products from this company with a high degree of probability.

Conclusions
The isolation of the L. monocytogenes outbreak strain in various food products from the same manufacturer, absence of the outbreak strain in a large number of food products collected during the outbreak from the same region, and subsequent epidemiologic findings suggested that the source of outbreak had been identified. Epidemiologic analysis did not provide the information needed to determine the outbreak source; thus, forensic microbiology based on WGS of L. monocytogenes isolates from patients and food became essential to take the appropriate countermeasures. Public health could benefit from continuous molecular surveillance of isolates from humans and food, which could allow for infectious disease outbreaks to be stopped before emergence.

Acknowledgments
We thank all partners who were engaged in the outbreak investigation, comprising local and state public health authorities, as well as the staff from official control laboratories, the European Union Reference Laboratory for Listeria monocytogenes in France and the Cantonal Laboratory Thurgau in Switzerland. We also thank Juliane Bräunig for her help and guidance throughout the outbreak investigation and our technical staff members Marett Splett, Corinna Maneck, Anne Stephan, Jane Kowall, Silvia Schmoger, Beatrice Baumann, Ernst Juncker, Ute Strutz, and Ilona Wermuth for their outstanding and unfailing work during the outbreak. Finally, we thank the staff of Eurofins Medigenomix for excellent technical support and bioinformatics analysis.

This work was funded by the German Federal Institute for Risk Assessment (project no. 47-003) and the Robert Koch Institute.

Dr. Kleta is a microbiologist in the Department of Biological Safety at the German Federal Institute for Risk Assessment in Berlin, Germany, and the head of the National Reference Laboratory for Listeria monocytogenes. Her research interests focus on food safety and molecular typing methods, especially on those for L. monocytogenes.

References

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Dengue Virus 1 Outbreak in Buenos Aires, Argentina, 2016

Estefanía Tittarelli, Silvina B. Lusso, Stephanie Goya, Gabriel L. Rojo, Mónica I. Natale, Mariana Viegas, Alicia S. Mistchenko, and Laura E. Valinotto

The largest outbreak of dengue in Buenos Aires, Argentina, occurred during 2016. Phylogenetic, phylodynamic, and phylogeographic analyses of 82 samples from dengue patients revealed co-circulation of 2 genotype V dengue virus lineages, suggesting that this virus has become endemic to the Buenos Aires metropolitan area.

Dengue virus (DENV) is a single-stranded RNA flavivirus primarily transmitted among humans by the *Aedes aegypti* mosquito. A substantial increase in dengue incidence has been observed in the past 2 decades in the Americas, and most of Argentina’s bordering countries have reported co-circulation of >1 dengue serotype (1). In Buenos Aires, Argentina, the presence of the *A. aegypti* mosquito vector has been reported since 1995; DENV-1 local transmission was detected for the first time in 2009. No new autochthonous cases were detected again until 2016, when the worst dengue outbreak in decades occurred in the Americas (2,3).

The Study

During December 2015–April 2016, we confirmed 2,306 cases of human infection with DENV-1 in the virology laboratory at Hospital de Niños R. Gutiérrez. Most (84.69%) cases occurred during February and March. Patient ages ranged from 0 to 93 years (median 30 years). Of the 2,306 laboratory-confirmed cases, 76.7% of patients reported no recent travel history outside the Buenos Aires metropolitan area within 15 days before the onset of fever (local cases). The remaining cases had recently returned from dengue-affected areas (imported cases).

To characterize the outbreak, we sequenced the DENV-1 envelope glycoprotein (E) gene of 82 positive samples from local and imported cases (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/23/10/16-1718-Techapp1.pdf). We used 3 phylogenetic inference methods that determined that the 82 sequences belonged to DENV-1 genotype V (online Technical Appendix Figure 1). We detected 145 mutations in 143 polymorphic sites, and we found 42 sites that were negatively selected with >2 of the assayed methods. We found no positively selected sites. The selection analysis resulted in an overall dN/dS of 0.05, which is consistent with our previous work on the 2009 outbreak sequences, where the overall dN/dS ratio was also <1 (4).

We found 18 amino acid substitutions in the E protein. Using the Meta-CATS (metadata-driven comparative analysis tool for sequences) statistical analysis tool (5), we found that 4 of these substitutions (S338L, R394K, V428L, and V436I) divided our sequences into 2 groups (p<0.01): 1 was related to the 2009 Buenos Aires outbreak and the other to sequences from Brazil (2010–2013). Additionally, we found within the Brazil group a subgroup of 10 sequences containing unique substitutions D235E, K325R, and K361R (p<0.01). Amino acid substitutions are not located on reported epitope positions. Glycosylation sites Asn-67 and Asn-153 were conserved in all the sequences we analyzed.

We performed phylodynamic and phylogeographic analyses on a total of 198 DENV-1 genotype V E-protein gene sequences from the Americas (82 obtained in this study and the rest retrieved from the NCBI Dengue Virus Resource) to analyze the origin, dynamics, and temporal-spatial diffusion process of the 2016 outbreak. We inferred that the most recent common ancestor was located in the Caribbean, with the highest state probabilities in the British Virgin Islands (0.54) and Puerto Rico (0.35), by the end of 1975 (95% HPD 1972–1979). The mean rate of nucleotide substitution was 6.95 × 10⁻⁴ substitutions/site/year (95% HPD 5.87 × 10⁻⁴–8.11 × 10⁻⁴), similar to previous reports (6–8).

A maximum clade credibility tree revealed the co-circulation of 2 lineages in Buenos Aires during 2016, characterized by the 4 amino acid substitutions described (online Technical Appendix Figure 2). One of the lineages has an inferred origin in Venezuela around 1999 (95% HPD 1998–2004) and arrived in Argentina around 2007 (95% HPD 2006–2007). We found 43 sequences from the 2016 outbreak, along with sequences from the 2009 outbreak...
previously described by our laboratory, in this lineage. The inferred origin for the second lineage is the British Virgin Islands around 1984 (95% HPD 1982–1985), later arriving in Brazil around 1999 (95% HPD 1996–2000). This lineage comprises 39 sequences from the 2016 outbreak, arranged in 3 subclades originated during 2012–2015.

Conclusions
Our continuous work in DENV diagnosis, surveillance, and research enabled us to characterize the serologic status of the population of Buenos Aires in 2009. We found that an unusually high percentage of the population had secondary DENV infections in what was considered at the time a non-endemic area; therefore, we proposed that cryptic DENV circulation causing inapparent infections might be affecting this area (9). We also described the phylogenetic and phylogeographic characteristics of the first DENV-1 outbreak in 2009; the circulating virus clustered in a monophyletic group within genotype V, which is the most predominant DENV-1 genotype in the Americas (10). In this study, we found that the virus in the 2016 outbreak is also genotype V DENV-1; surprisingly, phylogenetic studies revealed that 2 lineages were circulating concurrently. Both identified lineages are related to sequences from different neighboring countries, and we observed no monophyletic groups local to Buenos Aires or other provinces of Argentina. The co-circulation of 2 DENV lineages was recently reported in Brazil (11,12).

Our data suggest that DENV-1 is established in Buenos Aires and that this densely populated area is changing from one with sporadic outbreaks to an endemic zone. Of note, other arboviruses transmitted by the same mosquito vector, such as Zika and chikungunya, caused autochthonous cases in northern provinces of Argentina in 2016. We believe that the Buenos Aires metropolitan area is now a susceptible area for the emergence of other DENV serotypes, as well as other viruses transmitted by the same vector. Public health authorities should develop stronger prevention and control strategies to avoid future arbovirus outbreaks.

Ethics Statement
These results are part of a study approved by the Medical Ethics and Research Committees of “Ricardo Gutiérrez” Children’s Hospital, Buenos Aires, Argentina (IRB No. 10.46). We did not obtain informed consent because patient information was anonymized and deidentified before analysis.

Acknowledgments
We thank Karina Zacarias, Mónica González, Oscar Luna, Marina Maldonado, Jorgelina Carusso, Estela Chacón, Mariana Campal, Patricia Rivero, Cristían Díaz, Cristina Álvarez López, Mercedes Altube, MaríaElina Acevedo, Oscar Jacquez, and Erica Grandis.

This research was supported by the Agencia Nacional de Promoción Científica y Tecnológica under grant PICT3446/2014 and by the Programa Nacional de Control de Dengue, Ministry of Health of Argentina.

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Mild Illness during Outbreak of Shiga Toxin–Producing Escherichia coli O157 Infections Associated with Agricultural Show, Australia

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During a large outbreak of Shiga toxin–producing Escherichia coli illness associated with an agricultural show in Australia, we used whole-genome sequencing to detect an IS1203v insertion in the Shiga toxin 2c subunit A gene of Shiga toxin–producing E. coli. Our study showed that clinical illness was mild, and hemolytic uremic syndrome was not detected.

Shiga toxin–producing Escherichia coli (STEC) is a major cause of serious human gastrointestinal illness and has the potential to cause life-threatening complications, such as hemolytic uremic syndrome (HUS) (1). An average of 0.4 cases of STEC illness per 100,000 persons per year are reported to public health authorities in Australia (2). Disease severity can range from asymptomatic infection to serious and sometimes fatal illness, particularly in young children and the elderly (3,4).

Healthy ruminants, particularly cattle, are the reservoir for STEC (5). Human infection with STEC usually occurs as a result of inadvertent ingestion of fecal matter after consumption of contaminated food, water, or unpasteurized milk; contact with animals or their environments; or secondarily, through contact with infected humans (4,5). In the largest previously reported outbreak of STEC illness in Australia in 1995, which was associated with consumption of mettwurst (uncooked, semidy, fermented sausages), HUS developed in 23 of the 51 case-patients identified, and there was 1 death (6).

The Study
A multidisciplinary incident management team was established to investigate an outbreak of STEC illness associated with an annual agricultural show in Brisbane, Queensland, Australia, in August 2013 (online Technical Appendix). Shiga toxin gene subtyping and whole-genome sequencing (WGS) analysis was performed (online Technical Appendix). The incident management team defined primary and secondary outbreak cases (online Technical Appendix). Persons with laboratory-confirmed STEC infection associated with the outbreak and their household contacts were followed up until the point of microbiological evidence of clearance, which was defined as 2 consecutive negative stool samples collected ≥24 h apart (7).

Case-patients and contacts with a high risk for transmission (persons <5 years of age; persons unable to maintain good hygiene; or childcare, healthcare, aged care, or food preparation workers) were advised to avoid childcare and work settings in accordance with Queensland Health Guidelines (7). Enhanced surveillance measures were implemented to assist with case finding (online Technical Appendix). Medical practitioners were requested to avoid use of antimicrobial drugs for suspected case-patients with STEC infections because of previously reported associations between antimicrobial drug use and HUS (online Technical Appendix).

We developed a case–control study to obtain additional information related to animal contact, hand hygiene, and food consumption at the agricultural show (online Technical Appendix). We analyzed data by using Epi Info 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) (online Technical Appendix).

STEC identified from human, environmental, and animal samples were serotyped for O and H antigens (online Technical Appendix). Expression of Shiga toxin 1 (stx1) and stx2 genes was determined for selected isolates (online Technical Appendix). Shiga toxin gene subtyping and whole-genome sequencing (WGS) analysis was performed (online Technical Appendix).

During August 21–September 27, 2013, we identified 57 outbreak-associated laboratory-confirmed case-patients with STEC infection: 54 confirmed primary case-patients, 1 probable primary case-patient, and 2 secondary case-patients (Figure 1). Of the 57 case-patients, 32 (56%) were...
female. Case-patients ranged in age from 1 to 77 (median 9) years; 31 (56%) case-patients were <10 years of age. Median incubation period after attending the agricultural show was 4 (range 1–11, 25th–75th percentile 3–5) days.

Case-patients reported diarrhea (96%), abdominal pain (93%), bloody diarrhea (41%), and fever (32%) (Table 1). Seven case-patients were hospitalized. No cases of HUS or deaths were reported.

Public Health Units followed up 40 case-patients until microbiological evidence of clearance; the remaining case-patients were lost to follow-up. Median duration of STEC excretion among primary case-patients was 18 (range 2–52) days (Table 2). After 27 days and 6 recurrent stools positive for STEC, and after acute diarrheal illness had resolved, 1 child was given azithromycin on day 40 for 3 days to hasten decolonization. Two consecutive stool specimens obtained >48 h after treatment with antimicrobial drugs was stopped were negative for STEC in this child. This patient did not have any adverse effects from azithromycin treatment.

Forty-four of 55 primary case-patients and 28 household contacts who attended the agricultural show were included in the case–control study. Median age of case-patients was 8 (range 1–77) years, and median age of controls was 38 (range 1–70) years.

We showed by using univariate analysis that case-patients were not more likely than controls to have entered the animal nursery at the show. Case-patients were more likely than controls to have had contact with lambs or goats, fed the animals, or had their hands licked by animals (online Technical Appendix).

We identified the same multilocus variable number tandem repeat and stx subtype genotype of E. coli O157:H7 in human case-patients, animal bedding from the animal nursery before disposal, and fecal samples collected from lambs, goats, and calves (online Technical Appendix). WGS and read mapping to an E. coli O157 reference genome showed that of the human, animal, and environmental isolates analyzed, all contained an IS1203 v insertion that resulted in deletion of the first 494 bp of the stx2c subunit A gene (Figure 2). Expression of stx2 was not detected in these isolates by Immunocard STAT! EHEC (Meridian Bioscience, Cincinnati, OH, USA) and Shiga toxin Quik Chek (Alere, Waltham, MA, USA) lateral flow devices. No additional stx2 genes were identified, and no disruptions were detected in the stx1 gene regions of any of the isolates.

Table 1. Frequency of symptoms among persons, by age group, with cases of Shiga toxin–producing Escherichia coli illness associated with an agricultural show, Brisbane, Queensland, Australia, 2013

<table>
<thead>
<tr>
<th>Symptom (self reported)</th>
<th>Age group, y, no. positive/no. responded (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All 1–4</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>48/50 (96)</td>
</tr>
<tr>
<td>Bloody diarrhea*</td>
<td>19/46 (41)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>37/40 (93)</td>
</tr>
<tr>
<td>Fever</td>
<td>14/44 (32)</td>
</tr>
</tbody>
</table>

*Older children and adults were significantly more likely to report bloody diarrhea than children <5 years of age (p = 0.001 by χ² test for linear trend).
We found that STEC infection was associated with feeding lambs or goats, feeding animals, and having the hands licked by animals. The course of *E. coli* O157 infection was relatively mild; no cases of HUS were associated with this outbreak. Heiman et al. found that of 4,928 cases of 390 *E. coli* O157 illness outbreaks in the United States during 2003–2012, HUS was detected in 299 cases (6% of illnesses) (8). HUS cases with stx1/stx2–*E. coli* O157 isolates have been reported (9,10). We speculate that the absence of severe complications in this outbreak might have been caused, in part, by disruption of the stx2 subunit A gene by the IS1203v insertion, which resulted in lack of expression or a nonfunctional Stx2 toxin.

The proportion of case-patients reporting bloody diarrhea (19/46, 41%) was also lower than previously reported. Ethelberg et al. reported that 69% (56/81) of case-patients in Denmark infected with *E. coli* O157 had bloody diarrhea (11). A recent retrospective cohort study from England reported that 61% (2,027/3,323) of symptomatic case-patients infected with *E. coli* O157 had bloody diarrhea. Bloody diarrhea was reported to be a risk factor for HUS (odds ratio 2.10; p = 0.001) (12). In the outbreak we studied, children <5 years of age were less likely than older children and adults to report bloody diarrhea. STEC infection should be actively considered for young children with nonbloody diarrhea who were exposed to potential sources of STEC.

In this outbreak, 1 child was given azithromycin for 3 days to hasten decolonization some weeks after the acute diarrheal illness had resolved. Antimicrobial drugs are generally not recommended to hasten STEC decolonization because of major associations with HUS (13). Recommendations to avoid high-risk activities (such as childcare attendance) might place a major socioeconomic burden on STEC carriers and their families. Further studies are required to assess whether WGS can provide useful information for safe administration of antimicrobial drugs for treatment of acute illness caused by STEC, or when chronic shedding becomes established.

Our comprehensive study of a large outbreak *E. coli* O157 illness, characterized by an IS1203v insertion disrupting the stx2c subunit A gene, showed mild clinical illness and an absence of HUS. Further characterization by virulence studies on isolates with this stx2c subunit A gene disruption might provide further insights into the mild illness caused by this strain.

### Acknowledgments
We thank Clare Nourse and Joshua Francis for reviewing draft versions of this article and providing advice about case management; staff at the Metro North, Metro South, Gold Coast, and West Moreton Public Health Units for public health management of STEC cases; Metro North environmental health officers for site investigations of the agricultural show; Queensland Forensic and Scientific services for testing human and animal samples; Biosecurity Queensland inspectors and veterinary officers for performing field work for animal investigation; the Biosecurity Services Laboratory for testing animal samples; and the Royal National Agricultural and Industrial Association of Queensland for assistance with public health investigations.

Dr. Vasant is a public health physician at Queensland Health, Brisbane, Queensland, Australia. Her research interests include public health management of communicable and noncommunicable diseases, health of indigenous and minority communities, and public health response to climate change.

### References
In August and September 1967, an outbreak of a viral hemorrhagic fever occurred among laboratory workers in Marburg and Frankfurt, Germany, and Belgrade, Yugoslavia (now Serbia) who were processing kidneys from African green monkeys that had been imported from Uganda. (These kidneys were used in the production of polio vaccine.) Of 25 primary and 6 secondary cases, 7 were fatal.

A new virus, named Marburg virus, was isolated from patients and monkeys, and the high case-fatality ratio called for the best biocontainment of the day. The Centers for Disease Control and Prevention (CDC) borrowed a mobile containment laboratory from the National Institutes of Health and set it up in the CDC parking lot; it provided approximately biosafety level 2+ containment. A cell culture propagation of Marburg virus, from original monkey kidney cell culture propagation done at CDC in 1967, magnification ≈40,000x. Image courtesy of Frederick A. Murphy.

Sources

Enterovirus D68–Associated Acute Flaccid Myelitis in Immunocompromised Woman, Italy

Emanuela Giombini, Martina Rueca, Walter Barberi, Anna Paola Iori, Concetta Castilletti, Paola Scognamiglio, Francesco Vairo, Giuseppe Ippolito, Maria Rosaria Capobianchi, Maria Beatrice Valli

In Italy in 2016, acute flaccid myelitis developed in a woman who had received a hematopoietic stem cell transplant. Enterovirus D68 viral genome was detected in respiratory and cerebrospinal fluid samples, and the viral protein 1 sequence clustered with lineage B3. Immunocompromised adults may be at risk for enterovirus D68–associated neurologic complications.

Enteroviruses are the most common viruses circulating worldwide and lead to a broad spectrum of clinical illnesses: respiratory infection; hand, foot, and mouth disease; acute and chronic cardiac disease; meningitis; and encephalitis (1). Originally isolated in 1962 from respiratory specimens of children with severe pneumonia, enterovirus serotype D68 (EV-D68), formerly classified as rhinovirus 87, belongs(631,588),(682,598) to the enterovirus Dspecies (2). EV-D68 infection typically causes mild respiratory illness but occasionally may progress to more severe clinical syndromes (pneumonia, hepatitis, cardiomyopathy, and acute neurologic diseases including aseptic meningitis and polio-like paralytic disease).

Since its initial identification, EV-D68 has been rarely identified, but more recently, it has become increasingly recognized in the context of enhanced surveillance for polio-like diseases (3). The extent of EV-D68 circulation is underestimated because of the scarcity of laboratories equipped to detect it and poor awareness among physicians. However, in the past decade, EV-D68 has emerged as a major respiratory pathogen, especially in children (1,4). Moreover, concurrent with the unprecedented respiratory outbreak of EV-D68 in North America in 2014, an apparent increased incidence of acute flaccid myelitis (AFM), consistent with polio-like illness, has been reported in several US states (5). These neurologic cases have been temporally associated with EV-D68 infection, although virus sequences were detected almost exclusively in respiratory specimens. Concurrently, EV-D68 was circulating in Europe, where the disease burden was more moderate than in the United States (4). Indeed, EV-D68 has been only rarely detected in the cerebrospinal fluid (CSF) of children with neurologic involvement (6,7).

A direct causative role of the virus in neurologic disease needs further evidence. We report a fatal case of EV-D68 infection and AFM in an adult recipient of a hematopoietic stem cell transplant (HSCT).

The Case

In October 2016, a 55-year-old woman was admitted to the emergency unit of the Hematology Department of Policlinico Umberto I (Rome, Italy) for sudden acute weakness and limited mobility of the left arm. The woman had no history of preexisting neurologic disease or recent travel, but she had had follicular non-Hodgkin B-cell lymphoma since 2011, which evolved into a diffuse large B-cell lymphoma despite immunochemotherapy and corticosteroid treatment. She underwent 2 HSCTs: 1 autologous in 2011 and 1 allogeneic in 2013. Despite treatment with cyclosporine and methotrexate, acute graft-versus-host disease with liver and gut involvement (grade IV) developed and was treated with high-dose corticosteroids and extracorporeal photopheresis. Although the graft-versus-host disease initially evolved into a severe chronic form, 1 year after allogeneic HSCT, the patient’s clinical condition gradually improved, and the immunosuppressive therapy was slowly reduced. At the 2016 hospital admission, she was receiving only a minimal dose of corticosteroids (prednisone 10 mg/d) and mycophenolate (750 mg/d).

A few days before onset of neurologic illness, the patient had had mild fever without respiratory or gastrointestinal symptoms. At admission, she had no fever, and her lymphocyte count was within reference range (5.25 × 10⁹ cells/L). A few hours after admission, muscle weakness extended to the right arm, neck, and head and evolved into tetraplegia with proximal muscle involvement. No deficits of superficial sensitivity or dysphagia were recorded. Initial therapy at admission was acyclovir, trimethoprim/sulfamethoxazole, and ciprofloxacin. CSF analysis was consistent with aseptic meningitis with pleocytosis (130

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DOI: https://doi.org/10.3201/eid2310.170792
leukocytes/mm³, 92.6% mononucleocytes), mildly elevated glucose level (86 mg/dL), and protein concentrations within reference range (34 mg/dL). Magnetic resonance images of the brain and spinal cord (Figure 1) showed a bulging spinal tract rope at C2–D1 with hyperintensity in the central region, which increased after administration of gadolinium, involving mainly the medullar anterior horn and sparing the posterior columns and lateral and ventral fins. The findings fulfilled the US Centers for Disease Control and Prevention and the California Public Health Department criteria for AFM (8). The images showed no signs of white matter involvement.

Electromyograms showed signs of C4–C7 horn abnormality, more evident on the left side, with absence of F wave at the upper limbs. The patient’s neurologic function rapidly worsened to include respiratory muscles. Intubation and ventilator support failed to improve respiratory function, and the patient died 14 days after symptom onset.

Clinical specimens (CSF, blood, oropharyngeal swab, and feces) collected 3 days after admission were sent to the regional reference laboratory in Rome (National Institute for Infectious Diseases “Lazzaro Spallanzani”), where the CSF was immediately analyzed by FilmArray ME (bioMérieux, Marcy l’Etoile, France), a molecular test that can rapidly detect many neurotropic pathogens; the test was positive for enteroviruses only. To confirm the enterovirus diagnosis, a panel of molecular and serologic tests was performed. Enterovirus genomes were detected by a commercial reverse transcription quantitative PCR (REALQUALITY RQ-ENTERO; AB-Analatica, Padova, Italy) in CSF and oropharyngeal swab samples (cycle thresholds 36.61 and 34.94, respectively), but not in feces, consistent with the fact that respiratory samples are the best diagnostic specimens for this enterovirus. Both samples also produced positive results in 2 laboratory-developed reverse transcription PCRs targeting the 5′ untranslated region and viral protein 1 (VP1) (9). Screening of CSF for the presence of genomes of other known neurotropic viruses (flaviviruses, cytomegalovirus, Epstein-Barr virus, herpes simplex viruses 1 and 2, varicella zoster virus), and serologic tests (for coxsackieviruses, adenoviruses, polioviruses) yielded negative results.

The amplicons targeting the 5′ untranslated region and VP1 were sequenced by using the Sanger method. The genus/species classification of the enterovirus genotype as D68 was based on the web-based open-access Enterovirus Genotyping Tool version 0.1 (10). Phylogenetic analysis of VP1 was performed in the context of 918 EV-D68 worldwide sequences retrieved from GenBank. Clades were assigned according to Tokarz et al. (3). In the resulting maximum-likelihood tree (Figure 2), the virus involved in this case clustered with the recently described subclade B3, comprising also the viral sequences of the VP1 gene involved in the 2016 epidemic in the Netherlands (4); the second closest subclade was B1, which includes EV-D68 strains from respiratory specimens of patients with AFM from the 2014 outbreak in North America (11).

**Conclusions**

Since the large outbreak in 2014, EV-D68 infection has been recognized as a potential threat to patients with hematologic malignancies, especially HSCT recipients.
Evidence that infections for these patients could be associated with severe respiratory disease is increasing (4,12). As for other enteroviruses, infection with EV-D68 may be associated with neurologic features; and risk for severe illness is highest among children, elderly persons, and adults with underlying immune-compromising conditions. However, evidence addressing whether the EV-D68 infection is an incidental finding or a newly emerging cause of AFM is limited. The case reported here is 1 of only a handful of cases in which the EV-D68 genome was evidenced in CSF and not only in respiratory specimens, suggesting a possible causal association between EV-D68 and neurologic disease in adults. Recent findings in a mouse model provide evidence of a specific tropism of EV-D68 for spinal cord motor neurons, suggesting that direct viral injury, rather than a postinfection immune-mediated process, is the most likely mechanism of neuronal cell loss and paralysis (13). The phylogenetic analysis indicates that the strain detected in the patient described here, like one recently detected in a child with neurologic illness (14), is genetically linked to those involved in the recent outbreak in the Netherlands (4).

Immunocompromised adults, as well as children, may be at risk for neurologic complications from EV-D68 infection (15). This consideration, adding to the upsurge of recognized respiratory EV-D68 infections (4) and the scarcity of respiratory virus screening tests able to detect enteroviruses, highlights the value of including EV-D68 in the differential diagnosis for respiratory and neurologic complications in immunosuppressed patients (12).
Acknowledgment
We gratefully acknowledge the editing of the manuscript by Peter De Muro.

This study was supported by the Italian Ministry of Health, Ricerca Corrente fund, and was partially supported by Lazio Innova project FILM-E.

Dr. Giombini is a PhD researcher at the National Institute for Infectious Diseases “L. Spallanzani,” Rome, Italy. She has longstanding expertise in bioinformatics tools and phylogenesis and focuses her research on virus evolution and molecular epidemiology.

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Diagnosis of Fatal Human Case of St. Louis Encephalitis Virus Infection by Metagenomic Sequencing, California, 2016

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We used unbiased metagenomic next-generation sequencing to diagnose a fatal case of meningoencephalitis caused by St. Louis encephalitis virus in a patient from California in September 2016. This case is associated with the recent 2015–2016 reemergence of this virus in the southwestern United States.

St. Louis encephalitis virus (SLEV), in the Flaviviridae family, is an infectious RNA virus transmitted by Culex spp. mosquitoes (1,2). Clinical manifestations range from mild febrile illness to fatal neurologic disease. According to recent reports (3,4), SLEV reemerged in the summer of 2015 in California and Arizona, USA, after a documented 11-year absence of activity in California.

In June 2016, we launched a multi-institutional clinical study titled Precision Diagnosis of Acute Infectious Diseases (PDAID). This 1-year study aimed to enroll 300 patients to evaluate the clinical utility of a metagenomic next-generation sequencing (mNGS) assay for diagnosing infectious causes of meningitis and encephalitis from patient cerebrospinal fluid (CSF) samples (5,6). The mNGS assay uses an unbiased sequencing approach to comprehensively identify pathogens (viruses, bacteria, fungi, and parasites) in clinical samples (7). We report a fatal human case of SLEV infection diagnosed by mNGS in a PDAID study patient from California.

The Case
The case-patient was a 68-year-old man with a history of coronary artery disease, hypertension, and mantle cell lymphoma treated with 4 cycles of chemotherapy and granulocyte colony stimulating factor. He sought medical care at the end of August 2016 having had 2 days of fever (up to 39.4°C), chills, lethargy, and confusion. He had fallen twice because of dizziness and reported shortness of breath, cough, and new-onset urinary incontinence.

The patient was a retired oil-field worker living with his wife in Bakersfield, Kern County, California. He owned 1 dog and had frequent contact with his 10 grandchildren. His travel history was notable for a trip to “the mountains” in late April 2016 (Payson, Arizona, elevation 1,500 m).

The patient was admitted to the hospital in early September 2016. An initial workup, including magnetic resonance imaging of the brain, was unrevealing. Empirical therapy with vancomycin, meropenem, and levofloxacin was started after lung imaging revealed inflammatory pneumonitis. On hospitalization day 3, the patient became acutely hypoxic with worsening altered mental status (AMS), and he was intubated and transferred to the intensive care unit. A lumbar puncture revealed CSF pleocytosis (18 leukocytes/mm³; 35% monocytes, 33% lymphocytes, and 32% neutrophils); glucose and protein were within reference ranges. Empiric antibiotic therapy was continued, and acyclovir and antifungal therapy were added to his regimen. Repeat lumbar puncture performed on hospital day 9 showed persistent pleocytosis. All microbiologic test results for CSF, blood, and bronchoalveolar lavage were negative (Table 1), as was a workup for noninfectious causes (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/10/16-1986-Techapp1.pdf).

After enrolling the patient in the PDAID study, we analyzed CSF from hospitalization day 9 by mNGS testing at University of California, San Francisco (online Technical Appendix) (8). RNA and DNA sequencing libraries from CSF yielded 8,056,471 and 9,083,963 sequence reads, respectively. In the RNA library, 236,615 (2.9%) of the reads were identified as SLEV by using the SURPI+ (sequence-based ultra-rapid pathogen identification) computational pipeline (7), with recovery of 99.4%
Table 1. Microbiologic testing results for a patient with fatal mosquito-borne St. Louis encephalitis virus infection diagnosed by metagenomic sequencing, California, 2016*  

<table>
<thead>
<tr>
<th>Test</th>
<th>Hospitalization day sample collected</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial cultures</td>
<td>0, 2, 4, 7</td>
<td>Negative</td>
</tr>
<tr>
<td>Fungal cultures</td>
<td>0, 2, 4, 7</td>
<td>Negative</td>
</tr>
<tr>
<td>Mycobacterial culture</td>
<td>10</td>
<td>Negative</td>
</tr>
<tr>
<td>Aspergillus antigen EIA</td>
<td>5</td>
<td>Negative</td>
</tr>
<tr>
<td>Adenovirus PCR</td>
<td>19</td>
<td>Negative</td>
</tr>
<tr>
<td>CMV DNA quantitative PCR</td>
<td>4, 12</td>
<td>Negative</td>
</tr>
<tr>
<td>EBV DNA quantitative PCR</td>
<td>10</td>
<td>Negative</td>
</tr>
<tr>
<td>Enterovirus RNA</td>
<td>19</td>
<td>Negative</td>
</tr>
<tr>
<td>HSV-1 and HSV-2 PCR</td>
<td>12</td>
<td>Negative</td>
</tr>
<tr>
<td>HHV-6 PCR</td>
<td>19</td>
<td>Negative</td>
</tr>
<tr>
<td>HIV RNA quantitative PCR</td>
<td>5</td>
<td>Negative</td>
</tr>
<tr>
<td>HSV DNA quantitative PCR</td>
<td>9</td>
<td>Negative</td>
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<tr>
<td>Leptospira DNA</td>
<td>14</td>
<td>Negative</td>
</tr>
<tr>
<td>Parvovirus B19 DNA</td>
<td>19</td>
<td>Negative</td>
</tr>
<tr>
<td>VZV DNA, qualitative PCR</td>
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<td>Negative</td>
</tr>
<tr>
<td>Cryptococcal antigen</td>
<td>5</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>CSF studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV 1 and 2 PCR</td>
<td>3</td>
<td>Negative</td>
</tr>
<tr>
<td>Fungal culture</td>
<td>3</td>
<td>Negative</td>
</tr>
<tr>
<td>Bacterial culture</td>
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</tr>
<tr>
<td>Coccioides Ab CF, ID</td>
<td>8</td>
<td>Negative</td>
</tr>
<tr>
<td>CMV PCR</td>
<td>9</td>
<td>Negative</td>
</tr>
<tr>
<td>EBV PCR</td>
<td>3, 9</td>
<td>Negative</td>
</tr>
<tr>
<td>HHV-6 PCR</td>
<td>3</td>
<td>Negative</td>
</tr>
<tr>
<td>JC polyomavirus DNA, PCR</td>
<td>9</td>
<td>Negative</td>
</tr>
<tr>
<td>Mycobacterial culture</td>
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<tr>
<td>Mycobacterium tuberculosis DNA PCR</td>
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<tr>
<td>Meningoencephalitis antibody panel†</td>
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<td>VDRL</td>
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<td>VZV Ab IgG</td>
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<tr>
<td>West Nile virus RNA</td>
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<td>Negative</td>
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<tr>
<td>mNGS for pathogen detection</td>
<td>9</td>
<td>SLEV</td>
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<tr>
<td><strong>Respiratory secretion testing‡</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>4, 5, 8</td>
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</tr>
<tr>
<td>Fungal culture</td>
<td>5, 8</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Respiratory virus panel§</td>
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<tr>
<td>Mycoplasma pneumoniae PCR</td>
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<td>HSV-1 and HSV-2 PCR</td>
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<td>Negative</td>
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<tr>
<td>CMV PCR</td>
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<td>Negative</td>
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<tr>
<td>Pneumocystis DFA</td>
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<tr>
<td>Mycobacterial culture</td>
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<td>Legionella culture and urinary Ag</td>
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<td>Negative</td>
</tr>
<tr>
<td>Nocardia culture</td>
<td>8</td>
<td>Negative</td>
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<tr>
<td><strong>Serologic testing</strong></td>
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<tr>
<td>Coccioides IgG/IgM</td>
<td>4, 9</td>
<td>Negative</td>
</tr>
<tr>
<td>Coccioides complement fixation</td>
<td>8</td>
<td>Negative</td>
</tr>
<tr>
<td>HCV Ab</td>
<td>9</td>
<td>Negative</td>
</tr>
<tr>
<td>HBV, core Ab and hepatitis B e Ab</td>
<td>9</td>
<td>Negative</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis quantifieron gold</td>
<td>8</td>
<td>Negative</td>
</tr>
<tr>
<td>Q fever antibody</td>
<td>8</td>
<td>Negative</td>
</tr>
<tr>
<td>Rapid plasma reagin</td>
<td>10</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Ab, antibody; Ag, antigen; BAL, bronchoalveolar lavage; CF, complement fixation; CMV, cytomegalovirus; DFA, direct fluorescent antigen; DNA, deoxyribonucleic acid; EBV, Epstein-Barr virus; EIA, enzyme immunoassay; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV-6, human herpesvirus 6; HIV, human immunodeficiency virus, HSV, herpes simplex virus; ID, immunodiffusion; IFA, indirect fluorescent antibody; mNGS, metagenomic next-generation sequencing; NP, nasopharyngeal; RNA, ribonucleic acid; SLEV, St. Louis encephalitis virus; VRDL, Venereal Disease Research Laboratory; VZV, varicella zoster virus.

†Includes IgG and IgM testing for West Nile virus; California encephalitis virus; Eastern equine encephalitis virus; St. Louis encephalitis virus; Western equine encephalitis virus; lymphocytic choriomeningitis virus; herpes simplex virus types 1 and 2 (HSV-1 and 2); adenovirus; influenza A; influenza B; measles (IFA); mumps (IFA); varicella-zoster Ab CF; coxsackie A types 2, 4, 7, 9, 10, and 16; coxsackie B types 1, 2, 3, 4, 5, and 6; echovirus types 4, 7, 9, 11, and 30; and CMV.

‡Testing performed on bronchoalveolar lavage unless noted otherwise.

§Detects the following viruses: influenza A; influenza A H1 seasonal; influenza A H3 seasonal; influenza A 2009 H1N1; influenza B; respiratory syncytial virus A and B; parainfluenza viruses 1–4; human metapneumovirus; human rhinovirus; adenovirus serogroups C and B/E; coronaviruses NL63, HKU1, 229E, and OC43.
of the predicted 10,936-bp virus genome. Subsequent mNGS testing of the patient’s CSF from hospitalization day 3 also was positive for SLEV.

The patient’s SLEV genome sequence was >99% identical with previously sequenced 2014–2015 SLEV isolates from mosquitoes in California and Arizona (4). Phylogenetic analysis placed the patient’s strain in a cluster containing these isolates and viruses sequenced from mosquitoes in Argentina in 1978 and 2005 (9) (Figure). The patient’s SLEV was genetically distinct from the 2003 Imperial Valley strain that had been circulating in California before an 11-year absence (12), suggesting that he was infected by the 2015–2016 reemergent genotype currently circulating in the southwestern United States (3,4). Furthermore, the patient’s SLEV genome was closely related to a strain sequenced from a mosquito pool collected in June 2016 from Kern County (Figure, panel A), with 99.9% pairwise nucleotide identity and only 5 single-nucleotide variants across the genome.

After extensive discussion with his wife regarding the patient’s SLEV diagnosis and poor prognosis, the patient was transitioned to comfort care on hospitalization day 23 and died the following day. Autopsy revealed residual mantle cell lymphoma and bronchopneumonia consistent with infection or chemical pneumonitis from aspiration. The diagnosis of SLEV meningoencephalitis was subsequently confirmed by positive reverse transcription PCR and virus culture testing from multiple laboratories (Table 2). However, follow-up testing for SLEV from the patient’s CSF and serum was negative.

Conclusions
We present a case of SLEV infection in an elderly immunocompromised patient hospitalized with fever and AMS.
and who experienced critical respiratory failure. Most SLEV infections are asymptomatic; when infections are symptomatic, clinical features include fever, lethargy, and confusion (1), with potential complications including sepsis, gastrointestinal hemorrhage, pulmonary embolism, and bronchopneumonia. In hindsight, SLEV infection is consistent with the patient’s clinical presentation, with pneumatic and respiratory decompensation possibly resulting from aspiration during the patient’s AMS from viral meningonecephalitis. Deaths from SLEV infection during the first 2 weeks are generally from encephalitis, whereas later deaths are more often caused by complications of hospitalization, such as this patient’s bronchopneumonia.

Routine diagnosis of SLEV is challenging because serologic testing is only performed by specialized reference laboratories, the period of viremia is brief, and molecular testing by reverse transcription PCR is not widely used. Clinicians in California might fail to consider SLEV when examining a patient with nonspecific febrile illness, especially given the lack of virus or disease activity in the state during 2004–2015. Antibody testing can be complicated by the absence of seroreactivity in elderly and immunocompromised patients, as observed in the case of this patient (Table 1), as well as potential cross-reactivity with other flavivirus infections, such as dengue, Zika virus, and West Nile virus (3).

The identification of SLEV infection in CSF by using a panpathogen metagenomic sequencing assay is another demonstration of the power of an unbiased approach to infectious disease testing (5–7), although challenges remain with respect to test availability, interpretation, and validation (8). No antiviral therapy for SLEV has been proven to be efficacious, although interferon-α has been tried (7). With a laboratory sample-to-reporting time of 4 days, earlier sample submission might have spared our patient from the side effects of antimicrobial drug therapy, costly laboratory testing, and invasive procedures. Importantly, the family obtained reassurance and closure from communication of an established diagnosis.

During summer 2016, SLEV was reported in mosquitoes from 7 counties in California, including Kern County, where the patient resided (4). According to his wife, the patient often sat outdoors during the few weeks before hospitalization, although she did not recall his reporting any mosquito bites. Nevertheless, we believe he most likely contracted SLEV in California, because his history of travel to Arizona 5 months prior was not consistent with the incubation period of the disease (4–21 days); mosquitoes are less prevalent at the higher altitudes of Payson, Arizona; and the patient’s SLEV sequence was most closely related to a strain from a June 2016 Kern County mosquito pool. Given the reemergence of SLEV in the southwestern United States, clinicians from affected areas will need to maintain a high index of suspicion for this disease, particularly during local community outbreaks or high SLEV activity detected through mosquito surveillance efforts.

Acknowledgments

We thank staff at the California Department of Health for their assistance in this study, including Kristina Hsieh and Artem Muradyan for validating and performing the SLEV reverse transcription PCR assay and Chris Preas and Peter Patiris for performing IgM and plaque-reduction neutralization serologic tests for SLEV and West Nile virus. We also thank the Centers for Disease Control and Prevention for confirmatory testing of the patient sample for SLEV.

The study was supported by the California Initiative to Advance Precision Medicine (C.Y.C., H.A.S., M.R.W., S.M.); an award from Abbott Laboratories, Inc. (C.Y.C.); and philanthropic grants from the Schwab Foundation (C.Y.C.) and the Sandler and William K. Bowes Foundations (C.Y.C., H.A.S., M.R.W., S.M.).

Table 2. Results of follow-up confirmatory testing for SLEV after mNGS result for a patient with fatal mosquito-borne St. Louis encephalitis virus infection diagnosed by metagenomic sequencing, California, 2016*

<table>
<thead>
<tr>
<th>Test (hospital day sample collected)</th>
<th>Laboratory</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLEV, RT-PCR (9)</td>
<td>UCSF research lab</td>
<td>Positive</td>
</tr>
<tr>
<td>SLEV, RT-PCR (3,9)</td>
<td>CDPH</td>
<td>Positive</td>
</tr>
<tr>
<td>SLEV, RT-PCR (3,9)</td>
<td>CDC</td>
<td>Positive</td>
</tr>
<tr>
<td>Viral culture (3,9)</td>
<td>CDPH</td>
<td>Positive, confirmed as SLEV by RT-PCR</td>
</tr>
<tr>
<td>SLEV, IgG/IgM antibody (3,9)</td>
<td>Quest Diagnostics</td>
<td>Negative, &lt;1:10</td>
</tr>
<tr>
<td>SLEV, PRNT for neutralizing antibodies (9)</td>
<td>CDPH</td>
<td>Negative, &lt;1:10</td>
</tr>
<tr>
<td>WNV, IgM</td>
<td>CDPH</td>
<td>Negative, &lt;1:10</td>
</tr>
<tr>
<td>WNV, PRNT for neutralizing antibodies (9)</td>
<td>CDPH</td>
<td>Negative, &lt;1:10</td>
</tr>
<tr>
<td>Serum studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLEV, RT-PCR (23)</td>
<td>CDPH</td>
<td>Negative</td>
</tr>
<tr>
<td>SLEV, IgM antibody (23)</td>
<td>CDPH</td>
<td>Negative, &lt;1:10</td>
</tr>
<tr>
<td>WNV, IgM antibody (23)</td>
<td>CDPH</td>
<td>Negative, &lt;1:10</td>
</tr>
<tr>
<td>SLEV, PRNT for neutralizing antibodies (23)</td>
<td>CDPH</td>
<td>Negative, &lt;1:10</td>
</tr>
<tr>
<td>WNV, PRNT for neutralizing antibodies (23)</td>
<td>CDPH</td>
<td>Borderline positive, 1:10 (normal &lt;1:10)</td>
</tr>
</tbody>
</table>

*Tests were performed after mNGS testing of patient CSF was positive from aliquots collected on hospital days 3 and 9. CDPH, California Department of Public Health; CSF, cerebrospinal fluid; mNGS, metagenomic next-generation sequencing; PRNT, plaque-reduction neutralization testing; RT-PCR, reverse transcription PCR; SLEV, St. Louis encephalitis virus; UCSF, University of California, San Francisco; WNV, West Nile virus.
C.Y.C. is the director of the UCSF–Abbott Viral Diagnostics and Discovery Center and receives research support from Abbott Laboratories, Inc. C.Y.C., S.N.N., and S.M. are co-inventors of the sequence-based ultra-rapid pathogen identification computational pipeline (known as SURPI+) and associated algorithms; a patent pending for SURPI+ has been filed by the University of California, San Francisco.

Dr. Chiu is an associate professor at the University of California, San Francisco, who heads a translational research laboratory focused on clinical metagenomic assay development for infectious diseases and characterization of emerging outbreak viruses.

References

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We identified Usutu virus (USUV) RNA in 6 pools of mosquitoes trapped in northern Israel during 2014–2015. These Israeli strains were most similar to strains identified in Senegal and Germany, which further elucidates common ancestry and evolutionary dynamics of USUV. Our findings suggest that human infection with USUV might occur in Israel.

Usutu virus (USUV) is a vectorborne flavivirus first isolated in South Africa in 1959 (1). The reservoirs for USUV, which are similar to those for West Nile virus (WNV), include numerous species of birds, and it is transmitted primarily by Culex spp. mosquitoes (2). Circulation of USUV has been reported in several countries in Africa, and 2 human cases of infection were identified in Africa in 1981 and 2004 (3). Since 1996, when USUV was first detected outside Africa in a blackbird in Italy, the virus has been identified in mosquitoes and birds in several countries in Europe (4). In recent years, there has been increasing evidence for human infection with USUV (4), and USUV antibodies and USUV RNA have been detected in blood donors (5,6).

A study published in 2016 characterized USUV phylogeny on the basis of available USUV strains from Africa and Europe (7). This study suggested that multiple introductions of USUV into central Europe from Africa could be confirmed and that Senegal was a possible origin for epizootics in central Europe.

WNV is the only flavivirus detected in Israel. WNV circulates in mosquitoes and birds in this country and was responsible for ≈1,400 cases of human infections during 2000–2012 (8). Because Israel is located on a central bird migration path between Africa and Eurasia (9), circulation of USUV in this area is also plausible. We used the WNV national mosquito surveillance system in Israel as a source for identification of USUV strains circulating in mosquitoes in Israel during 2014–2015.

The Study
During 2014–2015, a total of 53,890 mosquitoes were trapped and grouped into 1,471 pools of ≤50 mosquitoes/pool. We tested RNA extracted from mosquito pools for USUV RNA by using quantitative reverse transcription PCR and specific primer–probe sets for the USUV non-structural protein 5 (NS5) gene as described (10). We detected USUV RNA in 6 pools, 5 from 2015 and 1 from 2014. Although mosquito specimens were collected from trapping sites throughout Israel, all USUV RNA–positive pools were captured in northern Israel (Figure 1) and belonged to 3 mosquito species (Table).

We then performed amplification of an 845-nt sequence containing part of the envelope, membrane, and premembrane genes of USUV as described (11). Phylogenetic analysis showed that sequenced USUV strains from Israel clustered with a USUV strain isolated from Cx. neavei mosquitoes in 2007 in Senegal (Figure 2, panel A).

We then performed phylogenetic analysis of part of the NS5 gene because a previous study showed that this sequence exhibits a phylogenetic signal similar to the complete genome (7), which enables rapid characterization of circulating virus strains. Results (Figure 2, panel B) showed that USUV sequences from Israel closely resemble sequences of a USUV strain isolated from a bird in Germany in 2016, which was recently shown to belong to a putative novel USUV lineage called Europe 5 (11).

Conclusions
Despite its identification in 1959, USUV was not considered a potential public health concern until the early 2000s, when the first large USUV outbreak in birds occurred in Austria (10). Since that time, several human cases were identified in Europe and showed a correlation with increasing numbers of birds infected with USUV (4). It is possible that, similar to WNV outbreaks in Europe, the United States, and Israel during 1990–2010, which were occasionally preceded by large numbers of WNV infections in birds (12), USUV detection in birds and mosquitoes might be the first indication of future USUV outbreaks in humans. In this study, we identified
that USUV RNA is present in mosquitoes in Israel, which suggested that USUV infection in humans might also occur in Israel.

USUV RNA has been detected primarily in *Cx. pipiens* and *Cx. perexigus* mosquitoes in Spain (13), in *Cx. pipiens* and *Cx. torrentium* mosquitoes in Germany (14), and in *Ae. albopictus* and *Cx. pipiens* mosquitoes in Italy (15). Our finding of USUV RNA in *Cx. perexigus*, *Cx. pipiens*, and *Ae. albopictus* mosquitoes also suggests that USUV is transmitted by these 3 mosquito species in Israel.

Recently, reconstruction of the evolutionary history and dispersal of USUV has identified 6 distinct lineages of USUV. Nevertheless, Engel et al. concluded that limited numbers of USUV isolates from Africa and lack of data for countries located between Africa and Europe might obscure additional spatial movements (7).

Our phylogenetic analysis of the NS5 gene (Table) showed that USUV strains detected in Israel belong to the USUV Europe 5 lineage, mostly resembling a strain isolated in Germany in 2016. However, phylogenetic analysis on the basis of part of USUV structural proteins (Figure 1) indicates that virus strains from Israel are similar to USUV isolated from mosquitoes in Senegal in 2007, which was shown to be part of the Africa 3 lineage (11).

Because Israel is located on a bird migration path between Africa and Eurasia, USUV strains isolated in Germany, which are part of the Europe 5 and Africa 3 lineages, might have arrived from Africa through Israel, thus explaining the relative similarity of the strains in Israel to both lineages. Identification of more isolates from the Middle East and in-depth sequence analysis are needed to examine the geographic spread of the virus and further decipher its evolutionary history.

Our results showed that all 6 USUV-positive pools were captured in 3 mosquito species in northern Israel (Figure 1). Because most WNV-positive pools detected throughout Israel every year since 2000 belonged to the same mosquito species as those identified for USUV (16), the geographic discrepancy between circulation of WNV and USUV might be caused by different bird species carrying USUV or WNV. Future studies should examine the circulation of USUV in dead birds, as was demonstrated in several countries in Europe (11) and compare bird species carrying USUV with bird species carrying WNV in Israel and those carrying USUV in Europe.

Given the history of flaviviruses that were responsible for major outbreaks worldwide after decades of silent circulation, such as WNV and Zika virus, the accumulating evidence of increased activity of USUV in Europe is alarming. Therefore, investigation of USUV in mosquitoes in Israel is essential, not only because of public health concerns but also because of a need to understand the kinetics and penetration routes of USUV from Africa into Europe, Asia, and the Middle East.

This study was supported by internal sources of the Central Virology Laboratory, Chaim Sheba Medical Center, Ramat Gan, Israel.

Dr. Mannasse is a researcher at the National Zoonotic Viruses Reference Laboratory at the Ministry of Health, Tel Hashomer, Israel. Her research interests include clinical virology and phylogeny of arboviral diseases.
Usutu Virus RNA in Mosquitoes, Israel, 2014–2015

References


Table. Analysis of mosquitoes for Usutu virus, northern Israel, 2014–2015*

<table>
<thead>
<tr>
<th>Collection site no.</th>
<th>Virus name</th>
<th>Location of collection site</th>
<th>Date of sample collection</th>
<th>Mosquito species</th>
<th>MLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Usutu, 269 m/2015/Israel</td>
<td>Sdeh Eliahu</td>
<td>2015 Jun 30</td>
<td>Culex perexigus</td>
<td>0.0047</td>
</tr>
<tr>
<td>2</td>
<td>Usutu, 550 m/2015/Israel</td>
<td>Midrach Stream</td>
<td>2015 Sep 8</td>
<td>Cx. perexigus</td>
<td>0.0025</td>
</tr>
<tr>
<td>3</td>
<td>Usutu, 558 m/2015/Israel</td>
<td>Yefatchel</td>
<td>2015 Sep 8</td>
<td>Cx. pipiens</td>
<td>0.0138</td>
</tr>
<tr>
<td>4</td>
<td>Usutu, 569 m/2015/Israel</td>
<td>Yefatchel</td>
<td>2015 Sep 8</td>
<td>Cx. pipiens</td>
<td>0.0138</td>
</tr>
<tr>
<td>5</td>
<td>Usutu, 593 m/2015/Israel</td>
<td>Haifa</td>
<td>2015 Sep 21</td>
<td>Aedes albopictus</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Usutu, 610 m/2014/Israel</td>
<td>Kiyvat Ata</td>
<td>2014 Oct 2</td>
<td>Cx. pipiens</td>
<td>0.0041</td>
</tr>
</tbody>
</table>

*Virus-positive mosquitoes were identified by using real-time reverse transcription PCR. MLE for infection rate was calculated by using the EpiTools epidemiologic calculators method (http://epitools.ausvet.com.au/content.php?page = PPVariablePoolSize). MLE, maximum likelihood estimate.

Figure 2. Phylogenetic tree of Usutu virus strains, Israel, 2014–2015. A) Structural protein genes. B) Nonstructural protein 5 genes. Analysis was conducted by using the maximum-likelihood method implemented in MEGA 6.0 software (http://www.megasoftware.net/). Robustness of branching pattern was tested by using 1,000 bootstrap replications. Percentage of successful bootstrap replicates is indicated at nodes (only values >70% are shown). Diamonds indicate virus strains sequenced in this study. Reference strains are indicated by GenBank accession numbers and country and year of isolation. Scale bars indicate nucleotide substitutions per site.


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Takaaki Tanaka, Tomohiro Oishi, Ippei Miyata, Shoko Wakabayashi, Mina Kono, Sahoko Ono, Atsushi Kato, Yoko Fukuda, Aki Saito, Eisuke Kondo, Hideto Teranishi, Yuhei Tanaka, Tokio Wakabayashi, Hiroto Akaike, Satoko Ogita, Naoki Ohno, Takashi Nakano, Kihei Terada, Kazunobu Ouchi

We evaluated isolates obtained from children with *Mycoplasma pneumoniae* infection throughout Japan during 2008–2015. The highest prevalence of macrolide-resistant *M. pneumoniae* was 81.6% in 2012, followed by 59.3% in 2014 and 43.6% in 2015. The prevalence of macrolide-resistant *M. pneumoniae* among children in Japan has decreased.

Since the early 2000s, macrolide-resistant *Mycoplasma pneumoniae* isolates have been identified in Japan. We previously reported the results of our national surveillance study that investigated the prevalence of macrolide-resistant *M. pneumoniae* infection among children in Japan during 2008–2012 (1). An *M. pneumoniae* pandemic occurred in Japan during 2010–2012, especially among children. Similar pandemics also occurred in other countries (2). However, the prevalence of macrolide-resistant *M. pneumoniae* infection also gradually increased at the same time in Asia, including Japan (1,2). We investigated the prevalence of macrolide-resistant *M. pneumoniae* infection after the pandemic.

The Study

Children with respiratory tract infections who visited 68 medical institutions in Japan were classified according to district: 1) Kyushu (population 14 million); 2) Chugoku-Shikoku (11 million); 3) Kinki (20 million); 4) Kanto-Chubu (62 million); and 5) Tohoku-Hokkaido (14 million) (Figure). Most patients had been enrolled in the Atypical Pathogen Study Group before the 2010–2012 pandemic; however, some did not participate in our previous study reported in 2013 (1). Here, we report the data for January 2008–December 2015.

As previously reported (1), pediatricians collected nasopharyngeal swab samples and sputum samples, when available, from children with respiratory tract infections. Participants’ parents provided informed consent before sample collection. The Ethics Committee of the Kawasaki Medical School (Kurashiki, Japan) approved the study protocol on December 8, 2014 (no. 286–3).

Nasopharyngeal swab specimens were collected with a sterile swab (Nippon Menbo, Saitama, Japan). After collection, each swab was placed into 3 mL of Universal Vial Transport Medium (Becton, Dickinson and Company, Sparks, MD, USA) and transported at room temperature within 2 days to Kawasaki Medical School by a parcel delivery system. Crude DNA extracts were obtained with the following procedure: 300 µL of resuspended transport medium was transferred into a 1.5-mL microtube centrifuged at 4°C, 20,000 × g for 30 min, after which 285 µL of supernatant was discarded; the remainder was transferred into a thin-wall 200-µL PCR tube after resuspension with 85 µL lysis buffer by gentle pipetting. This suspension was incubated at 55°C for 60 min, followed by 100°C for 10 min before cooling to 4°C. The composition of the lysis buffer was Tris-HCl (pH 8.3) 2 mmol/L, KCl 10 mM, MgCl₂ 0.045 mM, Triton X-100 0.45%, Tween 20 0.45%, and RNA-grade Proteinase K (Thermo Fisher Scientific Inc., Waltham, MA, USA) 0.4 µg/µL. *M. pneumoniae* DNA was detected by real-time PCR targeting a conserved part of the gene coding for the P1 adhesin (3).

We searched for mutations at sites 2063, 2064, and 2617 in domain V of 23S rRNA of *M. pneumoniae* using a direct sequencing method with isolates or samples with a positive PCR result, as reported previously (3). For this study, we investigated 1,448 samples obtained from patients in Japan who had respiratory tract infections; we detected *M. pneumoniae* DNA by real-time PCR and searched for mutations using a direct sequencing method.

The overall prevalence rate of macrolide-resistant *M. pneumoniae* in Japan was 70.2% (1,016/1,448) and ranged from 43.7% in Kyushu to 89.3% in Kanto-Chubu (Table 1). When divided into 3 time periods (prepandemic, pandemic, and postpandemic), the overall rate of macrolide-resistant *M. pneumonia* was substantially lower in the post epidemic period than in the pandemic period; macrolide-resistant *M. pneumoniae* isolates decreased in 4 of the 5 locations.

The peak rate of macrolide-resistant *M. pneumoniae* infection was 81.6% in 2012 (493/604) (Table 2). Rates of macrolide-resistant *M. pneumoniae* infection gradually decreased as follows: 65.8% (253/38) in 2013, 59.3% (16/27) in 2014, and 43.6% (65/149) in 2015. The most frequent mutation was A2063G mutation (95.8%), followed...
DISPATCHES

by A2063T (3.1%), A2064G (0.6%), A2063C (0.3%),
C2617G (0.2%), and C2617T (0.1%).

Conclusions
The prevalence of macrolide-resistant M. pneumoniae
infection was high during 2008–2012 but gradually de-
creased throughout Japan during 2013–2015. One reason
for this decrease might be the 2011 publication of guide-
lines for treating M. pneumoniae pneumonia (4). Because
of the higher prevalence of macrolide-resistant M. pneu-
moniae infection in children than in adults (5), respiratory
fluoroquinolone/tosufloxacin was recommended for use in
patients in whom M. pneumoniae pneumonia responded
poorly to macrolide treatment in these guidelines. After the
pandemic, the guideline committee addressed the concerns
of further accumulation of macrolide-resistant M. pneu-
moniae in children resulting from constant macrolide use
and teeth damage from tetracycline use in children <8 years
of age (4). Tosufloxacin was approved for use in children
in 2010 in Japan and has been used to treat M. pneumoniae
infection. Because the guidelines recommend tosufloxacin
as a second-line drug, pediatricians in Japan may be us-
ing the appropriate antimicrobial drugs for M. pneumoniae
infection in accordance with these guidelines, which might
have led to the decrease in incidence. Unfortunately, to our
knowledge, no reports have been published to support these
hypotheses. However, prescriptions for oral antimicrobial
drugs in Japan comprise most of the prescriptions for an-
timicrobial drugs (6); therefore, we believe that the rate
of macrolide-resistant M. pneumoniae might be affected by
changes in the use of oral macrolide agents.

In addition, the prevalence of macrolide-resistant M. pneumoniae
infection varies among countries: for example, 13.2% in the United States (7), 8.3% in France (8), and
3.1% in Germany (9). These variations might be attribut-
ed to differences in the number of prescription macrolide
agents among countries. Although accurately assuming the
number of prescription macrolide agents in each country is

Table 1. Mycoplasma pneumoniae infections diagnosed by real-time PCR and prevalence of macrolide-resistant M. pneumoniae, by
district, Japan

<table>
<thead>
<tr>
<th>District</th>
<th>Average age, y (range)</th>
<th>No. patients (M:F)</th>
<th>Macrolide resistance, % (no. positive/total no. patients)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyushu</td>
<td>6.4 (0–14)</td>
<td>239 (1:2:1)</td>
<td>–</td>
</tr>
<tr>
<td>Chugoku-Shikoku</td>
<td>7.3 (0–15)</td>
<td>623 (1:4:1)</td>
<td>68.6 (59/86)</td>
</tr>
<tr>
<td>Kinki</td>
<td>7.1 (1–15)</td>
<td>227 (1:1)</td>
<td>33.3 (7/21)</td>
</tr>
<tr>
<td>Kanto-Chubu</td>
<td>7.5 (0–13)</td>
<td>268 (1:1:1)</td>
<td>–</td>
</tr>
<tr>
<td>Hokkaido-Tohoku</td>
<td>7.7 (0–13)</td>
<td>91 (1:2:1)</td>
<td>84.2 (32/38)</td>
</tr>
<tr>
<td>Total</td>
<td>7.3 (0–15)</td>
<td>1,446 (1:3:1)</td>
<td>67.6 (98/145)</td>
</tr>
</tbody>
</table>

Table 2. Rates of macrolide-resistant *Mycoplasma pneumoniae* and point mutations in domain V of 23S rRNA, Japan

<table>
<thead>
<tr>
<th>Variable</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolide resistance, % (no. positive/total no. patients)</td>
<td>55.6 (10/18)</td>
<td>72.7 (8/11)</td>
<td>69.0 (80/116)</td>
<td>65.8 (319/485)</td>
<td>81.6 (493/604)</td>
<td>65.8 (25/38)</td>
<td>59.3 (16/27)</td>
<td>43.6 (65/149)</td>
<td>70.2 (1,016/1,448)</td>
</tr>
</tbody>
</table>

Point mutations, no. (%) |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A2063G</td>
<td>10 (100)</td>
</tr>
<tr>
<td>A2063C</td>
<td>0</td>
</tr>
<tr>
<td>A2063T</td>
<td>0</td>
</tr>
<tr>
<td>A2064G</td>
<td>0</td>
</tr>
<tr>
<td>C2617G</td>
<td>0</td>
</tr>
<tr>
<td>C2617T</td>
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</tr>
</tbody>
</table>

difficult, we can estimate the macrolide resistance rate of *Streptococcus pneumoniae* among those countries. A recent report supported the hypothesis that antimicrobial selection pressure results in clonal expansion of existing resistant strains (10). In Japan and China, which have a high prevalence of macrolide-resistant *M. pneumoniae* (3,5), the rates of macrolide-resistant *S. pneumoniae* also are very high (11,12). In countries with low rates of macrolide-resistant *M. pneumoniae*, such as the United States, France, and Germany (7,8,9), the prevalence rates of macrolide-resistant *S. pneumoniae* are low (13,14). However, the mechanisms of macrolide resistance are difficult to compare between *Streptococcus* and *Mycoplasma* (15). Therefore, we also interviewed the pediatricians who collected the samples for this study, and analysis of their responses is ongoing. Upon completion of these interviews, we will be able to report patients’ background characteristics, such as previous use of macrolides and medical examination histories; this information may provide further insight into the decreased prevalence of macrolide-resistant *M. pneumoniae* infection.

In summary, the prevalence of macrolide-resistant *M. pneumoniae* infection in children in Japan was high and increased between 2008 and 2012 but declined thereafter. Careful continuous monitoring of macrolide-resistant *M. pneumoniae* infection rates in Japan and other countries is needed.

Acknowledgments

We thank Reiji Kimura and Mika Suwa for their technical assistance and all the clinicians who participated by taking samples in the Atypical Pathogen Study Group.

Dr. Tanaka is a senior lecturer in the Department of Pediatrics at Kawasaki Medical School. His primary research interest is vaccines for travelers.

References


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September 2016: Antimicrobial Resistance

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- Virulence and Evolution of West Nile Virus, Australia, 1960–2012
- Phylogeographic Evidence for 2 Genetically Distinct Zoonotic Plasmodium knowlesi Parasites, Malaysia
- Hemolysis after Oral Artemisinin Combination Therapy for Uncomplicated Plasmodium falciparum Malaria
- Human Babesiosis, Bolivia, 2013
- Assessment of Community Event–Based Surveillance for Ebola Virus Disease, Sierra Leone, 2015
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Epidemiology of Reemerging Scarlet Fever, Hong Kong, 2005–2015

Chun Fan Lee, Benjamin J. Cowling, Eric H. Y. Lau

Annual incidence of scarlet fever in Hong Kong remained elevated after an upsurge in 2011. Incidence increased from 3.3/10,000 children ≤5 years of age during 2005–2010 to 18.1/10,000 during 2012–2015. Incidence was higher among boys and was 32%–42% lower in the week following school holidays.

Scarlet fever, caused by group A Streptococcus (Streptococcus pyogenes), was a common infectious disease in children before the early 20th century (1) and a major cause of death, with a case-fatality risk >30% (2,3). Various factors, including improvements in hygiene and the introduction of effective antibiotics, led to the disappearance of scarlet fever as a major cause of pediatric deaths by the mid-20th century (4). However, reemerging cases of scarlet fever were reported in China in 2011 and the United Kingdom in 2014 (5,6). Hong Kong also experienced an upsurg in scarlet fever cases in 2011, with a >10-fold increase over the previous incidence rate (7,8). The reason for the surge is unclear. One report has suggested that toxin acquisition and multidrug resistance might have contributed (9). Since 2011, the reported number of scarlet fever cases in these locations has remained at elevated levels (10,11). We analyzed the patterns in scarlet fever incidence in Hong Kong during 2005–2015, including the upsurge since 2011.

The Study
Scarlet fever is a notifiable disease in Hong Kong. We collected individual data, including age, sex, dates of illness onset, and travel history, from 7,266 local case-patients ≤14 years of age (with 3,304 having laboratory-confirmed cases) reported to the Department of Health during 2005–2015. The 2011 upsurge was characterized by a sharp peak (Figure 1, panel A). During that year, 1,438 cases (incidence 17.5/10,000 children ≤14 years of age) were reported, exceeding the total number of 1,117 cases (average incidence 2.1/10,000 children ≤14 years of age) in the previous 6 years (2005–2010). Since then, the annual number of reported cases has remained at a relatively high level, with an average of 14.5 cases/10,000 children ≤14 years of age during 2012–2015. The elevated pattern was more apparent in children ≤5 years of age; among this age group, annual incidence averaged 3.3/10,000 children during 2005–2010, jumped to 23.9/10,000 in 2011, and dropped slightly to 18.1/10,000 during 2012–2015.

We investigated the effect of various epidemiologic and meteorologic variables on scarlet fever incidence by using hierarchical multivariable negative binomial regression, accounting for autocorrelation and annual and bimodal seasonal trends by using Fourier terms (i.e., including sine and cosine terms in the model) (12). Specifically, we modeled the age- and sex-specific weekly number of scarlet fever cases by age and sex of the patient; linear time trend; average temperature, relative humidity, and rainfall in the previous week (Figure 1, panel B); and school holidays in the previous week. We divided age into 4 groups reflecting the type of school attended: 0–2 years (nursery), 3–5 years (kindergarten), 6–11 years (primary school), and 12–14 years (junior high school). In Hong Kong, school holidays include Christmas (~2 weeks), Chinese New Year (~10 days), Easter (~10 days), and summer vacation (~7 weeks) (Figure 1, panel A). We included the logarithm of the population size for each age–sex group as an offset term. We first fitted a model with age, sex, and a linear time trend variable for the whole study period, allowing for change in slope after the upsurge, and found a significant change in the linear time trend after the 2011 upsurge (p<0.001). Therefore, we divided the time-series into preupsurge (2005–2010 [311 weeks]) and postupsurge (2012–2015 [209 weeks]) periods, excluding data in 2011 as a window period of transition.

We then added seasonality to the model in each period (model I; online Technical Appendix Table, https://www.cdc.gov/EID/article/23/10/16-1456-Techapp1.pdf). Seasonal trends were similar in both periods, with a more pronounced bimodal pattern after the upsurge (Figure 2). The trough ended in early September, when school begins, then disease activity increased to its peak in January, followed by a milder peak in June. Previous studies have indicated a similar bimodal seasonal pattern (6,13).

The final model also included the variables of school holidays and meteorologic factors in the preceding week (Table). This model fitted the scarlet fever incidence satisfactorily, especially for the age groups of 3–5 years and 6–11 years (online Technical Appendix Figure). We observed no significant linear trend in scarlet fever incidence before the upsurge but a mild decreasing trend after the upsurge.
of an 8% decrease per year. In general, boys had a higher IRR than girls at all age groups, and children 3–5 years of age had the highest IRR, followed by those 6–11 years of age and then those 0–2 years of age, for both sexes. We found a significant age–sex interaction in the postupsurge period. Specifically, boys had a higher risk for scarlet fever than girls, with an adjusted IRR of 1.08 (95% CI 0.87–1.34) at 0–2 years of age, 1.47 (95% CI 1.32–1.65) at 3–5 years of age, 1.31 (95% CI 1.18–1.46) at 6–11 years of age, and 2.01 (95% CI 1.26–3.20) at 12–14 years of age. Similar to what has been reported elsewhere (5,13), almost all reported cases in Hong Kong were among children. The relatively high incidence among children of kindergarten age corresponds to the start of schooling, consistent with studies in China (14). The new cohort of children in kindergarten might partly drive the major winter peak. Boys being more at risk than girls might be attributable to more physical interactions or poorer personal hygiene among boys (15).

In 2011, the outbreak reached its peak with 141 cases in the second half of June but sharply fell to 9 cases in the last week of August, at the end of the summer vacation (Figure 1, panel A). However, a clear upturn could be observed once the new school year started. We observed a similar pattern in each subsequent year. Also, school holidays were significantly associated with lower incidence, with IRRs of 0.68 (95% CI 0.55–0.85) before the upsurge (a 32% reduction) and 0.58 (95% CI 0.51–0.65) after the upsurge (a 42% reduction). Together with the observation that the prenursery-age children had a lower incidence, school is probably a major transmission site of scarlet fever because children began to increase their social contact substantially.
Reemerging Scarlet Fever, Hong Kong

Table. Estimated incidence rate ratios of various epidemiologic and meteorological factors affecting reemergence of scarlet fever, Hong Kong, 2005–2015*

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Linear time trend, per year</td>
<td>0.98 (0.95–1.02)</td>
<td>0.92 (0.89–0.94)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>M</td>
<td>1.33 (0.92–1.94)</td>
<td>1.08 (0.87–1.34)</td>
</tr>
<tr>
<td>Age group, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>3–5</td>
<td>2.78 (2.02–3.90)</td>
<td>3.17 (2.63–3.84)</td>
</tr>
<tr>
<td>6–11</td>
<td>1.29 (0.95–1.80)</td>
<td>2.06 (1.71–2.50)</td>
</tr>
<tr>
<td>12–14</td>
<td>0.13 (0.07–0.24)</td>
<td>0.14 (0.09–0.21)</td>
</tr>
<tr>
<td>Sex × age interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, 0–2 y</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Boys, 3–5 y</td>
<td>1.11 (0.72–1.68)</td>
<td>1.36 (1.07–1.74)</td>
</tr>
<tr>
<td>Boys, 6–11 y</td>
<td>1.07 (0.70–1.61)</td>
<td>1.22 (0.96–1.55)</td>
</tr>
<tr>
<td>Boys, 12–14 y</td>
<td>1.01 (0.44–2.36)</td>
<td>1.86 (1.12–3.15)</td>
</tr>
<tr>
<td>School holidays in the preceding week</td>
<td>0.68 (0.55–0.85)</td>
<td>0.58 (0.51–0.65)</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>0.991 (0.953–1.031)</td>
<td>0.963 (0.940–0.987)</td>
</tr>
<tr>
<td>Relative humidity, %</td>
<td>0.981 (0.972–0.990)</td>
<td>0.997 (0.992–1.002)</td>
</tr>
<tr>
<td>Rainfall, mm</td>
<td>1.009 (1.002–1.016)</td>
<td>0.998 (0.993–1.002)</td>
</tr>
</tbody>
</table>

*Incidence rate ratios estimated by negative binomial regression models also accounting for autocorrelation and annual and biannual seasonality using Fourier terms with periods of 1 year (T) and half a year (T/2), where T = 365.25/7 weeks. IRR, incidence rate ratio.

In the preupsurge period, relative humidity (adjusted IRR 0.981, 95% CI 0.972–0.990) and rainfall (adjusted IRR = 1.009; 95% CI 1.002–1.016) were significantly associated with incidence of scarlet fever, whereas temperature was not significantly associated. In contrast, in the postupsurge period, temperature had a significant effect on scarlet fever incidence (adjusted IRR 0.963; 95% CI 0.940–0.987), whereas relative humidity and rainfall had an insignificant effect.

Conclusions

Scarlet fever cases continued to occur in Hong Kong at elevated incidence rates for 5 consecutive years after a major epidemic in 2011. Scarlet fever incidence is higher among younger children entering schools and during school days. School-based control measures, especially for boys 3–5 years of age, could be particularly important in scarlet fever control. A limitation of our study is that we relied on reported cases to study scarlet fever epidemiology, and subclinical infections might have occurred. Moreover, some uncaptured or unobserved factors not considered in this study might have influenced the trend observed. Further community-based studies, including serologic studies, might further elucidate the epidemiology of this reemerging disease.

Acknowledgment

We thank the Centre for Health Protection, Department of Health, in Hong Kong for their kind support and assistance in collating the notification data.

This project was supported by the Harvard Center for Communicable Disease Dynamics from the National Institute of General Medical Sciences (grant no. U54 GM088558) and a commissioned grant from the Health and Medical Research Fund from the Government of the Hong Kong Special Administrative Region (grant no. HKS-16-E09). The funding bodies had no role in study design, data collection and analysis, preparation of the manuscript, or the decision to publish.

B.J.C. consults for Crucell NV and has received research funding from MedImmune Inc. and Sanofi Pasteur.

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References


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June 2015: Bacterial/Fungal Infections

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- Mycobacterium bovis in Panama, 2013
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- Invasion Dynamics of White-Nose Syndrome Fungus, Midwestern United States, 2012–2014
- Coccidioides Exposure and Coccidioidomycosis among Prison Employees, California, United States
- Prospective Multicenter International Surveillance of Azole Resistance in Aspergillus fumigatus
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- Drug Resistance–Associated Mutations in Mycoplasma genitalium in Female Sex Workers, Japan
- Lack of Protection Against Ebola Virus from Chloroquine in Mice and Hamsters
- European Rabbits as Reservoir for Coxiella burnetii
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https://wwwnc.cdc.gov/eid/content/21/6/contents.htm
Off-Label Use of Bedaquiline in Children and Adolescents with Multidrug-Resistant Tuberculosis

Jay Achar, Cathy Hewison, Ana P. Cavalheiro, Alena Skrahina, Junia Cajazeiro, Parpieva Nargiza, Krzysztof Herboczek, Assliddin S. Rajabov, Jennifer Hughes, Gabriella Ferlazzo, James A. Seddon, Philipp du Cros

We describe 27 children and adolescents <18 years of age who received bedaquiline during treatment for multidrug-resistant tuberculosis. We report good treatment responses and no cessation attributable to adverse effects. Bedaquiline could be considered for use with this age group for multidrug-resistant tuberculosis when treatment options are limited.

The World Health Organization (WHO) estimated that nearly half a million persons became infected with multidrug-resistant (MDR) tuberculosis (TB), defined as disease caused by Mycobacterium tuberculosis strains resistant to rifampin and isoniazid, in 2015 (1). Modeling studies suggest that ≈32,000 of these cases occurred in children <15 years of age (2). Although limited information is available on the burden of extremely drug-resistant (XDR) TB (MDR TB with additional resistance to a fluoroquinolone and a second-line injectable drug) among children, >33% of children with MDR TB are estimated to exhibit additional resistance to fluoroquinolone, a second-line injectable drug, or both (3). Once a child with MDR TB is given the correct diagnosis and started on therapy, treatment outcomes are good (4). However, multiple challenges exist for children and adolescents with this disease. First, poor access to effective regimens and difficulties in establishing laboratory diagnoses continue to lead to inappropriate management of disease among many children. Second, adverse effects from MDR TB treatments are common; in 1 cohort, >25% of children receiving an injectable drug suffered hearing loss (5). Third, for children and adolescents infected with more extensively resistant strains, treatment options are limited.

In 2013, following US Food and Drug Administration approval of bedaquiline (in 2012), the WHO released interim guidance on the use of this drug (6). Key determinants of eligibility to receive bedaquiline included the inability to construct an effective 4-drug regimen using other available drugs or diagnosis with disease caused by strains with fluoroquinolone resistance. Limited available data to inform the use of bedaquiline in children led to the WHO stating that “Use of the drug in pregnant women and children is not advised due to a lack of evidence on safety and efficacy.”

One large retrospective cohort analysis reported that up to two thirds of all patients with MDR TB might benefit from adding bedaquiline or delamanid to their treatment regimen (7). However, despite the US Centers for Disease Control and Prevention stating that bedaquiline use can be considered for children and adolescents when treatment options are limited, further studies to evaluate the drug in these groups have been slow to materialize. The bedaquiline compassionate use program conducted by the drug’s manufacturer, Janssen Pharmaceutical (Beerse, Belgium), excluded all patients <18 years of age (8). A Janssen-sponsored study (ongoing as of July 2017) will evaluate the antimycobacterial activity, pharmacokinetic profile, tolerability, and safety of bedaquiline among children and adolescents <18 years of age in South Africa, the Philippines, and Russia, with further sites planned in India (ClinicalTrials.gov nos. NCT02354014). Despite WHO guidance to avoid using bedaquiline in patients <18 years of age (6), some clinicians have done so when options were limited. The aim of this report was to describe experiences treating children and adolescents with MDR TB with drug regimens that included bedaquiline.

The Study

We collected data on patients <18 years of age from the TB treatment programs supported by Médecins Sans Frontières in South Africa, Tajikistan, and Uzbekistan and the National TB Programme in Belarus. During November...
2014–January 2017, a total of 27 children and adolescents began regimens containing bedaquiline (Table). Median age was 16 (range 10–17) years, and 15 (56%) were girls. Median weight was 50 (range 35–76) kg. No patients were HIV positive. One male patient had intrathoracic lymph node TB, and 26 patients had pulmonary TB. Diagnoses for 17 (63%) patients were confirmed by mycobacterial culture. Baseline sputum smears from 19 (70%) patients were positive for acid-fast bacilli. One boy had concomitant spinal TB osteomyelitis. Most patients (18/27, 67%) had XDR TB; 6 (22%) had MDR TB with fluoroquinolone resistance; and 3 (11%) had MDR TB with resistance to a second-line injectable drug. For the 10 patients without positive mycobacterial cultures, drug susceptibility was presumed from contact history. Thus, for all patients, the decision to use bedaquiline was based on confirmed or presumed extensive drug resistance that resulted in the inability to construct an effective treatment regimen.

The high proportion of patients with resistance to second-line drugs led to frequent use of repurposed drugs, such as linezolid (26/27, 96%) and clofazimine (26/27, 96%). Intravenous imipenem was used in some patients (4/27, 15%). Despite concerns about potential additive cardiac toxicity when combining bedaquiline and moxifloxacin, combined treatment was judged necessary for 6 (22%) children. Five of these 6 children also received clofazimine in their regimen. All cases except 1 received the recommended adult dosing regimen for bedaquiline (400 mg/d for 2 weeks, then 200 mg 3×/wk for 6 months). One 10-year-old girl (weighing 35 kg) received 300 mg/d (recommended on the basis of expert opinion) during her loading phase.

The mean duration of bedaquiline treatment of the 20 children and adolescents who completed therapy was 172 days. Regular (weekly for most) electrocardiogram monitoring was performed for all cases. Fridericia’s formula was used to correct the measured QT intervals (QTcF) for the heart rate, and cardiotoxicity was defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). Five patients had grade 3 or 4 prolongation: 2 received bedaquiline, clofazimine, and moxifloxacin and 3 received bedaquiline and clofazimine. Four patients experienced increases in QTcF >60 ms above baseline: 2 during the first month of treatment, 1 after 3 months of treatment, and 1 after 6 months of treatment. All 4 patients improved <1 month following electrolyte replacement, and no drug cessation was required. Recurrent prolongation of QTcF >500 ms was identified in 1 adolescent during the first 6 months of treatment and necessitated cessation of moxifloxacin and clofazimine after electrolyte replacement showed insufficient benefit. Following stoppage of these drugs, QTcF returned to normal. No patient experienced symptoms attributable to prolongation of QTcF during treatment with bedaquiline.

As of February 24, 2017, of the 23 patients who remained on treatment and had data available, all were culture negative; 14 of these 23 had been positive at baseline. No clinical signs suggestive of treatment failure were noted among patients of this cohort.

### Table. Demographic, treatment, and outcome characteristics of a cohort of 27 children <18 years of age receiving bedaquiline for the treatment of MDR TB

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Country</strong></td>
<td></td>
</tr>
<tr>
<td>Belarus</td>
<td>15 (56)</td>
</tr>
<tr>
<td>South Africa</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Tajikistan</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Uzbekistan</td>
<td>3 (11)</td>
</tr>
<tr>
<td><strong>Age, y, median (range)</strong></td>
<td>16 (10–17)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>15 (56)</td>
</tr>
<tr>
<td>Male</td>
<td>12 (44)</td>
</tr>
<tr>
<td><strong>Weight, kg, median (range)</strong></td>
<td>50 (35–76)</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m², median (IQR)</strong></td>
<td>18.5 (17.2–19.6)</td>
</tr>
<tr>
<td><strong>Cavities on baseline chest radiograph, n = 24</strong></td>
<td>9 (38)</td>
</tr>
<tr>
<td><strong>Baseline sputum smear positive</strong></td>
<td>19 (70)</td>
</tr>
<tr>
<td><strong>Baseline sputum culture positive</strong></td>
<td>17 (63)</td>
</tr>
<tr>
<td><strong>Baseline drug resistance pattern</strong></td>
<td></td>
</tr>
<tr>
<td>MDR TB</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pre-XDR TB</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Resistant to second-line injectable</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Resistant to fluoroquinolone</td>
<td>18 (67)</td>
</tr>
<tr>
<td>XDR TB</td>
<td></td>
</tr>
<tr>
<td>Resistant drugs,† median (IQR), n = 24</td>
<td>5 (5–6)</td>
</tr>
<tr>
<td>Drugs in initial treatment regimen, median (IQR)</td>
<td>6 (6–7)</td>
</tr>
<tr>
<td><strong>Drugs included in treatment regimen</strong></td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>26 (96)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>26 (96)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>4 (15)</td>
</tr>
<tr>
<td><strong>Bedaquiline treatment duration if completed, d, median (IQR), n = 20</strong></td>
<td>172 (168–178)</td>
</tr>
<tr>
<td><strong>Sputum culture negative at February 24, 2017, n = 23</strong></td>
<td>23 (100)</td>
</tr>
<tr>
<td><strong>Sputum culture negative after 24 wks of bedaquiline, n = 22‡</strong></td>
<td>22 (100)</td>
</tr>
<tr>
<td><strong>Reported adverse effects</strong></td>
<td></td>
</tr>
<tr>
<td>No grade 3 or 4</td>
<td>19 (70)</td>
</tr>
<tr>
<td>Grade 3 or 4, not caused by bedaquiline</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Grade 3 or 4, caused by bedaquiline</td>
<td>5 (19)§</td>
</tr>
</tbody>
</table>

*Values are no. (%) patients except as indicated; IQR, interquartile range; MDR TB, multidrug-resistant tuberculosis; XDR TB, extensively drug-resistant tuberculosis; QTcF, QT interval corrected using the Fridericia formula.
†Resistance among the following drugs were considered: isoniazid, rifampin, ethambutol, pyrazinamide, kanamycin, any fluoroquinolone, amikacin, and capreomycin.
‡Twenty-seven children completed 24 weeks of bedaquiline, but data were available for 22.
§All 5 were children who experienced prolongation of QTcF.

### Conclusions

Our experience suggests that bedaquiline can be used safely in children >12 years of age with appropriate monitoring...
and could be considered in younger children in select circumstances when benefits are likely to outweigh risks. Although treatment outcomes are preliminary, we report good responses to treatment with bedaquiline among a group of children and adolescents with advanced resistance to second-line drugs. Although prolongation of QTcF was noted in some (5/27) patients when concomitant cardiotoxic drugs were used, no patient required bedaquiline cessation.

Continued reluctance to use contact history for diagnosing advanced drug resistance and limited availability of drug susceptibility testing in children remain barriers for the consideration of new drugs and use of appropriate MDR TB regimens. In addition, restricted availability of delamanid in TB programs and the perceived age restriction on the use of bedaquiline has resulted in children failing to benefit from drugs that are being used safely and successfully in adults. Although the lack of pharmacokinetic data on bedaquiline in children and adolescents must be addressed, other second-line TB drugs have been recommended and prescribed despite insufficient data on pharmacokinetics. Expanding access to bedaquiline and delamanid for children could lead to the reduction in the need for second-line injectable drugs, which are strongly associated with irreversible toxicity (5). This experience supports similar recommendations given by the US Centers for Disease Control and Prevention (9) and an international group of pediatric TB experts (10).

This research fulfilled the exemption criteria set by the Médecins Sans Frontières Ethics Review Board for a posteriori analyses of routinely collected clinical data and thus did not require Médecins Sans Frontières Ethics Review Board review.

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References


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During 2016 in Guangzhou, China, we detected infectious avian influenza viruses (AIVs) in 39.8% of samples from chicken carcasses slaughtered at live poultry markets but none from carcasses supplied to supermarkets by facilities bypassing live poultry markets. Promoting supply chains with high biosecurity may reduce the risk for zoonotic AIV transmission.

Live poultry markets (LPMs) are hot spots for avian influenza virus (AIV) amplification among poultry and dissemination to humans (1). Direct contact with live poultry is a major route of zoonotic transmission (2), but field data are limited on risks from contaminated poultry carcasses (3). Control measures, including market rest-days and ban of live poultry stalls in urban areas with central slaughtering and sales of poultry carcasses, have been implemented in China (3,4). In the city of Guangzhou (population 14 million), 200,000 live poultry (5) and ≈81,000 freshly processed poultry carcasses are supplied daily. Live poultry is supplied from 6 wholesale LPMs to ≈600 retail LPMs (5). Poultry carcasses are supplied through 2 sources: 1) poultry sourced from multiple independent poultry farms and slaughtered at the wholesale LPMs or 2) private poultry slaughtering industries with integrated supply chains that bypass the LPM system (6).

Low pathogenic AIV replicates in the respiratory and gastrointestinal epithelium cells of infected birds; highly pathogenic AIV can replicate systematically in multiple tissues (7). In avian species, the lungs where AIV can replicate are entwined into the ribs and cannot be removed during poultry slaughtering; furthermore, AIV has been detected in air sacs from experimentally inoculated chickens (8). The mass slaughtering process also provides an opportunity for cross-contamination through common use of tools or water. Previous studies have reported detection of infectious highly pathogenic AIV (H5N1, H5N2, H5N3, and H7N1) and low pathogenic AIV (H9N2) subtypes from poultry meat after natural infections or after experimental inoculations (8–14). However, how frequently infectious AIV can be detected from processed poultry carcasses is unclear. We report the detection of viral RNA and infectious AIV from freshly processed chicken carcasses sold at different markets in Guangzhou, China.

The Study
During June–November 2016, we sampled fresh chicken carcasses supplied from the LPM system. Samples were collected twice each month from dressed poultry stalls within 1 wholesale LPM and from 3 retail LPMs located in different districts in Guangzhou. Dressed poultry or poultry carcasses are prepared similarly as in other countries (e.g., defeathered and eviscerated); however, the head and feet remain with the carcass. A total of 1,230 swabs were collected from the oropharynx, cloacal cavity, and visceral cavity of chicken carcasses supplied from the LPM system (Table 1). During July–December 2016, we also sampled chilled chicken carcasses supplied from the private poultry slaughtering industries that bypass the LPM system. Chicken carcasses were sampled from 3 different supermarket chains once each month; 147 swabs were collected in virus transport media (Table 1). The quantity of AIV viral RNA segment 7 (matrix gene) was determined by quantitative real-time reverse transcription PCR (qRT-PCR) (15) and the infectious virus dose was determined by titration in MDCK cells, which are not as sensitive for AIV as the embryonated chicken eggs and might underestimate the rate of positivity. Influenza A virus matrix gene–positive samples were subtyped using H5-, H7-, or H9-specific primers and probes by qRT-PCR (15); the design of the primers and probes cannot differentiate whether the multiple basic amino acids are present at the hemagglutinin cleavage site.

The AIV-positive rates detected from fresh chicken carcasses varied by market type (Table 1). Rates of positive

<table>
<thead>
<tr>
<th>Market Type</th>
<th>Positive Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPM system</td>
<td>39.8%</td>
</tr>
<tr>
<td>Retail LPMs</td>
<td>0%</td>
</tr>
</tbody>
</table>
viral RNA detected from oropharyngeal, cloacal, or visceral cavity samples of chicken carcasses sold at the retail markets were 20% higher than those collected from the dressed poultry stalls (all p<0.1 by Fisher exact test) and >50% higher than those collected from the supermarkets (all p<0.002 by Fisher exact test) (Table 1). Samples collected from retail markets had 9%–20% higher culture-positive rates than those collected from dressed poultry stalls (all p<0.2 by Fisher exact test); no culture-positive samples were identified from the supermarkets. qRT-PCR yielded higher rates of positivity than did the cell culture–based assay (all p<0.05 by Fisher exact test), possibly because of the inactivation of AIV during the scalding process, when the poultry carcasses are immersed in hot water (50°C–64°C) for 45–120 s to loosen the feathers.

We detected significantly higher viral loads in oropharyngeal swabs than in cloacal or visceral cavity swabs of chicken carcasses sold at the dressed poultry stalls or the retail markets (Figure). Most AIVs detected belonged to H9 subtype, which is consistent with our previous report (15). We found samples positive for H7 or for H7 and H9 subtypes at a frequency of up to 6.3% (oropharyngeal swabs at the retail markets) (Table 2). We detected more diverse hemagglutinin subtypes from the chicken carcasses sold at the retail market than sold elsewhere; specifically, we detected H5 subtype only at retail market B.

Conclusions
Our results agree with results from a previous study that reported detection of AIV RNA from chicken carcasses sold at retail and dressed poultry stalls in Guangzhou (3). However, the previous study did not provide data on virus viability. Our data demonstrate high levels (39.8% of 1,230 samples collected from carcasses) of contaminated chicken carcasses with infectious AIV supplied through the LPM system, either at the retail LPM or at the dressed poultry stalls. These results suggest potential infection risk for consumers through handling the poultry meat, contaminating other foods in the kitchen, or eating partially cooked poultry products. In contrast, we found no infectious AIV in the 147 chicken carcasses collected from supermarkets supplied through the integrated poultry production and slaughtering industries that bypass the LPM system. Chilling should not affect the sensitivity of qRT-PCR and might help sustain survival of infectious virus. Although we did not assess the potential difference in the slaughtering process at the private slaughtering industry and at LPMs, we believe qRT-PCR might still be sensitive enough to detect AIV-contaminated carcasses after extensive cleaning. Collectively, our results suggest that AIV amplification through poultry mixing and extended overnight stay within the wholesale or retail LPM system (1) might have contributed to contamination of carcasses.

Table 1. AIV detected from chicken carcasses sold at live poultry markets, dressed poultry stalls, or supermarkets, Guangzhou, Guangdong Province, China, June–December 2016

<table>
<thead>
<tr>
<th>Swab type</th>
<th>No. qRT-PCR positive/no. tested (%)</th>
<th>No. culture positive/no. tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dressed poultry stall†</td>
<td>Retail market‡</td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td>67/121 (55.4)</td>
<td>207/277 (74.7)</td>
</tr>
<tr>
<td>Cloacal</td>
<td>55/120 (45.8)</td>
<td>177/265 (66.8)</td>
</tr>
<tr>
<td>Visceral cavity</td>
<td>48/118 (40.7)</td>
<td>203/329 (61.7)</td>
</tr>
</tbody>
</table>

†AIV, avian influenza virus; qRT-PCR, quantitative real-time reverse transcription PCR.
‡All qRT-PCR–positive samples were tested for infectivity in MDCK cells, and all qRT-PCR–negative samples were assumed negative for AIV infectivity.
¶Chickens centrally slaughtered at the wholesale market are sold at designated dressed poultry stalls without live poultry on site.
§Data from 3 retail markets located at different districts in Guangzhou city.
#Comparison of AIV-positive rates between dressed poultry stalls and retail markets by Fisher exact test.

Table 2. AIV subtypes detected by qRT-PCR from swabs of chicken carcasses sold at live poultry markets and from chilled poultry supplied through slaughtering industries, Guangzhou, Guangdong Province, China, June–December 2016

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Wholesale market</th>
<th>Retail market</th>
<th>Supermarket</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OP</td>
<td>Cloacal</td>
<td>Visceral cavity</td>
</tr>
<tr>
<td>H9</td>
<td>56/67</td>
<td>39/55</td>
<td>32/48</td>
</tr>
<tr>
<td>H5</td>
<td>0/67</td>
<td>0/55</td>
<td>0/48</td>
</tr>
<tr>
<td>H7</td>
<td>0/67</td>
<td>0/55</td>
<td>2/48 (4.2)</td>
</tr>
<tr>
<td>H5+H9</td>
<td>0/67</td>
<td>0/55</td>
<td>0/48</td>
</tr>
<tr>
<td>H7+H9</td>
<td>2/67 (3.0)</td>
<td>3/55 (5.5)</td>
<td>2/48 (4.2)</td>
</tr>
<tr>
<td>H5+H7+H9</td>
<td>0/67</td>
<td>0/55</td>
<td>0/48</td>
</tr>
<tr>
<td>Non-H5/H7/H9</td>
<td>4/67 (6.0)</td>
<td>3/55 (5.5)</td>
<td>1/48 (2.1)</td>
</tr>
<tr>
<td>Low copy</td>
<td>3/67 (4.5)</td>
<td>10/55</td>
<td>11/48</td>
</tr>
<tr>
<td>(nontypeable)</td>
<td>(18.2)</td>
<td>(22.9)</td>
<td></td>
</tr>
</tbody>
</table>

*Values are no. positive/no. tested (%). AIV, avian influenza virus; OP, oropharyngeal; qRT-PCR, quantitative real-time reverse transcription PCR.
†Samples with matrix gene copy below linear range of quantification (2.903 log10 matrix gene copies/mL) and are negative for H5/H7/H9 by qRT-PCR.
Figure. Copies of avian influenza virus RNA (A) and infectious viral loads (B) detected from chicken carcasses sold at live poultry markets or supplied through slaughtering industries, Guangzhou, Guangdong, China, June–December 2016. The median value with interquartile range is shown for each dataset. Dotted lines represent the limit of linear range of quantification for viral RNA (2.903 log_{10} matrix gene copies/mL) (A) or the detection limit by TCID_{50} assay (1.789 log_{10} TCID_{50}/mL) in MDCK cells (B). p values from Kruskal-Wallis test followed by Dunn’s multiple comparison test are shown. AIV, avian influenza virus; TCID_{50}, 50% tissue culture infectious dose.
In a separate study conducted during June–November 2016, we detected similar rates of positive viral RNA from the oropharyngeal swabs (172 [47.8%] of 360) (p = 0.172 by Fisher exact test) from live poultry sold at the same wholesale market as that detected from the chicken carcasses at the dressed poultry stalls (67 [55.4%] of 121) (Table 1). The result further supports that the AIV prevalence in the source poultry determines the level of residual AIV found on chicken carcasses.

In conclusion, our data suggest that chicken carcasses may pose a substantial zoonotic risk for AIV infection even in the absence of direct contact with live poultry. Central slaughtering might not by itself eliminate zoonotic risk if the source poultry have high rates of virus carriage. The LPM system in China continues to provide venues and opportunities of poultry mixing from different sources that facilitate AIV persistence and amplification despite interventions, such as market rest-days and banning the holding of live poultry overnight, that aim to reduce such a risk (1). In this regard, promoting vertically integrated supply chains of farms and slaughterhouses with high biosecurity would be a promising effective control measure to reduce the risk for zoonotic transmission of AIV.

This study was supported by contract HHSN272201400006C from the US National Institute of Allergy and Infectious Diseases, National Institutes of Health; the Theme-based Research Scheme (project no. T11-705/14N) from the Government of Hong Kong Special Administrative Region; and AXA Research Award.

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References


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We report the experiences of 5 patients taking bedaquiline with delamanid in combination: 1 patient was cured; 3 culture converted, with 2 continuing and 1 changing therapy; and 1 died from respiratory insufficiency. For 2 patients, QT-interval prolongation but no arrhythmias occurred. Use of this therapy is justified for patients with limited options.
The following were considered adverse events: an absolute QT interval corrected with Fridericia’s formula (QTcF) prolongation of >500 ms; a QTcF increase of >60 ms over the baseline reading; cardiac arrest; ventricular tachycardia or atrial fibrillation; syncope; and events suggestive of arrhythmia, dizziness, seizures, and palpitations. To assess severity, we used the Common Terminology Criteria for Adverse Events version 4.0 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf).

Of the 428 patients with culture-confirmed MDR TB who were treated with bedaquiline, 5 received combined treatment with delamanid. Considering the long half-life of bedaquiline (>5 months), 2 additional patients could have also been considered to have combined treatment; they were treated with delamanid shortly after bedaquiline (5–6). On April 28, 2017, we obtained information on the patients’ last follow-up from the physicians managing their care, and this information was updated in the study database.

Bedaquiline and delamanid were given concurrently to 5 patients with pulmonary XDR TB who lived in Russia (2), India (2), or the Netherlands (1) (Table 1). Four were women and 1 was a man; patients were 17–43 years of age. All were HIV negative, and 2 were recreational drug users. All had previously been treated with TB drugs (range 1–8 treatments) for >30 days; 4 patients had drug treatment failures, and 1 had a relapse. Chest radiographs indicated that 3 patients had extensive bilateral cavities, 1 had bilateral lesions (without cavities), and 1 had monolateral cavitary lung disease. All patients were sputum-smear and culture positive for mycobacteria and had been potentially infectious for a mean of 65 weeks.

The resistance patterns of the isolated Mycobacterium tuberculosis strains were extensive, ranging from 5 to 10 drugs (Table 1). Salvage regimens were designed for each patient on the basis of their unique resistance patterns, which lead to their treatments including bedaquiline and delamanid (Table 2). All regimens included another QT-prolonging drug in addition to bedaquiline and delamanid: moxifloxacin (patients 1 and 3) or clofazimine (patients 2, 4, and 5). Patient exposure to bedaquiline was 155–427 days, for a total duration of TB treatment of 16–46 months. The total duration of hospital admission was 256–1,140 days.

As of April 28, 2017, patient 3 had been declared cured; patients 2, 4, and 5 were continuing therapy, although patient 2 was receiving a different drug regimen. Patient 1 had received 4 months of salvage therapy, but treatment failed, and she died from respiratory insufficiency. Patient 2 switched therapies because bedaquiline and delamanid had been already administered for a fixed period

| Table 1. Demographics and clinical history of patients with pulmonary extensively drug-resistant TB treated with bedaquiline and delamanid* |
|---|---|---|---|---|---|---|
| Pt no. | Country of birth/illness | Age, y/sex | Risk factor | Weeks | Weight at baseline (kg) | MDR TB treatment duration, mo | Length of hospital stay, d |
| 1 | India/India | 20/F | None | 1, failure | 200 | 54 (40) | 50 | NA |
| 2 | UK/the Netherlands | 31/F | Recreational drug user | 8, failure | 4 | 54 (68) | 21 | 567 |
| 3 | Russia/ Russia | 43/M | Recreational drug user | 1, failure | 62 | 54 (76) | 36 | 887 |
| 4 | Azerbaijan/ Russia | 17/F | None | 1, failure | 20 | 53 (51) | 16 | 256 |
| 5 | Tibet/India | 39/F | None | 2, relapse | 52 | 65 (60) | 18 | 1,140 |

*All patients were sputum smear and culture positive. Amk, amikacin; Amx/Clv, amoxicillin/clavulanate; Bdq, bedaquiline; c+, culture positive; Cfz, clofazimine; C1r, clarithromycin; Cm, capreomycin; Cs, cycloserine; Dlm, delamanid; E, ethambutol; Eto, ethionamide; Fq, fluoroquinolone; H, isoniazid; Hd, high dose; Km, kanamycin; Lfx, levofloxacin; Lzd, linezolid; MDR TB, multidrug-resistant tuberculosis; Mfx, moxifloxacin; Mpm, meropenem; NA, not available; PAS, para-aminosalicylic acid; Pt, patient; Pto, prothionamide; R, rifampin; Rfb, rifabutin; S, streptomycin; ss+, sputum smear positive; TB, tuberculosis; Trd, terizidone; Z, pyrazinamide.
of 168 days as recommended by WHO. With the exception of patient 1, who remained sputum-smear and culture positive, the other 4 patients’ sputum smears converted to negative after 18–435 days, and cultures converted after 28–218 days.

As recommended, all patients underwent QTcF-interval monitoring at baseline, at 2 weeks, and then monthly (4), even though no patient had a history of heart problems or electrocardiogram abnormalities. A QTc interval >500 ms is considered a risk factor for fatal arrhythmia; when this sign is found in patients, clinicians should either stop treatment with >1 QTc-prolonging drugs and start verapamil or watch and closely monitor. The baseline QTcF intervals were <500 (range 340–449) ms for all patients. Patients 1, 2, and 3 did not report adverse events for bedaquiline or delamanid, and their QTcF intervals remained below the threshold. Patient 5’s QTcF interval reached 520 ms at week 16, which required a dose adjustment and the introduction of verapamil (9–11). Patient 5’s treatment continued without further problems; she continued improving clinically, with improved chest radiograph findings and continuously negative sputum smears and cultures. Patient 4 had a QTcF interval of 509 ms twice. Each time the treating physician practiced closer clinical observation with more frequent electrocardiogram monitoring, and her QTcF interval normalized spontaneously without changes in treatment.

Conclusions

We report that of 5 patients receiving bedaquiline and delamanid in combination 2 had potentially life-threatening QTcF prolongation. The clinical centers took the necessary precautions and acted promptly to manage the problem, and no arrhythmias occurred (9–11). When patients received bedaquiline, delamanid, and another QTc-prolonging agent, clinically significant cardiac events and permanent discontinuation of bedaquiline and delamanid did not occur. For patient 1, additional resistance to ethionamide and linezolid was detected in a drug susceptibility test in the final phase. This treatment failure highlights that great care is needed when deciding drug regimens; the resistance threshold of both repurposed and new drugs still needs to be determined. Although these data are preliminary and more work is needed, the findings from this cohort suggest that providing bedaquiline and delamanid in combination as part of therapy against XDR TB is justified when clinical options are limited. Two ongoing randomized controlled trials (ClinicalTrials.gov nos. NCT02583048 and NCT02754765) have experimental arms containing these drugs in combination, so additional datasets will be available in the future.

Dr. Maryandychev is a professor and head of the Department of Phthisiopulmonology of Northern State Medical University in Arkhangelsk, Russia. His primary research interests are prevention, diagnosis, and treatment of tuberculosis, including new and repurposed TB drugs.

References


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Hantavirus Pulmonary Syndrome Caused by Maripa Virus in French Guiana, 2008–2016

Séverine Matheus, Hatem Kallel, Claire Mayence, Laetitia Bremand, Stéphanie Houcke, Dominique Rousset, Vincent Lacoste, Benoît de Thoisy, Didier Hommel, Anne Lavergne

We report 5 human cases of hantavirus pulmonary syndrome found during surveillance in French Guiana in 2008–2016; of the 5 patients, 4 died. This pathogen should continue to be monitored in humans and rodents in effort to reduce the occurrence of these lethal infections in humans stemming from ecosystem disturbances.

New World hantavirus pulmonary syndrome (HPS) is an emerging infectious disease caused by viruses of the family Bunyaviridae and genus Hantavirus (1). Hanta-viruses are transmitted to humans most frequently by inhalation of viral particles expired by chronically infected rodents. Since the first case reported in the United States in 1993, HPS has been reported in Argentina, Chile, Brazil, Uruguay, Paraguay, Bolivia, and many other countries in South and Central America (2,3).

The circulation of hantavirus in French Guiana, a French overseas department located in the Amazon rainforest on the northeastern corner of South America, was first suggested in a retrospective serologic survey that showed an antibody prevalence of 1.42% among a select population of 420 patients having symptoms consistent with HPS (4). In 2008, after publication of the antibody prevalence data, active surveillance for this emerging infectious disease in humans was implemented, and in that same year, the first native biologically confirmed human HPS case occurred (5). The novel hantavirus detected in that patient was closely related to the Rio Mamoré virus and was named Maripa virus (6). We report the results of this 8-year hantavirus surveillance in humans and rodents in French Guiana and an investigation for hantavirus in rodents.

The Study

During 2008–2016, samples from 151 patients having symptoms consistent with hantavirus disease were received for diagnosis at the Institut Pasteur de la Guyane, Cayenne, French Guiana. Patient symptoms were primarily fever, myalgia, headache, and cough; most patients were hospitalized. We conducted serologic and molecular investigations with patient samples, depending on the time of sample collection; for samples collected days 0–7 after illness onset, we performed both investigations, and for samples collected thereafter, only serology. These analyses led to the identification of 5 patients with acute hantavirus infection that occurred in August 2008, December 2009, December 2010, May 2013, and October 2016 and 3 patients with hantavirus IgG only.

All 5 patients with acute infection were men (Table) who did not have a history of travel outside French Guiana. Each arrived at the emergency department of Andrée Rosemon General Hospital in Cayenne with a rapid onset of acute lung injury requiring admission to the intensive care unit (ICU) for intubation and mechanical ventilation. Of the 5 patients, 3 had a history of other medical conditions: 2 had hypertension and 1 had diabetes. All 5 patients had the initial symptoms of fever and dyspnea, 4 had myalgia, 3 had cough, and 2 had diarrhea and vomiting. At admission to the ICU, all patients had the following clinical characteristics: hemodynamic changes, acute lung and kidney injury, lactic acidosis, elevated hematocrit, standard or low protein level, and thrombocytopenia (Table). Laboratory tests for infectious agents ruled out malaria, dengue, leptospirosis, Chagas disease, Q fever, cytomegalovirus, and HIV, and blood cultures for bacterial growth were all negative. Chest radiographs of all patients showed a bilateral alveolar infiltrate with pleural effusion. Heart sizes were within reference limits. At admission to the ICU, the patients received mechanical ventilation, fluid infusion, and catecholamines. Four of the 5 patients died within the first 24 hours after admission (case-fatality rate 80%). The surviving patient was discharged from the hospital 47 days after admission with complete clinical recovery (5).

We evaluated patient serum samples collected at admission to either the emergency department or the ICU to confirm a suspected hantavirus infection. We performed serologic analyses with an indirect ELISA using inactivated Sin Nombre virus (SNV) antigen designed to detect IgM and an ELISA using recombinant SNV antigen designed to detect IgG, both provided by the US Centers for Disease Control and Prevention (7). The results revealed the presence of IgM and IgG reactive against SNV antigens in all samples. We confirmed acute infection by detecting a fragment of the small (S) segment (encoding the nucleoprotein)
by reverse transcription PCR using consensus primers targeting New World hantavirus (8). We confirmed the Maripa virus sequence by sequencing the PCR products. Comparison of the partial sequences (393 bps excluding primers) obtained from the last 4 cases confirmed the closeness of these sequences with that obtained from the first case of Maripa virus identified in 2008 (5). Finally, we generated the complete RNA sequence of the S segment by using a Bayesian approach performed with Mr. Bayes 3.2.2 (9). All Maripa virus sequences identified in French Guiana clustered together within the Rio Mamoré and Anajatuba clades at the basal position of this group (Figure).

During the same period, we also found 3 patients (2 men and 1 woman) positive for hantavirus IgG and negative for virus genomic material by reverse transcription PCR. These serologic results reflected a previously cleared infection by another New World hantavirus circulating in the region causing no or mild clinical symptoms could not be ruled out.

We performed environmental investigations around the residences of the 5 patients with confirmed hantavirus infections. Patients 1, 3, and 4 were from different parts of the same municipality, Tonate-Macouria (Table), and

Table. Clinical characteristics of 5 male patients infected with Maripa virus, French Guiana, 2008–2016*.  

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Reference range</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Mean (min–max)</th>
<th>No. (%) patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year case reported</td>
<td></td>
<td>2008</td>
<td>2009</td>
<td>2010</td>
<td>2013</td>
<td>2016</td>
<td>56.2 (38.0–71.0)</td>
<td>13.4</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td>38</td>
<td>56</td>
<td>49</td>
<td>67</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of residence</td>
<td></td>
<td>Tonate-Macouria</td>
<td>Rémire-Montjoly</td>
<td>Tonate-Macouria</td>
<td>Tonate-Macouria</td>
<td>Iracoubo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of hospital admission after symptom onset</td>
<td></td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td></td>
<td>4.2 (2.0–7.0)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

| Characteristics at admission to ICU |  |  |  |  |  |  |  |  |
| Heart rate, beats/min | 140 | 150 | 140 | 168 | 170 | 154 (140–170) | 15 |
| Shock | Yes | Yes | Yes | Yes | Yes | Yes |  |  |
| Acute lung injury | Yes | Yes | Yes | Yes | Yes | Yes |  |  |
| Acute kidney injury | Yes | Yes | Yes | Yes | Yes | Yes |  |  |
| Urea nitrogen, mmol/L | 1.7–8.3 | 9.3 | 10.0 | 10.7 | 13.7 | 6.4 | 10.0 (6.4–13.7) | 2.6 |
| Creatinine, μmol/L | 62–106 | 192 | 174 | 196 | 196 | 126 | 176.8 (126.0–196.0) | 29.8 |
| Serum protein, g/L | 60–80 | 44.8 | 69.0 | 30.1 | 57.0 | 55.0 | 51.2 (30.1–69.0) | 14.6 |
| Lactate, mmol/L | 0.63–2.44 | 2.2 | 8.0 | 5.3 | 3.1 | 5.0 | 4.7 (2.2–8.0) | 2.2 |
| Leukocytes, ×10⁹ cells/L | 4–10 | 22.5 | 21.1 | 19.5 | 9.6 | 9.7 | 16.5 (9.6–22.5) | 6.3 |
| Platelet count, ×10⁹/L | 150–400 | 50 | 149 | 67 | 125 | 79 | 94.0 (50.0–149.0) | 41.5 |
| Hematocrit, % | 38–51 | 50.5 | 66.6 | 55.9 | 52.6 | 41.7 | 53.5 (41.7–66.6) | 9.0 |
| AST, IU/L | <37 | 17 | 49 | 10 | 18 | 17 | 22.2 (10.0–49.0) | 15.3 |
| ALT, IU/L | <40 | 31 | 47 | 24 | 58 | 29 | 37.8 (24.0–58.0) | 14.2 |
| Bilirubin, μmol/L | <17 | 3.4 | 8.2 | 6.0 | 6.0 | 4.6 | 5.6 (3.4–8.2) | 1.8 |
| CPK, U/L | <17–174 | 215 | 119 | 149 | 181 | 262 | 185.2 (119.0–262.0) | 55.9 |
| Troponin, μg/L | <0.1 ND | ND | ND | 0.20 | 0.06 | 0.25 | 0.17 (0.06–0.25) | 0.10 |
| CRP, mg/L | <10 | 192.0 | 166.0 | 93.3 | 92.4 | 154 | 139.5 (92.4–192.0) | 44.8 |

| Death | No | Yes | Yes | Yes | Yes | Yes | 4 (80) |
| Time of death after admission, h | NA | 2 | 6 | 16 | 14 | 9.5 (2.0–16.0) | 6.6 |

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; CRP, C-reactive protein; ICU, intensive care unit; max, maximum; min, minimum; NA, not applicable; ND, not determined.
Figure. Phylogenetic tree based on the 1,308-bp fragment of the small (S) segment of 58 hantaviruses, including the 5 Maripa hantavirus isolates identified in French Guiana, 2008–2016 (gray shading). Tree was constructed by using the general time-reversible plus gamma distribution plus invariable site model of nucleotide evolution. GenBank accession numbers of viruses are indicated. Support for nodes was provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probability >0.7. Scale bar indicates mean number of nucleotide substitutions per site.
potentially exposed to hantavirus at a forest edge (patient 1), a periurban area (patient 3), and a slash-and-burn agricultural field (patient 4). Patient 2 was from Rémire-Montjoly municipality, a suburb of Cayenne (the largest city in French Guiana), and patient 5 was from a slash-and-burn agriculture field in the Iracoubo municipality (Table). No secondary human cases were reported near these confirmed cases. We set up a large ecorepidemiologic survey in French Guiana and captured rodents around the patients’ homes to characterize rodent reservoirs (10). This survey led to the capture of 20 rodents of 5 different species. By using molecular approaches, we detected 4 Maripa virus–positive rodents: 2 *Oligoryzomys delicatus* (formerly named *O. fulvescens* in French Guiana) (11), 1 of which was found near the home of patient 4, and 2 *Zygodontomys brevicauda*, both found near the home of patient 2.

**Conclusions**

The results of a retrospective serologic study in French Guiana prompted us to pursue an active human surveillance program focused on patients with clinical symptoms consistent with hantavirus disease. This surveillance, conducted over 8 years, showed that human infection with Maripa hantavirus in this department is rare but associated with a high case-fatality rate. In the context of environmental perturbations with growing and unplanned urbanization and human populations that increasingly come in contact with wild mammalian fauna, surveillance in humans and investigations with rodent reservoirs should continue. These initiatives could help prevent the potential emergence of HPS in French Guiana.

**Acknowledgment**

We thank Damien Donato for his technical assistance.

This study was supported in part by the Centre National de Référence des Hantavirus Laboratoire Associé financed by the Institut Pasteur de la Guyane and Santé Publique France (Saint-Maurice, France). This study benefited from the RESERVOIRS program, which is supported by the European Regional Development Fund and Fonds Européen de Développement Régional, and received assistance from Région Guyane and Direction Régionale pour la Recherche et la Technologie and Investissement d’Avenir grants managed by the Agence Nationale de la Recherche (CEBA ANR-10- LABEX-25-01).

Dr. Matheus is a research assistant studying viral emergence, particularly that of hantaviruses, at the Institute Pasteur de la Guyane in French Guiana. She has research interests in the diagnosis and pathophysiology of arboviruses.

**References**


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Berlin Squirrelpox Virus, a New Poxvirus in Red Squirrels, Berlin, Germany

Gudrun Wibbelt,1 Simon H. Tausch,1 Piotr W. Dabrowski, Olivia Kershaw, Andreas Nitsche, Livia Schrick

Near Berlin, Germany, several juvenile red squirrels (Sciurus vulgaris) were found with moist, crusty skin lesions. Histology, electron microscopy, and cell culture isolation revealed an orthopoxvirus-like infection. Subsequent PCR and genome analysis identified a new poxvirus (Berlin squirrelpox virus) that could not be assigned to any known poxvirus genera.

The Eurasian red squirrel (Sciurus vulgaris) is the only species of tree squirrels endemic throughout most of Europe. Although they are usually abundant, red squirrels are endangered or extinct in some regions in Great Britain and Ireland that are co-inhabited by invasive eastern gray squirrels (Sciurus carolinensis), which were introduced from North America in the late 19th century. One major threat is the transmission of squirrelpox virus (SQPV) from the gray squirrel reservoir host to red squirrels, which succumb to lethal infections (1). SQPV had been assigned to the parapoxvirus family (2), but the latest viral genome data placed it in a separate clade within the poxvirus family (3). Recently, different poxviruses have been associated with similar lesions in American red squirrels (Tamiasciurus hudsonicus) from Canada (4), but except for a single case report from Spain (5), no poxvirus infections in squirrels have been reported in continental Europe.

The Study
In 2015 and 2016, at least 10 abandoned weak juvenile red squirrels were submitted to a sanctuary near Berlin, Germany. The animals had exudative and erosive-to-ulcerative dermatitis with serocellular crusts at auricles, noses, digits, tails, and genital/perianal regions. Skin specimens from affected animals were investigated by electron microscopy (EM) and PCR. Three animals that died under care were submitted for necropsy. We obtained samples of all organs for histological and PCR examination. We used 1 sample of a skin lesion for virus propagation in cell culture.

EM-negative staining of skin lesions from all animals led to the discovery of brick-shaped poxvirus particles with irregular threadlike surface fibers and an average size of 294 nm × 221 nm (Figure 1). Pathological findings of corresponding skin lesions were consistent with poxvirus infection (ballooning degeneration of epidermal keratinocytes, numerous intracytoplasmic inclusion bodies, epidermal ulceration with suppurative inflammation, and secondary bacterial infection). All inner organs had either no pathological changes or lesions unrelated to poxvirus infection.

To confirm the morphologic diagnosis, we extracted DNA from skin lesions and performed various PCRs. An orthopoxvirus (OPV)–specific PCR showed negative results (6); a parapoxvirus (PPV)–specific PCR (6), a leporipoxvirus-specific PCR (A. Nitsche and L. Schrick, unpub. data), and a poxvirus-screening PCR (7) were positive for some samples. Obtained sequence fragments indicated poxviral relatedness but did not allow for the assignment to a poxvirus genus. Thus, we performed massively parallel sequencing. We directly subjected DNA extracted from a skin lesion on the foot of a dead animal to Nextera XT Library preparation and sequenced it on an Illumina HiSeq 1500 instrument (Illumina, San Diego, CA, USA), yielding 7,242,301 paired-end reads (150 + 150 bases, rapid run mode). Mapping (8) the obtained reads to all poxvirus reference sequences available in GenBank in high-sensitivity mode provided no notable results, which pointed to a virus with a highly deviant genome. Therefore, we separated poxviral reads from background data using RAMBO-K version 1.2 (9) and assembled the resulting 1,520,811 reads (460-fold coverage after manual iterative mapping and scaffolding. We confirmed the genomic sequence by resequencing (Illumina MiSeq) of a Vero E6 cell-culture isolate obtained from a different skin specimen of the same animal. We named the new virus Berlin SQPV (BerSQPV), and uploaded the combined sequence information to GenBank (accession no. MF503315). Direct sequencing of DNA from skin samples of 3 other animals from the same origin yielded sequences with >99.9% identity to BerSQPV.

We compared characteristics of BerSQPV to related viruses and found that the EM structure shows features typical for OPV but the genome size of ≈143 kb is more consistent with PPV or SQPV from the United Kingdom (11) than with the large genome of OPV, whereas the guanine-cytosine (GC) content of 38.5% is more consistent

1These authors contributed equally to this article.
with OPV and leporipoxvirus than with PPV and SQPV from the United Kingdom. Therefore, we explored the genomic relationship of BerSQPV to other chordopoxviruses. Pairwise alignments of each of the chordopoxvirus genomes available in GenBank with the BerSQPV genome resulted in a pairwise identity of at most 47% to tanapox virus isolate TPV-Kenya (accession no. EF420156.1). The retrieved phylogenetic tree (Figure 2) demonstrates that BerSQPV cannot be assigned to any of the known poxvirus genera; moreover, it does not cluster with the only other squirrel poxvirus with a published genome sequence (11). Further phylogenetic analyses based on conserved single genes frequently used for poxvirus tree calculations (A3L, F10L+F12L, F13L, E13L, E9L [VACV Copenhagen nomenclature]) showed similar results (A56R was not used for tree calculations because this open reading frame is too divergent among the Chordopoxvirinae), with BerSQPV forming a unique branch (data not shown). In addition, any partial sequences of SQPV available in GenBank were aligned to BerSQPV, showing a maximal sequence identity of 64.3% to gene E9L (GenBank accession

**Table.** Results of PCRs of different tissues from 7 live and 4 deceased squirrels showing poxvirus lesions, Berlin, Germany*

<table>
<thead>
<tr>
<th>Year of sampling</th>
<th>Live/dead</th>
<th>Tissue</th>
<th>Cq BerSQPV</th>
<th>Cq c-myc</th>
<th>ΔCq (BerSQPV – c-myc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>Dead</td>
<td>Archived skin (paraffin)</td>
<td>23.7</td>
<td>34.8</td>
<td>−11.1</td>
</tr>
<tr>
<td>2015</td>
<td>Live</td>
<td>Crust†</td>
<td>12.5</td>
<td>17.7</td>
<td>−5.2</td>
</tr>
<tr>
<td>2015</td>
<td>Live</td>
<td>Crust†</td>
<td>14.8</td>
<td>18.3</td>
<td>−3.5</td>
</tr>
<tr>
<td>2015</td>
<td>Dead</td>
<td>Skin (foot)†</td>
<td>11.1</td>
<td>18.8</td>
<td>−7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin (tail)</td>
<td>9.7</td>
<td>17.9</td>
<td>−8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin (toe)†‡</td>
<td>10.1</td>
<td>18.6</td>
<td>−8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung</td>
<td>33.2</td>
<td>27.0</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>34.7</td>
<td>23.1</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>34.9</td>
<td>23.9</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain</td>
<td>33.9</td>
<td>24.5</td>
<td>9.4</td>
</tr>
<tr>
<td>2015</td>
<td>Dead</td>
<td>Skin (forefoot)†</td>
<td>10.9</td>
<td>18.2</td>
<td>−7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin</td>
<td>26.3</td>
<td>28.0</td>
<td>−1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung</td>
<td>33.6</td>
<td>23.1</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Negative</td>
<td>22.1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>38.3</td>
<td>23.9</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>Negative</td>
<td>24.1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Small intestine</td>
<td>Negative</td>
<td>21.8</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Large intestine</td>
<td>Negative</td>
<td>24.4</td>
<td>NA</td>
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<tr>
<td></td>
<td></td>
<td>Brain</td>
<td>Negative</td>
<td>25.3</td>
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</tr>
<tr>
<td>2015</td>
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<td>Crust</td>
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<td>23.2</td>
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<td>35.2</td>
<td>25.4</td>
<td>9.8</td>
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<td></td>
<td></td>
<td>Liver</td>
<td>Negative</td>
<td>20.8</td>
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<td>Spleen</td>
<td>34.0</td>
<td>25.1</td>
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<td>Kidney</td>
<td>Negative</td>
<td>25.9</td>
<td>NA</td>
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<td></td>
<td>Small intestine</td>
<td>36.4</td>
<td>21.6</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Large intestine</td>
<td>35.0</td>
<td>23.5</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain</td>
<td>Negative</td>
<td>24.6</td>
<td>NA</td>
</tr>
<tr>
<td>2016</td>
<td>Live</td>
<td>Crust</td>
<td>15.0</td>
<td>22.0</td>
<td>−7.0</td>
</tr>
<tr>
<td>2016</td>
<td>Live</td>
<td>Crust</td>
<td>12.1</td>
<td>18.6</td>
<td>−6.5</td>
</tr>
<tr>
<td>2016</td>
<td>Live</td>
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<td>14.1</td>
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<tr>
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<td>Crust</td>
<td>13.2</td>
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<tr>
<td>2016</td>
<td>Live</td>
<td>Crust</td>
<td>12.9</td>
<td>18.3</td>
<td>−5.4</td>
</tr>
</tbody>
</table>

*BerSQPV DNA was quantified in relation to cellular c-myc DNA; lower values for ΔCq indicate higher virus loads in a respective tissue. BerSQPV, Berlin squirrelpox virus; Cq, quantification cycle; NA, not applicable.
†Specimen applied to next-generation sequencing.
‡Specimen used to obtain the cell culture isolate.
no. AY340976.1), further emphasizing the uniqueness of this newly identified virus.

We designed a BerSQPV-specific quantitative PCR based on the genome sequence as a tool for future investigations (primer BerSQPV_F: ggAAGTTTCCCATACCAAATgA, primer BerSQPV_R: ATCTCAACCgCAGACgTA, probe BerSQPV_TM: FAM-ACgTTAATTgCgTA-ATT). Sensitivity was <10 genome equivalents per reaction amplifying plasmid dilution rows. We first validated the specificity in silico during the design process, revealing the highest identity of 88% to cowpox virus Kostroma (GenBank accession no. KY369926.1), with mismatches in crucial positions in the primer and probe binding sites. Squirrel poxvirus strain Red squirrel UK (GenBank accession no. NC_022563.1) showed only 84% identity, with additional mismatches in amplification-relevant positions. Practical PCR testing using DNA from cowpox, monkeypox, ectromelia, parapox-ORF, myxoma, avipox, and molluscipox viruses showed no cross-reactivity.

The new specific quantitative PCR was subsequently applied to DNA from skin lesions archived from 1 squirrel found dead in 2014 in the Berlin area, 2 live squirrels from...
2015, and 5 live squirrels from 2016, as well as various organs from 3 affected squirrels necropsied in 2015 (Table). Organ tissues yielded high BerSQPV DNA loads in the affected skin but low viral DNA loads for inner organs, findings in concordance with pathological findings, indicating the detection of viral DNA in the blood homogeneously distributed throughout the organs with specific tropism for the skin. Low virus loads in inner organs are usually observed in poxvirus infections that do not generalize. PCR results indicate that this virus has been circulating in the Berlin area over the past 10 years.

Conclusions
We describe a new poxvirus, BerSQPV, isolated from red squirrels in Berlin, Germany, that causes pathological changes consistent with other epidermal poxvirus infections. Genome analysis revealed a unique sequence within the poxvirus family, as BerSQPV is not clustering to other poxvirus genera, including UK SQPV from red squirrels from Great Britain. In contrast to UK SQPV, which resembles PPV ultrastructurally (2), the ultrastructure of BerSQPV is comparable to that of OPV. Two other poxviruses from tree squirrels with ultrastructural appearance similar to BerSQPV have been reported: a Eurasian red squirrel from Spain with epidermal poxvirus lesions (5) and American red squirrels from Canada (15). Although no sequence information is available for the SQPV from Spain, the partial sequence analysis of SQPV from Canada showed the virus to also be distinct from all known mammalian poxviruses but most closely related to PPV, followed by UK SQPV (15).

BerSQPV is suspected to have been circulating for several years among Eurasian red squirrels in the greater Berlin area. Although diseased animals in care were handled in close contact, caretakers have remained asymptomatic, suggesting a negligible risk for human infection. Further detailed characterization of the isolated virus is ongoing.

Acknowledgments
The authors are grateful to Tanya Lenn, who encountered squirrels with suspicious lesions for many years, for providing samples. Special thanks are given to Dagmar Viertel, Zoltan Mezoe, Doris Krumnow, and Anja Ostrowski and the technicians of the German Consultant Lab for Poxviruses (RKI) for their excellent technical assistance, as well as to Ursula Erikli for copyediting.

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References

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Summer Dreams

Veranja Liyanapathirana

Monsoon rains
Have come and gone
Quintessential
Remnants of summer
Rambutans and durians
Discarded peels
Bustles with activity
Lives of other kinds
Wriggling and swimming
Summer dreams
Of warm humid nights
Fanning away the creatures of nature
That annoying bustle of wings
Of lives that emerged from the discards
Creatures of nature
That fly in search of meals
Meals of red
Within the meal
Enveloped
In a protective sheath
Lies a being
Is it alive or is it not?
We argue in the summer’s heat
How to define life?
This being is a villain
A villain of a kind
Minute yet mighty
Astute and elusive
Adapting and evolving

Acknowledgment

Thanks to Samanthika Abeywickrama for the inspiration.
This poem was inspired by an online conversation with Dr.

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DOI: https://doi.org/10.3201/eid2310.171117
**Bedaquiline and Linezolid for Extensively Drug-Resistant Tuberculosis in Pregnant Woman**

Marie Jaspard, Elisabeth Elefant-Amoura, Isabelle Melonio, Inés De Montgolfier, Nicolas Veziris, Eric Caumes

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DOI: https://doi.org/10.3201/eid2310.161398

A woman with extremely drug-resistant tuberculosis treated with a drug regimen including linezolid and bedaquiline during her last 3 weeks of pregnancy gave birth to a child without abnormalities. No fetal toxicities were noted by 2 years after delivery. This drug combination might be safe during the late third trimester of pregnancy.

In 2014, a total of 480,000 cases of multidrug-resistant tuberculosis (MDR TB) were present worldwide; 9% of these cases were also resistant to fluoroquinolones and aminoglycosides (1 of the 3 available injectable second-line drugs), thereby defining them as extensively drug-resistant TB (XDR TB) (1). Pregnant women with untreated TB have a mortality rate of 40%, suggesting a severe disease course in these women (2). Considering the severity of XDR TB, pregnant women with this form of TB need to be effectively treated before delivery. However, treatment regimens are usually stopped for fear of fetal toxicity (3).

Bedaquiline efficacy has been demonstrated in MDR TB and is now recommended by the World Health Organization for the treatment of MDR TB (4). However, the half-life of bedaquiline in plasma is extremely prolonged, and serious side effects such as hepatitis and QT-interval prolongation can occur (5). Similarly, linezolid is an effective drug for treating XDR TB, but side effects such as neurotoxicity and hematologic toxicity frequently occur (6). Because no data are available on the treatment of pregnant woman with these drugs, bedaquiline and linezolid are not prescribed for this population.

We report the case of a pregnant woman with XDR TB treated with a regimen including bedaquiline and linezolid. In 2008, MDR TB was diagnosed in a 33-year-old woman in Georgia. She was given a 7-drug regimen including ethambutol, pyrazinamide, cycloserine, para-aminosalicylic acid (PAS), and kanamycin for 18 months. In 2012, she relapsed and was given a different regimen of drugs (pyrazinamide, cycloserine, PAS, amoxicillin/clavulanate, capreomycin, levofloxacin, and prothionamide) for the same duration. In 2014, she relapsed again but with an XDR strain and was prescribed pyrazinamide, cycloserine, PAS, amoxicillin/clavulanate, capreomycin, levofloxacin, prothionamide, clarithromycin, and clofazimine. Despite 4 months of this drug regimen, sputum test results were positive. She eventually went to France for treatment.

When she arrived at Hôpital Pitié Salpêtrière she was 31 weeks pregnant. She had not received any MDR TB drugs since the beginning of her pregnancy. Her clinical status was good, except for a chronic cough over the past 6 months without weight loss. The fetus had no abnormalities. The woman’s sputum had >100 acid-fast bacilli and was culture positive for a *Mycobacterium tuberculosis* strain exhibiting resistance to isoniazid, rifampin, fluoroquinolones (low-level resistance), ethambutol, ethionamide, and aminoglycoside but susceptibility to cycloserine, PAS, bedaquiline, and linezolid. A computed tomography scan showed a large cavity in her upper left lung.

On the basis of the patient’s resistance profile, she was given the following drug regimen at 36 weeks’ gestation: bedaquiline, linezolid (600 mg/d), PAS, cycloserine, and levofloxacin. The Reference Centre for Teratogenic Agents (Paris, France) and the manufacturer of bedaquiline approved giving this regimen because the fetus did not have major side effects.

Fetal status was monitored weekly. Delivery was planned at 39 weeks, and the patient gave birth to a healthy girl who was immediately separated from her mother because of the acid-fast bacilli in her mother’s sputum.

Mycobacterial analysis of the placenta was negative. The newborn underwent 3 gastric washings to collect biological samples for further TB testing. These samples were negative by acid-fast staining and mycobacteria culture. Tuberculin skin test results were negative, and radiographs of her chest and cardiac ultrasonography gave remarkable results. She did not have a QT prolongation or hepatitis.

After 24 months of therapy and lung surgery, the mother’s TB resolved. At 2 years of age, the child showed normal growth and did not have TB or signs of any clinical disorders, especially those of neurologic and cardiac conditions.

This report suggests that pregnant women with XDR TB can receive bedaquiline and linezolid during the last 3 weeks (late third trimester) of pregnancy without major side effects. Second-line therapy appears to be effective and safe during pregnancy and could be considered to treat some pregnant women with MDR TB. Indeed, in a study in Peru, no difference in efficacy was found when comparing non-pregnant women and pregnant women with MDR TB treated with second-line TB drugs (7). Also, long-term follow-up of children born to women given these treatments affirms the safety of second-line TB drugs during pregnancy (8,9).
However, treating pregnant women with XDR TB is more challenging. Our patient was given a regimen that included bedaquiline and linezolid, neither of which has data available on its safety during pregnancy. Even though the newborn was in good health at birth, no general conclusion could be drawn about the potential teratogenicity of these drugs because the treatment had been introduced only 3 weeks before delivery. In this single case, no specific maternal or fetal side effects were noticed, indicating the potential for using this drug combination. However, more data are needed to ensure the safety of these drugs during pregnancy.

Dr. Jaspard is a medical doctor in the Infectious Disease Department at Pitié Salpêtrière Hospital in Paris, France. Her research interests are medicinal treatments for multidrug-resistant tuberculosis.

References


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Mycobacterium riyadhense in Saudi Arabia

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DOI: https://doi.org/10.3201/eid2310.161430

We explored in detail the nationwide existence of Mycobacterium riyadhense in Saudi Arabia. In 18 months, 12 new cases of M. riyadhense infection were observed, predominantly among Saudi nationals, as a cause of pulmonary disease. M. riyadhense may be emerging as a more common pathogen in Saudi Arabia.

Infections caused by nontuberculous mycobacteria (NTM) appear to be emerging globally, but the definitive reasons for this are unclear. Advances in diagnostic technologies have led to the identification of >160 species of Mycobacterium, including several human pathogens. M. riyadhense is a slow-growing NTM identified as a cause of pulmonary and extrapulmonary illnesses in humans from Riyadh, Saudi Arabia (1,2). At least 8 clinical cases have been reported from France, Bahrain, Saudi Arabia, and South Korea, with 5 of the 8 cases in Saudi Arabia (1–6) (Table). M. riyadhense can be misidentified by commercially available line probe assays as M. tuberculosis complex, mostly because of confusing banding patterns (1). A recent nationwide study of NTM prevalence in Saudi Arabia showed no suspected cases of M. riyadhense, which could be due to limiting the screening to line probe assays (7).

To explore the presence of M. riyadhense in clinical settings in Saudi Arabia, we conducted a prospective study on a nationwide collection of isolates. Suspected NTM isolates reported as M. tuberculosis complex or Mycobacterium species with nonspecific banding pattern by line probe assays were subjected to different conservative gene sequencing to identify M. riyadhense.

During April 2014–September 2015, we collected 458 NTM isolates, with clinical and epidemiological data, from all 9 national referral laboratories in different provinces of Saudi Arabia. We formulated the isolate enrollment strategy to suspect M. riyadhense on the basis of previous studies (1,2). In brief, we conducted primary identification of the
isolates using line probe assay-Genotype MTBC (Hain Lifescience, Nehren, Germany). We further tested isolates that showed a nonspecific banding pattern (1,2,3) by using Genotype Mycobacteria CM and AS assays (Hain Lifescience). The Genotype Mycobacteria CM assay showed a specific banding pattern of 1,2,3,10,15,16 (1,2,3,10,16 in previous study) for a group of isolates; AS assay identified these isolates as *Mycobacterium* species. We subjected all isolates to partial sequencing of 16S rRNA, *rpoB* and *hsp65* genes using BigDye Terminator chemistry (Applied Biosystems, Foster City, CA, USA) (8–10). We then subjected the assembled sequences of all 3 genes to analysis via BLAST (https://blast.ncbi.nlm.nih.gov) and the EzTaxon database. We followed stringent identification criteria, requiring similarity ≥99% between isolate and reference strain for species confirmation.

We identified 14 isolates that fit the inclusion criteria; most were reported from the Central province, Riyadh, in Saudi Arabia, but the reason is unclear. Microbiological analysis showed slow-growing mycobacteria producing rough white colonies on LJ medium within 3–4 weeks of incubation at 37°C. Primary sequencing of the 16S rRNA gene showed 12 cases of *M. riyadhense* had a 99%–100% match with 3

<table>
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<th>Case</th>
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<th>Nationality</th>
<th>City</th>
<th>Region/ country</th>
<th>Specimen</th>
<th>Smear/ culture results</th>
<th>Clinical relevance†</th>
<th>Treatment†</th>
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<td>West/ Saudi Arabia</td>
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<td>West/ Saudi Arabia</td>
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<td>(8)</td>
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<td>++/+</td>
<td>Yes</td>
<td>INH/RIF/EMB/PZA</td>
<td>Cured</td>
</tr>
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</table>

*BA, bronchoalveolar lavage; NA, not available; +, positive; -, negative. Positive smearing results indicate the presence of acid-fast bacilli (AFB). Wherein, 10–99 AFB identified in 100 fields have been noted with (+), and 100–999 AFB in 100 fields correlates with (++). Positive culturing results highlight the presence of mycobacterial growth. CIP, ciprofloxacin; CLR, clarithromycin; EMB, ethambutol; INH, isoniazid; MX, moxifloxacin; PZA, pyrazinamide; RFP, rifampin. †Based on American Thoracic Society guidelines for pulmonary NTM disease/colonization ([https://www.thoracic.org/statements/resources/mtpi/nontuberculous-mycobacterial-diseases.pdf](https://www.thoracic.org/statements/resources/mtpi/nontuberculous-mycobacterial-diseases.pdf)).

Table. Summary of all reported *Mycobacterium riyadhense* infections in Saudi Arabia and other countries*
database strains (GenBank accession nos. JF896094, JF896095, and NR044449). On the other hand, rpoB and hsp65 sequences also showed 99%–100% similarity with other sequences (accession nos. EU921671, EU27644.1, JF86095 and NR 044449.1). The other closest species observed during the analysis were M. alsense, M. szulgai, and M. angelicum (98% similarity with 16S rRNA gene sequences); M. genavensis and M. simulans (96% similarity with hsp65 gene sequences); and M. lacus, M. intracellulare, and M. malmoense (94% similarity with rpoB gene sequences). Two isolates that matched inclusion criteria could not be identified as M. riyadhense; BLAST analysis showed the closest matching species as M. lacus DSM 44577(T), with 89% similarity. Two 16S rRNA gene sequences from this study were deposited in GenBank (accession nos. KX898970 and KX898971).

We identified 12 clinical cases of M. riyadhense infection, including pulmonary and extrapulmonary invasive infections, over a period of 18 months. Demographically, Saudi citizens dominated; 11 of 12 case-patients were male, and mean age was 50 years. Geographic distribution of cases showed 10 cases from Riyadh (Central province) and 2 from Dammam (Eastern province). Clinical data revealed 9 cases with pulmonary involvement and 3, including a pediatric case, with lymphadenitis. Of note, 75% of the respiratory cases were clinically relevant according to American Thoracic Society criteria for NTM pulmonary disease. Most patients recovered with isoniazid, rifampin, and ethambutol therapy (Table).

The lack of advanced molecular diagnostic tools in clinical laboratories in Saudi Arabia impedes the accurate identification of M. riyadhense. Without an accurate diagnosis, treatment is delayed. In this study, most of the patients were treated with standard TB regimens; some of them received clarithromycin, which did not appear to be highly effective (2). To date, no standard treatment regimen for M. riyadhense disease has been developed, likely due to its status as a rare species. In the cases reported here, patients generally responded well to the initial therapies, but drug resistance may challenge the empirical treatment used. A strain resistant to isoniazid is already reported from South Korea (3). We recommend that clinicians in Saudi Arabia be vigilant to the possible emergence of M. riyadhense as a more common pathogen.

Acknowledgments
We thank Abdulrahman Abdullah Korairi and Abdulhameed Alfagih for the immense support in data collection.

This study was supported by King Abdulaziz City for Science and Technology (KACST) under the National Science and Technology Initiative Program (12-MED-3172-20).

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References

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Carbapenemase VCC-1–Producing Vibrio cholerae in Coastal Waters of Germany

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DOI: https://doi.org/10.3201/eid2310.161625

During antimicrobial drug resistance testing for Vibrio spp. from coastal waters of Germany, we identified 4 nontoxigenic, carbapenem-resistant Vibrio cholerae isolates. We used whole-genome sequencing to identify the carbapenemase gene bla\textsubscript{VCC-1}. In addition, a molecular survey showed that more bla\textsubscript{VCC-1}–harboring isolates are present in coastal waters of Germany.

Mangat et al. recently identified a novel ambler class A carbapenemase (VCC-1) in nontoxigenic Vibrio cholerae isolated from imported retail shrimp from India intended for human consumption in Canada (1). Occurrence of bla\textsubscript{VCC-1}–harboring bacteria in seafood might be caused by uptake of V. cholerae in the aquatic environment. Lutz et al. reported worldwide distribution of V. cholerae non–O1/O139 strains in coastal waters with low salinity (2). Some of these strains were associated with wound infections and with diarrheal diseases after ingestion of contaminated seafood (3,4).

An antimicrobial resistance survey of potentially pathogenic Vibrio spp. recovered from coastal waters of Germany identified 4 carbapenem-resistant V. cholerae non–O1/O139 isolates (5). These isolates were detected in the Baltic Sea (VN-2997, Eckernförde) and North Sea (VN-2808, Büsum; VN-2825, Speicherkoog; VN-2923, unknown). We used whole-genome sequencing to examine the genetic basis of carbapenem resistance in these strains.

We isolated genomic DNA by using the Easy-DNA Kit (Invitrogen, Carlsbad, CA, USA). This DNA was used for preparation of libraries by using the Nextera-XT DNA Sample Preparation Kit (Illumina Inc., San Diego, CA, USA) and sequenced by using an MiSeq-benchtop sequencer, MiSeq-Reagent version 2 (300 cycles), and two 150-bp paired-end reads (Illumina Inc.). We then performed de novo assemblies of reads by using SPAdes version 3.5.0 (http://spades.bioinf.spbau.ru/release3.5.0/).

1Current affiliation: European Food Safety Authority, Parma, Italy.

We deposited sequences in GenBank and performed genome annotation by using the NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

We found bla\textsubscript{VCC-1} in all isolates on contigs of 2,135 bp (VN-2825, VN-2997), 2,139 bp (VN-2923), and 2,737 bp (VN-2808). The bla\textsubscript{VCC-1}–coding sequences and flanking nucleotide sequences were 100% identical among the strains, as determined by sequence alignments. We also identified the main characteristics of V. cholerae genomes (Table; online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/10/16-1625-Techapp1.pdf). Overall, the genomes belong to the same multilocus sequence type (ST), ST336 (adk 57, gyrB 76, mdh 14, metE 115, pntA 18, purM 1, pyrC 101) (6).

We performed functional studies of the entire bla\textsubscript{VCC-1}–harboring region (pVCC-1C, 2.7 kb) and bla\textsubscript{VCC-1} gene (pVCC-1G, 0.9 kb) of V. cholerae VN-2808 (online Technical Appendix Figure) by molecular cloning of PCR-amplified regions according to standard procedures (7). After transformation of Escherichia coli GeneHogs (Invitrogen, Darmstadt, Germany) and susceptibility testing for aztreonam, imipenem, and meropenem as described (5), both constructs showed decreased inhibition zone diameters compared with that for E. coli GeneHogs vector. We observed slightly reduced drug susceptibility levels or intermediate resistance levels, as observed in V. cholerae VN-2808 (online Technical Appendix Figure).

On the basis of these results, we conducted bla\textsubscript{VCC-1} screening of the V. cholerae collection at the German Federal Institute for Risk Assessment (Berlin, Germany). This collection contains 312 toxigenic and nontoxigenic isolates from human, environmental, and food origin obtained in Europe (n = 218), Africa (n = 20), Asia (n = 18), North America (n = 1), South America (n = 1), and of unknown origin (n = 54) during 1941–2015.

We performed PCR by using primers (bla\textsubscript{VCC-1}–forward/reverse: 5′-ATCTCTACTTCAACAGCTTTCG/CCTAGCTGCTTTAGCAATCAC-3′) with denaturation at 94°C for 120 s; 35 cycles of denaturation at 94°C for 15 s, annealing at 53°C for 30 s, and elongation for 210 s at 72°C; and a final elongation at 72°C for 1 min. This testing detected bla\textsubscript{VCC-1} in 3 (1.6%) ctx-negative, non–O1/O139 V. cholerae isolates obtained from waters of the port of Husum, Germany, on the North Sea during 2015. Sanger sequencing confirmed the presence of bla\textsubscript{VCC-1}. These 3 isolates belong to the new multilocus ST516. This type is divergent from ST336 only for the novel pyrC 150 variant, which was recently deposited in the PubMLST database (https://pubmlst.org).

In conclusion, this study showed the presence of 7 VCC-1 carbapenemase-producing V. cholerae at different locations on the coastline of Germany. The bla\textsubscript{VCC-1} flanking manual.html).
genetic sequences were identical in the 4 sequenced *V. cholerae* from Germany and appeared to be different from the strain isolated in Canada, which probably originated in India (online Technical Appendix Figure). This finding suggests that *bla*<sub>VCC-1</sub> was acquired by *V. cholerae* from a yet unknown progenitor on at least 2 occasions. Strains from Germany probably belong to the autochthonous microflora and represent an environmental reservoir of carbapenem resistance in coastal waters. *bla*<sub>VCC-1</sub>-encoding *V. cholerae* might be taken up by mussels, shrimps, and fish and then enter the food chain.

Because carbapenems are needed for treatment of severe infections with multiderug-resistant bacteria in humans, the presence of bacteria with acquired, and thereby potentially transferable, carbapenemases in environments near human activities is a major public health concern (8). To date, acquired carbapenemases were detected mainly in clinical isolates and only rarely in environmental and foodborne bacteria (9,10). Exposure of humans to carbapenemase-producing pathogenic bacteria by ingestion of contaminated food products or by direct contact with contaminated water might pose a threat to public health. Our findings indicate that surveillance for antimicrobial drug resistance should be extended to locations of human activities and foods of aquatic origin.

This study was supported by the Federal Ministry of Education and Research (VibrioNet grant 01K1015A). The German Research Program Auswirkungen des Klimawandels auf Wasserstraßen und Schifffahrt was supported the Federal Ministry of Transport and Digital Infrastructure.

The positions and opinions in this article are those of the authors alone and are not intended to represent the views or scientific works of the European Food Safety Authority.

Dr. Hammerl is a research scientist and deputy at the National Reference Laboratory on Antimicrobial Resistance, Department of Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany. His primary research interests are microbiological and molecular tracing of foodborne pathogens and antimicrobial resistance.

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3. Deshayes S, Daurel C, Cattoir V, Parienti JJ, Quilici ML, de La Blanchardière A. Non-O1, non-O139 *Vibrio cholerae* bacteraemia:
We isolated East/Central/South African genotype chikungunya virus during the 2016 epidemic in Rio de Janeiro, Brazil. Genome sequencing revealed unique mutations in the nonstructural protein 4 (NSP4-A481D) and envelope protein 1 (E1-K211T). Moreover, all Brazil East/Central/South isolates shared the exclusive mutations E1-M407L and E2-A103T.

Chikungunya virus (CHIKV) is an alphavirus (family Togaviridae) transmitted by Aedes aegypti and Aedes albopictus mosquitoes. Chikungunya fever is characterized by fever, intense polyarthralgia, headache, joint swelling, and rash. Polyarthralgia can persist for several months after the acute phase (1). The 3 main CHIKV genotypes are Asian, West African, and East/Central/South African (ECSA), in addition to the ECSA-derived Indian Ocean lineage (1). Genetic analysis of CHIKV genomes has shown substitutions in envelope (E) 1 and E2 proteins that affect virus adaptability to Aedes mosquitoes. For example, E1-K211E and E2-V264A mutations have been reported to increase CHIKV fitness in Aedes aegypti (2), whereas E1-A226V and E2-L210Q mutations have been associated with improved adaptability to Aedes albopictus mosquitoes (3,4). The E1-T98A mutation enhances the vector-adaptability effect of E1-A226V, which is otherwise restricted by epistatic interactions between E1-98T and E1-A226V (4).

Autochthonous transmission of CHIKV (Asian genotype) in Brazil first occurred in 2014 in Oiapoque, Brazilian Amazon, 1 year after CHIKV introduction in the Caribbean (1). Since late 2014, autochthonous cases of the ECSA genotype have been reported in northeastern Brazil (1), a region of sustained cocirculation of dengue virus (DENV) for decades and the epicenter of recent Zika virus outbreaks (5).

Until late 2015, only a few imported cases of CHIKV (Asian genotype) had been reported in Rio de Janeiro (6). However, in December 2015, ten autochthonous CHIKV transmissions were reported, followed by an increase to 11,602 by August 2016, which accounted for 88.9% of reported cases in the state. Of these, 1,868 have been laboratory confirmed as CHIKV, leading to the highest incidence of CHIKV infection in southeastern Brazil (7). Nevertheless, the CHIKV genotype associated with the epidemic in Rio de Janeiro remains unknown.

On March 16, 2016, the emergency laboratory of the Brazilian Army Institute of Biology at Rio de Janeiro collected blood samples from a 16-year-old girl and a 29-year-old man who sought care at the associated military hospital. The patients had fever (40°C) for 24–48 h, debilitating polyarthralgia, and myalgia. The man also had exanthema and itching. The diagnostic hypotheses were Zika, dengue, or chikungunya, given their similar clinical symptoms (5). Laboratory findings were unremarkable except for

Autochthonous Transmission of East/Central/South African Genotype Chikungunya Virus, Brazil

Marcela S. Cunha, Nádia V.G. Cruz, Laila C. Schnellrath, Maria Luiza Gomes Medaglia, Michele E. Casotto, Rodolpho M. Albano, Luciana J. Costa, Clarissa R. Damaso


DOI: https://doi.org/10.3201/eid2310.161855
leukopenia (2,630 cells/mm$^3$ [reference range 4,500–10,000 cells/mm$^3$]) in the man. The patients were not related, lived 13 km apart, and had no history of travel outside the region during the previous 30 days. Both reported persistent mild arthralgia since October 2016. They were informed about this study and provided oral consent.

Blood samples tested negative for CHIKV, DENV, and Zika virus by TaqMan-based real-time reverse transcription PCR (8). Nevertheless, virus isolation from blood-cell fraction was successful in Vero cells infected for 48 h (isolate RJ-IB1 from the girl and RJ-IB5 from the man). The isolates had similar growth kinetics in cell culture, reaching highest yields at 48 h after infection (Figure). Virus RNA isolated from cell supernatants tested positive for CHIKV by real-time reverse transcription PCR and negative for DENV and Zika virus (8).

We used RNA isolated from cell supernatants to sequence the RJ-IB1 and RJ-IB5 genomes. After cDNA synthesis, we amplified 4 overlapping fragments by PCR and sequenced them on an Illumina MiSeq platform after

**Figure.** Growth curve and phylogenetic analysis of RJ-IB1 and RJ-IB5 CHIKV isolates, Rio Janeiro, Brazil, 2016. A) Vero cell–amplified RJ-IB1 or RJ-IB5 was titrated and used to infect Vero cells in duplicates at a multiplicity of infection of 0.05. The resulting supernatants were harvested at 0, 24, 48, and 72 h after infection. The production of infectious progeny was determined by plaque assay in Vero cells. B) The full-genome sequences of RJ-IB1 and RJ-IB5 isolates and 53 CHIKV strains representing all known genotypes were aligned with MAFFT (http://mafft.cbrc.jp/alignment/software). Phylogeny inference was performed with MEGA 6 (http://www.megasoftware.net) opting for the neighbor-joining method and kimura-2p model of substitution. Numbers on branches indicate the percentage of bootstrap support from 1,000 replicates. Values >70% are shown. Similar tree topology was obtained with maximum-likelihood method opting for Tamura-Nei model of substitution. Isolates are identified by GenBank accession number, location, and year of CHIKV isolation; genotypes are indicated at right. CHIKV, chikungunya virus; ESCA, East/Central/South African genotype. Scale bar indicates nucleotide substitutions per site.
tagement-based library construction with the NexteraXT DNA Sample Prep kit (Illumina, San Diego, CA, USA). We used Geneious (http://www.geneious.com) for de novo genome assembly.

RJ-IB1 and RJ-IB5 mapped within the ECSA genotype with other isolates from northeastern Brazil (Figure). We found no evidence of intergenotypic recombination despite cocirculation of ECSA and Asian strains in Brazil since 2014. The coding region of the consensus genome sequences revealed mutations present in 98.3%–100.0% of the roads mapping to each mutated residue. We detected unique mutations in RJ-IB1 and RJ-IB5 that are absent in other CHIKV isolates: NSP4-A481D in the viral RNA polymerase and E1-K211T in an E1 polymorphic site. A K211E mutation in this same E1 site showed increased CHIKV adaptability to Aedes aegypti mosquitoes in a E1-226A background (2). Nonetheless, the effects of the E1-K211T substitution in Rio de Janeiro isolates are unknown. Furthermore, we detected unique substitutions in RJ-IB1 (E1-N335D) and RJ-IB5 (NSP2-L27I). Both Rio de Janeiro isolates and the ECSA isolate Bahia-08 share a NSP2-P352A substitution, which is not detected in other Brazilian ECSA isolates. In contrast, the Brazil ECSA subgroup shares 2 exclusive mutations (E1-M407L and E2-A103T) that are absent in other ECSA strains. RJ-IB1 and RJ-IB5 genomes do not have the vector-adaptive E1-A226V or E2-L210Q substitutions but do have the E1-T98A substitution also present in other ECSA isolates. We also detected minority variants within the viral population, particularly in RJ-IB1, which showed higher genetic diversity than RJ-IB5 (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/23/10/16-1855-Techapp1.pdf). Further investigation of plasma-isolated viruses is necessary to confirm the diversity of minority variants (9).

Genetic surveillance and screening for mutations that might alter CHIKV fitness in vertebrates or Aedes mosquitoes are crucial. Brazil reports widespread geographic distribution of Aedes aegypti mosquitoes, with substantial concentrations in northern regions. Conversely, Aedes albopictus mosquitoes are concentrated in the subtropical southeastern states (10), which can lead to selection of different adaptive mutations in circulating CHIKV strains.

Acknowledgments

We thank Amilcar Tanuri for the use of the Biosafety Level 3 facility and Nissin Moussatché for suggestions.

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior to C.R.D. M.S.C. and L.C.S. received postdoctoral fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, respectively. M.L.G.M. received a doctoral fellowship from Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro.

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Fluoroquinolone-Resistant *Alcaligenes faecalis* Related to Chronic Suppurative Otitis Media, Angola

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DOI: https://doi.org/10.3201/eid2310.170268

We found that 20 (10.6%) of 188 patients with chronic suppurative otitis media in Angola were co-colonized with fluoroquinolone-resistant *Alcaligenes faecalis*, commonly found in birds. A likely explanation for our findings was the use of bird feces by residents as a traditional remedy to prevent ear secretions caused by primary ear infection.

Chronic suppurative otitis media (CSOM) is a common condition in developing countries and in original populations such as the Inuit (1). Mainly, young children are reported to have CSOM; 30%-40% of the population are affected in some geographic areas (2). CSOM is associated with hearing loss caused by perforation of the tympanic membrane, which often leads to chronic infection of the middle ear. Overcrowding, poor hygiene, and low nutrition status, in addition to absence of modern healthcare systems, are contributing factors for CSOM (2). CSOM is caused by a polymicrobial infection including gram-negative species, dominated by *Pseudomonas aeruginosa* and *Proteus mirabilis*, in addition to gram-positive bacteria such as enterococci and staphylococci (3).

During studies of ear discharge caused by otitis media, we detected the gram-negative bacillus *Alcaligenes faecalis* in addition to the commonly isolated bacterial species (3). *A. faecalis* may reside in the human microbiome of the gastrointestinal tract but only occasionally causes disease. Most cases occur in immunocompromised hosts, but in rare instances, *A. faecalis* infection has been described in patients who had acute otitis media, peritonitis, and eye or urinary tract infections (4–6). *A. faecalis* is frequently found in bird fecal specimens and can also cause opportunistic infections in these animals (7).

We examined specimens from 188 patients who had ear discharge related to otitis media who attended an outpatient ear, nose, and throat clinic in Luanda, Angola, during January–December 2016. This study was approved by the Angolan Medical Council, the director of the Josina Machel Hospital, and the Luanda University Medical Faculty. After cleaning the ear canal with 70% ethanol, we collected discharge with a swab. For nasopharyngeal sampling, we inserted a swab into the nostril past the choana and touched the wall of the nasopharynx. We collected ear and nasopharyngeal samples and stored them in skim milk-tryptone-glucose-glycerol (STGG) medium (8) at −70°C (Public Health Laboratory, Luanda, Angola) before transport to the Riesbeck Laboratory (Malmö, Sweden). Clinical specimens were cultured and bacteria analyzed (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/10/17-0268-Techapp.pdf). We found that 20 (10.6%) patients were colonized by *A. faecalis* (Table). Among patients harboring *A. faecalis*, 14 were male (1–47 years of age) and 6 were female (2–23 years of age). *A. faecalis* was growing in polymicrobial communities, and *P. aeruginosa* was the predominant species in 10 (50%) of the patients. *Proteus mirabilis* was the second most common bacterium (n = 7), followed by Klebsiella spp. and gram-positive cocci. Eight of the patients had otalgia, and the duration of otorrrhea was >12 months in 4 of those patients.

We tested *A. faecalis* isolates against a series of selected antimicrobial drugs by using broth microdilution (online Technical Appendix Table) and found 100% were susceptible to amoxicillin/clavulanate, tetracyclines, and colistin. In addition, 90% were susceptible to gentamicin, tobramycin, and piperacillin/tazobactam, and 75% were susceptible to trimethoprim/sulfamethoxazole. By contrast, most isolates were resistant to the fluoroquinolones ciprofloxacin (100%) and levofloxacin (82.6%).

*A. faecalis* has been described in patients with CSOM (9), but for us, this observation was initially an enigma. We considered the possibility of a contaminant from the endogenous fecal microbiome. We found, however, that to prevent ear discharge, patients occasionally filled their external auditory canals with dove or pigeon feces. Some patients in the geographic area also used cockroach paste, palm oil, sweet olive oil, sewing machine oil, or breast milk to prevent ear discharge. The origin of *A. faecalis* from birds would be a likely explanation for the appearance of this particular bacterial species among this study cohort.

Topical treatment using antimicrobial drugs in combination with keeping the ear canal clean and dry is the mainstay of therapy for CSOM (10). It remains to be confirmed whether *A. faecalis* colonization plays a crucial role for disease progression or merely is a contaminant. However, the microbiological findings in this study should be a note of caution because all *A. faecalis* isolates we obtained were
resistant against the most commonly used fluoroquinolone, ciprofloxacin. An alternative strategy would therefore be to consider colistin as topical treatment or supplement with orally administered amoxicillin/clavulanic acid in the treatment of more severe cases. The supply of topical agents in Luanda is unknown; therefore, the optimal treatment of patients colonized with *A. faecalis* should be determined and appropriate supplies obtained.

This work was supported by grants from Foundations of Anna and Edwin Berger (to K.R.) and Gyllenstierna-Krapperup (to K.R. and Å.R.), as well as the Swedish Medical Research Council (grant no. K2015-57X-03163-43-4), and Skåne Council’s research and development foundation (to K.R.). Support was also obtained from Päivikki and Sakari Sohlberg Foundation (to T.P.), and the Paediatric Research Foundation (Helsinki, Finland) (to T.P.).

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### Table. Co-colonizing bacterial species, clinical characteristics, and demographics of 20 patients colonized with *Alcaligenes faecalis* who had ear discharge related to otitis media, Luanda, Angola, January–December 2016*

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Co-colonizing bacterial species</th>
<th>Patient age, y/sex</th>
<th>Otolgia</th>
<th>Otorrhoea type, duration, mo</th>
<th>Neighborhood of residence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pseudomonas aeruginosa, Escherichia coli,</em> <em>Proteus spp., Enterococcus faecalis,</em> <em>Enterococcus avium</em></td>
<td>10/M</td>
<td>No</td>
<td>Unilateral, 1</td>
<td>Coelho, Viana†</td>
</tr>
<tr>
<td>2</td>
<td><em>P. aeruginosa,</em> <em>Proteus mirabilis,</em> <em>Citrobacter freundii</em></td>
<td>8/F</td>
<td>No</td>
<td>Bilateral, &lt;0.5</td>
<td>Casenga, Casenga†</td>
</tr>
<tr>
<td>3</td>
<td><em>P. aeruginosa,</em> <em>P. mirabilis,</em> <em>Morganella morganii,</em> <em>Providencia rettgeri,</em> <em>E. faecalis</em></td>
<td>20/M</td>
<td>No</td>
<td>Bilateral, &gt;3</td>
<td>Tala-Hady, Casenga†</td>
</tr>
<tr>
<td>4</td>
<td><em>P. mirabilis,</em> <em>C. freundii,</em> <em>E. faecalis</em></td>
<td>8/M</td>
<td>No</td>
<td>Unilateral, &gt;0.5</td>
<td>Hoji-ya-Henda, Casenga†</td>
</tr>
<tr>
<td>5</td>
<td><em>P. aeruginosa,</em> <em>Staphylococcus saprophyticus</em></td>
<td>5/F</td>
<td>No</td>
<td>Unilateral, &gt;12</td>
<td>Bairro 6, Viana†</td>
</tr>
<tr>
<td>6</td>
<td><em>P. mirabilis,</em> <em>Corynebacterium striatum,</em> <em>Citrobacter amalonaticus</em></td>
<td>4/M</td>
<td>No</td>
<td>Bilateral, &gt;12</td>
<td>Antonove, Casenga†</td>
</tr>
<tr>
<td>7</td>
<td><em>Klebsiella pneumoniae,</em> <em>C. freundii</em></td>
<td>23/F</td>
<td>Yes</td>
<td>Unilateral, &gt;12</td>
<td>Rangel, Luanda</td>
</tr>
<tr>
<td>8</td>
<td><em>P. aeruginosa,</em> <em>Providencia stuartii,</em> <em>Stenotrophomonas maltophilia</em></td>
<td>17/M</td>
<td>Yes</td>
<td>Unilateral, &gt;6</td>
<td>Asa Branca, Casenga†</td>
</tr>
<tr>
<td>9</td>
<td><em>P. aeruginosa,</em> <em>K. pneumoniae,</em> <em>Enterobacter cloacae,</em> <em>Arthrobacter spp.</em></td>
<td>20/F</td>
<td>Yes</td>
<td>Unilateral, &lt;0.5</td>
<td>Asa Branca, Casenga†</td>
</tr>
<tr>
<td>10</td>
<td><em>P. aeruginosa,</em> <em>P. mirabilis,</em> <em>K. pneumoniae,</em> <em>M. morganii</em></td>
<td>22/M</td>
<td>Yes</td>
<td>Unilateral, &gt;12</td>
<td>Casenga, Casenga†</td>
</tr>
<tr>
<td>11</td>
<td><em>P. aeruginosa,</em> <em>P. stuartii,</em> <em>Citrobacter koseri</em></td>
<td>7/F</td>
<td>No</td>
<td>Unilateral, &gt;12</td>
<td>Sambizanga, Luanda</td>
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<tr>
<td>12</td>
<td>No other species†‡</td>
<td>13/M</td>
<td>No</td>
<td>Unilateral, &gt;12</td>
<td>Saurimo, Saurimo§</td>
</tr>
<tr>
<td>13</td>
<td><em>M. morganii</em></td>
<td>5/M</td>
<td>Yes</td>
<td>Unilateral, &gt;12</td>
<td>Pimba, Saurimo§</td>
</tr>
<tr>
<td>14</td>
<td><em>Arthrobacter spp.</em></td>
<td>6/M</td>
<td>No</td>
<td>Bilateral, NA‡</td>
<td>Saurimo, Saurimo§</td>
</tr>
<tr>
<td>15</td>
<td><em>P. aeruginosa,</em> <em>Streptococcus constellatus</em></td>
<td>14/M</td>
<td>Yes</td>
<td>Unilateral, &gt;12</td>
<td>Saurimo, Saurimo§</td>
</tr>
<tr>
<td>16</td>
<td><em>P. mirabilis,</em> <em>Arthrobacter spp., E. faecalis</em></td>
<td>1/M</td>
<td>Yes</td>
<td>Unilateral, &gt;3</td>
<td>Viana, Viana†</td>
</tr>
<tr>
<td>17</td>
<td><em>P. aeruginosa,</em> <em>Klebsiella oxytoca</em></td>
<td>2/M</td>
<td>Yes</td>
<td>Unilateral, &gt;3</td>
<td>Sanzala, Viana†</td>
</tr>
<tr>
<td>18</td>
<td><em>Arthrobacter spp.</em></td>
<td>ND/M</td>
<td>Yes</td>
<td>Unilateral, &gt;12</td>
<td>Tshinganja 2, Saurimo§</td>
</tr>
<tr>
<td>19</td>
<td><em>Arthrobacter spp.,</em> <em>P. stuartii</em></td>
<td>47/M</td>
<td>No</td>
<td>Unilateral, &gt;12</td>
<td>Estalagem, Viana†</td>
</tr>
<tr>
<td>20</td>
<td><em>Enterococcus faecium</em></td>
<td>2/F</td>
<td>NA</td>
<td>NA</td>
<td>NA‡</td>
</tr>
</tbody>
</table>

* A. faecalis isolated from external ear discharge except as indicated. NA, not applicable; ND, no data.
† Province Lunda.
‡ A. faecalis isolated from nasopharyngeal swab.
§ Province Lunda Sul.
†† Province Namib.

### References


We investigated an outbreak of exanthematosus illness in Maceió by using molecular surveillance; 76% of samples tested positive for chikungunya virus. Genetic analysis of 23 newly generated genomes identified the East/Central/South African genotype, suggesting that this lineage has persisted since mid-2014 in Brazil and may spread in the Americas and beyond.

 Dengue virus (DENV), Zika virus (ZIKV), and chikungunya virus (CHIKV) co-circulate in Brazil, are predominantly transmitted by Aedes aegypti mosquitoes, and cause similar clinical symptoms upon infection, complicating epidemiologic surveillance. Brazil harbors the highest diversity of CHIKV in the Americas; both the Asian and the East/Central/South African (ECSA) lineages circulate in the country (1). Despite high prevalence of CHIKV in Brazil (352,773 notified cases during January 2016–May 2017) and its widespread distribution (2), little is known about its transmission. We report a molecular and genomic investigation of an outbreak of CHIKV infection in Maceió, Alagoas state, Northeast Brazil.

During March 30–May 3, 2016, ≈12,000 patients visited 2 private hospitals in Maceió; roughly 70% of them had exanthematos illness symptoms compatible with DENV, CHIKV, or Zika virus infection. We analyzed 273 randomly chosen samples by using molecular diagnostics and virus discovery methods. The study was approved by the Faculty of Medicine from the University of São Paulo Review Board, and we obtained informed consent from all participants.

Analyzed samples were from patients who were on average 37 years of age (range 1–86 years); 175 (64%) were female, and 198 (73%) resided in Maceió municipality. Diagnostic tests for DENV, ZIKV, and CHIKV confirmed that 208 (76%) were positive for CHIKV RNA (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/10/17-0307-Techapp1.pdf). In addition, 66 (24%) were positive for Zika virus RNA and 36 (13.2%) were co-infected with CHIKV and Zika virus, consistent with Zika virus circulation in Northeast Brazil in mid-2016 (3). We detected no DENV infections. Cycle threshold (Ct) values for CHIKV RNA–positive samples were lower (average Ct = 24.6) than those for ZIKV (average Ct = 33.5).

We applied a metagenomics next-generation sequencing protocol to 38 randomly chosen CHIKV RNA–positive samples (4) (online Technical Appendix). We recovered 23 CHIKV genomes (>4,000 bp) by using the MiSeq Sequencer (Illumina, Inc., San Diego, CA, USA); mean genome coverage was 72× and mean depth coverage 207× (online Technical Appendix Table 1). We also
included 2 CHIKV RNA–positive samples from João Pessoa, Paraíba state. We did not detect the E1-A226V adaptive mutation associated with large outbreaks in Asia (5) in the strains we analyzed. We then appended sequences to publicly available data (659 CHIKV isolates) and used maximum-likelihood and Bayesian phylogenetic analysis to identify the origins of the outbreak (online Technical Appendix).

On the basis of available sequences of isolates from the Americas, the Maceió sequences we analyzed fell within a single strongly supported monophyletic clade (bootstrap support = 99%, posterior support = 1.00) that belongs to the ECSA genotype (Figure). Genetic analysis suggests the outbreak most likely originated from transmission cycles not previously identified in Northeast Brazil and not from a separate introduction into the Americas. Before August 2015, no CHIKV infections had been reported in Alagoas (Figure). Molecular dating analysis indicates that the outbreak was caused by a single founder strain that is estimated to have arrived in Alagoas around late April 2015 (95% Bayesian credible interval July 2014–October 2015), possibly a few months before the earliest reports of CHIKV there (Figure). Our reconstruction of the history of the ECSA genotype in Brazil using a phylogeographic approach (6) further suggests that this lineage was introduced into Alagoas from the neighboring Bahia state, which experienced a CHIKV epidemic during January–August 2015 (7).

The brief to negligible period of undetected transmission of CHIKV in Alagoas is consistent with past
reports (8) and in contrast with the unrecognized circulation in the region of Zika virus, which typically causes milder symptoms (3). The most common clinical signs and symptoms for the sequenced CHIKV cases were fever (87%), arthralgia (70%), headache (44%), exanthema (30%), and myalgia (26%) (Technical Appendix Table 2). CHIKV infection is often characterized by prolonged periods of disability. Further investigation is needed to study potential differences in the effects of CHIKV and Zika virus infection on public health, as well in pathology and innate and adaptive immune responses to each genotype.

The unrecognized transmission of the CHIKV ECSA genotype in Northeast Brazil is unique in the Americas. The spread of this genotype in this region will be mediated by several factors, including herd immunity, vector suitability, and human mobility. Serologic and molecular surveys in human and mosquito populations are required to characterize the factors involved in transmission and the extent of cross-protection of the Asian and the ECSA genotypes in the Americas. Although CHIKV ECSA has been found only in *Ae. aegypti* mosquitoes (9), a recent study has shown that *Ae. albopictus* mosquitoes in Brazil are also highly competent in CHIKV ECSA transmission (10). Given the widespread distribution of both vectors in the Americas (1), it is possible that the ECSA lineage may spread to other regions in the Americas and beyond. A better understanding of the transmission dynamics of CHIKV, DENV, and Zika virus in the Americas is essential to fully understand the risk of arbovirus-associated congenital anomalies, Guillain-Barré, and other neurological syndromes.

**Acknowledgments**

We thank all participants in this study and Luciano Monteiro da Silva Patricia Chefre, Renata Palma, and Suzana Santos for support. We also thank Illumina, Inc., Sage Science, Inc., Promega Biotecnologia do Brasil, Ltda, and Greiner Bio-One Brasil Produtos Médicos Hospitalares Ltda for the donation of reagents and plastics for this project.

This work was supported by FAPESP #2016/01735-2 and CNPq #400354/2016-0. A.C.d.C. is funded by FAPESP #2012/03417-7 and 2017/00021-9. N.R.F. is funded by a Sir Henry Dale Fellowship (Wellcome Trust/Royal Society Grant 204311/Z/16/Z). This research received funding from the ERC under the European Union’s Seventh Framework Programme (FP7/2007-2013), grant agreement no. 614725-PATHPHYLDYN; and the US Agency for International Development Emerging Pandemic Threats Program-2 PREDICT-2 ( Cooperative Agreement No. AID-OAA-A-14-00102).

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Familial Transmission of emm12 Group A Streptococcus

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DOI: https://doi.org/10.3201/eid2310.170343

Incidence and severity of invasive group A Streptococcus infections are of increasing concern in France and worldwide. The risk for secondary infection of close contacts is known but rarely described. We report a case of intrafamilial and life-threatening transmission of emm12 group A Streptococcus.

In recent years, incidence and severity of invasive group A Streptococcus infections (iGAS) has increased in Europe and worldwide (1). The Centers for Disease Control and Prevention surveillance reports attest to this increase in the United States (2). In northern France, an increase in emm1 iGAS was reported (3). Streptococcus pyogenes can spread from infected persons to close contacts, especially if >24 hours is spent with an infected person (4). However, transmission of life-threatening infections remains a relatively rare event. We report a case of intrafamilial transmission of iGAS.

On August 17, 2016, a 67-year-old woman was admitted to the Centre Hospitalier de Roubaix (Roubaix, France) for knee pain and necrotic zones on her thigh. Her medical history consisted of treated hypertension. At admission, her temperature was 36.3°C, blood pressure 110/80 mm Hg, pulse rate 74 beats/min, blood leukocyte count 8,530 cells/µL (89% polymorphonuclear), and C-reactive protein 281 mg/L. Her health condition quickly deteriorated; she had disseminated intravascular coagulation and blood pressure of 70/40 mm Hg, despite appropriate hemodynamic care. She experienced toxic shock and multiorgan system failure. A revision surgery was necessary but not possible because of heavy bleeding, hemodynamic instability, metabolic acidosis, acute renal failure, and hyperkaliemia. She died on August 18. Cultures of necrotized tissues and blood samples yielded S. pyogenes.

On August 21, the index case-patient’s husband, who was 66 years of age, was admitted to Centre Hospitalier Régional Universitaire de Lille (Lille, France) with a 2-day history of bursitis of the right elbow. He had been treated during the 2 days by his family’s physician with intravenous amoxicillin/clavulanic acid (1 g 3×/d), pristinamycin (1 g 3×/d), and NSAIDs. At hospital admission, his temperature was 36.5°C, blood pressure 95/55 mm Hg, and pulse rate 95 beats/min, blood leukocyte count 22,300 cells/µL (89% polymorphonuclear), and C-reactive protein 511 mg/L. Mobilization of the elbow was possible but limited by major edema to the axilla; severe blistering of the elbow was visible. We drained his forearm surgically to treat extensive cellulitis and diagnosed superinfected bursitis. We stopped pristinamycin and NSAIDs, increased the intravenous amoxicillin/clavulanic acid dose (to 2 g 3×/d), added linezolid for antitoxic action (600 mg 2× in 24 h), and provided 14 hyperbaric oxygen therapy sessions. On August 23, the wound condition improved and C-reactive protein decreased (211 mg/L), but a wide erythema was still visible. Clinical outcome was favorable, and we discharged the patient on September 5. Culture of the deep tissue samples yielded S. pyogenes. The strain was susceptible to amoxicillin, so we continued it (2 g 3×/d) until September 11.

Strains isolated from both patients were the same strain of S. pyogenes emm12 (online Technical Appendix Tables 1, 2, https://wwwnc.cdc.gov/EID/article/23/10/17-0343-Techapp1.pdf). The couple were caregivers for their granddaughter, and they met their 2 adult children and son-in-law several days each week, so we evaluated these close contacts. The son and daughter had had a sore throat 9 days before onset of illness in the mother and were treated empirically by their general practitioner with amoxicillin/clavulanic acid (1 g 2×/d for 6 d). We prescribed cefuroxime axetil (250 mg 2×/d for 10 d). Buccal swabs cultured remained negative.

The risk for iGAS infection in close contacts of patients was reviewed in 2016 (5): the evidence was based on 13 instances of transmission published in 4 separate studies covering 5,858 household contacts. The annual risk among close contacts was 151 times greater than the risk for sporadic disease and comparable to that estimated for meningococcal disease. However, the benefit from
antimicrobial drug prophylaxis is not known (5), and guidelines vary among countries. In the United Kingdom, prophylaxis is recommended for exposed mothers or babies during the neonatal period, for symptomatic close contacts, or for the entire household if there is >1 case (6). In Canada, prophylaxis is recommended for persons who had close contact with a person with a confirmed severe case during a specified period (7); in France and the United States, prophylaxis is recommended for close contacts with risk factors for invasive infections (8,9). In the cases we report here, the second case-patient did not receive prophylaxis because of the short period between the 2 cases.

Both case-patients received NSAIDs during the onset of the disease. The role of these drugs in streptococcal infection outcome is frequently discussed; they seem to cause an increase of severe infection, most probably in children (10).

These cases highlight that different life-threatening transmissible types of S. pyogenes are circulating in the same area and that transmission can occur rapidly. Clinician and family education about prophylaxis and symptoms requiring medical care is needed.

Acknowledgments

We thank the staff of the Centre National de Référence des Streptococques and C. Poyart for their assistance in characterization of the group A streptococcal isolates.

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Six-Month Response to Delamanid Treatment in MDR TB Patients

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Delamanid, recently available for the treatment of multidrug-resistant tuberculosis (MDR TB), has had limited use outside clinical trials. We present the early treatment results for 53 patients from 7 countries who received a delamanid-containing treatment for MDR TB. Results show good tolerability and treatment response at 6 months.

Outcomes of conventional 18–24-month regimens for multidrug-resistant tuberculosis (MDR TB) (1,2) and extensively drug-resistant tuberculosis (XDR TB) (3,4) are notoriously poor. Two recently marketed drugs, delamanid (5–7) and bedaquiline (8), represent hope for better outcomes. Médecins Sans Frontières (MSF) supported national TB programs to introduce delamanid according to World Health Organization recommendations (9) for patients lacking 4 effective second-line drugs in the regimen or at high risk for poor treatment outcomes. Delamanid was preferred over bedaquiline to treat TB in patients with hepatitis C (because of less potential hepatic toxicity with delamanid), patients who are taking antiretroviral drugs (because delamanid produces fewer interactions), or patients previously exposed to bedaquiline (and who had previous treatment failure) or clofazimine (because of potential cross resistance with bedaquiline). We present interim treatment response and safety data for patients treated with delamanid within MSF-supported programs.

This retrospective study comprises all patients started on MDR TB regimens containing delamanid in MSF-supported sites before March 1, 2016. Routine programmatic data were collected on site. Information on serious adverse events (SAEs) was retrieved from a central pharmacovigilance database. The study was approved by the relevant health ministries and meets the criteria of the MSF Ethics Review Board for exemption from ethics review.

We defined culture conversion as 2 consecutive negative culture results 1 month apart for culture-positive patients at start of delamanid treatment. We defined patients as having a favorable interim treatment response at 6 months if they completed 24 weeks of delamanid and culture converted or remained culture negative; we classified patients who did not meet these criteria as having an unfavorable interim treatment response. We used unadjusted bivariate odds ratios with 95% CIs to express the magnitude and precision of associations between outcomes and risk factors (the small number of records precluded a multivariable analysis). We defined SAEs as deaths irrespective of cause, hospitalizations, events leading to disability or congenital malformation, and events considered life threatening or otherwise medically noteworthy.

During February 6, 2015—February 29, 2016, a total of 53 patients from 7 countries (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/23/10/17-0468-Techapp1.pdf) started a delamanid-containing regimen (Table). Of these, 46 (86.8%) received delamanid through a compassionate-use program. Most patients had been treated previously with second-line drugs (48/53, 90.6%), experienced MDR TB treatment failures (32/53, 60.4%), exhibited resistance to second-line TB drugs (41/51, 80.4%), or had extensive pulmonary disease (40/45, 88.9%). Almost all patients (52/53, 98.1%) received delamanid for an indication of <4 effective drugs in the regimen.

<table>
<thead>
<tr>
<th>Variable/WHO case definition</th>
<th>No. (%) patients or median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>No. (%) patients or median (IQR)</td>
</tr>
<tr>
<td>Male</td>
<td>36 (67.9)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (32.1)</td>
</tr>
<tr>
<td>Age at delamanid start, y</td>
<td>29.5 (21.0–43.0)</td>
</tr>
<tr>
<td>14–17</td>
<td>11 (20.8)</td>
</tr>
<tr>
<td>HIV co-infected, n = 48</td>
<td>8 (16.7)</td>
</tr>
<tr>
<td>HCV co-infected, n = 42</td>
<td>8 (19.0)</td>
</tr>
<tr>
<td>Malnutrition,† n = 51</td>
<td>21 (41.2)</td>
</tr>
<tr>
<td>Serum albumin at delamanid start, g/L, n = 46</td>
<td>37.6 (32.0–37.6)</td>
</tr>
<tr>
<td>New case</td>
<td>4 (7.5)</td>
</tr>
<tr>
<td>Relapse</td>
<td>5 (9.4)</td>
</tr>
<tr>
<td>Treatment after being lost to follow-up</td>
<td>5 (9.4)</td>
</tr>
<tr>
<td>Treatment after failure</td>
<td>32 (60.4)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (13.5)</td>
</tr>
<tr>
<td>Previously treated</td>
<td>49 (92.4)</td>
</tr>
<tr>
<td>With first-line drugs only</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>With second-line drugs</td>
<td>48 (97.9)</td>
</tr>
<tr>
<td>MDR TB confirmed</td>
<td>51 (96.2)</td>
</tr>
<tr>
<td>Drug resistance subgroups among confirmed MDR TB</td>
<td></td>
</tr>
<tr>
<td>MDR TB only‡</td>
<td>10 (19.6)</td>
</tr>
<tr>
<td>Pre–XDR TB FQ</td>
<td>6 (11.8)</td>
</tr>
<tr>
<td>Pre–XDR TB Inj</td>
<td>8 (15.7)</td>
</tr>
<tr>
<td>XDR TB</td>
<td>27 (52.9)</td>
</tr>
<tr>
<td>Radiograph features</td>
<td></td>
</tr>
<tr>
<td>Bilateral, n = 45</td>
<td>35 (77.8)</td>
</tr>
<tr>
<td>Cavities, n = 43</td>
<td>26 (60.5)</td>
</tr>
<tr>
<td>Bilateral or cavity, n = 45</td>
<td>40 (88.9)</td>
</tr>
<tr>
<td>Culture positive at delamanid start</td>
<td>37 (69.8)</td>
</tr>
</tbody>
</table>

*HCV, hepatitis C virus serology; HIV, human immunodeficiency virus; MDR TB, multidrug-resistant tuberculosis; pre–XDR TB FQ, MDR TB with fluoroquinolone resistance; pre–XDR TB Inj, MDR TB with resistance to injectable drugs; WHO, World Health Organization; XDR TB, extensively drug-resistant tuberculosis.
†Malnutrition: either BMI <18.5 kg/m², mid-upper arm circumference <16cm, or weight <50 kg in 3 patients from South Africa without height measurement.
‡Without resistance to fluoroquinolone or injectable drugs.
A total of 31 SAEs were reported in 14 patients (26.4%); most common were hepatotoxicity (5), electrolyte imbalance (5), and QT prolongation (3). The most frequent contributing factors reported were TB disease (6), hepatitis C infection (6), and non–anti-TB drugs, including antiretroviral drugs (ARVs) (8). A possible relation to any TB drug was reported in 80.6% (25/31) of events, including a possible relation to delamanid in 58.6% (18/31). Causes of the 7 reported deaths were advanced TB (2), encephalitis in an untreated HIV patient (1), traumatic pneumothorax (1), sepsis in an HIV patient (1), respiratory failure related to end-stage hepatitis (1), and sudden death of unknown cause (1); a possible relationship to anti-TB drugs was initially reported in the last 2 cases. In 1 patient with hepatitis C and liver cirrhosis, all drugs were permanently discontinued due to hepatotoxicity. No other permanent discontinuation of delamanid was reported (online Technical Appendix Table 2).

Of the patients who were culture positive at delamanid start, 67.6% (25/37) culture converted by 6 months. At 6 months, 73.6% (39/53) of patients had a favorable response, 13.2% (7/53) had died, 7.5% (4/53) remained culture positive, 3.8% (2/53) were lost to follow-up, and 1.9% (1/53) were declared to have a failure in treatment as a result of an SAE. Factors associated with unfavorable response in a univariate analysis were age >35 years (odds ratio [OR] 5.62, 95% CI 1.47–21.57; p = 0.012); hepatitis C infection (OR 7.78, 95% CI 1.45–41.78; p = 0.017); smear positivity at delamanid start (OR 5.21, 95% CI 1.35–20.06; p = 0.016); and serum albumin <34 g/L (OR 7.14, 95% CI 1.35–20.06; p = 0.016); and serum albumin <34 g/L (OR 7.14, 95% CI 1.35–20.06; p = 0.016). Smear-positive patients had a mean (SD) duration of therapy of 21.4 (11.4) months vs. 5.8 (4.0) months for smear-negative patients (p = 0.009). At 6 months, 73.6% (39/53) of patients had a favorable response in a univariate analysis were age >35 years (odds ratio [OR] 5.62, 95% CI 1.47–21.57; p = 0.012); hepatitis C infection (OR 7.78, 95% CI 1.45–41.78; p = 0.017); smear positivity at delamanid start (OR 5.21, 95% CI 1.35–20.06; p = 0.016); and serum albumin <34 g/L (OR 7.14, 95% CI 1.35–20.06; p = 0.016). Smear-positive patients had a mean (SD) duration of therapy of 21.4 (11.4) months vs. 5.8 (4.0) months for smear-negative patients (p = 0.009).

These preliminary results indicate good tolerability and interim treatment response to delamanid at 6 months in a narrow and difficult-to-treat cohort of patients for whom delamanid was preferred to bedaquiline, most of whom had previously failed MDR TB treatment and had extensive disease. Delamanid was used in preference to bedaquiline in this group of patients, despite the programmatic availability of bedaquiline, which may explain the frequency of adverse events in relation to hepatitis C and HIV co-infection, comorbidities that influence this choice, further supporting the need for essential monitoring and treatment of hepatitis C and HIV in MDR TB patients. Limitations of this study include its small numbers and retrospective nature, and data on delamanid treatment outcomes and safety in programmatic conditions with larger indications deserve further studies.

Acknowledgments
The authors thank Nana Kiria, Lusine Yeghiazaryan, Virginia de Azevedo, and the MSF-TB working group: Krzysztof Herboczek, Alex Telnov, Mathieu Bastard, Helena Huerga, and Jay Achar.

C.H., N.L., and F.V. report grants from UNITAID, outside the submitted work. MSF received a donation of delamanid from Otsuka Company.

Dr. Hewison is a medical doctor working with Médecins Sans Frontières since 1997, a tuberculosis advisor in the medical department of MSF since 2005, and chair of the endTB medical committee. Her primary research interest is all aspects of diagnosis and treatment of MDR TB, particularly the effectiveness and safety of new anti-TB drugs.

References
**Mycobacterium orygis**

**Lymphadenitis in New York, USA**

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DOI: https://doi.org/10.3201/eid2310.170490

We report a case of lymphadenitis caused by *Mycobacterium orygis* in an immunocompetent person in Stony Brook, New York, USA. Initial real-time PCR assay failed to provide a final subspecies identification within the *M. tuberculosis* complex, but whole-genome sequencing characterized the isolate as *M. orygis*.

Genomic analysis has previously shown that the *Mycobacterium tuberculosis* (MTB) complex comprises >8 distinct subgroups: *M. tuberculosis*, *M. africanum*, *M. canetti*, *M. bovis*, *M. caprae*, *M. pinnipedii*, *M. microti*, and *M. mungi* (1). *M. orygis* was first characterized in Africa and South Asia in 2012 based on examination of 22 isolates selected for the similarity of their IS6110 restriction fragment length polymorphism patterns to previously described oryg bacilli (2). Eleven of these isolates were from animals (a cow, a rhesus monkey, and types of antelope including oryx), and 11 were from humans (9 from South Asia). On the basis of single-nucleotide polymorphism (SNP) and region of difference (RD) analysis, van Ingen et al. concluded that these mycobacteria belonged to a phylogenetically distinct lineage of the clonal MTB complex (2). *M. orygis* is also distinguished by a mutation in gene *Rv2042c* (2) and a G1113A mutation in the *gyrB* gene (3).

We report a case of lymphadenitis caused by *M. orygis* in an immunocompetent person in Stony Brook, New York, USA. During July 2015, we diagnosed pneumonia in the upper lobe of the right lung in a woman, 71 years of age, who had a remote history of lymphoma. The condition was characterized by enlarged lymph nodes. The patient was born in Pakistan, moved to India at age 1, and emigrated to the United States ≈25 years before onset; her preimmigration TB skin test was <5 mm (bacillus Calmette-Guérin vaccinated), and chest radiograph results were negative. She drank unpasteurized milk while living in India.

We completed positron emission and computed tomography scans by using intravenous F-18 fluoro-2-deoxyglucose that detected hypermetabolic foci in the right axilla, subpectoral, subcarinal, and para hilar regions. Quantiferon-TB Gold in-tube system (Quest Diagnostics, Inc., Lyndhurst, NJ, USA) test result was positive (TB antigen minus nil value 3.58 IU/mL, mitogen minus nil value 8.5 IU/mL). Three induced sputum samples for acid-fast bacilli smear and cultures were negative. Because of the patient’s history of lymphoma, we biopsied the subpectoral lymph node. Histopathology revealed diffuse large caseating granulomas with extensive central necrosis, small lymphocytes, plasma cells, and histiocytes. Grocott’s Methenamine Silver Stain (Ventana Medical Systems, Inc. Tucson, AZ, USA), and acid-fast bacilli stains did not detect organisms. Bacterial and fungal cultures were negative.

*Mycobacterium* Growth Indicator Tube (MGIT) system turned positive on day 29, and the isolate was identified as MTB complex by probe hybridization (Hologic, Inc. San Diego, CA, USA). Further testing at the New York State Department of Health with real-time PCR using primers/probes specific for 4 MTB regions of difference yielded an inconclusive pattern (4). Results were positive for RD1 and RD4, but negative for RD9 and RD12; subspecies identification was initially reported as “inconclusive” because this pattern did not match the signature patterns used to determine MTB complex species with this assay.

We performed a whole-genome sequencing assay that confirmed the absence of RD9 and RD12 and identified the isolate as *M. orygis*, as reported by Shea et al (5). This isolate belonged to Spoligotype International Type 587, contained the specific *gyrB* SNP at position 1113 (3), and lacked resistance-associated mutations, suggesting susceptibility to all tested current antituberculosis agents. The patient received first-line, 4-drug therapy.

*M. orygis* infections in humans have been rarely reported. In Australia, of 1,763 case-patients diagnosed with MTB complex infection, 8 causative pathogens were identified as *M. orygis*; all of the patients were born in India (6). In New Zealand, Dawson et al. used advanced molecular techniques to demonstrate a transmission of *M. orygis* from a human, who emigrated from India, to a cow (7).

*M. orygis* infection may be underreported in the literature because cases may be identified as MTB complex or misidentified as *M. africanum* or *M. bovis* (8). Through whole-genome sequencing, the New York State Department of Health identified 8 additional cases of *M. orygis* of 6,322 MTB complex isolates from New York tested (3 pulmonary, 2 lymph node, and 2 abscess samples) that were received during 2005–2016 but were initially misidentified (data not shown). All patients were from India, Pakistan, or Nepal and had moved to the United States. SNP analysis indicated that the *M. orygis* isolates were genetically similar, but all were distant from other members of the MTB complex (Figure) and contained the G1113A mutation in *gyrB*.
The number of SNPs separating the 8 *M. orygis* isolates was 106–323, which excludes their belonging to an epidemiologic transmission cluster (9) and strongly suggests that the infections were independently acquired.

We found no previous reports of *M. orygis* originating in the Americas; the most notable epidemiologic risk factor in this patient was prior residency in India, where *M. orygis* was found in a variety of animals (10). Because all organisms in the MTB complex have a distinct host preference, it is possible that *M. orygis* is presently in animals and few cases occur in humans, similar to *M. bovis*. This case demonstrates the value of molecular methodologies such as whole-genome sequencing for providing more detailed insight into the clinical and epidemiologic aspects of the MTB complex.

Dr. Marcos is an associate professor of clinical medicine at Stony Brook University in New York, USA. His research interests include epidemiology of emerging infectious diseases, tickborne diseases, and global health.

References

Ross River Virus Seroprevalence, French Polynesia, 2014–2015

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DOI: https://doi.org/10.3201/eid2310.170583

Ross River virus (RRV), spread by Aedes and Culex mosquito species (1). Symptoms of RRV infections mainly consist of fever, arthralgia, and rash. RRV was first isolated in North Queensland in 1959 and has become the most common arboviral disease in Australia (2). RRV outbreaks were reported during 1979–1980 in Pacific Island countries and territories (PICTs) including Fiji, Cook Islands, American Samoa, and New Caledonia (3).

In French Polynesia (FP), a territory of the Pacific region with ≈270,000 inhabitants, dengue virus (DENV) was the only arbovirus detected until identification of the Zika virus (ZIKV), then chikungunya virus (CHIKV), causing outbreaks during 2013–2014 and 2014–2015, respectively (3). Although RRV infections have never been reported in FP, a serosurvey of blood donors during 2011–2013 suggested that RRV has circulated without being detected (4). In this study, we report additional evidence of RRV circulation in FP based on further screening of blood samples collected during 2014–2015 and previously used for a ZIKV serosurvey (5).

We tested 3 groups of participants randomly sampled in FP for the presence of anti-RRV IgG by using recombinant antigen-based indirect ELISA (patent no.WO2012076715A1) and microsphere multiplex immunoassay (patent no. WO2013083847A2) (4,5). The first group consisted of 196 participants recruited during February–March 2014 in 8 of the most inhabited islands of the 5 FP archipelagos (archipelagos listed in parentheses): Tahiti and Moorea (Society), Rangiroa and Makemo (Tuamotu), Nuku Hiva and Hiva Oa (Marquesas), Rurutu (Austral), and Rikitea ( Gambier). The second group included 700 participants recruited during September–November 2015 on the 2 most inhabited islands of FP: Tahiti and Moorea. The third group consisted of 476 schoolchildren recruited during May–June 2014 on Tahiti. The Ethics Committee of French Polynesia approved recruitment of participants and processing of blood samples (approval no. 60/CEPF-06/27/2013). We analyzed seroprevalence data by using the Fisher exact test, and considered p values <0.05 as statistically significant.

RRV seropositivity rates among participants sampled in the 5 archipelagos in 2014 ranged from 16% to 49% (average 35%), and were significantly different between the Society and Marquesas (p = 0.036), Tuamotu and Marquesas (p = 0.001), and Tuamotu and Austral-Gambier (p = 0.002) Islands (Table). In the Society Islands, screening of additional participants in 2015 did not lead to a significant difference in RRV seroprevalence (18%) compared with the 1 observed in the participants recruited in 2014 (27%) (p = 0.125). In contrast, RRV seroprevalence among schoolchildren (1%) was significantly lower than in the general population from the Society Islands, during both 2014 and 2015 (p<0.0001). We compared 2 groups in the general population in 2014 and 2015. Rates of participants whose samples were RRV–seropositive and who were born or arrived in FP before
1982 (respectively, 39% and 19%) and thus potentially exposed to the last reported epidemic in the Pacific, were not significantly different from those who were born or arrived during or after 1982 (respectively, 28% and 15%).

Although no RRV outbreaks were reported in the PICTs after 1980, identification of RRV infections among travelers returning from Fiji between 1997–2009 suggested subsequent circulation of the virus in the Pacific (6,7). In a serosurvey conducted in American Samoa in 2010, the finding that 63% of participants born after 1980 and who had lived their whole lives in the territory were seropositive for RRV also supported this assumption (8). In our study, detection of RRV seropositive participants who were born or arrived in FP from 1982 shows that the virus probably circulated after the end of the 1979–1980 epidemic in the Pacific. This finding corroborates data previously obtained regarding blood donors (4).

In this study, we also provide evidence that RRV circulated in all archipelagos. The overall seroprevalence rate of 35% found in the general population in 2014 is consistent with the rate of 34.40% previously obtained in blood donors (4), with both groups of participants mainly including adults (median 41 and 36 years, respectively). The lower seroprevalence found in schoolchildren (median 11 years) compared with the general population in 2015 (median 43 years) is consistent with previous age-stratified studies showing that RRV seroprevalence increases with age (1). Nevertheless, because the seroprevalence observed in children is lower in FP than in endemic Australian areas (9), another reasonable explanation is that RRV circulated poorly in FP during the 16 years before the study. This observation is also supported by the small number of seropositive participants who arrived or were born after 2000 in FP, among the general population in 2014 (3/18) and 2015 (0/54) (data not shown).

Increasing evidence that RRV circulated silently in several PICTs, in the absence of marsupial animal reservoirs (8), supports the need for enhanced laboratory and epidemiologic surveillance. Moreover, clinicians should be aware of the potential for RRV infections to occur. As illustrated with ZIKV and CHIKV, tropical islands are new hubs for emerging arboviruses and neither diseases nor places should be neglected (10).

Acknowledgments
We thank the staff from the Ministries of Health and Education of French Polynesia, the municipalities selected for the study, and the Institut Louis Malardé for their contribution in this work. We also thank all of the participants.

This study was partially funded by the Contrat de Projet Etat-Pays (convention no. 7331/MSS/DSP du 31/08/12 modifiée) and the French Government’s Investissement d’Avenir Programme (Labex Integrative Biology of Emerging Infectious Diseases, IBEID, grant no. ANR-10-LABX-62-IBEID).

Dr Aubry is a research scientist at the Institut Louis Malardé, Tahiti, French Polynesia. Her research focuses on the epidemiology in the Pacific region and genetic evolution of various arboviruses including dengue, Zika, chikungunya and Ross River viruses.

References

Table. Seropositivity for Ross River virus among participants randomly recruited in French Polynesia from the general population during February–March, 2014 and September–November, 2015, and from schoolchildren during May–June, 2014*

<table>
<thead>
<tr>
<th>Sampled population, time of sampling</th>
<th>Median age (range), y</th>
<th>No. seropositive/No. born or arrived in French Polynesia before 1982 (% [95% CI])</th>
<th>No. seropositive/No. born or arrived in French Polynesia after 1982 (% [95% CI])</th>
<th>Total no. seropositive/Total no. tested (% [95% CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>February–March 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuamotu Islands</td>
<td>39 (7–86)</td>
<td>6/28 (21 [6–37])</td>
<td>2/21 (10 [4–27])</td>
<td>8/49 (16 [6–26])</td>
</tr>
<tr>
<td>Marquessas Islands</td>
<td>45 (10–82)</td>
<td>15/32 (47 [30–64])</td>
<td>9/17 (53 [29–77])</td>
<td>24/48 (49 [36–62])</td>
</tr>
<tr>
<td>Austral–Gambier Islands</td>
<td>38 (7–84)</td>
<td>15/26 (58 [39–77])</td>
<td>8/23 (35 [15–54])</td>
<td>23/49 (47 [35–58])</td>
</tr>
<tr>
<td>Total</td>
<td>41 (7–86)</td>
<td>45/115 (39 [30–48])</td>
<td>23/81 (2 [19–38])</td>
<td>68/196 (35 [27–43])</td>
</tr>
<tr>
<td>September–November 2015</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Society Islands</td>
<td>43 (4–88)</td>
<td>77/397 (19 [16–23])</td>
<td>46/303 (15[11–19])</td>
<td>123/700 (18 [15–21])</td>
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<tr>
<td>Schoolchildren</td>
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<td></td>
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<tr>
<td>May–June 2014</td>
<td>11 (6–16)</td>
<td>0</td>
<td>6/476 (6 [0–2])</td>
<td>6/476 (1 [0–2])</td>
</tr>
</tbody>
</table>

*CIs were calculated by using the Fisher exact test.

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Unrecognized Subclinical Infection with Tickborne Encephalitis Virus, Japan

Kentaro Yoshii, Reiji Kojima, Hiroshi Nishiura

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DOI: https://doi.org/10.3201/eid2310.170918

During early 2017, we conducted a seroepidemiologic investigation for tickborne encephalitis virus among 291 Japan Self-Defense Forces members in Hokkaido. Two (0.7%) tested positive. Neither had clinically apparent symptoms after removing ticks.

Tickborne encephalitis virus (TBEV; genus Flavivirus, family Flaviviridae) persists in ticks and wild animals, including wild rodents and shika deer (1,2). Geographically, the virus is widely spread across Eurasia and annually causes ≈10,000 clinically apparent cases in humans (3). In Japan, tickborne encephalitis is notifiable. Two confirmed cases, both in Hokkaido in the northernmost prefecture, have been reported. The first confirmed case was serologically diagnosed in 1993 (4); the second case was confirmed in 2016.

During the 20-year gap between cases, serologic and virologic surveys of wild animals (5,6) clarified that TBEV has been maintained in animal populations, especially in wild rodents in Hokkaido. Given the continued ecologic findings of virus activity in animals, it is plausible that humans have acquired TBEV infection, especially persons frequently exposed to ticks, including Japan Self-Defense Forces (JSDF) members of the Northern Army. We report the result from a pilot seroepidemiologic study of JSDF members conducted to determine the presence of unrecognized infections and to crudely measure the frequency.

We recruited 291 JSDF members who belong to the Northern Army and who received tick bites during ground activities (7). During their general health screening in February and March 2017, participants were asked to provide an additional 2 mL of serum for laboratory testing and to answer a questionnaire about the frequency of tick bites. We conducted neutralizing antibody testing using the virus isolated from Hokkaido in 1993 (4). We determined a serum sample to be TBEV positive if ≥50% plaque reduction compared with healthy human control serum was observed. We defined neutralizing titer as the reciprocal of the highest dilution of serum. To differentiate TBEV infection from Japanese encephalitis virus (JEV) infection, which occurs in southwestern Japan, we also conducted neutralization testing for JEV on all TBEV positive samples.

The Medical Ethics Committees at the Graduate School of Medicine, Hokkaido University and JSDF Sapporo Hospital approved this study. The research team explained to participants that the enrollment was voluntary and gave participants the right to withdraw. We obtained written informed consent from participants, and no names were assigned to serum samples or questionnaires.

Participants ranged in age from 35 to 54 years (mean 43.3 years). A total of 288 (99.0%) of the 291 participants were men. Ninety-two (31.6%; 95% CI 26.5%–37.1%) participants appeared to have been infected with TBEV; both were negative for JEV, the only other flavivirus in Japan. The TBEV-positive participants were men 42 and 48 years of age who had been bitten 3 and 1 times, respectively, within the previous 10 years (Table). Neither man complained of symptoms of TBEV infection, such as high-grade fever, headache, nausea, or paralysis after tick removal; however, the 48-year-old participant noted right knee joint pain, inguinal lymph node swelling, and low-grade fever, which he attributed to the remaining body part of a tick.
These 2 unrecognized subclinical TBEV infections were serologically diagnosed, demonstrating that humans who are particularly at risk for tick bites are partly asymptotically infected with TBEV in Hokkaido. Because flaviviruses are known to serologically cross-react with other close flaviviruses (8), we tested serum against JEV, the only other endemic flavivirus in Japan, and successfully excluded its possibility. The antibody titer was lower than that in persons with clinically apparent cases (e.g., >1,600), perhaps because the virus replication was limited among subclinical cases or antibody had decayed since infection.

Our findings echo similar cross-sectional survey results among persons recently bitten by ticks in Xinjiang and Inner Mongolia, China (9). Although the estimated frequency in Japan was as low as 0.7%, this figure should not be regarded as small, considering that >30,000 persons serve in the Northern Army. In addition, frequently bitten persons include not only JSDF members but also dairy farmers, foresters, and hikers. Serological surveys with greater sample size and broader scope of study participants are needed to identify persons at high risk for infection and determine the pros and cons of specific countermeasures, including vaccination (10). Such surveys also are needed to measure the virulence of TBEV of the so-called Far-Eastern subtype because the detection of subclinical or mild cases may lead to an overall decrease in its case-fatality risk, which is perceived as high (7).

This work was supported in part by the Japan Agency for Medical Research and Development, the Japan Science and Technology Agency’s Core Research for Evolutional Science and Technology (CREST) program (JPMJCR1413), and Research Institute of Science and Technology for Society (RISTEX) program for Science of Science, Technology and Innovation Policy, and the Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (KAKENHI) 16KT0130 and 16K15032.

Dr. Yoshii is an associate professor at the Graduate School of Veterinary Medicine at Hokkaido University. His primary research interests include virology of tickborne encephalitis.

References


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bla<sub>CTX-M-27</sub>—Encoding Escherichia coli Sequence Type 131 Lineage C1-M27 Clone in Clinical Isolates, Germany

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Author affiliation: Justus Liebig University, Giessen, Germany

DOI: https://doi.org/10.3201/eid2310.170938
We examined extended-spectrum β-lactamase–producing isolates from livestock, humans, companion animals, food, and the environment during 2009–2016 in Germany for the presence of CTX-M-27 allele within *Escherichia coli* sequence type (ST) 131. *E. coli* ST131 C1-M27 was exclusively present in humans; its incidence increased from 0% in 2009 to 45% in 2016.

During the past 20 years, *Escherichia coli* sequence type (ST) 131 has emerged as a prevalent vehicle for extended-spectrum β-lactamases (ESBL) worldwide. Particularly prevalent are isolates of the clade ST131 C/H30R, which frequently are associated with urinary tract infections and bacteremia (1,2). Although the ESBL production of the predominant subgroup ST131 C2/H30Rx is conferred by the CTX-M-15 allele, the emerging subgroup C1 often is associated with other CTX-M alleles, such as CTX-M-14 and CTX-M-27 (3). An increase in C1/H30R ST131 isolates was initially reported among clinical isolates in Japan; most of those were identified as members of the recently defined clade C1-M27 (90% of C1/H30R) (3). More recently, a dramatic rise from 0% to 65% in the incidence of ST131 C1/H30R *bla*$_{CTX-M-27}$ isolates in the fecal carriage of children in France during 2010–2015 was reported (4). In addition, ST131 isolates harboring *bla*$_{CTX-M-27}$ have been reported sporadically from other countries (3). We examined ESBL-producing isolates from livestock, humans, companion animals, food, and the environment during 2009–2016 in Germany for the CTX-M-27 allele.

We analyzed a representative subset of 953 sequenced isolates from a collection of 4,386 nonrepetitive ESBL-producing *E. coli*, which were obtained through 2 national research networks investigating the incidence of antimicrobial resistance: ESBL and Fluoroquinolone Resistance in Enterobacteriaceae (RESET) and German Center for Infection Research (DZIF) in Germany (online Technical Appendix 1, https://wwwnc.cdc.gov/EID/article/23/10/17-0938-Techapp1.xlsx). In silico multilocus sequence typing (MLST) identified 159 (17%) of the 953 isolates as ST131 (online Technical Appendix 2). The most prevalent ESBL genes in the studied isolates were *bla*$_{CTX-M-15}$ (73 [46%]), followed by *bla*$_{CTX-M-27}$ (24 [15%]), *bla*$_{CTX-M-1}$ (18 [11%]), *bla*$_{CTX-M-14}$ (15 [9%]), and others (*bla*$_{CTX-M-3/11/17/24/36/47}$) (10 [6%]). Because recent reports have documented an increase in the number of C1-M27 clade isolates in Japan and France, we investigated *bla*$_{CTX-M-27}$–encoding ST131 isolates in more detail. All ST131 isolates with *bla*$_{CTX-M-27}$ were of serogroup O25b and harbored a *fimH30* allele, except for 1 isolate that was of serogroup O16 and carried a *fimH41* allele. Recently, the M27PP1 prophage-like region was defined as a specific marker for C1-M27 clade (3,4). This region was present in 23 of 24 *bla*$_{CTX-M-27}$–harboring isolates. Phylogenomic analysis revealed that these 23 isolates belong to clade C1/H30R (online Technical Appendix 2 Figure).

We identified contigs with F1:A2:B20 plasmid replicons in sequences from 20 of 24 isolates; the remaining isolates harbored contigs with F1:A6:B20, F1:A2:B20, F1:A2:B–, and F29:A–B10 plasmid incompatibility groups. We sequenced the genome of 1 representative isolate (H105) to completion (GenBank accession numbers: chromosome, CP021454; plasmid, CP021871) and confirmed that the *bla*$_{CTX-M-27}$–encoding contig was indeed part of a plasmid harboring the F1:A2:B20 replicon (5). This plasmid is highly conserved in isolates of ST131 and is probably ancestral to the C1/H30R clade because it is present in all the *bla*$_{CTX-M-27}$–positive ST131 isolates, regardless of whether they harbor antimicrobial resistance genes (5).

Core genome phylogenetic comparisons of all C/H30R ST131 isolates, based on alignment to the closed genome of *E. coli* ST131 lineage C1-M27 isolate H105, revealed an average of 292 single-nucleotide polymorphisms (SNPs). In contrast, isolates within the C1-M27 clade were separated by <100 SNPs. Comparative analyses of 13 isolates reported from Japan showed that these isolates share ≈85% of the genome with those from Germany. Isolates from both countries exhibit an average difference of 59 SNPs, indicating clonality and possible evolution from a single common ancestor (online Technical Appendix 2). Metadata of the C1-M27 isolates showed 19 of 24 isolates were obtained in 2015 and 2016, indicating recent emergence.

Our results provide evidence for the recent emergence of ST131 subgroup *fimH30-O25b*, clade C1-M27, harboring *bla*$_{CTX-M-27}$ in Germany and reinforce observations made elsewhere. The data suggest an ongoing shift in CTX-M alleles associated with ST131 infections worldwide that now warrants further attention.

**Acknowledgments**

We thank Christina Gerstmann and Natalia Lest for excellent technical assistance and members of the RESET (http://www.reset-verbund.de/) and German Center for Infection Research (http://www.dzif.de/en/) networks consortia for providing isolates. This study was supported by grants from the Bundesministerium für Bildung und Forschung within the framework of the RESET Research Network (contract no. 01KI1013G/01KI1313G) and the German Center for Infection Research (DZIF/grant no. 8000 701–3 Helmholtz-Zentrum für Infektionsforschung to T.C. and C.I. and, T06.001/8032808811 to T.C.).

Mr. Ghosh is a PhD fellow at the Institute for Medical Microbiology, Justus Liebig University Faculty of Medicine. His primary research interests include investigating the transmission and epidemiology of important resistance genes and mobile genetic element using next-generation sequencing.
Angiostrongylus cantonensis: Eosinophilic Meningitis in an Infant, Tennessee, USA

Tim Flerlage, Yvonne Qvarnstrom, John Noh, John P. Devincenzo, Arshia Madni, Bindiya Bagga, Nicholas D. Hysmith

In 2016, a 12-month-old, fully vaccinated boy was admitted to a hospital in Memphis, Tennessee, USA, for evaluation of 18 days of daily fever, irritability, decreased oral intake, and emesis. His medical history was unremarkable, and he had no known contact with sick persons. He had not traveled outside the area comprising southwestern Tennessee and northwestern Mississippi. Wild rats had been observed in and around the home, and rat droppings had been found in the child’s bed. Raccoons were seen on the property; however, contact, either direct or through fomites such as latrines, was not reported. During a 17-day period, 2 evaluations by his primary care physician and 4 emergency department visits resulted in the diagnosis of fever of unknown origin and inpatient admission.

A cerebrospinal fluid (CSF) sample taken by lumbar puncture on day 20 of illness showed eosinophilic-predominant pleocytosis, mild hypoglycorrhachia, and a mildly elevated protein level (Table). Magnetic resonance imaging of the brain and spine showed scattered areas of restricted diffusion throughout the brain parenchyma, leptomeningeal enhancement, and multifocal nodular enhancement along the ventral portion of multiple spinal levels. Serologic testing was negative for Toxocara canis/cati, Strongyloides stercoralis, Ehrlichia chaffeensis, Rickettsia rickettsiae, Epstein-Barr virus, HIV, and Toxoplasma gondii; a rapid plasma reagin was also negative. Tuberculin skin testing was negative. Results of CSF PCR for Streptococcus pneumoniae, herpes simplex virus, and enteroviruses were negative; CSF cryptococcal antigen testing was also negative. Due to concern for infection with Baylisascaris procyonis, the raccoon roundworm, physicians prescribed albendazole and dexamethasone. The patient’s temperature returned to normal, and his symptoms resolved. Upon discharge, he was to complete 3 weeks of albendazole and tapering doses of corticosteroids. Attending physicians repeated lumbar punctures on days 28, 41, and 56 (Table).

Physicians sent samples (CSF and serum) taken on day 20 to the Centers for Disease Control and Prevention (Atlanta, GA, USA) to test for B. procyonis roundworms and samples taken on day 56 to test for Angiostrongylus cantonensis, the rat lungworm. Results were negative for B. procyonis but positive for A. cantonensis. In addition, serum samples obtained at the time of the initial lumbar puncture were positive for A. cantonensis antibodies by investigational whole-worm Western blot.

The first documented human infection with A. cantonensis worms occurred in 1944 in Taiwan. Since then, >2,800 cases among humans have been reported; most have been in Southeast Asia and the Pacific islands (1); online Technical Appendix, https://www.cdc.gov/EID/article/23/10/17-0978-Techapp1.pdf). In the late 1950s, the first report of human A. cantonensis infection in the

References
United States occurred in Hawaii. *A. cantonensis* worms have since become endemic to wide-ranging tropical and subtropical locales in the Western Hemisphere, including the Hawaiian Islands (2), the Caribbean Islands (3), and South America (4).

The first report of the rat lungworm in the continental United States was in 1987, when Kim et al. found that 18% of rats sampled on necropsy in New Orleans, Louisiana, were infected with the nematode (5). First-stage *A. cantonensis* larvae from these rats produced infections in native gastropods, providing the potential for these parasites to become endemic to the region. A report ≈15 years later documented infection in vertebrates not only in New Orleans but also in other areas of Louisiana and Mississippi. *A. cantonensis* worms are now considered to be endemic to Louisiana (5). Infection has since been documented in rats (6), gastropods (7), and vertebrates (8) across a large area of the southern United States, from Oklahoma (6) to Florida (7,8).

Soon after the initial recognition in local animal reservoirs, the first reported *A. cantonensis* infection in a human acquired in the continental United States occurred in an 11-year-old boy residing in New Orleans. Since then, 3 additional cases have been reported in an 11-month-old, a 12-month-old, and a 19-month-old, all of whom resided in Houston, Texas, and had not traveled (9).

*A. cantonensis* infection causes a self-limited illness in which headaches, nonfocal neurologic findings, and cranial nerve involvement are the most common signs and symptoms. Optimal therapy has not been clearly defined, and symptomatic management is an option for this self-limited illness. When therapy is prescribed, corticosteroids alone or in combination with antihelminth medications are most commonly used. In a prospective study that followed up on 3 previous studies, Chomtongkol et al. confirmed that a 2-week course of corticosteroids shortened the duration of headache and reduced the need for repeated lumbar puncture (10). The study concluded that corticosteroids plus albendazole was no better than corticosteroids alone.

International shipping and the ability of *A. cantonensis* worms to use diverse species of gastropods as intermediate hosts have all contributed to this parasite becoming a pathogen of increasing public health concern (5). Angiostrongyliasis should be considered in the differential diagnosis of prolonged fever of unknown origin with compatible clinical and laboratory findings.

Dr. Flerlage is a second-year fellow in a combined fellowship program for training in pediatric infectious diseases and critical care medicine at University of Tennessee/St. Jude Children’s Research Hospital. His primary research interest is acute lung injury caused by respiratory viruses in immunocompromised patients.

### References


### Table. Results of cerebrospinal fluid testing for 12-month-old boy with meningoencephalitis, Memphis, Tennessee, USA, 2016*

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<th>Test</th>
<th>Reference range</th>
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</tr>
<tr>
<td></td>
<td>547</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>41</td>
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<tr>
<td></td>
<td>2,979</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>4</td>
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<tr>
<td>Polymorphonuclear cells, %</td>
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<tr>
<td>Lymphocytes, %</td>
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<tr>
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</table>

*NA, not applicable.

---

**Table. Results of cerebrospinal fluid testing for 12-month-old boy with meningoencephalitis, Memphis, Tennessee, USA, 2016**

- **Leukocytes, cells/mm³**: Reference range is 0–8, with values of 547, 55, 2,979, and 115 on days 20, 28, 41, and 56, respectively.
- **Polymorphonuclear cells, %**: Reference range is 0–1, with values of 0, 4, 20, and 4 on days 20, 28, 41, and 56, respectively.
- **Lymphocytes, %**: Reference range is 0–5, with values of 44, 80, 65, and 6 on days 20, 28, 41, and 56, respectively.
- **Eosinophils, %**: Reference range is 0–5, with values of 20, 10, 5, and 11 on days 20, 28, 41, and 56, respectively.
- **Monocytes, %**: Reference range is 0–5, with values of 20, 6, 10, and 21 on days 20, 28, 41, and 56, respectively.
- **Erythrocytes**: Reference range is 40–70, with values of 0–14, 14–45, and 45 on days 20, 28, and 56, respectively.
- **Glucose, mg/dL**: Reference range is 15–45, with values of 67, 74, and 104 on days 20, 28, and 56, respectively.
- **Protein, mg/dL**: Reference range is 15–45, with values of 67, 74, and 104 on days 20, 28, and 56, respectively.
- **Gram stain**: Reference range is NA, with values of 0, 0, 0, and 0 on days 20, 28, 41, and 56, respectively.
- **Bacterial culture**: Reference range is NA, with values of Sterile, Sterile, Sterile, and Sterile on days 20, 28, 41, and 56, respectively.
Dengue Virus Exported from Côte d’Ivoire to Japan, June 2017


DOI: https://doi.org/10.3201/eid2310.171132

Since April 2017, a dengue fever outbreak has been ongoing in Côte d’Ivoire. We diagnosed dengue fever (type 2 virus) in a traveler returning to Japan from Côte d’Ivoire. Phylogenetic analysis revealed strain homology with the Burkina Faso 2016 strain. This case may serve as an alert to possible disease spread outside Africa.

In recent decades, dengue virus (DENV) infection has been spreading worldwide. Although in Africa the leading cause of acute febrile illness is still malaria, dengue has recently gained momentum (1). Dengue has been reported in 34 African countries, although it has probably been underreported because of the lack of diagnostic testing and systematic surveillance in Africa (2). Four types of virus have been isolated; the most endemic to Africa is DENV type 2 (DENV-2), followed by DENV-1 (2). The first reported case of DENV-1 infection occurred in a young soldier from Abidjan, Côte d’Ivoire, in 1999 (3). At that time, no other similar cases or epidemics in Abidjan had been reported. In 2008, a closely related strain, DENV-3, was isolated from visitors to Côte d’Ivoire (4,5). In 2010, dengue fever was biologically confirmed for 7 patients who had never been in a dengue-endemic area, and DENV-3 was confirmed by reverse transcription PCR for 4 of these patients (6). A prospective study in Abidjan also revealed that DENV-3 had been the cause of febrile illness during 2011–2012 (7). Thus, DENV-3 may have circulated widely in Côte d’Ivoire, especially in Abidjan. During the 2016 outbreak in Burkina Faso, DENV-2 infection was detected in 2 travelers returning from Burkina Faso to France (8). During August–November 2016, the World Health Organization reported 1,061 probable dengue cases and 15 deaths from dengue (9). We report a case of dengue fever exported to Japan from Abidjan in 2017.

On June 19, 2017, a man in his early 50s sought care at the Center Hospital of the National Center for Global Health and Medicine, Tokyo, Japan, for fever, chills, headache, and mild joint pain. In June 2013, he had traveled to Abidjan for business, and on June 13, 2017, he returned to Japan. He had been vaccinated for yellow fever. He had noticed a high fever in the morning and sought care the same evening.

Physical examination revealed body temperature of 39.3°C, mildly hyperemic conjunctiva, and a slight rash on his trunk. His blood biochemistry profile showed 3,640 leukocytes/L, hemoglobin level 13.5 g/dL, and 151 × 10^9 thrombocytes/L. Results of a rapid diagnostic test for malaria (BinaxNOW Malaria; Alere, Waltham, MA, USA) were negative. A thin-coated peripheral blood smear with May-Grünwald Giemsa stain showed no Plasmodium parasites. Results of a dengue rapid diagnostic test (Dengue Duo NS1 Ag + Ab Combo; Alere) were negative for IgM and IgG but positive for nonstructural protein 1 antigen. Serum samples obtained on June 19 and 26 were sent for real-time reverse transcription PCR to the National Institute of Infectious Diseases, Tokyo, where DENV-2 RNA was detected.

The patient’s signs and symptoms resolved spontaneously in a week; his lowest thrombocyte count was 99 × 10^9 thrombocytes/L. On June 19, a diagnostic test for DENV IgM (Dengue Virus IgM Capture ELISA; Focus Diagnostics, Cypress, CA, USA) yielded negative results; however, on June 26, positive results indicated seroconversion.

Phylogenetic analysis of the DENV envelope gene indicated that the sequence of DENV-2 obtained from the patient belonged to the cosmopolitan genotype and was 99% identical with the envelope gene of DENV-2 strains from the 2016 dengue epidemic in Burkina Faso (GenBank accession nos. LC206003, KY627763, and KY627762) (Figure). The sequence of DENV-2 from the patient also showed 97% identity...
Figure. Comparison of dengue virus type 2 from a patient returning from Abidjan, Côte d’Ivoire, to Japan (arrow) with reference dengue virus sequences. Virus lineages are shown on right. Phylogenetic trees were constructed by using the neighbor-joining method. The maximum composite likelihood method was used, and rates among sites were uniform. Analyses were performed by using MEGA6 software (http://megasoftware.net). Scale bar indicates substitutions per nucleotide position.
with that of the DENV-2 strains from the 1983 (accession no. EU056810) and 1986 (accession nos. HM234642 and GU131843) epidemics in Burkina Faso. Strains of DENV-2 from the 2005 epidemic in Ghana (accession no. EU005258) shared 95% identity with that of the patient reported here.

Phylogenetic analysis indicated that the dengue virus genome sequence in this case is highly homologous with recent strains in Africa, especially from the 2016, 1986, and 1983 outbreaks in Burkina Faso. Similar strains of DENV-2 have repeatedly caused outbreaks in West Africa (8). As of April 2017, DENV-2 and DENV-3 have been isolated from patients in the ongoing outbreak in Abidjan (J0). Although no cases have been reported outside Abidjan, the case reported here may be a sentinel case, serving as an alert to the possibility of disease spread outside Africa.

This work was supported by grants from the Japan National Center for Global Health and Medicine (grant nos. 27-6001 and 29-1018).

Dr. Suzuki is a medical doctor at the National Center for Global Health and Medicine, Disease Control and Prevention Center, in Shinjuku-ku, Tokyo, Japan. His main research interest is tropical infectious diseases.

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LETTER

Etymologia: Creutzfeldt-Jakob Disease

Douglas John Lanska

Author affiliations: VA Medical Center, Tomah, Wisconsin, USA; University of Wisconsin, Madison, Wisconsin, USA

DOI: https://doi.org/10.3201/eid2310.171142

To the Editor: The recent etymologia article on Creutzfeldt-Jakob disease by Henry and Murphy (1) does not accurately reflect current understanding of the contributions of Creutzfeldt and Jakob. Although Jakob had reported that Creutzfeldt’s earlier case was a “noso- logically very closely connected if not identical affection” (2), Creutzfeldt himself later reported that “his case did not bear any resemblance to the cases described by Jakob” (3). As discussed by neuropathologist Edgar Peirson Richardson, Jr., in 1977, “Did Creutzfeldt and Jakob describe CJD? … Creutzfeldt probably did not—Jakob to the contrary notwithstanding—and Creutzfeldt is said to have disagreed with the identification of his case with Jakob’s cases. Jakob’s cases, on the other hand, can more readily be fitted into current concepts of the disease without undue strain” (4). In 1982, neuropathologist Colin L. Masters and pediatrician D. Carleton Gajdusek concurred with Richardson: “We agree with Richardson (1977) that Creutzfeldt’s case probably can be excluded from classification as a spongiform encephalopathy on the basis of his own clinical and pathological descriptions, although a specific alternative
diagnosis cannot be made” (5). In a later article, Richardson and Masters further noted that Creutzfeldt’s case showed no indication of spongiform change, and the character of the lesions was not characteristic of “CJD” (6). In contrast, Jakob clearly described cases of “CJD”: based on reexamination of the original pathologic slides preserved at the University of Hamburg, several of Jakob’s cases were consistent with the clinical picture of “CJD” and showed characteristic pathologic findings of spongiform encephalopathy (5). Finally, although Walther Spielmeyer first used the term “Creutzfeldt-Jakob disease” in 1922, his decision to emphasize Creutzfeldt was likely because Creutzfeldt was then working in Spielmeyer’s laboratory; other early terms for the disease gave credit preferentially or solely to Jakob.

References

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Zika: The Emerging Epidemic


In 1947, in a small forest in Uganda, near the capital, Kampala, a filterable transmissible agent was found in a sentinel rhesus macaque with a high fever. The agent was later identified as a virus and named Zika after the forest in which it was found. For the next 60 years, the virus was largely ignored by scientists because very few human cases were identified. However, in the summer of 2015, in several cities in eastern Brazil, a Zika epidemic was identified concurrently with a markedly increased number of infant microcephaly cases in maternity wards. Before long, Zika virus infection during pregnancy was identified as the cause of microcephaly, the virus was confirmed to be spreading among >40 countries in the Americas, and numerous public health agencies were faced with an emergency about how best to protect a generation of children conceived during the Zika outbreak.

The journey taken by Zika virus from Africa, across the Pacific, to the World Health Organization–declared Public Health Emergency of International Concern in February 2016 is chronicled by New York Times writer Donald G. McNeil, Jr., in Zika: The Emerging Epidemic. The book starts with the discovery of Zika virus in 1947 and traces the early research on human infections, the 2007 outbreak in Micronesia, the 2013 outbreak in French Polynesia, and the long 2015–2016 epidemic wave of Zika virus infection that occurred in the Americas. The book is organized chronologically and is current through June 2016.

McNeil’s book is thorough in its timeline and excellent at describing the political history of public health management for Zika. This history includes the blaming of an insecticide for the initial cases of Guillain-Barré syndrome in French Polynesia in 2013, the US Centers for Disease Control and Prevention’s initial handling of evidence of a sexual transmission route for Zika virus, and the controversy of whether to recommend delaying pregnancy in Zika-endemic areas where travel advisories were already in place. McNeil presents convincing arguments at the end of the book for why the recommendations to delay pregnancy should have been stronger, clearer, and more prompt. The book also catalogs a range of alternative hypotheses that emerged in the media before a scientific consensus formed that Zika did in fact cause microcephaly.

The book contains some poignant individual stories that humanize the toll of the epidemics in the Pacific and the Americas. McNeil correctly calls attention to Guillain-Barré syndrome, which typically does not generate headlines the way that a photo of a microcephalic infant does. In some instances, anecdotes about newspaper editors and public officials draw attention away from the victims of the epidemic.

The book sometimes strays into a narrative of epidemiologist or journalist as hero, a convention that should be avoided. It contains a small number of scientific inaccuracies (e.g., biological classification of Toxoplasma, risk for death from dengue virus), and academic readers would be wise to follow their noses to the source material, much of which McNeil lists by chapter at the end of the book. The book is very readable, as expected from a New York Times writer, and it successfully presents a current-events summary of the 2015–2016 Zika epidemic and what the world’s public health systems did and did not do to minimize the risk for those in its path.

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DOI: http://dx.doi.org/10.3201/eid2310.171139

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Correction: Vol. 23, No. 6

In Invasive Serotype 35B Pneumococci Including an Expanding Serotype Switch Lineage, United States, 2015–2016 (S. Chochua et al.), serotype 35B was incorrectly described as the most common cause of invasive pneumococcal disease in children and adults. It is among the most common causes, but not the most common. The article has been corrected online (https://wwwnc.cdc.gov/eid/article/23/6/17-0071_article).
Art critic Ossian Ward has written that Wassily Kandinsky strove “to evoke sound through sight and create the painterly equivalent of a symphony that would stimulate not just the eyes but the ears as well.” One of the great artists of the early 20th century, Kandinsky was born on December 4, 1866, in Moscow to well-educated, upper-class parents. From a young age, he exhibited interest in and sensitivity to sound and color. Although he attended drawing and music classes during his childhood, Kandinsky pursued law, ethnography, and economics when he enrolled at the University of Moscow in 1886. A decade later he abandoned a career in teaching law to attend art school in Munich, Germany, where he met other like-minded young artists and co-founded “Phalanx,” an art school that spurned traditionalist approaches and conventions.

Ward notes that Kandinsky is believed to have had synesthesia and to have the experience of hearing color and seeing sound. Synesthesia is a mingling of sensory input in which stimulation in one sense, such as hearing, concurrently and consistently stimulates a sensation in another sense, e.g., vision or taste. Kandinsky often drew from the lexicon of music in naming or describing his works as compositions, impressions, and improvisations. Neurologist and author Richard E. Cytowic states that “Kandinsky was among the first to step off the path of representation that Western art had followed for 500 years, and his model for this new ‘symbolic’ form of art was music.”

For the last 11 years of his life, Kandinsky lived in Paris. During those years, according to his Centre Pompidou biography, “Kandinsky painted and drew prolifically, putting together an important body of work in which the common factor is the inspiration of images from biology, forms resembling embryos, larvae, or invertebrates, a minuscule population embodying the living.” This body of work, dubbed biomorphic abstraction, contrasts with the artist’s earlier abstract compositions that often featured straight lines, precise circles, and sharp angles.1

Art historian and painter Hajo Düchting

According to the Tate Museum, biomorphic—derived from the Greek words “bios,” which means life, and “morphe,” which means form—was initially used during the 1930s “to describe the imagery in the more abstract types of surrealist painting and sculpture” (http://www.tate.org.uk/art/art-terms/b/biomorphic).

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DOI: https://doi.org/10.3201/eid2310.AC2310

Chaos in Form and Color Yields to Harmony

Byron Breedlove


Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 23, No. 10, October 2017 1763
observed that Kandinsky’s “basic geometrical forms dissolve into an unbelievable variety of shapes among which biomorphic ones predominate over those derived from geometrical shapes.”

A splendid example of Kandinsky’s biomorphic art, Colorful Ensemble, this month’s cover image, shows that regardless of this shift in approach, the artist’s lifelong passion for infusing his art with musical themes and imagery continued to be at the heart of his work. What at first viewing appears to be frenzied, haphazard chaos yields to a pervading sense of harmony. The neutral background issues a tranquil invitation to peer more carefully at the mass of teeming shapes and bold primary colors enclosed by the heart-shaped figure.

Kandinsky studs the blue border with small bejeweled images, miniature constellations of color and harmony that may be separating from the whole or moving toward absorption. Densely packed scores of perfect circles create a textured mosaic. Curious interspersed forms evoke musical imagery: the head of a guitar, a stringed harp, and breath marks. Other shapes within the painting resemble biologic structures, including flagella, ribosomes, and genetic material found in bacteria.

Kandinsky created his biomorphic art during a time of crucial discovery and innovation in treating bacterial infections. The physicist Ladislaus Laszlo Marton had recently examined biologic specimens with an electron microscope and published the first electron micrographs of bacteria. Alexander Fleming had discovered penicillin just a decade earlier, and antibiotics were for the first time being widely used to treat bacterial infections.

Bibliography

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etymologia revisited

*Mycobacterium* [(mi’-ko-bak-tēr-eəm), from the Greek—myces (fungus) and baktērion (little rod)]

The only genus of bacteria in the family *Mycobacteriaceae*. In 1882, German scientist Robert Koch reported the discovery of a bacillus from the lung tubercles that caused tuberculosis. Earlier, Norwegian researcher G.H.A. Hansen had identified a similar microbe which caused leprosy. In 1896, the genus name *Mycobacterium*, from the Middle Latin noun meaning fungus rodlet, was proposed to include these new pathogens, *M. tuberculosis* and *M. leprae*. The name does not mean that mycobacteria are fungi; rather, the tubercle bacilli grow on the surface of liquid media as moldlike pellets when cultured. The nonmotile, acid-fast, aerobic organisms in this genus cause numerous human and animal diseases.

Emerging Infectious Diseases

Upcoming Issue

- Pregnant Women Hospitalized with Chikungunya Virus Infection, Colombia, 2015
- Legionnaires’ Disease Outbreaks and Cooling Towers, New York City, New York, USA
- Legionnaires’ Disease Outbreak Caused by Persistent Endemic Strain of Legionella sp., New York City, New York, USA, 2015
- Antimicrobial Nonsusceptibility of Gram-Negative Bacilli in the Veterans Health Administration System, United States, 2003–2013
- Mycoplasma genitalium Infection among Adults Reporting Sexual Contact with Infected Partners, Melbourne, Australia, 2008–2016
- Drug-Resistant Tuberculosis among Children, China, 2006–2015
- High-Level Fosfomycin Resistance in Vancomycin-Resistant Enterococcus faecium
- Long-Term Viruria in Zika-Infected Pregnant Women
- Highly Pathogenic Avian Influenza A(H7N9) Virus, Tennessee, USA, 2017
- Pulmonary versus Nonpulmonary Nontuberculous Mycobacteria, Ontario, Canada
- Changing Demographics and Prevalence of Body Lice among Homeless Persons, Marseille, France
- Bartonella quintana and Typhus Group Rickettsiae Exposure among Homeless Persons, Bogotá, Colombia
- Virulence of Japanese Encephalitis Virus Genotypes I and III, Taiwan
- Paracoccidioidomycosis after Highway Construction, Rio de Janeiro, Brazil
- Detection of Spotted Fever Group Rickettsia DNA by Deep Sequencing
- The Breadth of Viruses in Human Semen
- Dengue Virus Type 2 in Travelers Returning to Japan from Sri Lanka, 2017
- Autochthonous Leprosy without Armadillo Exposure, Eastern United States
- Zika Virus Outbreak, Dominica, 2016
- Blood Culture-Negative Endocarditis, Morocco
- Zika Virus Persistence and Higher Viral Load in Cutaneous Capillaries Than in Venous Blood

Complete list of articles in the November issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

October 3–7, 2017
ID Week
San Diego, CA, USA
http://www.idweek.org/

November 5–9, 2017
ASTMH
American Society for Tropical Medicine and Hygiene
66th Annual Meeting
The Baltimore Convention Center
Baltimore, MD, USA
http://www.astmh.org/

December 5–8, 2017
6th National Congress of Tropical Medicine and International Symposium on HIV/aids Infection
9th National Congress of Microbiology and Parasitology
80th Anniversary of the Institute of Tropical Medicine Pedro Kouri
Havana, Cuba

Feb. 1–3, 2018
8th Advances in Aspergillosis
Lisbon, Portugal
www.AAA2018.org

March 1–4, 2018
18th International Congress on Infectious Diseases (ICID)
Buenos Aires, Argentina
http://www.isid.org/icid/

Announcements
To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.
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.

You must be a registered user on http://www.medscape.org. If you are not registered on http://www.medscape.org, please click on the “Register” link on the right-hand side of the website.

Only one answer is correct for each question. Once you successfully answer all post-test questions, you will be able to view and/or print your certificate. For questions regarding this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@medscape.net. American Medical Association’s Physician’s Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please go to https://www.ama-assn.org. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for AMA PRA Category 1 Credits™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the AMA PRA CME credit certificate, and present it to your national medical association for review.

Article Title

Fatal Rocky Mountain Spotted Fever along the United States–Mexico Border, 2013–2016

CME Questions

1. You are advising a clinic located near the US–Mexico border regarding management of Rocky Mountain spotted fever (RMSF). According to the case series by Drexler and colleagues, which of the following statements about clinical presentation and diagnosis of RMSF in patients with recent exposure in northern Mexico is correct?
   A. All 4 patients initially presented with rash
   B. All 4 patients had several nonspecific findings at presentation, including fever, headache, nausea, vomiting, or myalgia
   C. None of the patients had thrombocytopenia early during their illness
   D. Two cases did not meet at least 1 laboratory criterion for a confirmed spotted fever rickettsiosis

2. According to the case series by Drexler and colleagues, which of the following statements about the clinical course and management of RMSF in patients with recent exposure in northern Mexico is correct?
   A. Of the 4 patients, 2 had respiratory failure
   B. Death occurred 2 to 3 months after the onset of illness
   C. Cutaneous necrosis of the extremities was not reported
   D. All patients received a nontetracycline-class antibiotic within the first week of illness, and only 1 patient received doxycycline at any time during their course

3. Which of the following statements about the clinical implications of analysis of this series of 4 fatal cases of RMSF in patients with recent exposure in northern Mexico is correct?
   A. Doxycycline should be delayed until appearance of rash or availability of confirmatory laboratory testing
   B. Clinicians on both sides of the US–Mexico border should consider RMSF in patients with rapidly progressing febrile illness and recent exposure in northern Mexico
   C. Complete blood counts and hepatic function panels are not helpful in distinguishing RMSF from arboviral infections
   D. RMSF diagnosis is straightforward based on characteristic features
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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Only one answer is correct for each question. Once you successfully answer all post-test questions, you will be able to view and/or print your certificate. For questions regarding this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@medscape.net. American Medical Association’s Physician’s Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please go to https://www.ama-assn.org. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for AMA PRA Category 1 Credits™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the AMA PRA CME credit certificate, and present it to your national medical association for review.

Article Title

Enteric Infections Circulating during Hajj Seasons, 2011–2013

CME Questions

1. You are seeing a 30-year-old man who just returned from Hajj yesterday. He reports a 3-day history of diarrhea with malaise and abdominal cramping. You consider where the apparent gastroenteritis infection might have originated. What was the most common country of origin for such infections in the current study by Abd El Ghany and colleagues?
   A. Pakistan  
   B. Saudi Arabia  
   C. Bangladesh  
   D. India

2. Which of the following statements regarding clinical characteristics of patients with gastroenteritis in the current study is most accurate?
   A. The median age of patients was 40 years  
   B. Women represented nearly half of all patients in 2013  
   C. The most common symptom was watery diarrhea  
   D. Most cases featured bloody stools and fever

3. You send a sample of the patient’s stool to the laboratory for analysis. Which of the following pathogens was responsible for most cases of gastroenteritis in the current study?
   A. Virus  
   B. Bacteria  
   C. Parasite  
   D. Mixed infection with different organisms

4. What was the most common single bacterial pathogen represented in the current study sample?
   A. Escherichia coli  
   B. Salmonella spp.  
   C. Shigella spp.  
   D. Campylobacter spp.
Global Health Security Special Issue
Supplement to *Emerging Infectious Diseases* December 2017

The upcoming *Emerging Infectious Diseases* supplement on global health security highlights how CDC remains a trusted partner and leader in establishing a worldwide platform to stop infectious diseases from crossing borders and threatening the health, safety, and security of Americans.

The supplement includes contributions from experts across the globe and highlights the importance of sustained, lifesaving investment in global health security initiatives. With more than 70% of countries still underprepared to contain outbreaks, this timely series of articles illustrates work being done to close the gaps that leave us all vulnerable to dangerous and deadly epidemics.

The online release begins in September with the lead article: *US Centers for Disease Control and Prevention and its Partners’ Contributions to Advance Global Health Security*. The print edition of this special issue will be published in December 2017.
Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

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.

Summary of Authors’ Instructions

Author’s Instructions. For a complete list of EID’s manuscript guidelines, see the author resource page: http://wwwnc.cdc.gov/eid/page/author-resource-center.

Manuscript Submission. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

Manuscript Preparation. For word processing, use MS Word. Set the document to show continuous line numbers. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, and figure legends. Appendix materials and figures should be in separate files.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author’s mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

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 typing new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and public health. Topics may include science and the human condition, the unanticipated side of epidemiologic 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.

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.

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 are made 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.

Etymology. Etymology (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.
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