Unexpected Hazards

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The CDC Yellow Book is available in print through Oxford University Press and online at www.cdc.gov/yellowbook.
Clinical Characteristics of \textit{Corynebacterium ulcerans} Infection, Japan


SYNOPSIS

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Learning Objectives

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

• Assess the demographic and clinical characteristics of patients with \textit{Corynebacterium ulcerans} infection, based on a case series of 34 patients in Japan from 2001 to 2020

• Compare clinical characteristics between patients with respiratory and nonrespiratory symptoms of \textit{Corynebacterium ulcerans} infection and among 3 severity subgroups of patients with respiratory symptoms, based on a case series of 34 patients in Japan from 2001 to 2020

• Determine the clinical and treatment implications of clinical characteristics, treatment-related factors, and outcomes of \textit{Corynebacterium ulcerans} infection, based on a case series of 34 patients in Japan from 2001 to 2020

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Corynebacterium ulcerans is a closely related bacterium to the diphtheria bacterium C. diphtheriae, and some C. ulcerans strains produce toxins that are similar to diphtheria toxin. C. ulcerans is widely distributed in the environment and is considered one of the most harmful pathogens to livestock and wildlife. Infection with C. ulcerans can cause respiratory or nonrespiratory symptoms in patients. Recently, the microorganism has been increasingly recognized as an emerging zoonotic agent of diphtheria-like illness in Japan. To clarify the overall clinical characteristics, treatment-related factors, and outcomes of C. ulcerans infection, we analyzed 34 cases of C. ulcerans that occurred in Japan during 2001–2020. During 2010–2020, the incidence rate of C. ulcerans infection increased markedly, and the overall mortality rate was 5.9%. It is recommended that adults be vaccinated with diphtheria toxoid vaccine to prevent the spread of this infection.

Diphtheria is an upper respiratory tract illness caused by toxin-producing Corynebacterium diphtheriae bacteria, and it is characterized by sore throat, fever, and formation of a pseudomembrane on the tonsils, pharynx, or both, along with nasal discharge. C. diphtheriae can also infect the skin, causing open sores or ulcers. However, diphtheria skin infections rarely result in any other severe disease (1). C. ulcerans is a closely related bacterium to C. diphtheriae, and some strains produce toxins that are very similar to diphtheria toxin (2,3). C. ulcerans is widely distributed in the environment and is considered one of the most harmful pathogens to livestock and wildlife. This bacterium can cause cutaneous inflammation, including mastitis, in dairy cows (4–6). C. ulcerans has been increasingly recognized as an emerging zoonotic agent of diphtheria-like illness in the world (7–18).

Infections caused by these 2 bacteria are difficult to distinguish clinically, and the World Health Organization (WHO) treats infections caused by toxin-producing C. ulcerans as part of the diphtheria case definition (19). C. diphtheriae is thought to be transmitted only among humans, but C. ulcerans can be transmitted to humans by nonhuman mammals and thus should be treated as a zoonosis (7–18). Dogs and cats as companion animals are considered the major causes of transmission to humans. Although there have been several reports of individual cases of C. ulcerans infection (20–24), information on clinical features, treatment-related factors, and outcomes is limited. In this study we elucidate the clinical features, treatment-related factors, and outcomes of C. ulcerans infection cases in Japan during 2001–2020.

Methods
This study was a retrospective, observational, national survey of C. ulcerans infections in Japan since the first reported case of this infection (25). The data acquisition period was 20 years, from February 2001 through December 2020. The institutional review board of St. Luke’s International Hospital (Tokyo, Japan) approved this cross-sectional, survey-based study (approval no. 19-R055).

Patients and Setting
The National Institute of Infectious Disease (NIID) has comprehensively organized research and controlled clinical practice in C. ulcerans infectious diseases in Japan. However, in Japan, C. ulcerans infection is not included in the diphtheria case definition, nor is it required to be reported in all cases, so there is no obligation to report. However, because C. ulcerans produces diphtheria toxin, it has clinical manifestations similar to those caused by C. diphtheriae, for which all cases must be reported in Japan (19). Therefore, Japan’s Ministry of Health, Labour and

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Welfare (MHLW) urged health management departments and hospitals throughout the country to call attention to the need to identify the causative bacterium in patients showing clinical symptoms similar to diphtheria. MHLW has also published diagnostic criteria for *C. ulcerans* to assist clinicians in classifying *C. diphtheriae* and *C. ulcerans* (26). Under those circumstances, information from doctors who treated patients with suspected diphtheria symptoms and requests for pathogen diagnosis were sent to NIID. Therefore, the data included in this analysis came from attending physicians who, at the time of care, chose to investigate and report cases as *C. ulcerans* infections.

**Data Collection**

The following parameters were recorded: age; sex; date of infection; location of patient’s origin; whether there was a companion animal; whether there was any interaction with animals, such as breeding livestock animals, or whether the patient had lived in an environment involving contact with animals; presence or absence of bacterial isolation from patients and related animals; and clinical symptoms (throat pain, nasal discharge, pseudomembrane, fever, headache, dyspnea, hoarseness, and abscess). In addition, we collected data on vital signs (heart rate, systolic blood pressure, temperature, and respiratory rate), laboratory data (leukocyte counts, platelet counts, creatinine kinase levels, C-reactive protein [CRP] levels), types of antibiotics administered, presence or absence of administration of diphtheria antitoxin, and outcomes (days of hospitalization, days of mechanical ventilation, and survival or death).

**Diagnosis of *C. ulcerans* Infection**

The diagnostic criteria for the cases collected in this study used the *C. ulcerans* diagnostic criteria of MHLW (26). Accordingly, several conditions must be met: the infection manifests the same clinical symptoms as respiratory diphtheria with intractable pharyngeal pseudomembrane formation, and gram-positive rods are isolated from local areas, such as the pharynx and nasal cavity, and identified as *C. ulcerans*; the isolated bacterium is *C. ulcerans* alone, or *C. ulcerans* is the main component; detection of the diphtheria toxin gene and its toxin activity have been confirmed from this isolated strain by a functional test (i.e., an Elek test or equivalent test) (1, 26); and cutaneous signs and symptoms are present and *C. ulcerans* is identified as the causative agent of local lymphadenopathy and abscesses (27, 28).

**Definitions of Symptoms**

*C. ulcerans* infections are classified into respiratory and nonrespiratory manifestations. We defined respiratory symptoms as dyspnea, hoarseness, sore throat, cough, fever, and (occasionally) white pseudomembrane of the nasopharynx and laryngeal vestibule. Nonrespiratory symptoms were defined as skin infections and abscesses, or symptoms in patients who did not show respiratory symptoms. We further classified both types of symptoms as mild (resolving on outpatient visits), moderate (requiring hospitalization), or severe (requiring hospitalization and further ventilator support). The definitions of all cases included in this study were those we described previously as consistent with diagnosis of *C. ulcerans* infection.

**Treatment of *C. ulcerans* Infection**

For treatment of *C. ulcerans* infection, administration of antibiotics to which *C. ulcerans* is susceptible, such as macrolides and penicillins, is effective. In severe cases, symptomatic treatment for diphtheria pneumonia and administration of diphtheria antitoxin are effective for ventilated patients. The antitoxin used for *C. ulcerans* infection and for diseases caused by *C. diphtheriae* is delivered from the nearest national stockpile. However, depending on the distance, sometimes immediate delivery cannot be achieved.

**Primary Data Analysis**

We compared patients’ characteristics, treatment-related factors, and outcomes between the respiratory symptoms group and nonrespiratory symptoms group by using the Mann-Whitney U test or Fisher exact test, as appropriate. We used quantitative properties in the calculation basically as they are and quantified qualitative properties by scoring and then analyzed them. In the respiratory symptoms group, we compared mild, moderate, and severe cases. Regarding the collection of clinical data, we did not impute missing data. We performed statistical analysis by using JMP Pro statistical software version 14 (SAS Institute). We considered 2-sided p values <0.05 to be statistically significant.

**Results**

**Demographic and Clinical Characteristics of Patients**

A total of 34 patients from 34 hospitals were identified during the 20-year study period (Appendix Table, https://wwwnc.cdc.gov/EID/article/29/8/22-0058-App1.pdf). The reports of *C. ulcerans* infections came from a wide range of areas, and there was no regional bias (Figure 1). Furthermore, when we
compared the number of *C. ulcerans* cases every 5 years, we found the number of cases during 2001–2010 was stable (4 total cases), but the number of cases during 2011–2015 was 7 and during 2016–2020 was 19. Therefore, compared with the number of cases during 2001–2010, the number during 2011–2015 was 1.75 times higher and during 2016–2020 was 4.75 times higher (Figure 2).

The symptoms of *C. ulcerans* infection were respiratory in 23 (67.7%) patients and nonrespiratory in 11 (32.3%) (Table 1). The median age of patients was 58 years, and 61.3% of patients were women. Almost all patients (97.1%) had contact with animals. The mortality rate was 5.9%.

**Details of *C. ulcerans* Infection Cases**

We divided the clinical characteristics of *C. ulcerans* patients into respiratory and nonrespiratory groups. The characteristics of respiratory symptoms include formation of pseudomembrane in addition to dyspnea, hoarseness, sore throat, and fever. In general, among the patients evaluated in our study, the pseudomembrane was often attached to the nasopharynx (Figure 3, panels A, B; Figure 4). Moreover, in severe cases, bronchoscopy showed a pseudomembranous material obstructing the bronchi (Figure 3, panel C).

Patients with pseudomembranes with bronchial obstruction were characterized by atelectasis (i.e., when part or all of the lung was devoid of air and collapses) on radiographs (Figure 4, panel A). A common cause of atelectasis was bronchial obstruction. Atelectasis spreads throughout the lungs as symptoms worsen (Figure 4, panels B, C). As lung function declines, ventilators and extracorporeal membrane oxygen therapy are required to save the patient’s life. Complications after *C. ulcerans* treatment have been reported (29); in that particular case, the patient reported dyspnea, and a thick pseudomembrane was
found in the larynx. *C. ulcerans* producing diphtheria toxin was detected in the pseudomembranes. Antibiotic treatment improved airway symptoms, but sudden cardiac arrest occurred, followed by dyspnea and seizures. Afterward, the patient’s general condition stabilized, but she remained unconscious.

In contrast, nonrespiratory *C. ulcerans* patients evaluated in our study had local lymph node abscesses near the trauma, parotid abscesses, axillary abscesses, cervical lymph node abscesses, plantar skin ulcers, subcutaneous abscesses, and mandibular abscesses. Symptoms such as abscess, thigh abscess, and purulent lymphadenitis in the right neck were observed (Appendix). MRI images of a patient’s elbow showed abscesses in the axillary and parotid lymph nodes (Figures 4, panels E–F).

**Comparing Clinical Characteristics between Respiratory and Nonrespiratory Symptoms Groups**

Patients in the respiratory symptoms group were significantly older than patients in the nonrespiratory group (64 [interquartile range (IQR) 54–72] years vs. 38 [IQR 21–61] years; p = 0.03). When we compared the 3 severity classifications for respiratory and nonrespiratory symptoms, we found the group with respiratory symptoms had 6 patients with mild, 7 patients with moderate, and 10 patients with severe symptoms. In contrast, in the nonrespiratory symptom group, there were 5 mild cases, 6 moderate cases, and 0 severe cases. The differences in the number of mild, moderate, and severe cases of symptom severity in the 2 groups were significant (p<0.01) (Table 1). Leukocyte counts and CRP levels were relatively higher in the respiratory group than in the nonrespiratory group (p = 0.07 for both) (Table 1).

**Comparing Clinical Characteristics among the 3 Severity Groups**

Within the group showing respiratory symptoms, we compared clinical characteristics for the mild, moderate, and severe subgroups (Table 2). Among the respiratory group patients with pseudomembrane, 5 had mild cases, 6 had moderate cases, and 10 had severe cases. Among cases with respiratory symptoms, pseudomembrane-positive patients accounted for 83.3% of mild cases, 85.7% of moderate cases, and 100% of severe cases. Laboratory data showed a significant difference among the 3 subgroups in CRP levels (4.7 mg/dL [IQR 0.9–6.1 mg/dL] in mild, 7.7 mg/dL [IQR 1.8–12.6 mg/dL] in moderate, and 21 mg/dL [IQR 11.7–25.4 mg/dL] in severe cases; p = 0.02). Macrolide antibiotics, which are effective for *C. ulcerans*, were mainly used for mild cases. As the severity increased, many additional antibiotics, such as penicillin, cephalosporin antibiotics, and quinolone, were used. Regarding the length of hospital stay, we observed a significant difference between moderate cases (7 [IQR 7–10] days) and severe cases (29 [IQR 20–56] days; p<0.01). Diphtheria antitoxin was administered only to 4 severe case-patients (cases 5, 24, 29, and 33).

Two deaths from *C. ulcerans* infection occurred among the severe case-patients (20%; cases 5 and 18). Case-patient 5 was administered 5,000 IU of diphtheria antitoxin on her second day of hospitalization. *C. ulcerans*, which had been detected in the pseudomembrane, became negative in culture 1 week later, but the patient died on the 21st day of hospitalization without improvement in her severe pneumonia. Case-patient 18 was not administered antitoxin. She was administered antibiotics but died on the third day of her hospitalization from severe dyspnea caused by a pseudomembrane obstructing her airway (30).

**Discussion**

To compare our findings to those from other countries, we reviewed reports on diphtheria from the United Kingdom (31,32) and Belgium (33). Because of the history of the diphtheria pandemic in Eastern Europe in the late 1980s, surveillance of reports of *C. diphtheriae* infection are still underway in Europe, and cases continue to be identified. Therefore, the literature cases from this region during that period also contain reports of disease caused by the diphtheria toxin–producing *C. diphtheriae* and *C. ulcerans* (31). In Japan, the most recent case report of *C. diphtheriae* infection was in 2000 (34), and since then, the number of *C. ulcerans* infections have been increasing.
as shown in our study (Figure 2). The annual trend in the number of cases of C. ulcerans infection in the United Kingdom has also increased over the past few decades (31,32). In contrast, in Belgium, the number of cases reported during 2010–2017 hardly increased (33) and has remained fairly constant.

When we compared the age of patients with C. ulcerans infection, we found that in the United Kingdom, 60% of those affected are <15 years of age, whereas in Belgium, 90% of those affected are >45 years of age. In Japan, as in Belgium, 80% of those infected are >45 years of age. The sex ratio of patients showed similar trends in all countries; women accounted for 75% of infections in the United Kingdom, 77% in Belgium, and 67% in Japan. Martini et al. (33) argued that women are more likely to be patients because they tend to have more contact with companion animals than men.

When we compared transmission routes of C. ulcerans, we found that in the past in the United Kingdom, infections were mainly caused by cattle and poorly sterilized dairy products, but in recent years,
infections have been mainly caused by companion animals such as cats and dogs. The same trends occurred in Belgium and Japan. The change over time in the source of \textit{C. ulcerans} infection in the United Kingdom indicates that this infection is not limited to persons involved in livestock farming and that the general public can become infected (32). We speculate that this change contributed to the recent increase in \textit{C. ulcerans} infections in the United Kingdom. Because \textit{C. ulcerans} infections in countries such as the United Kingdom, Belgium, and Japan are suspected to be transmitted from companion animals, not only physicians but also veterinarians who examine companion animals should be informed about \textit{C. ulcerans} infection (8,11,16).

Regarding the prognosis of \textit{C. ulcerans} infection, mortality rates were 6% in the United Kingdom during 1986–2017 (31,32) and 5.9% in Japan during 2001–2020. No deaths from \textit{C. ulcerans} infections were reported in Belgium during 2010–2017 (33). Further details of the course of fatal cases of \textit{C. ulcerans} infection in the United Kingdom show that all of the fatal cases were in women ≥70 years of age who had respiratory symptoms, the death of nearly one third of patients overall was possibly associated with delayed administration of antitoxin, and the death of nearly two thirds of patients overall may have been associated with delayed diagnosis of diphtheria (31,32). By comparison, 2 fatal cases in Japan occurred in women 57 and 66 years of age who had respiratory symptoms; 1 death may have been attributable to delay in administration of antitoxin, and the other death may have been because the patient was diagnosed with \textit{C. ulcerans} infection too late.

When we compared cases of \textit{C. ulcerans} infection in the United Kingdom and Japan in terms of deaths, we observed a similar course of death in both countries. Administration of antitoxin is the primary treatment for diphtheria, but because diphtheria antitoxin is a preparation made from horse serum immunized with diphtheria toxin, its administration may be accompanied by adverse events, such as serum sickness. The decision requires judgment in considering the risks and benefits of antitoxin administration. In the United Kingdom and Japan, 94% of patients with \textit{C. ulcerans} infection survive. In both cases, administration of macrolide antibiotics is the main treatment method, whereas diphtheria antitoxin is administered to severely ill patients (31,32).

When we compared the symptoms caused by \textit{C. ulcerans} infection, we found that ≈80% of patients in the United Kingdom had respiratory symptoms and ≈20% had nonrespiratory symptoms. We noted the same tendency in cases in Japan (respiratory symptoms in 66% of patients and nonrespiratory symptoms in 34%). In Belgium, on the contrary, prevalence of nonrespiratory symptoms were as high as 64%, and respiratory symptoms were observed in 36% of cases (31–33). The different proportions of respiratory and nonrespiratory symptoms of \textit{C. ulcerans} infection in the 3 countries may be related to the immunologic status of patients with respect to diphtheria toxin. In our study, the patients with respiratory symptoms were mostly elderly and severely ill, whereas the patients with nonrespiratory symptoms were relatively young, and few were severely ill (Table 1). In Japan, young persons have high levels of antibody titers against diphtheria toxin, but this antibody titer declines with age (35). Patients with high antibody titers who have \textit{C. ulcerans} may show nonrespiratory symptoms without exacerbation of respiratory symptoms. Different diphtheria toxoid vaccination schedules in the United Kingdom and Belgium may also influence symptoms after \textit{C. ulcerans} infection (37,38).

We also considered the status of vaccination for \textit{C. ulcerans} infections. The diphtheria vaccine in Japan became available in 1948, and after several changes in the inoculation content, the formulation now in use was implemented in 1995. The current vaccination schedule in Japan is to inoculate 3 times

**Figure 3.** Endoscopic images of the pharynx and bronchi of patients with \textit{Corynebacterium ulcerans} infection, Japan, 2001–2020. A. B) Posterior wall of the pharynx has a yellowish white pseudomembrane. Arrows indicate the white pseudomembrane attached to the pharynx (case no. 21, from Dr. Toyoshima, Japanese Red Cross Ise Hospital, Mie, Japan). C) Pseudomembrane on the bronchi. Arrows indicate the pseudomembrane attached to the bronchi (case no. 29, from Dr. Hayashi, Maebashi Red Cross Hospital, Gunma, Japan).
at intervals of 3–8 weeks starting at 3 months of age and to give the fourth inoculation 1 year after the third inoculation. At 11 years of age, children receive a fifth boost and are not vaccinated after that point (35). In Japan, to understand the state of immunity to diphtheria toxoid, a certain number of persons are randomly selected from prefectures nationwide and their antibody titers are measured every 4–5 years. That survey is commissioned by the government of Japan and is conducted by NIID and local health authorities. According to those survey data, the proportion of persons in their 50s who still had antibody titers at a level protective against diphtheria decreased to ≈10% (36). When we interviewed patients in our study and asked about their vaccination status, most of the patients >60 years of age, except for the 20-year-old patient in case 13 and the 6-year-old patient in case 14, so vaccination status was usually unknown. In addition, the average age of patients in our study with severe respiratory symptoms was 67 years (Table 2), and patients born before 1948 had not been vaccinated. We hypothesize that persons in the older age group are inadequately vaccinated or unvaccinated against diphtheria toxin and that the characteristics of \textit{C. ulcerans} infection are related to the vaccination system in Japan.

According to reports on \textit{C. ulcerans} infection in the United Kingdom and Belgium, many patients, especially those who died or were severely ill, were unvaccinated or inadequately vaccinated (31–33).
The vaccination schedule in the United Kingdom is to inoculate 4 times until 5 years of age; at the age of 14, persons receive a fifth booster and are not vaccinated after that point (37). The vaccination schedule in Belgium is to inoculate 6 times until at the age of 14–16 years. Adults in Belgium are recommended to be vaccinated with a diphtheria toxoid-containing vaccine every 10 years (38). When we compared the diphtheria toxoid vaccination schedule in the United Kingdom and Belgium with Japan, we found that the United Kingdom schedule is very similar to that of Japan, but the Belgium schedule is similar to the WHO-recommended schedule and the US Advisory Committee on Immunization Practices schedule and includes vaccination for adults. This difference may explain why the number of patients with \textit{C. ulcerans} infection in Belgium has remained constant over the past decade or so (38).

In light of those findings, it appears that \textit{C. ulcerans} infections tend to affect generations with reduced levels of diphtheria antitoxin antibodies. Moreover, because the risk for severe disease from \textit{C. ulcerans} infections increases with age, we recommend that adults be vaccinated with diphtheria toxoid vaccine to prevent the spread of this infection. In fact, the European Union (which includes Belgium), the US Centers for Disease Control and Prevention, and WHO recommend that adults be vaccinated with a diphtheria toxoid–containing vaccine every 10 years after completing the initial vaccination series in childhood (1,19,35,38).

Among the limitations of this research, we summarized clinical data on 34 cases of \textit{C. ulcerans} infection reported in Japan over a 20-year period, and we reported the clinical features, treatments performed, and prognoses of these cases. However, not all cases were captured, and some data on the reported cases may have been incomplete, which may affect the reliability of our findings. Therefore, it is necessary to verify our findings with more case information in the future.

In Japan, diphtheria caused by diphtheria toxin–producing \textit{C. diphtheriae} occurred in ≤100,000 patients around 1945, and ≤10% of them died. This form of diphtheria was significantly reduced by regular vaccination with the diphtheria toxoid vaccine, and the last such case was reported in 2000 (34). Meanwhile, \textit{C. ulcerans} infections have been increasing over the past 20 years and have replaced disease

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mild symptoms, n = 6†</th>
<th>Moderate symptoms, n = 7‡</th>
<th>Severe symptoms, n = 10§</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54 (28–61)</td>
<td>62 (51–76)</td>
<td>67 (62–72)</td>
<td>0.07</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>3 (60.0)</td>
<td>3 (42.9)</td>
<td>1 (10.0)</td>
<td>0.11</td>
</tr>
<tr>
<td>F</td>
<td>2 (40.0)</td>
<td>4 (57.1)</td>
<td>9 (90.0)</td>
<td>0.29</td>
</tr>
<tr>
<td>Vital signs on admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>37 (36.6–37.4)</td>
<td>38 (37.6–38.8)</td>
<td>38 (37.5–38.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>Pseudomembrane</td>
<td>5 (100)</td>
<td>6 (85.7)</td>
<td>10 (100)</td>
<td>0.33</td>
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<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leukocytes, cells/mm$^3$</td>
<td>9,500 (6,700–14,800)</td>
<td>14,350 (10,363–23,550)</td>
<td>18,900 (13,400–22,600)</td>
<td>0.26</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL</td>
<td>4.7 (0.9–6.1)</td>
<td>7.7 (1.8–12.6)</td>
<td>21 (11.7–25.4)</td>
<td>0.02</td>
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<tr>
<td>Treatment antibiotic (no. cases)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Penicillins</td>
<td>None</td>
<td>Penicillin G (1),</td>
<td>Subbacitamp/ampicillin (6),</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sulbacitamp/ampicillin (2),</td>
<td>piperacillin (2)</td>
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<tr>
<td>Macrolides</td>
<td>Erythromycin (1),</td>
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<td>Erythromycin (2),</td>
<td></td>
</tr>
<tr>
<td></td>
<td>clarithromycin (2)</td>
<td>clarithromycin (3),</td>
<td>azithromycin (3)</td>
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<td>Cephalosporins</td>
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<td>clindamycin (1)</td>
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<tr>
<td>Quinolones</td>
<td>Ceftriaxone (1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Other</td>
<td>Levofoxacin (2)</td>
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<td></td>
<td></td>
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<tr>
<td>Diphtheria antitoxin</td>
<td>0</td>
<td>0</td>
<td>4 (40.0)</td>
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<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hospital days</td>
<td>0</td>
<td>7 (7–10)</td>
<td>29 (20–56)</td>
<td>&lt;0.01</td>
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<tr>
<td>Ventilator days</td>
<td>0</td>
<td>0</td>
<td>12 (5–42)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>2 (20.0)</td>
<td>2 (20.0)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Data are medians (interquartile range) for continuous variables and no. (%) for categorical variables.
†Missing data for mild cases: age (n = 1), sex (n = 1), heart rate (n = 6), systolic blood pressure (n = 6), body temperature (n = 4), respiratory rate (n = 6), pseudomembrane (n = 1), leukocytes (n = 3), platelets (n = 6), C-reactive protein (n = 3), treatment (n = 3), hospital days (n = 3), ventilator days (n = 3).
‡Missing data for moderate: heat rate (n = 5), systolic blood pressure (n = 5), body temperature (n = 3), respiratory rate (n = 5), leukocytes (n = 3), platelets (n = 6), T-bilirubin (n = 5), creatine (n = 4), C-reactive protein (n = 3), treatment (n = 1).
§Missing data for severe: heart rate (n = 6), systolic blood pressure (n = 6), body temperature (n = 4), respiratory rate (n = 6), leukocytes (n = 4), platelets (n = 6), T-bilirubin (n = 6), creatine (n = 5), C-reactive protein (n = 4), treatment (n = 1), ventilator days (n = 1).
cases caused by *C. diptheriae*. Clinicians and various local hygiene agencies have been alerted to this kind of infection. However, the law does not require all cases to be reported. Given the increased number of cases revealed in our study and the WHO position of considering *C. ulcerans* infections to be diphteria, we suggest that all *C. ulcerans* cases should be included with the infections that currently must be reported immediately.

**Acknowledgments**

We thank Shouji Asakura, Tetsu Aizawa, Teruyuki Ishii, and Takehiko Morioka for their contributions to this article.

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**References**


For many people, the prolonged period of social distancing during the coronavirus disease pandemic felt frightening, uncanny, or surreal. For Ron Louie, the sensation was reminiscent of a moth taking refuge in its cocoon, slumbering in isolation as he waited for better days ahead.

In this EID podcast, Dr. Ron Louie, a clinical professor in Pediatrics Hematology-Oncology at the University of Washington in Seattle, reads and discusses his poem about the early days of the pandemic.

Visit our website to listen: https://go.usa.gov/x6W9A
Healthcare-Associated Infections Caused by Mycolicibacterium neoaurum

Kate Shapiro, Shane J. Cross, Ted H. Morton, Hiroto Inaba, Ashley Holland, Francisca R. Fasipe, Elisabeth E. Adderson

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Release date: July 14, 2023; Expiration date: July 14, 2024

Learning Objectives

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

• Assess the demographic and clinical characteristics of Mycolicibacterium neoaurum infection, based on a case report of a child with leukemia and catheter-related bloodstream infection and a case series of 36 previously reported episodes of M. neoaurum infection
• Evaluate the diagnosis and management of Mycolicibacterium neoaurum infection, based on a case report of a child with leukemia and catheter-related bloodstream infection and a case series of 36 previously reported episodes of M. neoaurum infection
• Determine the clinical implications of demographic and clinical characteristics, diagnosis, and management of Mycolicibacterium neoaurum infection, based on a case report of a child with leukemia and catheter-related bloodstream infection and a case series of 36 previously reported episodes of M. neoaurum infection

CME Editor
Susan Zunino, PhD, Technical Writer/Editor, Emerging Infectious Diseases. Disclosure: Susan Zunino, PhD, has no relevant financial relationships.

CME Author
Laurie Barclay, MD, freelance writer and reviewer, Medscape, LLC. Disclosure: Laurie Barclay, MD, has no relevant financial relationships.

Authors
Kate Shapiro, MD; Shane J. Cross, PharmD; Ted H. Morton, PharmD; Hiroto Inaba, PhD; Ashley Holland, MSN; Francisca R. Fasipe, MD; and Elisabeth E. Adderson, MD.
**Mycolicibacterium neoaurum** is a rapidly growing mycobacterium and an emerging cause of human infections. *M. neoaurum* infections are uncommon but likely underreported, and our understanding of the disease spectrum and optimum management is incomplete. We summarize demographic and clinical characteristics of a case of catheter-related *M. neoaurum* bacteremia in a child with leukemia and those of 36 previously reported episodes of *M. neoaurum* infection. Most infections occurred in young to middle-aged adults with serious underlying medical conditions and commonly involved medical devices. Overall, infections were not associated with severe illness or death. In contrast to other mycobacteria species, *M. neoaurum* was generally susceptible to multiple antimicrobial drugs and responded promptly to treatment, and infections were associated with good outcomes after relatively short therapy duration and device removal. Delays in identification and susceptibility testing were common. We recommend using combination antimicrobial drug therapy and removal of infected devices to eradicate infection.

Comprehensive phylogenetic and genomic studies support the division of the genus *Mycobacterium* into 5 main clades: the emended genus *Mycobacterium*, *Mycolicibacter* gen. nov., *Mycolicibacillus* gen. nov., *Mycolicibacterium* gen. nov., and *Mycobacteroides* gen. nov. (1). *Mycolicibacterium* spp. include rapidly growing mycobacteria (RGM) that are not part of the *Mycobacterium abscessus* complex (i.e., *Mycobacteroides* gen. nov., which includes *M. abscessus*, *M. chelonae*, *M. franklinii*, *M. immunogenum*, and *M. saapaulense*).

*Mycolicibacterium* spp. are considered to have low pathogenicity, but some are associated with human infection. *Mycolicibacterium neoaurum*, originally described by Tsukamura in 1972, is derived from the Greek word for new gold because of distinctive yellow-orange colonies (2). Since its identification, increasing numbers of case reports and small case series of invasive *M. neoaurum* infections have been described. Infections are likely underrecognized because many laboratories do not identify all mycobacteria at the species level. Bacteremia might also be missed or isolates inappropriately dismissed as contaminants because mycobacteria might require longer culture incubation than conventional bacterial pathogens, and they are ubiquitous in the environment.

Although best practices for treating infections caused by more commonly reported RGM species are now recognized, our understanding of disease spectra and best infection management strategies for rarer RGM species, such as *M. neoaurum*, remains incomplete. Therefore, to improve clinical awareness of *M. neoaurum*, we summarized demographic and clinical characteristics of 36 previously reported episodes and report an additional case of *M. neoaurum* infection.

**Methods**

We obtained demographic and clinical characteristics of the patient in the reported case from health information records. We searched PubMed and Embase (https://www.embase.com) databases by using the terms neoaurum, *Mycobacterium neoaurum*, and *Mycolicibacterium neoaurum*. We cited cases that reported individual clinical data and were published in any year or language. Patient characteristics were summarized by using descriptive statistics. Analyses were performed by using Stata version 16.1 software (StataCorp LLC).

**Results**

**Case Report**

A 21-month-old boy with low-risk B cell acute lymphoblastic leukemia (ALL) in remission was treated by using the St. Jude Children’s Research Hospital TOTAL Therapy Study 17 protocol (ClinicalTrials.gov identifier NCT03117751), which is similar to the low-risk arm of the TOTAL Therapy Study 16 protocol (identifier NCT00549848) (3). In brief, remission induction consists of prednisone, vincristine, daunorubicin, and pegylated asparaginase, then cyclophosphamide, cytarabine, and mercaptopurine. Consolidation therapy consists of 4 courses of high-dose methotrexate and mercaptopurine. Continuation therapy consists of 120 weeks of mercaptopurine, dexamethasone, vincristine, and methotrexate interrupted by 2 reinduction cycles with dexamethasone, vincristine, and pegylated asparaginase. Triple intrathecal therapy with methotrexate, dexamethasone, and cytarabine was provided to control leukemia in the central nervous system.

During week 13 of continuation therapy, the patient had a fever of 103°F, cough, coryza, anorexia, and diarrhea and was hospitalized 1 day after onset of those signs and symptoms. His medical history was remarkable because of episodes of mucositis associated with chemotherapy, a recent respiratory syncytial virus upper respiratory tract infection, and distant placement of a subcutaneous port (SCP) for intravenous access. He received trimethoprim/sulfamethoxazole (TMP/SMX) for *Pneumocystis* pneumonia prophylaxis. He lived with his family in an urban area, and no history of difficulties accessing his SCP or erythema, discharge, or tenderness at the SCP insertion site had been observed. His physical
SYNOPSIS

examination was unremarkable except for mild pallor. His blood leukocyte count was 10.9 × 10^3 cells/μL (reference range 6.0–17.0 × 10^3 cells/μL), consisting of 77% neutrophils, 9% lymphocytes (absolute lymphocyte count 0.97 × 10^3 cells/μL [reference range 1.20–4.00 × 10^3 cells/μL]), and 13% monocytes. He was mildly anemic; hemoglobin level was 10.7 g/dL (reference range 11.3–12.3 g/dL). Serum C-reactive protein was elevated at 10.7 mg/L (reference range <5.0 mg/dL), aspartate aminotransferase level was 136 U/L (reference range 10–50 U/L), and alanine aminotransferase level was 520 U/L (reference range ≤50 U/L). Serum bilirubin was within reference range. A respiratory PCR panel was positive for respiratory syncytial virus.

The patient’s symptoms resolved overnight, and he was discharged. One bacteria species was isolated after a 5-day incubation of blood cultures obtained from the patient’s SCP at hospital admission by using the BacT/ALERT automated microbial detection system (bioMérieux). The organism was initially reported as a gram-positive coccobacillus but stained weakly, prompting acid-fast bacillus (AFB) staining, which gave positive results. M. neoaurum was identified initially by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and confirmed by 16S rRNA sequencing; both of those analyses were performed at the Mayo Clinic Laboratories (Rochester, MN, USA). The Mayo Clinic Laboratories also performed antimicrobial drug susceptibility testing by using the broth microtiter dilution method. Results were reported as MICs (in μg/mL) and interpreted according to Clinical and Laboratory Standards Institute guidelines (4).

The patient was readmitted for further bacteremia evaluation 9 days after initial blood cultures were obtained. M. neoaurum was again isolated from blood drawn from the SCP and catheter tip, but blood cultures obtained from a peripheral vein were sterile. Results of chest radiograph were unremarkable. We initiated empirical therapy with intravenous imipenem/cilastatin, oral azithromycin, and oral ciprofloxacin. We removed the SCP on hospital day 3, and 3 blood cultures obtained after port removal were sterile. We inserted a central catheter line peripherally on hospital day 6. Antimicrobial drug susceptibility tests showed that the M. neoaurum isolate was susceptible to cefoxitin, imipenem, ciprofloxacin, moxifloxacin, amikacin, tobramycin, doxycycline, TMP/SMX, and linezolid and resistant to clarithromycin. The MIC for tigecycline was 0.12 μg/mL. We ultimately treated the patient with imipenem/cilastatin, azithromycin, and ciprofloxacin for 16 days, then with TMP/SMX and ciprofloxacin for 26 days, and he remained well 15 months later.

Literature Review

We found 238 articles in the literature and included 31 reports describing 36 cases in this review (Appendix Table, https://wwwnc.cdc.gov/EID/article/29/8/23-0007-App1.pdf). Including the case report we described, the median age of patients was 46 (interquartile range [IQR] 25–59) years, and 19 (51%) were female. All but 1 patient had serious underlying chronic medical conditions: malignancy (n = 13, 35%), cardiovascular disease (n = 9, 24%), chronic renal insufficiency (n = 6, 16%), diabetes (n = 6, 16%), and gastrointestinal disorders (n = 4, 11%). Some patients had indwelling central venous catheters (CVCs) (n = 19, 51%) or other foreign bodies, such as prosthetic valves (n = 3), pacemakers (n = 2), peritoneal dialysis catheters (n = 2), hemodialysis catheter (n = 1), and an orthopedic external fixation device (n = 1).

The most common manifestation of infection was bacteremia (n = 22, 59%); a total of 11 patients had central line-associated bacteremia, 8 had CVC-related bacteremia, 1 had bacteremia associated with a pacemaker lead infection, and 1 had bacteremia from a hemodialysis fistula (5). Bacteremia occurred in 1 patient without a CVC who had undergone liver transplantation. Pneumonia occurred in 4 patients, 3 of whom had underlying pulmonary disease. Skin and soft tissue infections were reported in 3 patients, and postsurgical infections were found in 2 patients (a pacemaker pocket infection and infection at a pin exit site). Both patients with endocarditis had histories of intravenous drug abuse and had undergone previous mitral valve replacement. Both patients with peritonitis had indwelling peritoneal dialysis catheters. Other infections included single episodes of granulomatous meningitis and urinary tract infection.

Delays in identifying M. neoaurum were common. The median time to reporting positive cultures was 4.5 (IQR 1–10) days. Most isolates were identified as gram-positive or gram-variable coccobacilli or bacilli. In 1 case, AFB staining was delayed, leading to preliminary identification of the isolate as Rhodococcus sp. (6). In 24 cases for which the method of definitive identification was reported, investigators used chromatography (n = 6); matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (n = 4); or sequencing of 16S rRNA (n = 15), the β subunit of RNA polymerase rpoB gene (n = 1), or 65-kDa heat shock protein gene hsp65 (n = 8). In 2 cases, a specific mycobacterial PCR was used for identification.
Of 21 isolates tested, all were susceptible to doxycycline, linezolid, and moxifloxacin according to the published report or Clinical and Laboratory Standards Institute broth microdilution interpretive criteria for RGM. Most isolates were susceptible to amikacin (17 of 18 isolates), cefoxitin (12 of 13), ciprofloxacin (15 of 16), imipenem (14 of 15), and meropenem (3 of 3) (Table) (6–23). Isolates were less reliably susceptible to TMP/SMX (10 of 15 isolates) and clarithromycin (7 of 15). Patients were treated with a variety of antimicrobial agents and regimens for a median duration of 6 (IQR 5–13) weeks; 3 patients received no antimicrobial drug therapy, and treatment details were not reported for 1 case. Combination antimicrobial drug therapy was used initially in 25 (74%) and, ultimately, 26 (76%) patients. Most (16 of 19) patients with CVC-associated bacteremia had their CVC removed. Infection management involving other medical devices often included device removal. However, several infections were treated only with antimicrobial drugs, including 1 of 2 cases of endocarditis, 1 of 2 infections associated with peritoneal dialysis catheters, 1 pacemaker pocket infection, and 1 pin tract infection. The patient with meningitis died; the long-term outcome of another patient with a urinary tract infection was not reported. Otherwise, infections in all patients were cured. One patient with CVC-related bacteremia who was treated medically had a relapse that was successfully treated by CVC removal and a second course of antimicrobial drugs. The relative risk for relapse among patients with CVC-associated infections who had their CVC removed was 0.083 (95% CI 0.0041–1.6860; p = 0.105); the number needed to treat for 1 patient to benefit was 2.9.

Other Reports

In addition to the individual cases in this review, a case series of 4 patients with *M. neoaurum* bacteremia has been reported (24). Their median age was 54 years, and 3 were male. All 4 patients were immunocompromised (3 with hematologic malignancies, 1 with a solid tumor) but not neutropenic. Three patients were treated by catheter removal and a combination of antimicrobial drugs. In 1 case, the isolate

<table>
<thead>
<tr>
<th>Reference Method</th>
<th>Antimicrobial drug susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case report Broth microdilution</td>
<td>Amikacin, S; cefoxitin, 1, S; ciprofloxacin, S; clarithromycin, 8, S; doxycycline, S; imipenem, S; linezolid, S; meropenem, S; moxifloxacin, S; tigecycline, S; TMP/SMX, S</td>
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<td>(6) Agar diffusion</td>
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<td>(7) Etest</td>
<td>Amikacin, S; clarithromycin, S; TMP/SMX, S</td>
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<tr>
<td>(8) Disk diffusion</td>
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<td>(9) Etest</td>
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<td>(11) Disk diffusion</td>
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<td>(12) Broth microdilution</td>
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<td>(14) Not reported</td>
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<tr>
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<tr>
<td>(21) Broth microdilution</td>
<td>Amikacin, S; cefoxitin, S; doxycycline, S; imipenem, S; linezolid, S; moxifloxacin, S; TMP/SMX, S</td>
</tr>
<tr>
<td>(22) Disk diffusion</td>
<td>Amikacin, S; cefoxitin, S; doxycycline, S; imipenem, S; linezolid, S; moxifloxacin, S; TMP/SMX, S</td>
</tr>
<tr>
<td>(23) Etest</td>
<td>Amikacin, S; cefoxitin, S; doxycycline, S; imipenem, S; linezolid, S; moxifloxacin, S; TMP/SMX, S</td>
</tr>
</tbody>
</table>

*Values for each antimicrobial drug are MICs in μg/mL. Etest, bioMérieux. I, intermediate; S, sensitive; R, resistant; TMP/SMX, trimethoprim/sulfamethoxazole.

†MICs were interpreted according to broth microdilution criteria in the Clinical and Laboratory Standards Institute guidelines for rapidly growing mycobacteria (4).
was considered a contaminant and not treated. All infections were cured. In another report, 2 of 28 patients (both children) with cancer had bacteremia attributed to *M. neoaurum* (25).

**Discussion**

Previous reports have described *M. neoaurum* infections as primarily affecting immunocompromised persons. However, infections that we described in our case report and literature review might be more appropriately considered healthcare-associated infections, because most patients were not immunocompromised but had medical devices or had undergone invasive procedures before infections developed. In our study, 3 patients with pulmonary infections had conditions that predisposed them to anatomic lung abnormalities and infections caused by other mycobacteria species (26). Furthermore, 1 patient with a skin and soft tissue infection had a history of penetrating trauma, but 2 others with this condition did not report trauma. However, injury might not have been recalled, or *M. neoaurum* inoculation might have occurred through an unrecognized skin break. In a single-center study of cutaneous nontuberculous mycobacteria infections, histories of trauma, surgical procedure, or environmental exposure to mycobacteria were common among patients; *M. neoaurum* caused 2 of 78 infections (27). As the population of persons with chronic medical conditions increases, more *M. neoaurum* infections will likely be recognized.

We found that 1 infection in our case series occurred in a previously healthy, 25-year-old woman who showed signs of pulmonary disease that was AFB smear positive; the infecting organism was confirmed as *M. neoaurum* by 16S rRNA sequencing (18). Although this finding suggests that *M. neoaurum* might cause occasional disease in healthy persons, the patient might have had an unrecognized risk factor for mycobacterial infection, such as interferon gamma receptor 1 deficiency, which would only become apparent over time (28). The patient responded to antimicrobial drug therapy, but her long-term outcome was not reported.

One patient in our review who had several serious medical comorbidities had rapidly progressive dementia and diagnostic imaging studies suggestive of recurrent ischemic stroke (29); an autopsy revealed granulomatous meningitis. Results of conventional diagnostic microbiology were uninformative, but broad-range bacterial rDNA PCR amplified a product that was 99% homologous to *M. neoaurum* DNA. However, histopathologic stains did not reveal AFB, cultures were sterile, and the patient met criteria for an alternative diagnosis of probable Creutzfeldt-Jacob disease, suggesting that PCR might have been falsely positive (29,30). Except for this case, all patients in our review were promptly cured of their infections, and no patient required intensive care or died. Thus, in contrast to other RGM species, *M. neoaurum* appears to have low virulence and is associated with limited illness and death (26).

*M. neoaurum* has been isolated from soil, tap water, fish, domesticated animals, and animal products (31–33). Infections are presumed to result from exposure of susceptible hosts to organisms in the environment (34). Nosocomial infections caused by RGM are not uncommon and are often related to contamination of medical devices, wounds, or aqueous solutions. An outbreak of *Mycobacterium mucogenicum* and *M. neoaurum* bacteremia among patients with hematologic malignancies has been reported (7). *M. mucogenicum* and other nontuberculous mycobacteria, but not *M. neoaurum*, were isolated from the hospital water system (water tanks, showers, wash basins). Environmental measures, such as cleaning or replacing fixtures, general cleaning, chlorinating the water supply, and minimizing stagnation, reduced but did not eliminate water contamination. After changes were made to protocols for the care of CVCs, however, no further cases were reported. Environmental samples from the hospital and home environment of a patient with pulmonary *M. neoaurum* infection were similarly analyzed (35). Again, other mycobacteria were isolated from these sources, but *M. neoaurum* was not identified. Therefore, additional studies will be needed to elucidate the pathogenesis and risk factors for *M. neoaurum* infection.

Delays in identifying *M. neoaurum* and obtaining susceptibility test results pose challenges to microbiologists and clinicians. The median time to initial culture positivity in this series was >4 days and as high as 10 days, which might exceed the usual incubation duration for blood cultures, leading to premature no growth determinations (6,36). Many hospital laboratories no longer routinely speciate bacteria or perform susceptibility testing, and delays in appropriate treatment might be compounded by the need to send isolates to a reference laboratory. In this case series, empirical therapy was often directed at more common RGM. In some cases, susceptibility testing was not performed, and therapy success was judged by the patient’s clinical response. The quality of care for patients with RGM infections might be improved by educating laboratory personnel regarding characteristics of less commonly identified RGM, developing protocols that promote rapid identification, and...
The optimal type and duration of antimicrobial drug therapy for *M. neoaurum* infections has not been established. *M. neoaurum* bacteria are resistant to most antituberculosis medications. In 1 study, all 46 *M. neoaurum* isolates tested were susceptible to amikacin, cefotixin, ciprofloxacin, doxycycline, imipenem, linezolid, moxifloxacin, and TMP/SMX, but only 8% were susceptible to clarithromycin (37). Isolates in our case series were also susceptible to most tested antimicrobial drugs but less consistently than previously described (37). Of note, most reports included in our study did not describe the methodology used for testing susceptibilities or used methods that were not recommended. In particular, macrolide susceptibility might have been overestimated if prolonged incubation was not used to detect inducible macrolide resistance (38). Furthermore, whereas most *M. neoaurum* infections have responded well to therapy, a formal correlation between antimicrobial drug susceptibility and clinical outcomes has not been made. As in our case report, other clinicians have frequently used a combination of agents that often include a macrolide, a fluoroquinolone, or both. Although initial combination therapy might be desirable, ≥25% of patients in our case series received monotherapy or were treated by device removal alone, and 1 patient with bacteremia recovered without treatment.

The ability of RGM to cause medical device infections and subsequent need for device removal to eradicate infection has been attributed in part to RGM biofilm formation (39). Not all RGM produce biofilms, however, and biofilm formation by *M. neoaurum* has not been specifically investigated (40–42). Failure to remove CVCs in patients with catheter-associated bacteremia was associated with treatment failure in our case series; however, the small number of cases precludes a precise estimate of risk. Good outcomes were reported in some cases when it was not feasible to remove devices. The ideal duration of antimicrobial drug therapy is also uncertain; ≥4 weeks has been recommended for patients with other RGM infections (39). For *M. neoaurum* infections described in this report, patients with bacteremia treated with antimicrobial drugs for ≤4 weeks had outcomes equivalent to those receiving a longer course.

RGM treatment for patients with underlying medical disorders might be challenging because of antimicrobial drug resistance, relatively high rates of adverse events, and some medications having multiple and serious drug interactions. In our case report, the patient required ongoing treatment for ALL that included mercaptopurine, methotrexate, dexamethasone, and vincristine. The availability of a relatively large number of antimicrobial drugs to which his isolate was susceptible permitted us to continue his chemotherapy without substantial disruption. Imipenem and cilastatin do not have notable interactions with those chemotherapeutic agents. Although TMP/SMX might theoretically exacerbate myelosuppression by mercaptopurine, the combination is commonly used during ALL treatment, and we felt this treatment would be manageable. However, clarithromycin (a strong cytochrome P450 3A4 inhibitor) has potentially severe interactions with vincristine and dexamethasone and is withheld typically for a specified period before and after administration of vincristine. Ciprofloxacin used in combination with dexamethasone might increase the risk for tendinitis or tendon rupture. During ALL induction therapy, patients are usually prescribed concurrent levofloxacin (for antibacterial prophylaxis) and corticosteroids for several weeks, and we have not observed frequent or severe adverse effects (43). We believe that, with careful observation, benefits of this regimen exceeded risks in our patient.

In conclusion, we established that infections caused by the emerging RGM pathogen *M. neoaurum* occurred in patients with diverse demographic characteristics, but almost all cases were healthcare associated. In contrast to isolates of other RGM species, *M. neoaurum* isolates were generally susceptible to tested antimicrobial drugs; a notable exception was clarithromycin. We recommend using combination antimicrobial drug therapy and removal of infected devices, although a shorter treatment duration than is generally recommended for RGM might be effective for *M. neoaurum* infections. We found that delays in identification of isolates and susceptibility testing occurred, but outcomes of most infections were good.

**Acknowledgments**

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**About the Author**

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References


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Poliomyelitis is a highly infectious disease, caused by poliovirus serotypes 1, 2, and 3, that primarily affects children <5 years of age. The main risk factors for poliovirus transmission are low immunization coverage, poor sanitation, and high population density. Since the worldwide launch of the Global Polio Eradication Initiative (GPEI) in 1988, polio cases have declined by >99%. Strategies to reduce the number of polio cases globally have focused on achieving high polio vaccine coverage and implementing robust acute flaccid paralysis (AFP) surveillance.

Administration of the injectable inactivated polio-virus vaccine (IPV) or live attenuated oral poliovirus vaccine (OPV, Sabin-strain virus types) can prevent poliomyelitis. IPV induces humoral protection, whereas OPV induces humoral and mucosal immunity and limits viral shedding, reducing person-to-person transmission. However, in areas with low vaccination coverage and poor sanitation, using OPV may exceptionally result in the emergence of vaccine-derived polioviruses (VDPVs). VDPVs are classified as cVDPV (circulating VDPV, when there is evidence of community transmission), iVDPV (immunodeficiency-associated VDPV, isolated from persons with primary immunodeficiencies), or aVDPV (ambiguous VDPV, isolated from persons without immunodeficiency or from wastewater samples without evidence of transmission). aVDPVs are generally considered to highlight risk for poliomyelitis reemergence in countries with low polio vaccine coverage.

Guatemala implemented wastewater-based poliovirus surveillance in 2018, and three genetically unrelated vaccine-derived polioviruses (VDPVs) were detected in 2019. The Ministry of Health (MoH) response included event investigation through institutional and community retrospective case searches for acute flaccid paralysis (AFP) during 2018–2020 and a bivalent oral polio/measles, mumps, and rubella vaccination campaign in September 2019. This response was reviewed by an international expert team in July 2021. During the campaign, 93% of children 6 months to <7 years of age received a polio-containing vaccine dose. No AFP cases were detected in the community search; institutional retrospective searches found 37% of unreported AFP cases in 2018–2020. No additional VDPV was isolated from wastewater. No evidence of circulating VDPV was found; the 3 isolated VDPVs were classified as ambiguous VDPVs by the international team of experts. These detections highlight risk for poliomyelitis reemergence in countries with low polio vaccine coverage.

Poliomyelitis is a highly infectious disease, caused by poliovirus serotypes 1, 2, and 3, that primarily affects children <5 years of age. The main risk factors for poliovirus transmission are low immunization coverage, poor sanitation, and high population density. Since the worldwide launch of the Global Polio Eradication Initiative (GPEI) in 1988, polio cases have declined by >99%. Strategies to reduce the number of polio cases globally have focused on achieving high polio vaccination coverage and implementing robust acute flaccid paralysis (AFP) surveillance.

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Author affiliations: Pan American Health Organization, Washington, DC, USA (R. Rodríguez, G. Rey-Benito, M.C. Freire, A.-E. Chévez, E. Cain, A.P. Villalobos Rodríguez); Ministry of Public Health and Social Assistance, Guatemala City, Guatemala (E. Juárez, M.O. Bautista Amézquita, L. Castillo Signor, L. Mendoza, C.A. Ovando, H. de J. Barillas Mayorga, E. Gaitán, A. Paredes, L. Gobern); US Centers for Disease Control and Prevention, Atlanta, Georgia, USA (C.F. Estívariz, S.J. Miles, L. Sayyad, H. Belgasmi-Allen); Pan American Health Organization, Guatemala City (C. Cajas, O. Orantes, C. Jarquin, M. Rondy); Poliovirus Regional Reference Laboratory, Malbrán Institute, Buenos Aires, Argentina (M.C. Freire); National Health Laboratory, Guatemala City (L. Mendoza)

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1These first authors contributed equally to this article.
be of low public health significance; however, they can still be an indicator of low vaccination coverage and poor sanitation, which can create the conditions for the emergence and circulation of potentially more dangerous cVDPVs. In the past decade, cVDPV outbreaks have caused >2,700 poliomyelitis cases globally (7–10).

In Guatemala, the last case of clinical poliomyelitis was detected in 1990 (11); the country routinely vaccinates children against poliomyelitis with trivalent IPV and bivalent (serotypes 1 and 3) OPV (bOPV) (12). Because of low vaccination coverage (<90% with third dose of polio-containing vaccine in 2017) and poor AFP surveillance indicators, the Regional Certification Commission (RCC) classified Guatemala as a high-risk country for polio reemergence in 2018. To complement AFP surveillance, the Pan American Health Organization’s (PAHO) Technical Advisory Group recommended implementing wastewater-based environmental surveillance in high-risk settings in the Americas in 2016 (13). After that recommendation was published, the Guatemala Ministry of Health (MoH) implemented poliovirus environmental surveillance in 2 urban municipalities in Guatemala in November 2018 (14).

The detection of 3 VDPVs from wastewater sampled in January, March, and December 2019 led the Guatemala MoH to implement a series of activities to classify these events and minimize the risk for transmission in the population (Figure). In July 2021, a team of international experts evaluated that response, according to the Global Polio Eradication Initiative’s (GPEI) Poliovirus Outbreak Response Assessment (OBRA) guidelines (15). We present the results of the investigation conducted by the MoH and the OBRA evaluation of their response.

**Methodology**

**Environmental Surveillance, VDPV Detection, and Genomic Sequencing**

Guatemala MoH implemented monthly collection of wastewater samples in November 2018. Three sampling sites in 2 municipalities were selected for logistical and financial reasons. The selected municipalities, Villa Nueva (VNA) and San Juan Sacatepéquez (SJS), were of high risk for poliovirus transmission (16): high population density (1,573 inhabitants/km² in VNA and 943 inhabitants/km² in SJS), OPV coverage <90% in 2014–2017, underperforming AFP surveillance indicators (<1 case/100,000 children <15 years of age), and poor sanitation conditions. In each municipality, the MoH revised wastewater networks to define 3 sampling points that would lead to a maximum population size unaffected by polluting industrial plants.

During November 2018–July 2021, trained MoH staff monthly collected a 1 L wastewater sample from each site and transported them in cold chain (2°C–8°C) the same day to the National Health Laboratory. Samples were stored at −80°C, then shipped at −20°C to the US Centers for Disease Control and Prevention (CDC) reference laboratory for polioviruses to be processed and tested for the presence of poliovirus (16–18). All poliovirus isolates underwent genomic sequencing and analysis of the region coding the viral protein 1 surface protein for nucleotide substitutions compared with the parent Sabin strains.

**Event Investigation**

To classify the isolated VDPVs, the MoH relied on preexisting national protocols. Within 72 hours of receiving the notification of VDPV detection in wastewater samples, MoH personnel conducted health facility and community-based AFP case search in the areas surrounding the sampling sites. In each site, after obtaining verbal consent from parents, they also collected and tested stool samples for poliovirus from 20 healthy children <5 years of age without a history of OPV vaccination in the previous 30 days.

MoH epidemiologists retrospectively reviewed medical records from all 42 national hospitals for patients admitted during January 2018–December 2020 who had diagnoses compatible with polio or AFP, identified by codes from the International Classification of Diseases, 10th Revision (ICD-10). The retrospective record search was conducted in 2 phases during July 2019–December 2020. Finally, MoH epidemiologists revised hospital-based information systems to identify reported cases of primary immunodeficiency in 2018–2019 in patients residing in the affected municipalities.

**Routine AFP Surveillance**

During 2017–2020, a total of 42 reporting hospitals conducted AFP surveillance in Guatemala. The surveillance system was classified as good quality if it reported ≥1 AFP case/100,000 children <15 years of age/year. Of reported AFP cases, ≥80% should have adequate sample collection and 80% should include an investigation conducted within 48 hours (19).

**Assessment of Polio Immunization Coverage and Supplementary Immunization Activities**

Since 2016, Guatemala routinely vaccinates children with 1 IPV dose at 2 months of age, 2 bOPV doses at 4 and 6 months of age, and 2 bOPV booster doses at 18 months and 4 years of age (12). A nationwide catch-up vaccination campaign for bOPV...
and measles, mumps, rubella (MMR) was already planned for September–October 2019 for children 1–6 years of age. The detection of VDPVs led officials to lower the eligibility age to 6 months for bOPV and mobilize additional funding to support vaccination activities, a nationwide communication campaign, and technical support at the national and local level.

We calculated routine vaccination coverage during 2017–2020 using the number of third doses of bOPV registered in the national electronic immunization system (individual personal data) as numerator. This national system is exclusively used by MoH vaccination centers and may include social security institute centers on some occasions (based on personal willingness to share data). We calculated the bOPV vaccination campaign coverage using the number of doses registered in an electronic immunization system designed for the vaccination campaign (aggregated data). We estimated population denominators using national census-based projections.

OBRA Implementation

In July 2021, an OBRA team virtually evaluated the MoH’s response to 3 VDPV detections in 4 key components: response planning and coordination, AFP surveillance sensitivity, vaccination, and health promotion and social mobilization. The team consisted of vaccination, epidemiologic surveillance, and laboratory experts from PAHO and CDC, as well as a national facilitator from the National Polio Elimination Certification Committee (NCC). The assessment involved reviewing technical documentation such as AFP and environmental surveillance protocols and vaccination reports; conducting interviews with national and local authorities, health personnel, and the National Polio Eradication Commission; and participating in work sessions with the OBRA team to discuss the data and provide recommendations. The assessment results were communicated to the MoH and local public health authorities through a virtual debriefing in August 2021 and a written report in September 2021.
Ethics Considerations
The Guatemala MOH determined that the community survey, including collection of stool specimens, did not constitute human subjects research. We obtained verbal consent from household members who participated in the community survey and from parents of children <5 years of age who provided a specimen. The information obtained would be used to guide the public health response to a potential outbreak and did not involve risk to participants’ health.

Results

Environmental Surveillance, VDPV Detection, and Genomic Sequencing
During November 2018–July 2021, we collected 192 wastewater samples in SJS and VNA. We detected ≥1 enterovirus in 97% of samples collected in SJS and in 96% of samples collected in VNA. We isolated Sabin-like vaccine poliovirus in 83 samples (43%), Sabin type 1 poliovirus in 20% of samples and type 3 in 33%.

We detected 3 genetically unrelated VDPVs in 2019 (Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/8/23-0236-App1.pdf). We identified a VDPV3 with 11 nt changes from Sabin type 3 in a January 2019 sample from Aldea Cruz Blanca, SJS, and a VDPV1 with 11 nt changes from Sabin type 1 in a March 2019 sample from Platanitos. We reported detecting those 2 VDPVs to the Guatemalan MoH on July 1, 2019. We isolated a VDPV1 with 10 nt changes from Sabin type 1 in a sample collected in December 2019 in Aldea Cruz Blanca, SJS, and reported it to the MoH in May 2020. The VDPVs were not genetically linked to any previously sequenced VDPV1 or VDPV3 worldwide. As of July 2021, no other VDPVs have been detected.

Event Investigation
We identified no cases of AFP from community case searches in which 1,580 children were screened. We isolated no VDPVs from stool samples collected among 61 healthy children sampled (20 children each for the first 2 VDPV detections and 21 children for the third detection).

We conducted a retrospective case review during January 1, 2018–August 31, 2020, in 2 phases after the VDPV notifications in July 2019 and May 2020; we reviewed 3,342,166 records from 42 national hospitals. The reviewers identified 7,318 (0.2%) persons with paralysis diagnoses, of whom 150 (2.1%) met the case definition for AFP. Of those, 56 (37%) cases had not been reported to the AFP surveillance system. No potential cases of primary immunodeficiency from the affected municipalities were found from national hospital-based information systems.

Routine AFP Surveillance
During 2017–2020, AFP yearly incidence met the target of 1 case/100,000 children in 2019. In 2017, 2018, and 2020, AFP incidence rate was 0.6–0.9 cases/100,000 children. Overall, 164 (76%) of 214 cases reported in 2017–2020 had an adequate sample collected, and 87 (41%) were investigated within 48 hours of being reported (Table 1).

Vaccination Coverage and OPV/MMR

Supplementary Immunization Campaign
During 2017–2020, the percentage of children <1 year of age who received 3 doses of polio vaccine as 2 doses of IPV and 1 dose of bOPV was 84.8%–89.8% at the national level (Table 2). Vaccination coverage for that vaccination series in the health areas where VDPVs were isolated was 62.1%–75.3% during the same period.

During the national bOPV/MMR vaccination campaign conducted in September–November 2019 in response to the VDPV detections, 93.0% of children 6 months to <7 years of age received 1 dose of bOPV. The campaign coverage reached 94.8% among children 6 months to <7 years of age in VNA and 91.2% in SJS.

OBRA Results
The 2021 OBRA conducted in Guatemala found no evidence of circulating VDPVs in Guatemala and that the MoH response planning and coordination after VDPV identification in 2019 environmental surveillance samples was appropriate. The 3 VDPVs detected in wastewater samples were classified as aVDPVs.

Discussion
In Guatemala, the environmental surveillance of poliovirus in wastewater detected 3 genetically unrelated

<table>
<thead>
<tr>
<th>Table 1. Surveillance indicators for acute flaccid paralysis, Guatemala, 2017–2020*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>2017</td>
</tr>
<tr>
<td>2018</td>
</tr>
<tr>
<td>2019</td>
</tr>
<tr>
<td>2020</td>
</tr>
</tbody>
</table>

*Information not systematically collected before 2018. Source: Department of Epidemiology, Guatemalan Ministry of Health. NA, not available.
VDPVs in 1 year. After careful revision of the MoH response and investigation, an international team of experts classified those 3 events as aVDPVs.

Although the Americas region was declared free from polio in 1994, recent events remind us that sustaining this status is a continuous challenge. In June 2022, a VDPV2 was isolated in an unvaccinated person in New York, New York, USA, and classified as cVPDV2 after being isolated from wastewater (8). This virus, genetically linked to other VDPV2 viruses identified in Israel and the United Kingdom (20), demonstrates the global threat that importation of poliovirus from anywhere in the world represents. In March 2023, a case of VDPV1 was detected in an unvaccinated child from a community with low vaccination coverage in Peru (21). The virus presented 31 nt changes compared with the type 1 Sabin virus and was not genetically linked to any VDPV1 identified in the world. This case is an example of the imminent risk for reemergence of poliomyelitis through VDPVs emerging in communities with low OPV vaccination coverage.

The OBRA team concluded that there was enough evidence to consider that none of the 3 VDPVs isolated in Guatemala had given rise to a clinical case of poliomyelitis in the country; however, those findings highlight the need to improve vaccination coverage and AFP surveillance to prevent or timely detect poliovirus reemergence. Through repeated workshops using the OBRA report structure (AFP surveillance, environmental surveillance, immunizations, and community mobilization), the Guatemala MoH translated results and recommendations from the evaluation into a roadmap to mitigate the risk for polio reemergence.

In Guatemala, 1 of 3 AFP cases detected through retrospective hospital-based case search had not been identified through routine surveillance. Such an underperforming surveillance system could place the country at risk of failing to detect clinical poliomyelitis in a timely manner. To improve AFP surveillance, the MoH implemented a daily zero-notification process, in which hospitals daily ascertain the absence of AFP cases in their admitted patients. After the OBRA evaluation, MoH strengthened the national surveillance team through increased staff and interinstitutional (national laboratory, epidemiology department and national immunizations program) training supported by national and international experts. MoH and non-MoH hospitals made efforts to improve AFP notification; AFP notifications increased to 0.9 cases/100,000 children in 2021 and 1.2 cases/100,000 children in 2022. Environmental surveillance continued through monthly wastewater sample collection in the same sampling sites; as of December 2022, no further VDPV or WPV had been detected.

National vaccination coverage with 3 doses of polio vaccine was 85% in 2018, the year before the VDPV isolations; at that time, coverage was 73% in VNA and 64% in SJS. Vaccination coverage in areas with poor access to clean water allowed for VPDVs to emerge and be detected in wastewaters. The first two VDPVs were notified in July 2019, which was 2 months before the launch of a planned national catch-up bOPV/MMR vaccination campaign. Notification of the VDPVs in accordance with international health regulations led to international awareness as well as technical and financial assistance from PAHO, the United Nations Children’s Fund, and GPEI for the MoH to successfully implement its vaccination campaign, reaching 93% of children 6 months to <7 years of age vaccinated with a dose of bOPV. In accordance with GPEI guidelines for polio outbreak response, vaccination campaigns should be organized after VDPV is classified as cVDPV (22). After the isolation of the third VDPV and its notification in May 2020, case investigation led to its classification as aVDPV; no further vaccination campaign was organized. Since the OBRA evaluation, declining polio vaccination coverage during the COVID-19 pandemic (7,23,24) has built a pool of susceptible children representing a risk for polio reemergence (25,26). National vaccination coverage for 3 doses of polio-containing vaccine in Guatemala has declined from 89% in 2020 to 76% in 2022 (27). Routine immunization programs in Guatemala must be intensified through outreach strategies and a better understanding of local issues contributing

### Table 2. Polio vaccination coverage in children <1 y of age, Guatemala, 2017–2020*

<table>
<thead>
<tr>
<th>Year</th>
<th>Dose</th>
<th>Population</th>
<th>No. (%) vaccinated</th>
<th>Population</th>
<th>No. (%) vaccinated</th>
<th>Population</th>
<th>No. (%) vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>Polio 3</td>
<td>8,339</td>
<td>6,282 (75.3)</td>
<td>6,159</td>
<td>3,824 (62.1)</td>
<td>381,396</td>
<td>342,437 (89.8)</td>
</tr>
<tr>
<td>2018</td>
<td>Polio 3</td>
<td>8,296</td>
<td>6,055 (73.0)</td>
<td>6,141</td>
<td>3,942 (64.2)</td>
<td>382,841</td>
<td>324,761 (84.8)</td>
</tr>
<tr>
<td>2019</td>
<td>Polio 3</td>
<td>7,252</td>
<td>5,495 (75.8)</td>
<td>5,856</td>
<td>4,056 (69.3)</td>
<td>366,448</td>
<td>320,963 (87.6)</td>
</tr>
<tr>
<td>2020</td>
<td>Polio 3</td>
<td>6,671</td>
<td>5,570 (83.5)</td>
<td>5,513</td>
<td>4,252 (77.1)</td>
<td>340,876</td>
<td>304,898 (89.4)</td>
</tr>
<tr>
<td>2019</td>
<td>bOPV</td>
<td>57,979</td>
<td>54,942 (94.8)</td>
<td>41,639</td>
<td>37,969 (91.2)</td>
<td>2,649,334</td>
<td>2,463,881 (93.0)</td>
</tr>
</tbody>
</table>

*The 2019 nationwide bOPV campaign targeted children 6 mo to >7 y of age. Polio 3 indicates a third dose of oral or injectable polio-containing vaccine. bOPV, bivalent oral poliovirus vaccine. Source: National Immunization Program, Guatemala Ministry of Health.
to low vaccination coverage. Since November 2022, MoH has implemented local workshops to identify reasons for low performance of vaccine preventable disease surveillance and immunization programs. During those 2-day workshops, participants from local and central MoH, municipalities, community leaders, and health service users have jointly developed mitigation plans using a problem tree analysis methodology.

Because of an elevated risk for polio reemergence, adequate response preparedness is essential for timely response to poliovirus detection. The OBRA team concluded that the outbreak response planning and implementation of activities in Guatemala were adequate according to international standards defined by GPEI. Existing, regularly updated guidelines and periodic outbreak preparedness training were key elements that enabled the Guatemala MoH to respond adequately to these events.

In conclusion, the detection of 3 unrelated VDPVs in Guatemala confirmed the strong risk for poliomyelitis emergence in sites with low vaccination coverage. Although no evidence of a circulating VDPV was found, improving polio vaccination coverage is critical to prevent new VDPV emergences and spread. Strengthening AFP surveillance for timely detection of clinical cases is essential for a rapid response to be implemented in all areas of the country. In high-risk countries, complementing AFP surveillance with risk-assessed environmental surveillance is of great value for early detection of reverted vaccine viruses before they generate poliomyelitis cases or can circulate widely in the community.

Acknowledgments
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About the Author
Dr. Rodríguez is the post-VDPV detection team coordinator for Poliovirus Outbreak Response Assessment in Guatemala, where he was previously PAHO/WHO advisor. His research interests include vaccine preventable diseases, program evaluation, community surveillance for health risks, and vital statistics analysis. Dr. Juárez is an epidemiologist at Hospital de Amatitlán in Amatitlán, Guatemala, and member of the Guatemalan National Polio Eradication Certification Committee Secretariat, 2013–2021. Her primary research interests are public health and immunization.

References
13. Regional Certification Commission for Polio Eradication. 8th meeting of the Regional Certification Commission for the Polio Endgame in the Region of the Americas—final
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Reported Legionnaires’ disease cases began increasing in the United States in 2003 after relatively stable numbers for more than 10 years. This rise was most associated with increases in racial disparities, geographic focus, and seasonality. Water management programs should be in place for preventing the growth and spread of Legionella in buildings.

In this EID podcast, Albert Barskey, an epidemiologist at CDC in Atlanta discusses the increase of Legionnaires’ disease within the United States.

Visit our website to listen: https://go.usa.gov/xuD7W
Carbapenem-resistant Enterobacterales (CRE) are classified as critical priority bacterial pathogens by the World Health Organization (1). Klebsiella pneumoniae is a major cause of neonatal infections in low- and middle-income countries (2,3). In a national population-level analysis, K. pneumoniae accounted for 26% of invasive neonatal infections in South Africa during 2014–2019 (4). Resistance mechanisms to carbapenems include enzymatic inactivation, changes to outer-membrane permeability, and efflux pump upregulation (5). Several outbreaks of K. pneumoniae infection in neonatal units have been investigated in South Africa since 1992 (6–11). Some of those studies used molecular typing methods, such as multilocus sequence typing and pulsed-field gel electrophoresis (PFGE), that lack sufficient resolution to distinguish between clonal strains (8–12). Whole-genome sequencing (WGS) is a powerful tool to investigate healthcare-associated pathogens such as K. pneumoniae and has been widely used in combination with epidemiologic information to track outbreaks and transmission routes of pathogens (13–17).

In December 2019, the National Institute for Communicable Diseases (NICD) in South Africa was notified of 8 cases of culture-confirmed carbapenem-resistant Klebsiella pneumoniae (CRKP) bloodstream infections in a neonatal unit at a provincial tertiary hospital. The outbreak began with 4 cases reported in October 2019. The objectives of this investigation were to verify the existence of an outbreak; describe the antimicrobial susceptibility profiles, resistance mechanisms, and transmission dynamics of CRKP clones in circulation; and monitor the incidence of carbapenem-resistant Enterobacterales (CRE) bloodstream infections in neonatal units in South Africa during 2014–2019 (4). Resistance mechanisms to carbapenems include enzymatic inactivation, changes to outer-membrane permeability, and efflux pump upregulation (5). Several outbreaks of K. pneumoniae infection in neonatal units have been investigated in South Africa since 1992 (6–11). Some of those studies used molecular typing methods, such as multilocus sequence typing and pulsed-field gel electrophoresis (PFGE), that lack sufficient resolution to distinguish between clonal strains (8–12). Whole-genome sequencing (WGS) is a powerful tool to investigate healthcare-associated pathogens such as K. pneumoniae and has been widely used in combination with epidemiologic information to track outbreaks and transmission routes of pathogens (13–17).

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CRKP infections within 1 year of instituting outbreak interventions.

Materials and Methods

Hospital Setting and Surveillance
Tembisa Provincial Tertiary Hospital is located in the Tembisa township in Gauteng Province, South Africa; the township has a population size of 2.5 million. The hospital also serves the communities of Midrand and Diepsloot and as a referral site for 20 clinics in the surrounding area, spanning 3 municipalities (the cities of Johannesburg, Ekurhuleni, and Tshwane). Annually, the hospital sees >280,000 patients. The hospital facility has 840 beds in total, 704 for adults, 71 for newborn babies, and 64 for pediatric patients; the average daily admission is 150 patients. The hospital’s neonatal unit consists of wards A and B. Neonates are assigned to a section within a ward depending on age and the acuity of their condition at admission. Ward A has high-care (A1), low-care (A2), and isolation rooms; the approved bed capacity is 40. Ward B consists of a kangaroo mother care section (designed to increase skin-to-skin contact between mother and infant for preterm or low birthweight infants), a neonatal intensive care unit, a low-care unit, and a pediatric intensive care unit that is separated from the other rooms by a door. Ward B has a total of 31 beds. The intended medical staff complement during a day shift in ward A was 8 doctors, with an intended bed-to-doctor ratio of 5:1 and an intended bed-to-nurse ratio of 3:1. One medical officer was usually on night call for all the neonatal wards. Ward B had 3 doctors and 4 nurses, with an overall bed-to-staff ratio of 4:4:1.

Coincidentally, enhanced surveillance for neonatal infections as part of the Baby GERMS-SA study was conducted at this hospital during October 1, 2019–September 30, 2020 (18). In brief, neonates with culture-confirmed bloodstream infections or meningitis were enrolled into the surveillance program if they met the case definition (i.e., a neonate <28 days of age from whom a pathogen was isolated from blood or cerebrospinal fluid). The diagnostic laboratory submitted the corresponding bacterial and fungal isolates to the NICD for further characterization. Demographic, clinical, and outcome data were retrospectively abstracted from their imaged medical records. In addition, admissions and patient bed-days were recorded by month for the neonatal unit.

Outbreak Investigation
A multidisciplinary investigation team consisting of members of the NICD, Infection Control Service Laboratory (National Health Laboratory Service), the Gauteng Provincial Department of Health, and the National Department of Health was assembled in January 2020. To estimate baseline rates of bloodstream infections, we obtained a line list from the NICD surveillance data warehouse for infants ≤6 months of age admitted to Tembisa Provincial Tertiary Hospital with blood cultures positive for any bacterial or fungal organism during January 2017–September 2019. We excluded organisms considered commensals by the US Centers for Disease Control and Prevention National Healthcare Safety Network (19). We regarded blood cultures with the same organism isolated within 21 days of the first positive culture as duplicates and excluded those. We defined an outbreak as an increase of ≥100% in the number of observed cases of CRKP infection in a month above the expected (average) number in the preceding 2 months. On March 11, 2020, before the initial investigation closeout, the World Health Organization declared the global COVID-19 pandemic (20). We calculated monthly bloodstream infection rates during the follow-up period, March 2020–May 2021, using the same methods used in the initial outbreak investigation.

External Infection Prevention and Control Audit Process
During the initial investigation, we conducted an external infection prevention and control (IPC) audit in January 2020 using the National Department of Health standardized Infection Control Assessment Tool (21). We compiled recommendations for immediate, mid-term, and long-term interventions. A similar internal audit by the hospital response team was conducted in August 2020 to monitor improvements in adherence to IPC measures. Specific IPC measures had been implemented early after the observed increase in the number of cases. For instance, all colonized and infected babies were cohorted or isolated in separate sections of the ward. Additional cleaning of the unit was conducted several times beginning in November 2019. Hand hygiene was monitored for both staff and parents entering the unit. When the newborn unit reached 100% bed capacity, maternity cases were rerouted to other facilities, although this intervention could not be sustained during the COVID-19 pandemic.

Cases and Isolates
We defined a case as culture-confirmed bloodstream infection caused by *K. pneumoniae* resistant to any carbapenem (i.e., meropenem, imipenem, doripenem, or ertapenem) in an infant ≤6 months of age. The case
K. pneumoniae Infections in Neonatal Unit

definition was not restricted to neonates <28 days of age because babies who stayed in hospital beyond the neonatal period remained at risk for infection. We analyzed all isolates submitted to NICD from this hospital during October 1, 2019–February 29, 2020, including those submitted for Baby GERMS-SA surveillance. The hospital provided monthly infection reports to NICD, listing cases of CRKP infection with dates of birth, birthweights, dates of specimen collection, and outcome information. We conducted the follow-up analysis in May 2021, 18 months after the outbreak started in October 2019.

**Microbiological Analysis**

We confirmed species-level identification using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, https://www.bruker.com) and performed antimicrobial susceptibility testing using the MicroScan WalkAway 96-plus system with the NM44 card (Siemens Healthineers, https://www.siemens-healthineers.com). We interpreted MICs for the tested antimicrobial agents according to Clinical and Laboratory Standards Institute guidelines and defined multi-drug-resistant isolates as those with nonsusceptibility to ≥1 agent in ≥3 antimicrobial classes (22). We screened for carbapenemase genes and strain relatedness using real-time multiplex PCR and PFGE and performed WGS to determine sequence types, identify acquired antimicrobial resistance genes, and confirm presence of plasmid replicons, O antigen locus types, and K locus types. We compared core genome single-nucleotide polymorphism (SNP) distances and epidemiologic information to investigate the transmission events of CRKP in the neonatal unit (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/23-0484-App1.pdf).

**Results**

**Baseline, Outbreak, and Follow-Up Periods**

During January 2017–September 2019, a total of 1,771 positive blood cultures were reported from infants ≤6 months of age at the hospital. Of those, 864 (49%) blood cultures yielded probable pathogenic organisms; 724 were in neonatal wards A and B. We excluded 144 duplicates from the analysis. Of the remaining 580 isolates, 428 were from patients with single-isolate bloodstream infection episodes and were included in the analysis. K. pneumoniae accounted for 29% (122/428) of cases, *Staphylococcus aureus* 36% (153/428), and *Acinetobacter baumannii* 27% (116/428). Of the 122 cases of *K. pneumoniae* isolated during the January 2017–September 2019 baseline period, 8% (10/122) were CRKP; 5 of those cases were diagnosed in January 2019 (Figure 1).

The number of CRKP bloodstream infection cases increased during October 1, 2019–February 29, 2020; a total of 31 cases and 15 deaths were reported. Most cases (n = 14) were reported in December 2019; in the preceding 2 months only 3 non-CRKP cases were diagnosed (average 1.5 cases/month) (Figure 2).

During the follow-up period (March 2020–May 2021), 299 positive blood cultures with probable pathogenic organisms were obtained from infants admitted to the neonatal unit (wards A and B). *K. pneumoniae* accounted for 38 of those episodes (13%); of those, 29% (n = 11) isolates were carbapenem-resistant (Appendix).

**External IPC Audit Findings and Internal Follow-Up Audit**

The initial external IPC auditors found that neonatal unit ward A exceeded the approved bed capacity by 60% (64 admissions in a ward with 40 approved
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ward A had 13 nurses per shift and a bed-to-nurse ratio of 5:1. Occupancy increased to 180% above the approved capacity during the internal audit in August 2020. Attrition occurred at a rate of 16 nurses and 13 clinical staff over a period of 7 months. Adherence to hand hygiene was 95% during the external audit and 100% during the follow-up internal audit. Hand hygiene adherence remained at >96% a year later in May 2021, maintained through a peer-monitoring system. Liquid hand soap with an antimicrobial agent, alcohol-based antiseptic, and hand lotions (aqueous cream) were initially not available for staff, but availability improved on follow-up. Alcohol-based antiseptic dispensers were available at each bed during the follow-up audit. Handwashing supplies were ordered, and stock levels were monitored. Elbow-operated taps were installed to improve quality of hand hygiene practices. Unannounced IPC audits were performed, but no direct observations were reported. The overall score for sterilizing and disinfecting instruments improved from 27% to 47% in August 2020. Policies or standard operating procedures (SOPs) were subsequently developed, and staff members signed to demonstrate their understanding of SOPs. The unit had no designated area for mixing standard intravenous fluids because of infrastructural challenges, single-dose vials were not used, and no SOP for multidose vials was in place in the ward (Appendix Table). Most external audit recommendations were implemented. For example, an integrated IPC and occupational health and safety team was established to maximize human resource capacity and budget allocations and to strengthen infection surveillance. However, some crucial initial IPC audit recommendations could not be sustained because of COVID-19 demands (e.g., diverting patients to neighboring hospitals when ward capacity was reached and converting the milk room into a kangaroo mother care unit). Although an antimicrobial stewardship committee was established, implementing its recommendations was delayed because attention and resources were diverted to the COVID-19 response.

Isolates and Cases

During October 2019–February 2020, a total of 31 laboratory-confirmed cases of CRKP bloodstream infections were reported in the neonatal unit. Of those, we did not have isolates for 2 cases (Figure 3), but laboratory reports indicated that these isolates were resistant to ertapenem, imipenem, and meropenem. The NICD reference laboratory received 34 isolates for the remaining 29 cases. Of those, 2 cases had 3 isolates each and 1 case had 2 isolates. The first isolate (i.e., the isolate with earliest specimen collection date) per case from 29 cases was selected for molecular characterization. Twenty-seven isolates were confirmed as *K. pneumoniae* subspecies *pneumoniae*; antimicrobial susceptibility profiling and genomic characterization was performed. We excluded 2 isolates identified as *Pseudomonas aeruginosa* that were likely contaminated during shipping.

Antimicrobial Susceptibility

All isolates were multidrug-resistant. In total, 89% (24/27) of the isolates were resistant to ertapenem and 81% (22/27) were resistant to meropenem (Figure 3).

Genomic Characterization

We identified 3 clonal clusters consisting of 6 sequence types from 27 isolates by using core genome maximum-likelihood phylogeny (Appendix Figure 1). Sequence type (ST) 152 accounted for 59% (16/27)
of the cases, followed by ST307 (6/27; 22%) and ST17 (2/27; 7%); ST25, ST45, and ST297 each accounted for a single case (1/27; 4%). We found that 94% (26/27) of the isolates carried carbapenemase genes. All isolates within ST152 clone harbored the blaNDM-1 gene and contained CoIRNAI, IncFIB(K), IncFIB(pB171), and IncFII(Yp) plasmid incompatibility groups. Further analysis revealed that the blaNDM-1 gene was carried on the Escherichia coli B171 plasmid pB171 (accession no. AB024946). Phenotypic resistance to third and fourth generation cephalosporins was confirmed by the presence of extended-spectrum β-lactamases, such as blaTEM-1, blaTX-M-15, and other β-lactamase genes (blaTEM-1 and blaSHV-1) (Appendix Figure 1).

Table. Clinical characteristics of WGS-confirmed cases of carbapenem-resistant Klebsiella pneumoniae bloodstream infection in newborns admitted to the neonatal unit of a tertiary care hospital, South Africa, October 2019–February 2020*

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Age, d/sex</th>
<th>Weight at birth, g</th>
<th>Gestational age, wks</th>
<th>Ward†</th>
<th>Other pathogens</th>
<th>Outcome</th>
<th>Isolate</th>
<th>Collection date</th>
<th>ST</th>
<th>K type</th>
<th>O type</th>
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<td>307</td>
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<td>17</td>
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<td>2020 Feb 2</td>
<td>152</td>
<td>KL149</td>
<td>O4</td>
</tr>
</tbody>
</table>

*The specimen type used for all patients was blood culture. NR, not recorded; Pt, patient; ST, sequence type; WGS, whole-genome sequencing. †Wards A1, A2, and isolation are sections (cubicles) within ward A with a total approved bed capacity of 40.
The gene $\text{bla}_{\text{OXA-181}}$ was present in all isolates belonging to ST307. Three isolates carrying the $\text{bla}_{\text{OXA-181}}$ gene coharbored the $\text{bla}_{\text{OXA-48}}$ gene. All isolates within $\text{bla}_{\text{OXA-181}}$ ST307 clone shared ColKP3, IncFIB(Mar), and IncX3 plasmid incompatibility groups. Plasmid analysis showed that $\text{bla}_{\text{OXA-181}}$ gene in ST307 clone isolates was carried on $K.\text{pneumoniae}$ KP3 plasmid KP3-A (accession no. JN205800) (Appendix Figure 1).

Clinical Characteristics of 26 WGS-Confirmed Cases of CRKP

Of the 26 WGS-confirmed cases of CRKP, 67% (18/26) of the infants were boys; median age was 7 days (interquartile range 3–17 days). Birthweights ranged from 760 to 3,200 g; the median weight was 1,100 g (interquartile range 970–1595 g). Four of the 26 infants required resuscitation at birth. Invasive devices were inserted in all infants. Two infants had a record of underlying abnormalities (congenital anemia and neonatal seizures). Just over half (54% [14/26]) of infants died in hospital. Of these, 64% (9/14) had cultured isolates belonging to the ST152 clone (Table).

Genomic and Epidemiologic Links among Outbreak-Associated Isolates

The first case (Pt1) of CRKP BSI $\text{bla}_{\text{OXA-181}}$ ST307 clone was confirmed on October 10, 2019 in ward A. The $\text{bla}_{\text{OXA-181}}$ ST307 was responsible for 4 more cases (2 each in wards A and B) in late November and during the first week of December 2019. The last case belonging to $\text{bla}_{\text{OXA-181}}$ ST307 clone was identified in January 2020. The transmission network revealed that Pt1 was a possible index case. Although this patient was later transferred to an academic hospital for further treatment, this clone continued to disseminate within the neonatal unit. Five more cases were detected with the transmission stemming from the intermediate host with SNP differences of <10 among the clonal isolates (Figure 4, panel A).

The first case-patient (Pt4) of the major outbreak-related $\text{bla}_{\text{NDM-1}}$ ST152 clone was diagnosed on November 15, 2019, in ward A of the neonatal unit, followed by another case (Pt7) 15 days later in ward A. During December 11, 2019–February 2, 2020, a total of 14 more cases were isolated in the neonatal unit. Isolates from Pt14 and Pt17 were not considered part of the outbreak because they had >30 SNPs compared to other isolates. No further cases caused by the $\text{bla}_{\text{NDM-1}}$ ST152 clone were diagnosed after February 2020. Transmission network analysis identified Pt4 as the index case for the ST152 clone outbreak. However, Pt4 died on November 16, 2019, ten days before Pt7 was admitted, suggesting the role of an intermediate host or environmental source in the dissemination of $\text{bla}_{\text{NDM-1}}$ ST152 clone in the neonatal unit. Pt8's infection was diagnosed 2 days after the diagnosis of Pt7. Pt8 died a day after diagnosis. No further transmission from Pt7 or Pt8 to other patients could be established. Nine patients (Pt12–Pt19 and Pt22) in whom transmission stemmed directly from Pt4 were potentially infected through an intermediate source. Further dissemination of the ST152 clone was observed between Pt21, Pt23, and Pt26 in January 2020. These patients were admitted to ward A but moved between ward A and ward B. The transmission network revealed 2 possible events: first, from Pt21 to Pt24, who died a day after diagnosis; and second, that transmission occurred from Pt21 to Pt23, then from Pt23 to Pt26. However, Pt26 was admitted to ward A, 15 days after Pt23 was discharged from the hospital, suggesting the role of

---

**Figure 4.** Transmission networks among 22 patients with outbreak-associated $\text{bla}_{\text{OXA-181}}$ sequence type 307 (A) and $\text{bla}_{\text{NDM-1}}$ sequence type 152 (B) clones of $K.\text{pneumoniae}$ isolated from a neonatal unit during outbreak, South Africa, October 2019–February 2020. Question marks denote missing isolates; numbers along branches indicate number of single-nucleotide polymorphisms between index isolate and other isolates.
an intermediate in the spread of blaNDM-1 ST152 clone (Figure 4, panel B).

Discussion
We describe the successful integration of genomic and epidemiologic data in tracking an outbreak of CRKP infections in the neonatal unit of a South Africa hospital. The number of CRKP bloodstream infections increased substantially in the neonatal unit beginning in October 2019. Two outbreak-related clones (blaNDM-1 ST152 and blaOXA-181 ST307) cocirculated. The major outbreak clone blaNDM-1 ST152 accounted for >60% of deaths. Fine transmission networks identified possible index cases of blaOXA-181 ST307 and blaNDM-1 ST152 clones. The spread and dissemination of these clones from patient to patient might have been enabled by healthcare workers or environmental or fomite contamination, resulting from breaches in IPC measures.

Reports of CRE infections in Africa have increased markedly; the most commonly reported carbapenemases are blaNDM-1 and blaOXA-48 and variants (23–27). In this study, K. pneumoniae carrying blaNDM-1, blaOXA-48 and blaOXA-181 were dominant in the neonatal unit, which was consistent with previous reports (23–27). The blaNDM-1 gene was first described in South Africa in an Enterobacter cloacae strain in 2011 (28). Recent data from South Africa sentinel surveillance of CRE bloodstream infections have shown a change in the distribution of carbapenemase genes (29). Previously, blaNDM-1 was the most commonly detected gene in Enterobacteriaceae, followed by blaOXA-48 (25). However, beginning in 2016, blaOXA-48 and variants began to dominate, followed by blaNDM-1 (29). This change is worrisome because blaOXA-48 and variants are more difficult to identify using phenotypic laboratory methods. Most outbreaks reported in South Africa are also associated with blaOXA-48 and variants (30–32). The emergence of blaNDM-1 K. pneumoniae as a cause of outbreaks is concerning because once inserted into drug-resistant plasmids, virulence determinants render these strains highly resistant, virulent, and easily transmissible regardless of the clone (33).

The first laboratory-confirmed outbreak of blaOXA-181 K. pneumoniae in South Africa was described in 2015 from a hematology unit in Cape Town (30). After that, Strydom et al. (31) identified K. pneumoniae superclone ST307 carrying blaOXA-181 on a self-transmissible plasmid IncX3 (p72_X3_OXA181) as the source of a CRKP outbreak that spread across multiple wards in a Pretoria hospital during September 2015–December 2016. Furthermore, blaOXA-181 was endemic among patients colonized with K. pneumoniae in a KwaZulu-Natal hospital intensive care unit, and the spread was enabled by plasmid replicon E. coli p010-B-OXA181 (32). A combination of genomic and epidemiologic data was used either to track outbreaks or reconstruct transmission events that occurred during an outbreak (14,16,34–36). In our study, blaNDM-1 and blaOXA-181 K. pneumoniae strains were responsible for outbreaks that occurred simultaneously in the neonatal unit. The resistant genes were spread by transmissible plasmids E. coli B171 plasmid pB171 (blaNDM-1) and K. pneumoniae KP3 plasmid KP3-A (blaOXA-181). A recent report describing an outbreak of CRKP bloodstream infections in a neonatal unit in another South Africa hospital showed that blaOXA-48 and variants K. pneumoniae were responsible for the outbreak (11). Although the main objectives of the outbreak investigation by Essel et al. (11) were to confirm the outbreak and assess the IPC program, the molecular typing technique used has low discriminatory power to distinguish genetically related isolates. Lowe et al. (37) documented the rapid spread of ST307 clone carrying blaOXA-181 in >40 hospitals across 3 provinces in South Africa, highlighting the critical need for more enhanced genomic surveillance of K. pneumoniae ST307 super clone in healthcare settings.

Given the rapid transfer and acquisition of endemic carbapenemase genes, IPC measures are critical to preventing and managing outbreaks. Both IPC audits flagged <50% adherence to instrument sterilization and disinfection procedures. Therefore, breaches in aseptic techniques during invasive medical device insertion or maintenance practices might have been involved in the causal pathway to neonatal bacteremia. Inadequate staffing, exceeded bed capacity, aging and undermaintained hospital infrastructure, and general lack of institutional support for IPC and antimicrobial stewardship initiatives also contribute to the spread of healthcare-associated infections (38). Dramowski et al. presented a framework for prevention of healthcare-associated infections in neonates and children, which highlighted the need for a nationally endorsed prevention strategy to ensure that children in South Africa receive safe and high-quality care (39).

The strength of this study was involvement of multiple stakeholders who enabled different segments of the outbreak investigation. Genomic and epidemiologic data were used to confirm the existence of the outbreak, identify the sources of the outbreak, and reconstruct probable transmission events. The first limitation of our study is that we conducted a search for cases of bloodstream infection among infants <6 months of age through a laboratory audit, but we did not extend this search to the rest of the hospital.
Second, not all isolates from laboratory-confirmed CRKP cases were available for molecular characterization. Finally, genetic links among isolates from infected neonates and contaminated environment, fomites, or colonized healthcare workers could not be established due to incomplete data collection and contamination from redundant sources.

In conclusion, a combination of high-resolution WGS and epidemiologic data enabled a detailed description of this healthcare-associated infection outbreak in a neonatal unit and established transmission links. Continued monitoring of pathogens carrying endemic carbapenemases is necessary to prevent further reemergence of outbreaks. IPC measures complemented with adequate staffing levels, adherence to bed occupancy limits, improved neonatal unit infrastructure, and antimicrobial stewardship are key to sustainably reducing neonatal healthcare-associated infections.


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Nontuberculous mycobacteria (NTM) are ubiquitous, environmental, opportunistic microorganisms. Most NTM infections are acquired by inhalation, microaspiration, or direct inoculation (1). A recent meta-analysis using data from cultured samples found the global rate of change in NTM disease showed an increase of 4.1% per 100,000 persons per year (2). In the United States, 2 recent studies using medical claims data reported an increase in incidence of NTM disease by 7.5% per year (3,4). One study found the lowest prevalence of NTM pulmonary disease (NTM-PD) among Medicare beneficiaries in the US Midwest and classified the region at low risk for NTM-PD clustering (4). However, another study examined 5 US states, including Missouri, a state located in the midwestern United States with an estimated population of 6 million (5), reported an annual increase of 9.9% over a 6-year study period. Missouri showed the most yearly variability in NTM prevalence rates; some rates were almost 3 times lower than in the other states studied (6).

Geographic differences in distribution of NTM species likely related to local climate factors or population density variations have been observed worldwide (2,7–9). One study identified specific watersheds near densely populated areas in Colorado, USA, associated with increased risk for clustering of slow-growing NTM infections (10). A study in Queensland, Australia, found higher risk for Mycobacterium intracellulare infection associated with shallower soil depth and M. kansasii with higher soil density (11). Another study in Queensland examined the effects of climatic factors on infection trends and found slow-growing NTM incidence increased after a lag period of several months after heavy rainfall, possibly because of the time required for rain to disperse and transport bioaerosols (12). However, a prolonged lag period between exposure and disease manifestation is thought to be the norm in NTM disease, so quantifying the effects of individual climatic events is difficult.

Furthermore, extreme weather events such as heavy rainfall, flooding, and drought likely influence the prevalence of additional environmental
organisms (13). One study reported increased cases of NTM infections associated with higher numbers of hurricanes affecting the state of Florida (14). The proportion of dry to wet areas in Missouri is similar to those of other states considered highly burdened with NTM-PD (4). Also, similar to other midwestern states, natural disasters such as tornadoes and floods are common, and earthquakes occur periodically (15). Flooding is one of the deadliest severe weather hazards in Missouri because the state is traversed by the Mississippi, Missouri, and White Rivers and their basins (15). Using Missouri Department of Health and Senior Services (MDHSS) NTM surveillance data, we aimed to identify spatial clusters of NTM infections and correlate them with sociodemographic factors and seasonal flooding patterns to identify factors associated with higher rates of infection. Washington University (St. Louis, MO, USA) and MDHSS institutional review boards approved this study.

**Methods**

**Patient Population**
NTM infection is a reportable condition in Missouri. The surveillance database contains patient sex assigned at birth, date of birth, residential address or postal (ZIP) code, specimen source of the culture, collection date, NTM species isolated, and date on which positive result was reported. We extracted all reports of NTM infections from the MDHSS communicable disease surveillance database collected during January 1, 2008–December 31, 2019.

**Definitions**
To be included, cases needed to have ≥1 mycobacterial culture positive for an NTM species and residential address or postal code for the sample donor. We excluded duplicate cultures and those positive for *M. gordonae*, given its low pathogenicity (16). We defined extrapulmonary NTM infection as a positive culture from a nonrespiratory specimen. For NTM-PD, we applied the microbiologic diagnostic criteria recommended by current guidelines developed by leading international respiratory medicine and infectious diseases societies for defining a case: 2 positive sputum cultures with the same NTM species or a single positive culture obtained through bronchoscopy (17). For subanalyses, we grouped NTM into slow-growing and rapid-growing species (18). We based annual incidence rates on the number of persons in a calendar year positive for an NTM isolate (infection rate) or fulfilling disease criteria (pulmonary and extrapulmonary disease rates) divided by the population of Missouri in the year of sampling, according to 2010–2019 US Census data (5). We calculated index rates using only the first NTM-positive culture from each individual participant.

**Descriptive Analysis**
For categorical variables, we summarized descriptive statistics for persons with NTM cultures using sample proportions. For continuous variables, we used sample medians and interquartile ranges (IQRs).

**Spatial Statistical and Multilevel Analyses**
Using spatial and space-time scan statistics (19,20) based on census tract–level coordinates and counts (i.e., centroids of census tracts, NTM cases, background population sizes), we applied a Poisson model to detect geographic hotspots of higher-than-expected NTM infections. We defined hotspots as areas in which NTM infection rates were significantly higher than the statewide average and elsewhere in Missouri. To infer the statistical significance of each potential cluster, we applied a circular window with a varied radius (≤50% of the total at-risk population in the study area) to scan the study area and generate 999 Monte Carlo permutation datasets for computing the statistics.

Because we considered the general population at risk, a multilevel framework (individual patients nested in their residential counties) was necessary to control bias from potential correlations of patients residing in the same county. We performed multilevel Poisson regression analysis to generate the predicted county-level incidence rates of NTM infection, quantify the small-area geographic variation in NTM infections, and identify neighborhood characteristics associated with NTM infections. The terms small-area and neighborhood refer to census tracts in cluster analyses and counties in multivariate multilevel Poisson regression. To remove potential bias from small populations in some counties when estimating county-level NTM infection rates, we used multilevel modeling–based prediction (adjusting for age, sex, and race and ethnicity) instead of observed values to report the smoothed rate. For multilevel Poisson regression analysis of predicted county-level incidence rates, we adjusted the model for demographics only to generate smoothed small-area incidence rates. To quantify small-area geographic variations in NTM infections and identify neighborhood characteristics associated with NTM infections, we fit multilevel Poisson regression to a...
single multivariate model to estimate county-level variation in NTM incidence (random effect measured by median rate ratio [MRR]) and potential associations of county-level factors (fixed effects measured by rate ratio [RR]).

We further integrated the NTM county-population dataset with neighborhood contextual measures, including dates of flooding events in specific counties during 2008–2019, rural–urban context (defined as rural, urban, or metropolitan using the rural–urban continuum area code from the US Department of Agriculture; https://www.ers.usda.gov/data-products/rural-urban-continuum-codes), county-level percentage of population below federal poverty line, and county-level percentage of non-Hispanic Black population. We did not include other minorities, which represented <5% of the state population, in the analyses (5). We also used an adjusted multivariate multilevel model for individual-level demographics (age, sex, and race and ethnicity). We reported MRR, a measure of geographic heterogeneity with a value $\geq 1$ (21), because it reflects the average difference between a pair of counties randomly selected from the study area, with a higher value indicating more small-area variation.

We analyzed geographic clusters by using SaTScan software version 9.7 (https://www.satscan.org) and managed datasets and performed multilevel modeling in SAS version 9.4 (SAS Institute Inc., https://www.sas.com). We visualized identified census tracts included in the significant clusters and predicted/smoothed county-level incidence rates by using the ArcGIS software package version 10.6.1 (ESRI https://www.esri.com).

Results
Cohort Characteristics
We identified 14,203 mycobacterial cultures reported to MDHSS during the study period, of which 10,996 met the inclusion criteria. After excluding 77 duplicates and 1,450 M. gordonae isolates, we included 9,469 culture-positive samples from 5,288 persons in the analyses. Median age of persons with NTM infection was 67 years (IQR 54–76 years); 52.1% were White and 52.7% female. A total of 3,292 (62%) persons provided respiratory cultures and 481 (9.1%) extrapulmonary cultures; culture source was unknown for 1,515 (28.6%). Smoothed median rate of NTM infection was 68.04 (IQR 59.65–81.12)/100,000 persons for the study period, and compared with the 2008 baseline, yearly rate of infections had increased 5.7% by 2010 and 12.2% by 2019.

The 5 most frequently isolated NTM species were M. avium (60.1%), M. fortuitum (8.3%), M. abscessus (6.5%), M. chelonae (5.6%), and M. kansasii (3.8%). Among isolates, 72% were slow-growing NTM; median time to positivity from culture collection was 20 days (IQR 13–30 days) (Table 1). The proportion of new isolates per NTM species remained stable during 2008–2019, except for M. avium, which exhibited a positive but not statistically significant increase (19.6%, $p = 0.067$) (Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/8-230378-App1.pdf).

Using the standardized population of Missouri, we estimated an age-adjusted rate of NMT infection of 84.80/100,000 persons (82.28 for men, 87.09 for women) and 29.87/100,000 persons for NTM-PD (25.68 for men, 33.85 for women). By type of NTM, age-adjusted rates were 24.04/100,000 persons for rapid-growing infections and 60.76/100,000 persons for slow-growing infections (Appendix Table 1).

Pulmonary and Extrapulmonary Nontuberculous Mycobacterial Disease
Among all NTMs detected in respiratory specimens, 1,875 (56.9%) fulfilled the microbiologic diagnostic criteria for NTM-PD. Patients with NTM-PD were more commonly female (58% vs. 43.5%; $p <0.001$), older (median age 70 vs. 59 years; $p <0.001$), and infected with slow-growing NTM (83.9% vs. 48.6%) compared with those with extrapulmonary disease (Appendix Table 2). Among the 669 (35.7%) patients with NTM-PD who provided >1 respiratory sample with the same NTM species isolated, the predominant NTM species was M. avium (81.2%), followed by M. kansasii (4.19%) and M. abscessus (3.9%). Among the 481 (9.1%) patients with extrapulmonary infection, the most commonly isolated NTM species were M. avium (37.9%), M. fortuitum (17.2%), M. chelonae (14.9%), and M. abscessus (8.1%).

Geographic Variation and Clustering of NTM Infections
We excluded data from the 4.95% of geocoded locations outside the state of Missouri from further analyses. During the study period, the counties with the highest incidence were all rural: Buchanan (171.46/100,000 persons), Cape Girardeau (134.81/100,000 persons), and Sullivan (121.1/100,000 persons); those findings were mainly driven by high NTM-PD incidence rates in Cape Girardeau (90.45/100,000 persons) and Buchanan County (58.9/100,000 persons). By comparison, incidence of NTM infections in the 2 most populous metropolitan areas were 111.1/100,000 persons for St. Louis County, in which St. Louis is located, and...
Nontuberculous Mycobacteria, Missouri, USA

95.91/100,000 persons for Jackson County, in which Kansas City is located. The 3 counties with the highest incidence rates of extrapulmonary NTM cases were Sullivan (12.8/100,000 persons), McDonald (11.8/100,000 persons), and Jackson (10.6/100,000 persons) (Figure 1). Using spatial and space-time scan statistics across the state, we identified hotspots of significantly higher-than-expected NTM infection, NTM-PD, and extrapulmonary disease (Figure 2). Persons living in Cape Girardeau County were 3.62 times more likely to have any NTM infection and 4.52 times more likely to have slow-growing NTM infection than persons living elsewhere in Missouri.

Multilevel Analysis of Contextual and Individual Characteristics of NTM Infections
Multilevel Poisson analysis (Table 2) showed substantial small-area geographic variation in NTM infections across counties (variance 0.32, MRR 1.73; p<0.001). Risk for NTM infections was significantly higher in counties with >5 floods per year than in those with no flooding (RR 1.38, 95% CI 1.26–1.52) but not in counties with the highest poverty rates (highest vs. lowest quartiles incomes, RR 0.78, 95% CI 0.54–1.13) or highest percentages of non-Hispanic Black population (highest vs. lowest quartiles, RR 0.84, 95% CI 0.58–1.21). Compared with metropolitan counties, both rural (RR 2.82, 95% CI 1.90–4.19) and urban (RR 2.08, 95% CI 1.53–2.82) counties had higher risks for NTM infection. Compared with persons ≤20 years of age, risk for NTM infection was significantly higher among persons 20–49 years of age (RR 7.23, 95% CI 5.11–10.2) and 50–64 years of age (RR 26.7, 95% CI 18.9–37.7), and even more so among persons ≥65 years of age (RR 76.8, 95% CI 54.6–108.2). Of note, risk of NTM infection was lower among women than men (RR 0.94, 95% CI 0.89–0.99) but higher among non-Hispanic Black persons than among non-Hispanic White persons (RR 2.62, 95% CI 2.31–2.98).

Table 1. Characteristics of 5,288 patients with NTM infections, Missouri, USA, 2008–2019*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
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</tr>
<tr>
<td>Median age, y (IQR)</td>
<td>67 (54–76)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>2,785 (52.7)</td>
</tr>
<tr>
<td>M</td>
<td>2,498 (47.2)</td>
</tr>
<tr>
<td>Not recorded</td>
<td>5 (0.1)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
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<tr>
<td>Non-Hispanic White</td>
<td>2,753 (52.1)</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>459 (8.7)</td>
</tr>
<tr>
<td>Asian</td>
<td>91 (1.7)</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>1,985 (37.5)</td>
</tr>
<tr>
<td>NTM characteristics</td>
<td></td>
</tr>
<tr>
<td>Median NTM rate (IQR)†</td>
<td>68.04 (59.65–81.12)</td>
</tr>
<tr>
<td>Slow-growing mycobacteria</td>
<td>3,806 (72.0)</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>4,752 (60.13)</td>
</tr>
<tr>
<td>M. kansas</td>
<td>305 (3.86)</td>
</tr>
<tr>
<td>Rapid-growing mycobacteria</td>
<td>1,482 (28.0)</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>660 (8.35)</td>
</tr>
<tr>
<td>M. chelonae</td>
<td>230 (6.69)</td>
</tr>
<tr>
<td>M. abscessus</td>
<td>196 (5.7)</td>
</tr>
<tr>
<td>Median time to culture positivity, d</td>
<td>20 (13–30)</td>
</tr>
<tr>
<td>(IQR), n = 4,956</td>
<td></td>
</tr>
</tbody>
</table>

*Values are no. (%) patients except as indicated. NTM, nontuberculous mycobacteria; IQR, interquartile range.
†Over study period, per 100,000 population.

Discussion
We identified clusters of NTM infections in Missouri associated with sociodemographic factors and flooding. In counties where NTM infection rates were 3–4 times those for the rest of the counties, higher-than-expected rates were associated with older age, rurality, non-Hispanic Black race, male sex, and higher numbers of annual floods. Of note, overall average NTM incidence rate in Missouri was higher in our study than previously reported in large national datasets (3,22). This discrepancy might be related to differing sources of NTM reporting, because previous studies relied on International Classification of Disease (ICD) codes, which have low sensitivity, to identify NTM cases, not the mandatory laboratory reporting that our study used (23).

Figure 1. Smoothed county-level incidence rates of nontuberculous mycobacterial (NTM) infections, by infection site, Missouri, USA, 2008–2019. A) All NTM; B) pulmonary NTM; C) extrapulmonary NTM.
In keeping with the known epidemiology of NTM infections, most cultures in our study came from respiratory sources (17). NTM-PD rates approximated those previously described in epidemiologic studies from large administrative healthcare sources (3,4,6). More than half the persons in our study were ≥65 years of age and female, both factors significantly associated with NTM-PD. Of note, on the basis of the multilevel Poisson analysis, women had a slightly lower risk of NTM infection than men. Like increased NTM infections reported worldwide (2), M. avium was the most common NTM species in both pulmonary and extrapulmonary infections and the only NTM species that exhibited an increasing incidence over time. Reasons for this reported increase are likely multifactorial and include better mycobacterial diagnostic tools, increased NTM disease awareness, and extreme weather events disrupting the NTM ecologic niche (13). However, unlike in other locations worldwide, the number of M. abscessus infections reported in Missouri during the study period remained stable.

We found that counties in Missouri with >5 flooding events per year had a 38% higher rate of NTM infections than those without flooding. A study conducted in Florida found higher numbers of hurricanes, which can lead to flooding, associated with higher numbers of NTM infections (14). Those findings support the hypothesis that trends in flooding events may correlate with NTM infection rates, possibly because disruptions in the ecosystem of environmental mycobacteria from extreme weather events could increase human exposure and risk for potential infection.

Our study was limited by its retrospective design and use of mandatory laboratory reporting data. Lack of clinical data did not enable us to differentiate between disease and colonization; for this reason, we used the term NTM infection throughout the text and used NTM-PD only when patients fulfilled microbiologic diagnostic criteria for NTM disease. In addition, the MDHSS NTM surveillance database was not routinely queried for inconsistencies; hence, incomplete data on key variables could have introduced bias. However, except for 37% missing or unknown entries for race, missingness was <5% for key variables and unlikely to have biased analyses. Furthermore, other environmental factors identified in previous studies (11,12) could have influenced NTM infection rates, but we focused on a factor, flooding, that had not been studied before.
Table 2. Results of multilevel Poisson regression analyses of risk for nontuberculous mycobacterial infection, Missouri, USA, 2008–2019

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rate ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed effects</td>
<td>Referent</td>
</tr>
<tr>
<td>County-level flooding</td>
<td>Referent</td>
</tr>
<tr>
<td>No flooding</td>
<td>Referent</td>
</tr>
<tr>
<td>1–3 times</td>
<td>1.19 (1.11–1.29)</td>
</tr>
<tr>
<td>4–5 times</td>
<td>1.29 (1.17–1.43)</td>
</tr>
<tr>
<td>&gt;5 times</td>
<td>1.38 (1.26–1.52)</td>
</tr>
<tr>
<td>County rural–urban context</td>
<td>Referent</td>
</tr>
<tr>
<td>Rural</td>
<td>Referent</td>
</tr>
<tr>
<td>Urban</td>
<td>Referent</td>
</tr>
<tr>
<td>Metro</td>
<td>Referent</td>
</tr>
<tr>
<td>County-level poverty levels</td>
<td>Referent</td>
</tr>
<tr>
<td>1st quartile (lowest)</td>
<td>Referent</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>0.88 (0.62–1.25)</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>0.71 (0.49–1.03)</td>
</tr>
<tr>
<td>4th quartile (highest)</td>
<td>0.78 (0.54–1.13)</td>
</tr>
<tr>
<td>County-level ratio non-Hispanic Black</td>
<td>Referent</td>
</tr>
<tr>
<td>1st quartile (lowest)</td>
<td>Referent</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>1.17 (0.81–1.69)</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>1.09 (0.76–1.56)</td>
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<tr>
<td>4th quartile (highest)</td>
<td>0.84 (0.58–1.21)</td>
</tr>
<tr>
<td>Patient age group, y</td>
<td>Referent</td>
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<tr>
<td>&lt;20</td>
<td>Referent</td>
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<tr>
<td>20–49</td>
<td>7.23 (5.11–10.2)</td>
</tr>
<tr>
<td>50–64</td>
<td>26.7 (18.9–37.7)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>76.8 (54.6–108.2)</td>
</tr>
<tr>
<td>Sex</td>
<td>Referent</td>
</tr>
<tr>
<td>Male</td>
<td>Referent</td>
</tr>
<tr>
<td>Female</td>
<td>0.94 (0.89–0.99)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td>Referent</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>Referent</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>2.62 (2.31–2.98)</td>
</tr>
<tr>
<td>Others</td>
<td>32.2 (30.3–34.2)</td>
</tr>
</tbody>
</table>

Random effect

Variance 0.32, p<0.001

In conclusion, we identified increasing rates of NTM infection over time. NTM infection clustering in Missouri was associated with older age, rurality, and higher rates of annual flooding events. Further investigation is warranted to determine the degree to which extreme weather events contribute to the increasing incidence and prevalence of NTM-PD worldwide. In addition, clinicians and public health professionals should be aware of the increased risk for NTM infections, especially in locations with environments similar to those described here.

This study was financially sponsored by Insmed Incorporated.

C.M.-C. reports a Centers for Disease Control and Prevention subaward, a vendor/individual agreement with Wayne State University, and serves as associate editor for Open Forum Infectious Diseases, outside the submitted work. C.M.-C. also reports research grants from the Centers for Disease Control and Prevention and Insmed Incorporated.

In the United States, the incidence and prevalence of nontuberculous mycobacterial lung disease have increased over the past decade, with rates particularly high in the Midwest and West regions. This study aimed to investigate the geographic distribution of nontuberculous mycobacterial infections in Missouri, USA, using a multilevel Poisson regression analysis of nontuberculous mycobacterial lung disease in Missouri, USA, 2008–2019.

In conclusion, we identified increasing rates of NTM infection over time. NTM infection clustering in Missouri was associated with older age, rurality, and higher rates of annual flooding events. Further investigation is warranted to determine the degree to which extreme weather events contribute to the increasing incidence and prevalence of NTM-PD worldwide. In addition, clinicians and public health professionals should be aware of the increased risk for NTM infections, especially in locations with environments similar to those described here.

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Author contributions: study concept and design, C.M.-C. and G.T.; acquisition, analysis, or interpretation of data, C.M.-C., M.A.C., M.L., M.A., G.T., T.C.B., A.S.; drafting of the manuscript, C.M.-C. All authors take responsibility for the accuracy of the data presented.

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Hunting, preparing, and selling bushmeat has been associated with high risk for zoonotic pathogen spillover due to contact with infectious materials from animals. Despite associations with global epidemics of severe illnesses, such as Ebola and mpox, quantitative assessments of bushmeat activities are lacking. However, such assessments could help prioritize pandemic prevention and preparedness efforts.

In this EID podcast, Dr. Soushieta Jagadesh, a postdoctoral researcher in Zurich, Switzerland, discusses mapping global bushmeat activities to improve zoonotic spillover surveillance.

Visit our website to listen: https://bit.ly/3NJL3Bw
Reproduction Number [ɪˈpɹə-də-kɔʃən ˈnɜm-bər]

Vijay Sharma, Rajnish Sharma, Balbir B. Singh

The basic reproduction number ($R_0$, pronounced R naught) is derived from demography terminology used to estimate the overall population reproduction rate. $R_0$ is an essential metric in the study of epidemics. This value measures the estimated number of new cases of an infection caused by an infectious person in a population of disease-susceptible person.

The effective reproduction number ($R_e$) is similar to $R_0$, but $R_e$ measures the number of persons infected by infectious person when some portion of the population has already been infected. This idea can be traced back to the work performed by Richard Bockh, Alfred Lotka and others.

A modern application of $R_0$ in epidemiology was reported in 1952 when George Macdonald constructed population models about the spread of malaria. Macdonald used the notation $Z_0$ instead of $R_0$ to differentiate it from the preceding demography terminology. The notation $R_0$ was adopted instead of $Z_0$ during the Dahlem conference in 1982 (Figure).

Sources

Waterborne Infectious Diseases Associated with Exposure to Tropical Cyclonic Storms, United States, 1996–2018

Victoria D. Lynch, Jeffrey Shaman

In the United States, tropical cyclones cause destructive flooding that can lead to adverse health outcomes. Storm-driven flooding contaminates environmental, recreational, and drinking water sources, but few studies have examined effects on specific infections over time. We used 23 years of exposure and case data to assess the effects of tropical cyclones on 6 waterborne diseases in a conditional quasi-Poisson model. We separately defined storm exposure for windspeed, rainfall, and proximity to the storm track. Exposure to storm-related rainfall was associated with a 48% (95% CI 27%–69%) increase in Shiga toxin–producing *Escherichia coli* infections 1 week after storms and a 42% (95% CI 22%–62%) increase in Legionnaires’ disease 2 weeks after storms. Cryptosporidiosis cases increased 52% (95% CI 42%–62%) during storm weeks but declined over ensuing weeks. Cyclones are a risk to public health that will likely become more serious with climate change and aging water infrastructure systems.

Tropical cyclones are a seasonal occurrence in the Eastern United States, where they cause widespread destruction and endanger public health (1–3). Among many storm-related hazards, extreme flooding is a concern because it can lead to the contamination of recreational, irrigation, and drinking water sources (4–6) and might increase risks for transmission of waterborne infectious diseases (7). Elevated case counts and outbreaks have been attributed to individual storms (8), but the effect of tropical cyclones on specific waterborne infections has not been evaluated over multiple storm seasons. Understanding waterborne pathogen transmission is a pressing public health challenge because the burden of disease will likely increase in conjunction with an aging population (9), deteriorating drinking and wastewater treatment systems (10), and increased storm-related flooding due to climate change (11).

Bacterial, parasitic, and viral pathogens cause ≈7.15 million cases of waterborne disease annually in the United States (12). Infections are typically mild but can lead to life-threatening enteric or respiratory illness for immunocompromised, young, or elderly persons (13,14). Cyclonic storms drive transmission because floodwater mobilizes pathogens in the environment and inundates water system infrastructure, which causes further contamination through ineffective treatment or sewage overflows (15,16). After cyclonic storms, high pathogen loads frequently are detected in floodwater (17,18) and in environmental and drinking water sources (19–21). Floods also can contaminate irrigation water used on crops (22); therefore, flood-driven contamination can influence transmission of pathogens that are predominantly foodborne.

However, contamination does not necessarily lead to transmission; although extreme weather events have been associated with gastrointestinal illness or specific outbreaks (23–25), some storms have been found to have no effect on incidence of cases (26). Those inconsistent associations reflect the relevance of pathogen-specific factors, particularly pathogen biology and primary reservoirs, in determining the effects of storms on transmission.

Pathogens that form oocysts or are members of biofilm communities persist in environmental waters for weeks, which can increase the likelihood of transmission (27,28), whereas pathogens that do not persist in the environment might be flushed from waterways by flooding (29). Pathogen biology also affects the efficacy of water treatment; in particular,
Cryptosporidium and Legionella are resistant to common decontamination methods (30,31), whereas Giardia is readily removed from water (32).

Cyclonic storms can also lead to different types of contamination depending on the land use and drinking water or sanitation infrastructure of affected regions. Cattle and poultry are the primary reservoirs for several gastrointestinal pathogens, and flooding near livestock production can contaminate drinking water sources with animal waste (33). Flooding near livestock production is of particular concern in rural agricultural regions where many persons rely on private wells that are untreated and vulnerable to inundation (34). On the other hand, storms in densely populated areas often lead to floodwater contaminated with human sewage (35). Urban flooding also can damage water treatment or distribution systems that serve entire cities, leading to large outbreaks (36).

The effect of cyclonic storms on waterborne disease also might depend on storm characteristics that determine the extent of flooding and destruction. Storms are generally defined by windspeed and rainfall, factors that are often weakly correlated with each other upon landfall (37) and lead to different conditions in affected areas. Slow-moving storms tend to cause greater accumulation of rain and more severe flooding, whereas tropical cyclones with high windspeeds might bring less rain but cause wind-related property or infrastructure destruction (1,38). Storm type also could dictate disaster management decisions and individual-level responses, such as the ability to comply with evacuation orders. In addition, storm severity influences healthcare-seeking behavior and healthcare infrastructure. Storm-related disruptions might dissuade persons with mild or moderate conditions from seeking care (39), whereas catastrophic storms can prevent persons with urgent needs from accessing healthcare systems (40).

Storm severity is projected to increase with atmospheric warming, so developing a thorough understanding of storm effects on waterborne diseases could aid climate change adaptation and public health policies. Previous research has largely focused on specific storms and outbreaks or on nonspecific gastrointestinal illness; however, associations over multiple storm seasons have not been thoroughly examined. In this study, we examined the effects of tropical cyclones on waterborne infectious diseases over more than a decade and determined whether those associations varied by pathogen or type of storm exposure.

Methods

Data

Case data

We used surveillance data from the National Notifiable Diseases Surveillance System (NNDSS; https://www.cdc.gov/nndss) to identify weekly cases of cryptosporidiosis, giardiasis, Legionnaires’ disease, Escherichia coli infections, salmonellosis, and shigellosis during 1996–2018 for each US state. Those infections are caused by parasitic (Cryptosporidium and Giardia), biofilm-forming bacterial (Legionella), and enteric bacterial (E. coli, Salmonella, Shigella) pathogens that can lead to severe gastrointestinal or respiratory illness. Of the 6 E. coli strains, NNDSS only tracks Shiga toxin–producing E. coli (STEC) infections.

The data consist of laboratory-confirmed cases from hospitalizations, emergency department visits, and primary care visits that are reported to local health departments and compiled by state health departments to submit to the Centers for Disease Control and Prevention (CDC), which manages the NNDSS and case definitions (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/8/22-1906-App1.pdf). We restricted our analyses to the 30 states and Washington, DC, that experienced ≥1 tropical cyclone during the study period and to June–November, the months of the Atlantic storm season. We also used US Census data (9) to determine county and state populations during the study period.

Storm Data

We obtained storm track, windspeed, and rainfall data for tropical cyclones that made landfall in the United States during 1996–2018 from the hurricane-exposure version 0.1.1 and hurricaneexposedata version 0.1.0 packages in R (R Foundation for Statistical Computing, https://www.r-project.org). For each county, we defined the primary exposure day as the day with the shortest distance between the county center and the storm track. We used storm track and surface windspeed data from the National Hurricane Center’s HURDAT-2 dataset (https://www.nhc.noaa.gov/data) and included maximum and sustained windspeeds on the primary exposure day. We used rainfall data from the North American Land Data Assimilation System 2 (https://ldas.gsfc.nasa.gov/nldas) and included in our dataset the total daily rainfall in each exposed county from 5 days before to 3 days after the primary exposure day. To inform the selection of exposure variables used in the analysis, we assessed correlations among

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distance, wind, and rainfall variables, including total and daily maximum rainfall.

**Storm Exposure Definition**
Informed by the correlation analysis of storm variables, we defined county-level exposure to storms according to total rainfall, maximum sustained windspeed, and distance from the storm track. In the primary analysis, we defined exposure separately for each variable and repeated the analyses using several exposure thresholds. We considered counties exposed when they experienced 50, 75, or 100 mm of total rainfall associated with the storm or were within 500, 250, or 150 km of the storm track. The National Oceanic and Atmospheric Administration categorizes cyclones as tropical storms or hurricanes on the basis of windspeed (https://www.nhc.noaa.gov/climo); consistent with those definitions, we considered counties exposed to tropical storms when maximum sustained windspeeds were ≥34 knots but <64 knots (gale-force wind on the Beaufort scale) and exposed to hurricanes when maximum sustained windspeeds were ≥64 knots. We assessed correlations among the exposure thresholds. To determine state-level exposure, we calculated the percent of the state population in exposed counties during storm weeks and classified the state as exposed if 75%, 50%, 25%, 5%, or any (>0%) of the population was exposed; we repeated the analysis for each of those population thresholds.

In the secondary analysis, we combined storm exposure variables to describe categories of cyclonic storms. We categorized storms as high rain–high wind if total rainfall was ≥100 mm and windspeed was ≥64 knots; as high rain–low wind if total rainfall was ≥100 mm and windspeed was ≥34 but <64 knots; and as low rain–low wind if total rainfall was <100 mm and windspeeds were ≥34 but <64 knots. We did not include a low rain–high wind category because no storms met that definition. We considered counties exposed to a specific storm type if the storm met both the rainfall and windspeed criteria. Hurricane-force winds are rare and usually affect a small proportion of a state’s population (Appendix Table 2); therefore, we defined state population exposure thresholds only by rainfall exposure, as in the primary analysis. We considered a state exposed to a given storm type if it met the rainfall-based population exposure threshold (e.g., for a 25% population-exposure threshold, ≥25% of the state’s population had to be exposed to storm-related rainfall) and any of the counties were exposed to the given storm type.

**Statistical Analysis**
We modeled the association between exposure to tropical cyclones and case rates by using a conditional quasi-Poisson model (Appendix), which accounted for overdispersion in the case data (41). We compared case rates in weeks with and without storms across matched strata based on state and week of the year. That structure addressed potential confounding due to variation among states (i.e., different state policies regarding storm preparedness or case reporting) and controlled for seasonality. We modeled cyclonic storm occurrence as a binary exposure variable and lagged from 0 to 3 weeks to account for the incubation periods of the pathogens and the potential for delays in seeking healthcare after destructive storms. The model included a flexibly adjusted term for year to control for long-term trends that could affect storm exposure or waterborne infectious disease transmission. We used annual state population as an offset to obtain the rate of cases and we modeled case rates for each pathogen separately. We repeated the analysis for all exposure definitions and population exposure thresholds. We used the Bonferroni-Holmes method to adjust 95% CIs for multiple comparisons. Finally, we repeated the method with counties stratified by drinking water source or for rural or urban location (Appendix).

**Results**
The number of cases reported to NNDSS varied by pathogen, and most infections involved enteric bacteria (Table 1). Most infections peaked in the late summer or early fall, but the amplitude of seasonality differed among pathogens and by geographic region (Figure 1). Cryptosporidiosis exhibited the strongest and most consistent seasonality; cases peaked in September in all geographic regions. In most states, Legionnaires’ disease and parasitic infections displayed only a moderate increase during summer months (Appendix Figure 1). Enteric bacterial infections were more common across all states, and salmonellosis showed a strong summer seasonality in most states (Appendix Figure 2). During 1996–2018, Legionnaires’ disease and cryptosporidiosis cases increased, and giardiasis decreased, in all geographic regions; the other infections were relatively consistent over time (Appendix Figure 3). The burden of disease also varied by geographic region; salmonellosis and shigellosis cases were more common in the Southeast, but Legionnaires’ disease was concentrated in the Mid-Atlantic region (Appendix Figure 4). E. coli infections, cryptosporidiosis, and giardiasis were all more common in the Upper Midwest and New England states than in other geographic regions (Appendix Figure 4).
Wind, rainfall, and distance variables were not highly correlated, but different measures of the same variable, such as maximum rainfall and total rainfall, were correlated (Appendix Figure 4). Among the storm variable thresholds used to determine county-level exposure, hurricane- and gale-force wind exposure were not highly correlated ($r = 0.21$), but >50-mm, >75-mm, and >100-mm rainfall exposure thresholds were highly correlated ($r = 0.50–0.72$) (Appendix Figure 5). Using the most inclusive storm exposure threshold, gale-force wind, 134 cyclonic storms occurred during the study period (Table 2). Those storms affected 2,363 counties in 30 states and Washington, DC, over 177 weeks. Counties with the greatest number of weeks of gale-force wind exposure storms were concentrated along the coast, particularly in North and South Carolina (Figure 2). Exposure to >75 mm of rainfall was most common in South Florida but was overall more

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. (% cases in NNDSS)</th>
<th>Pathogen type</th>
<th>Incubation period, d (range)†</th>
<th>Estimated cases attributed to waterborne transmission, %‡</th>
<th>Years reported in NNDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legionella</td>
<td>77,765 (3.8)</td>
<td>Biofilm-forming bacteria</td>
<td>5–6 (2–10)</td>
<td>97</td>
<td>1996–2018</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>151,573 (7.4)</td>
<td>Parasite</td>
<td>7 (2–12)</td>
<td>43</td>
<td>1998–2018</td>
</tr>
<tr>
<td>Giardia</td>
<td>297,379 (14.6)</td>
<td>Parasite</td>
<td>7 (1–14)</td>
<td>44</td>
<td>2002–2018</td>
</tr>
<tr>
<td>STEC</td>
<td>128,332 (6.3)</td>
<td>Enteric bacteria</td>
<td>0.5–4 (0.5–10)</td>
<td>5</td>
<td>1996–2018</td>
</tr>
<tr>
<td>Salmonella</td>
<td>964,235 (47.3)</td>
<td>Enteric bacteria</td>
<td>0.5–2 (0.5–16)</td>
<td>6</td>
<td>1996–2018</td>
</tr>
<tr>
<td>Shigella</td>
<td>421,369 (20.4)</td>
<td>Enteric bacteria</td>
<td>1–3 (0.5–7)</td>
<td>4</td>
<td>1998–2018</td>
</tr>
</tbody>
</table>

† According to D.W.K. Acheson (42).
‡ According to S.A. Collier et al. (12).

Figure 1. Average weekly cases by geographic region in a study of waterborne infectious diseases associated with exposure to tropical cyclonic storms, United States, 1996–2018. Graphs indicate weekly number of cases per 1,000,000 populations for the following waterborne diseases: A) cryptosporidiosis; B) giardiasis; C) Legionnaires’ disease; D) Escherichia coli infection; E) salmonellosis; and F) shigellosis. Not all infections were reported for the entire study period (Table 1). The shaded region represents the weeks encompassed in the annual Atlantic storm season, June 1–November 30. The geographic regions reflect the reporting areas used for infectious disease surveillance in the National Notifiable Diseases Surveillance System (https://www.cdc.gov/nndss). The New England region comprises the states of Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont; the Mid-Atlantic Region comprises New Jersey, New York, and Pennsylvania; the South-Atlantic Region comprises Delaware, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, West Virginia, and Washington, DC; the East-North Central Region comprises Illinois, Indiana, Michigan, Ohio, and Wisconsin; the East-South Central Region comprises Alabama, Kentucky, Mississippi, and Tennessee; and the West-South Central Region comprises Arkansas, Louisiana, Oklahoma, and Texas.
widespread and uniform than the wind and distance metrics (Figure 2). We noted no long-term trend in the number of cyclonic storms during the study period (Appendix Figure 6).

Cryptosporidiosis case rates greatly increased during storm weeks at low population exposure thresholds; storms that brought ≥75 mm of rainfall were associated with a 40% increase in case rates when any of the state’s population was exposed and a 52% increase when ≥5% of the population was exposed (Figure 3). Similar associations persisted across lagged exposures, but the effects were weaker, ranging from 12%–20% increases in the poststorm weeks (Appendix Table 3). Legionnaires’ disease case rates were also highly associated with storm exposure, but the effect was strongest 2 and 3 weeks after a storm and at higher population exposure thresholds (Figure 3). When 75% of the state population was exposed to a storm, case rates increased by 31% in lag week 1, 42% in lag week 2, and 39% in lag week 3 (Appendix Table 3). E. coli case rates exhibited a clearer peak and decline associated with lagged storm events. After an initial decrease during the storm week, case rates increased 48% in week 1 and 33% in week 2 post storm when 75% of the state’s population was exposed (Figure 3). Salmonellosis and giardiasis were not greatly associated with storm exposure, and shigellosis case rates slightly decreased during storm weeks (Figure 3).

### Table 2. Cyclonic storm exposure definitions used to assess waterborne infectious diseases associated with exposure to tropical cyclonic storms, United States, 1996–2018

<table>
<thead>
<tr>
<th>Storm exposure variables, definition</th>
<th>No. storms</th>
<th>No. counties affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total rainfall, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>98</td>
<td>2,165</td>
</tr>
<tr>
<td>75</td>
<td>96</td>
<td>2,041</td>
</tr>
<tr>
<td>100</td>
<td>87</td>
<td>1,732</td>
</tr>
<tr>
<td>Sustained wind gusts*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gale-force winds</td>
<td>134</td>
<td>1,025</td>
</tr>
<tr>
<td>Hurricane-force winds</td>
<td>31</td>
<td>136</td>
</tr>
<tr>
<td>Distance from storm track, km</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>134</td>
<td>2,363</td>
</tr>
<tr>
<td>250</td>
<td>134</td>
<td>2,179</td>
</tr>
<tr>
<td>150</td>
<td>117</td>
<td>2,072</td>
</tr>
</tbody>
</table>

*National Oceanic and Atmospheric Administration (https://www.noaa.gov) designates tropical storms as those with gale-force winds, defined as ≥34 knots to <64 knots, and hurricane-force winds as ≥64 knots.*

### Figure 2. Total number of weeks of storm exposure per county in a study of waterborne infectious diseases associated with exposure to tropical cyclonic storms, United States, 1996–2018.

Exposure is defined by 3 factors: A) distance, ≤500 km of storm track; B) cumulative rainfall of ≥75 mm; and C) sustained winds above gale-force, i.e., ≥34 knots.
The associations between storm-related rainfall and cryptosporidiosis, Legionnaires’ disease, and *E. coli* case rates were consistent across different exposure definitions (Figure 4). Storms with less (>50 mm) or more (>100 mm) rainfall were associated with substantial initial increases in cryptosporidiosis cases that attenuated over lag weeks 1–3. The strength of the association between Legionnaires’ disease case rates and storm exposure increased in conjunction with population exposure threshold and amount of rainfall (Figure 4). Similarly, the lagged increase in *E. coli* rates was more pronounced in storms with >100 mm of rainfall. The associations between case rates and storm exposure were similar when exposure was defined by distance from the storm track instead of rainfall (Appendix Figure 7). Stratifying exposure by drinking water source or rural or urban location also yielded similar results; the lagged effect on *E. coli* and Legionnaires’ disease rates was slightly more pronounced when restricted to rural or groundwater-reliant counties, but associations were otherwise consistent (Appendix).

Storm exposure defined by hurricane-force winds was associated with increased cryptosporidiosis case rates 2 and 3 weeks after storms, but otherwise had no effect on rates (Appendix Figure 8). Conversely, gale-force wind exposure was associated with decreased cryptosporidiosis and giardiasis rates during the storm week and had no effect in the lagged weeks after storms (Appendix Figure 8).

Combining wind and rainfall exposure in storm type categories supported the findings of the wind exposure analysis. High rain–high wind, high rain–low wind, and low rain–low wind storms were all associated with decreased giardiasis case rates during the storm week before returning to baseline 1 week poststorm (Figure 5). Consistent with the rainfall analysis, high rain–low wind storms were positively associated with cryptosporidiosis rates up to 2 weeks poststorm, but unlike for rainfall alone, cases also increased 3 weeks after high rain–high wind and low rain–low wind storms; a 58% increase in cryptosporidiosis rates when ≥5% of the population was exposed to high wind–high rain storms and a 17% increase after low rain–low wind storms (Figure 5).

**Discussion**

In this analysis, we found tropical cyclones were associated with waterborne diseases, although the effect magnitude varied by exposure. The associations also
differed among the specific pathogens; Legionnaires’ disease, *E. coli*, and cryptosporidiosis rates increased with rainfall, whereas salmonellosis, shigellosis, and giardiasis rates were unaffected, or decreased, during storm weeks. Those divergent associations likely reflect factors that mediate the relationship between storms and disease, including pathogen biology, transmission routes, and severity of infection.

Legionnaires’ disease and *E. coli* case rates consistently increased with rainfall and population exposure thresholds, but the timing of the effects differed between these infections. *E. coli* rates peaked 1 week after storms and returned to baseline by week 3, whereas Legionnaires’ disease rates were highest 3 weeks after storms. Those findings support microbiological studies that have analyzed bacterial counts in streams and water systems after specific hurricanes (43,44); elevated *E. coli* loads were reported 12–24 hours after a storm started, whereas *Legionella* increased 4–5 days later (43). *Legionella* are natural inhabitants of aquatic environments and replicate in water, typically in biofilm communities that colonize household plumbing and water infrastructure systems (13,45). Thus, the *Legionella* load can increase over time, whereas other bacterial pathogens that do not replicate in the environment typically have bacterial loads that peak after the initial contamination event and dissipate over time (46).

Cryptosporidiosis case rates also increased with storm-related rainfall but only at low population thresholds and concurrent with the storm week. Cryptosporidiosis cases were most common in the north-central Midwest, a region that infrequently experiences tropical storms or hurricanes severe enough to affect >25% of the population. The substantial increase in cases concurrent with storm weeks might be driven by several widespread outbreaks attributed to specific storm events that damaged water treatment facilities (47). *Cryptosporidium* is resistant to standard chemical disinfectants and is small enough to pass through sand filtration systems common in water treatment plants (29); thus, when the parasite contaminates water distribution systems that serve large populations, massive outbreaks can occur (8).

County-level exposure to heavy rainfall and cyclonic windspeed often were uncorrelated, which is characteristic of tropical cyclones (37), and the effect of extreme wind on cases differed from that of rainfall.

**Figure 4.** Average percent change in weekly case rates associated with exposure to storm-related rainfall in a study of waterborne infectious diseases associated with exposure to tropical cyclonic storms, United States, 1996–2018. Exposure is defined by 3 cumulative rainfall thresholds, ≥50 mm, ≥75 mm, or ≥100 mm; and for 2 population-exposure thresholds, 5% or 50% exposed. Estimates (shapes) and Bonferroni-corrected 95% CIs (bars) are reported for cryptosporidiosis, Legionnaires’ disease, and *Escherichia coli* infections for the week of the storm (week 0) and 1–3 weeks after the storm.
Waterborne Diseases Associated with Storms

for several infections. Gale-force wind was associated with a lagged increase in Legionnaires’ disease, but the effect on *E. coli* and cryptosporidiosis was minimal; hurricane-force wind was only associated with increased cryptosporidiosis rates 3 weeks after storms. Such attenuated effects could reflect the intricate, and possibly opposing, factors that influence transmission. High windspeeds are typically associated with destructive storms that can damage sanitation infrastructure, increasing the probability of transmission (18), but also could lead to population displacement (48), reducing the likelihood that persons will have contact with contaminated water. Extreme storms can also disrupt healthcare systems or alter healthcare-seeking behavior, which can lead to a reduction in detecting or reporting cases (49).

For areas that experienced both rainfall and cyclonic wind, we combined exposures into storm-type categories; the results underscored the necessity for pathogen-specific analyses and the limitations inherent in studying events that rarely occur. The high rain–high wind category encompassed the most devastating storms that occurred during the study period, including Hurricanes Katrina and Ivan, but represented a small fraction of all storms. Those events were associated with a substantial decrease in giardiasis but had no effect on Legionnaires’ disease. *Giardia* transmission often occurs in recreational waters, such as swimming pools and rivers, and might be thwarted during storm weeks, when the population is less likely to engage in recreational activities. On the other hand, the burden of Legionnaires’ disease was highest in regions that infrequently experience hurricane-force winds. High rain–high wind storms were associated with a substantial increase in cryptosporidiosis cases 3 weeks after storms, but the effect might have been driven by a 2-month span in 2008 when Texas experienced 2 hurricanes and a third tropical cyclone in succession and reported extremely high cryptosporidiosis case counts for an extended period.

Unlike the other infections, salmonellosis was unaffected by cyclonic storms at all population thresholds. *Salmonella* transmission is predominantly foodborne, and outbreaks attributed to contaminated

Figure 5. Average percent change in weekly waterborne infectious disease case rates associated with exposure to tropical cyclonic storm types, United States, 1996–2018. Exposure is defined by 3 categories according to rainfall and wind thresholds: high rain–high wind (red); high rain–low wind (yellow); and low rain–low wind (green). Estimates (shapes) and Bonferroni-corrected 95% CIs (bars) are reported for giardiasis, cryptosporidiosis, and Legionnaires’ disease at 2 population-exposure thresholds (shape) for the week of the storm (week 0) and 1–3 weeks after the storm. The population-exposure thresholds refer to the percentage of the state population exposed to storm-related rainfall only; no hurricane-force winds affected >25% of the state population.
food are common, particularly during the summer (42). The high frequency of salmonellosis outbreaks makes it difficult to detect elevated case counts associated with storms because comparison weeks for storms coincide with those for foodborne outbreaks. Storm-related rainfall was associated with a slight decrease in shigellosis at high population thresholds during storm weeks. Shigellosis is typically mild, and the negative association might reflect a reduction in seeking healthcare for minor illnesses after disruptive storm events.

Except for shigellosis, other disease cases studied exhibited a summer seasonality that coincided with the cyclonic storm season in the United States. However, the inconsistent associations between storms and specific pathogens demonstrated that the effects were not simply driven by overlapping seasonal patterns. Salmonellosis and *E. coli* cases peaked during the same weeks in most regions, but storm-related rainfall had no effect on salmonellosis and a strong positive effect on *E. coli*. This study demonstrated the need for more pathogen-specific analyses that combine microbiological water quality data from multiple sources with epidemiologic data.

One limitation of this study is the spatial mismatch between cases and storm data. Aggregating from county- to state-level storm exposure introduced the possibility of misclassification bias because state-level exposure might be inconsistent with the conditions experienced by cases. We aimed to address this limitation by repeating the analysis at several population thresholds to define exposure and by assessing the consistency of the associations. That type of nondifferential misclassification would also be biased toward the null and underestimate the associations (50). Another limitation resulted from the spatial resolution, which only enabled us to perform a rough estimate of the effect of storms stratified by drinking water source or rural or urban location using county-level averages. Highly resolved water source and location data could provide insight into the mechanisms underlying the associations between storms and some waterborne diseases.

In summary, we found that tropical cyclones represent a risk to public health in the United States, although findings for individual pathogens varied. The US sanitation infrastructure is aging (10), and the country will likely experience more severe storm-related flooding as a result of climate change (11). Thus, identifying the drivers of pathogen transmission, and opportunities for intervention, will be crucial to reducing disease burden after cyclonic storm events.

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### References

Waterborne Diseases Associated with Storms

etymologia revisited

Lassa Virus
[lah sa] virus

This virus was named after the town of Lassa at the southern end of Lake Chad in northeastern Nigeria, where the first known patient, a nurse in a mission hospital, had lived and worked when she contracted this infection in 1969. The virus was discovered as part of a plan to identify unknown viruses from Africa by collecting serum specimens from patients with fevers of unknown origin. Lassa virus, transmitted by field rats, is endemic in West Africa, where it causes up to 300,000 infections and 5,000 deaths each year.

References:

https://wwwnc.cdc.gov/eid/article/16/6/et-1606_article
Elimination of *Dirofilaria immitis* Infection in Dogs, Linosa Island, Italy, 2020–2022

Emanuele Brianti, Ettore Napoli, Giovanni De Benedetto, Luigi Venco, Jairo Alfonso Mendoza-Roldan, Angelo Basile, Marcos Antônio Bezerra-Santos, Jason Drake, Roland Schaper, Domenico Otranto

On Linosa Island, Italy, *Dirofilaria immitis* infection has been hyperendemic in dogs and seroprevalent among islanders. In 2020, we implemented a heartworm disease elimination program on Linosa Island. Of 54 dogs tested for *D. immitis* antigen and microfilariae, 28 had positive results and received treatment with oral doxycycline twice daily for 4 weeks plus topical imidacloprid/moxidectin monthly for 12 months. The 26 dogs with negative results received monthly topical imidacloprid/moxidectin as preventive. During month 1, the number of microfilariae was reduced by 76.5%. From month 2 on, all animals were microfilaria negative, and during months 3 to 9, the number of antigen-positive dogs decreased progressively. Treatment of positive dogs coupled with chemophylaxis for noninfected dogs was effective, protecting them from new infections. The elimination program reduced the risk for human infection, representing a One Health paradigm. Monitoring and chemophylaxis are advocated to maintain the status of heartworm disease–free area.

*Dirofilaria immitis* and *D. repens* (Spirurida, Onchocercidae) nematodes are among the most common species of filariae that cause diseases in dogs and other animals; both species infect humans (1,2). In dogs, *D. immitis* filariae cause the severe illness heartworm disease (HWD), whereas *D. repens* infection is less severe. Both *Dirofilaria* species are transmitted by mosquitoes; in Europe, the competent vectors are *Culex pipiens* and *Aedes albopictus* mosquitoes (3). However, the involvement of flying insects other than mosquitos has been recently hypothesized, including black flies belonging to the *Simulium turgicintum* complex (4) and *Culicoides paolae* biting midges (5).

Unlike *D. repens* filariae, which are more widely distributed in Europe, including the Italian Peninsula (1,6–8), *D. immitis* worms are more frequently recorded in central Europe (9), including regions in northern Italy (10,11). Nevertheless, autochthonous cases of HWD in dogs from central and southern Italy have been retrospectively reported from 2009 (1) through 2019 (12); highly endemic foci in southern regions of Italy have been described (13,14). That new epidemiologic scenario developed after the arrival of a new invasive mosquito species (i.e., *Ae. albopictus*) and the increased movement of animals throughout the country combined with lack of chemophylactic strategies for dogs from non–HWD-endemic regions (9,12,13). The island of Linosa, Italy, represents a paradigm of that scenario. A highly endemic focus of *D. immitis* filariae was recently described on the small and remote island located in the middle of the Mediterranean Basin, where 58.9% of dogs tested positive for HWD by modified Knott and SNAP 4Dx Plus tests (14). In the same epidemiologic context, 7.9% of human islanders tested positive for *D. immitis* antibodies (15), which emphasizes the role of *D. immitis*-infected dogs as a source for human infections in specifically isolated environments, thus advocating for treating infected animals in the context of One Health.

Traditionally, HWD is treated with melarsomine dihydrochloride, the sole registered heartworm adulticide drug (16,17). However, an alternative therapeutic approach, known as the slow-kill protocol, that combines macrocyclic lactones with doxycycline, targeting the bacterial endosymbiont *Wolbachia*, has been used successfully in experimentally and naturally infected dogs, (18–20). That protocol was recently recognized by the American Heartworm Society and the European Society of Dirofilariosis and Angiostrongylosis...
as an alternative strategy when treatment with melarsomine is either unavailable or contraindicated. Compared with the melarsomine treatment, doxycycline (10 mg/kg 2×/d) for 4 weeks combined with monthly administration of a topical formulation of 10% wt/vol imidacloprid and 2.5% wt/vol moxidectin for 9 months proved to be safe and effective for treating HWD in naturally infected dogs and for clearing dogs of circulating microfilariae (20).

Because of its geographic and epidemiologic peculiarities, Linosa Island offered an exceptional opportunity to use this elimination program for HWD. The program involved therapeutically and prophylactically administering the alternative protocol to all infected dogs and administering monthly preventive of 10% wt/vol imidacloprid plus 2.5% wt/vol moxidectin to all remaining uninfected dogs on the island. The study was complemented by an entomologic survey to assess mosquito vectors within this unique epidemiologic context.

The study was approved by the ethics committee of animal experiments of the Department of Veterinary Medicine, University of Bari, Italy (approval number 01/2021). It was conducted according to the VICH GL9 principles of Good Clinical Practice (21).

Methods

Animal Sampling and Diagnosis
In October 2020 (T0), we physically examined all 58 dogs on Linosa Island and recorded signalment and history (e.g., age, sex, breed, clinical signs, and treatments) in individual files. At T0, we collected blood samples from each dog and stored them in two 1-mL K₃EDTA tubes and in one 5-mL tube with clot activator. We used the Knott test (22) to detect and identify circulating microfilariae in whole-blood samples and a duplex real-time quantitative PCR (qPCR) to differentiate Dirofilaria species (23).

We analyzed serum samples for the presence of D. immitis–specific antigens by using the SNAP 4Dx Plus rapid ELISA (IDEXX, https://www.idexx.com). We considered dogs that were positive for D. immitis, either microscopically, serologically, or molecularly, to be infected and assigned them to the treatment group (G1); we assigned dogs that were negative to the prevention group (G2). Before the beginning of treatment, we performed cardiac ultrasonography for all D. immitis–infected dogs to detect adult parasites in the pulmonary arteries. We used an echocardiographic unit (Vivid-iQ; GE Healthcare, https://www.gehealthcare.com) equipped with dedicated multifrequency phased array transducers (6s-RS and M5-RS). In brief, we used the right parasternal long-axis view optimized for the right pulmonary vein and artery (standard view 1) and the right parasternal short-axis view optimized for the right pulmonary artery (standard view 2) to detect worms (24). We did not perform thoracic radiography because no facility was available on the island.

Treatment and Follow-up
D. immitis–infected dogs (G1) received doxycycline (Ronaxan; Boehringer Ingelheim, https://www.boehringer-ingelheim.com) (10 mg/kg orally 2×/d) for 4 weeks plus a monthly application of a spot-on formulation containing 10% wt/vol imidacloprid and 2.5% wt/vol moxidectin (Advocate; Elanco Animal Health, www.elanco.com) for 12 months. Dogs in the G2 group underwent monthly chemoprophylactic treatment with the same spot-on product used for the G1 dogs.

G1 dogs underwent follow-up examination at 1, 2, 3, 6, 9, 12 and 18 months (designated as T1, T2, T3, T6, T9, T12, and T18) after enrollment (Figure 1).

Figure 1. Schematic design of study of elimination of Dirofilaria immitis infection in dogs, Linosa Island, Italy, 2020, and follow-up examinations. G1, infected group; G2, noninfected group; T, time after start of elimination program, in months. Boldface indicates follow-up visits.
Elimination of *Dirofilaria immitis* in Dogs, Italy

At each follow-up visit, we clinically examined dogs and collected blood samples that we analyzed by Knott test (at T1, T2, and T18) or by SNAP 4Dx Plus test (at T1, T2, T3, T6, T9, T12, and T18). We repeated cardiac ultrasonography for G1 dogs at T3, T6, T9, T12, and T18. G2 dogs underwent clinical examination monthly, and we tested them for parasite antigenemia (by SNAP 4Dx Plus test) at T3, T6, T9, T12, and T18 (Figure 1).

Of the 58 dogs examined at T0, we included only 54 in the study because 2 dogs were going to be on the island for only a few weeks/months and the owners of the other 2 dogs did not consent to their study participation. For dogs that were permanently introduced onto the island after the beginning of the study, we performed clinical examination and specific testing (i.e., Knott test and testing for *D. immitis* circulating antigens) with owner consent and allocated them to G1 or G2 according to the test results. We advised owners to immediately report any clinical signs (i.e., cough, hemoptysis, syncope) that might appear during the study and to reduce physical activity for all infected dogs, regardless of symptoms, as much as possible.

**Mosquito Collection**

We collected mosquitoes from 5 locations where cases of *D. immitis* infection in dogs were detected (Figure 2). We trapped mosquitoes daily during October 2020–November 2021, 5:30 p.m.–9:00 a.m., by using 1 light trap per site, set ≈50 cm above the ground. We replaced net bags daily and stored insects in the net bags at −20°C according to collection site/day. After morphologically identifying mosquitoes to the species level (25), we further analyzed female mosquitoes with qPCR to detect and differentiate *Dirofilaria* spp.

**Molecular Diagnosis**

We extracted genomic DNA from samples of dog blood by using the commercial QIAamp DNA Micro Kit (QIAGEN GmbH, https://www.qiagen.com) and samples of mosquito abdomen and thorax by using an in-house method (26). We tested all DNA samples with qPCR by using 2 species-specific primer sets targeting partial cytochrome *c* oxidase subunit 1 for *D. immitis* DNA and the second internal transcribed spacer-2 of nuclear ribosomal DNA for *D. repens* DNA, as previously described (23). We tested all

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**Figure 2.** Linosa Island (Sicily, Italy) indicating the positions of infected dogs, noninfected dogs, and light traps used to capture mosquitoes. Inset shows location of Linosa Island.
DNA samples in duplicate and included positive and negative controls in each qPCR run.

**Results**

We detected *D. immitis* infection in 28 (51.9%) of 54 (33 male, 21 female) enrolled dogs (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/22-1910-App1.pdf). We found circulating microfilariae in 17 (60.7%) infected dogs and specific *D. immitis* antigens in 27 (96.4%). All microfilaremic animals except 1 had positive ELISA antigen test results (Appendix). Microfilariae were morphologically and molecularly identified as *D. immitis*.

At the time of enrollment (T0), we conducted echocardiography on 23 of 28 infected dogs and found *D. immitis* adults within the pulmonary arteries of 20 (Appendix). On T0, we allocated 28 dogs to G1 and 26 to G2. A total of 4 dogs (3 in G1 and 1 in G2) were unavailable for follow-up analysis because of events not associated with treatment (e.g., low owner compliance or neoplastic disease). During the study period, 5 new dogs were permanently introduced onto the island (3 in January 2021 and 2 in March 2021); they tested negative for *D. immitis* infection and were included in group G2.

At T1, the number of microfilaremic dogs was reduced by 76.5%; only 4 of 17 dogs were positive by Knott test. From T2 until the end of the study, no microfilariae were found in G1 dogs. By T1, 3 infected dogs had become antigen negative (no antigen detected) according to ELISA test (Appendix). From T3 on, the number of antigen-positive dogs decreased progressively (20 at T3, 10 at T6, and 2 at T9) (Appendix). All dogs in G1 were negative for *D. immitis* circulating antigens at the 1-year follow-up visit (T12, October 2021).

Cardiac ultrasonography showed filarial parasites in the pulmonary arteries in 14 dogs at T3, 9 dogs at T6, 6 dogs at T9, and 3 dogs at T12 (Appendix). At T18, all G1 dogs scored negative by Knott test and ELISA antigen test, and no parasites were detected by echocardiography. Group 2 dogs tested negative for *D. immitis* antigens at all scheduled follow-up visits.

In this study, treatment with doxycycline and 10% wt/vol imidacloprid plus 2.5% wt/vol moxidectin seemed to be safe, and no adverse events were observed. However, in 1 dog from the G1 group, a thromboembolic-like disorder with hindlimb paralysis and pain was observed a few days after the beginning of treatment. Doxycycline treatment was temporarily suspended, and fluid therapy was promptly administered in association with unfractionated heparin (200 U/kg subcutaneously every 8 h for 3 d) and prednisone (1 mg/kg 2×/d in gradually decreasing doses for 15 d), resulting in full recovery of that G1 animal after 5 days.

Another dog that was apparently healthy at T0 exhibited ascites and diffuse edema of the hindlimbs at the clinical examination conducted on T3. Ultrasonography revealed a high parasite burden within the pulmonary arteries, associated with posthepatic portal hypertension. Treatment with sildenafil (2 mg/kg 3×/d for ≥6 mo) was administered. At the T6 follow-up visit, the dog had improved clinically, although pulmonary hypertension was still present, but pulmonary hypertension was absent at the next follow-up visits.

During this study, we collected 359 mosquitoes (169 male and 190 female) belonging to 6 species, the most abundant of which was *C. pipiens* (Table). Of the 190 females collected, 53 were engorged, and none of the analyzed specimens scored positive to *Dirofilaria* spp. DNA.

**Discussion**

In a short time, *D. immitis* parasites were eliminated from Linosa Island after we combined an alternative adulticide protocol for infected dogs with administration of preventives to noninfected animals. The peculiar epidemiologic context of the island (i.e., geographically confined area, limited number of dogs, and absence of wild reservoirs) aided the campaign. After the HWD focus in southernmost Europe was described (14), we planned and successfully implemented our project. In contrast to programs targeting other mosquitoborne diseases, such as malaria,
this elimination campaign did not target the vector (27) and instead targeted the parasite specifically by eliminating the circulating juvenile forms and adult parasites in infected dogs while simultaneously protecting noninfected animals with chemoprophylaxis.

Treatment with doxycycline and imidacloprid/moxidectin was effective and safe for all of the dogs; side effects were observed in only 2 dogs, probably resulting from thromboembolism after worm death, which is a side effect associated with any adulticide protocol. Nevertheless, the risk for thromboembolism is also linked to patients’ excessive physical activity after treatment, which cannot be ruled out (28). Furthermore, protocols that cause the progressive, slow death of adult parasites expand the temporal window of thromboembolic risk compared with conventional treatment (24). A retrospective study suggests that the risk for thromboembolic events is higher 3 months after the start of the alternative treatment protocol than after start of the melarsomine protocol and that exercise restriction for treated animals is advisable for longer periods or until antigen test results are negative (28,29). Regardless, the alternative protocol has proven to be effective (20,24,30) and may result in fewer adverse events than those resulting from melarsomine alone (31,32) if exercise restriction and other precautions are adequately implemented.

For this study, we chose the combined doxycycline/moxidectin protocol over the conventional adulticide therapy, considering the potential adverse events associated with the sudden death of adult worms causing pulmonary thrombosis (32) and the lack of veterinary services available on the island. On the other hand, the adulticide effects of doxycycline on developing and adult stages of the parasite are slow (33), and macrocyclic lactones are effective against *D. immitis* L3 and L4 and kill only adult parasites after prolonged use (2). Whether the 2 drugs work better together to eliminate *D. immitis* parasites in a short time because of a cumulative or synergistic effect is not known.

Eliminating circulating microfilariae in infected dogs after the beginning of the study (T2) drastically reduced the number of dogs acting as reservoirs on the island and therefore the risk for *D. immitis* infection of mosquitoes. All of the actions contributed to breaking the life cycle of the parasite, a finding that was supported by the fact that none of the examined mosquitoes scored positive for *Dirofilaria* spp. DNA up to November 2021, unlike before the study had started a few months earlier (14).

The protocol that we used led to progressive reduction of detectable antigens in treated animals from T3 to T9 and to dogs testing negative at T12. This finding is similar or even better than what was recorded in previous studies that used the same protocol (20,34,35). Also, the conversion to the antigen-negative status observed was faster than that recorded with ivermectin alone (24,36) or with ivermectin (every 2 weeks for 6 months) and doxycycline (10 mg/kg/d orally for 30 d) in which only 73% or 80% of treated dogs reached antigen-negative status (30,31).

The use of macrocyclic lactones as adulticides has been discouraged by several authors because it may take many months for adult worms to die, enabling the disease to progress and allowing for the potential selection of macrocyclic lactone–resistant strains (37). Moreover, potential interference with antigen test results has also been suggested (37). Several studies have reported false-negative antigen test results in dogs and cats treated with macrocylic lactones, presumably resulting from formation of immune complexes and the consequent binding of the antigens (37–39). To overcome this problem, 2 consecutive negative antigen test results 6 months apart have been suggested as a valid criterion for considering a dog cleared of infection (34). Therefore, in this elimination program, all dogs were probably cured of infection because in no dog was antigen detected at T12 or 6 months after (T18). This finding is also strongly supported by ultrasonography in which no adult heartworms were observed at the T18 follow-up visit. Our data may also be useful in where *D. immitis* parasites are endemic and melarsomine is not commercially available (e.g., Brazil, eastern Europe).

Despite the presence of domestic and feral cats on Linosa Island (40), our study did not include those hosts because of lack of owner compliance and the difficulty of sampling these animals in a remote environment. However, considering the occurrence and possible role of cats as reservoirs of *D. immitis* parasites (41), further studies evaluating the prevalence of this parasite in cat populations on Linosa Island are warranted.

Considering the zoonotic potential of *D. immitis* worms, elimination of HWD in dogs is pivotal for reducing the risk for human infection. Indeed, in previous surveys conducted on the human population from Linosa Island, up to 7.9% of islanders were positive for circulating *D. immitis* antibodies (15), which represented one of the highest percentages of human exposure ever reported. Therefore, results from our study suggest that adulticide treatment of all infected dogs, coupled with prevention for noninfected dogs, not only protects animals from HWD but may also potentially reduce the risk for human infection. Such an
approach represents a paradigm for the One Health concept. Continuous monitoring and chemoprophylaxis of the canine population on the island and of all newly introduced dogs are highly recommended to maintain the status of HWD-free area.

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References


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Many serious human pathogens result from zoonotic transmission, including 61% of known human pathogens and 75% of emerging human pathogens (1). For example, rabies virus is transmitted by saliva of infected animals (2). The plague bacteria (*Yersina pestis*), the causative agent of the largest documented pandemic in human history that reduced the population of Europe by 30%–50%, was transmitted from rats to humans by fleas (3). Other zoonoses include Ebola virus (4), tularemia (*Francisella tularensis*) (5), and tuberculosis (6). The SARS-CoV-2 pandemic, thought to have a bat reservoir, has stimulated renewed emphasis on zoonotic pathogen surveillance (7,8).

Natural history museums are repositories of biologic information in the form of voucher specimens that represent a major, underused resource for studying zoonotic pathogens (9–13). Originally, specimens were archived as dried skin and skeletal vouchers or preserved in fluids (ethanol) after fixation with formalin or formaldehyde. Now, best practices include preserving specimens and associated soft tissues in liquid nitrogen (−190°C) or mechanical freezers (−80°C) from the time they are collected (14). Those advances in preservation make it possible to extract high-quality DNA and RNA that can be used for pathogen surveillance. For example, retroactive sampling of archived tissues from the US Southwest found that Sin Nombre virus, a New World hantavirus, was circulating in wild rodent populations almost 20 years before the first human cases were reported (15).

It is critical to develop a range of tools for extracting pathogen information from museum-archived samples. Targeted sequencing using probe enrichment has become the tool of choice for medical genomics (16), population genetics (17), phylogenetics (18), and ancient DNA (19,20). Those methods are designed to enrich small amounts of DNA target from a background of contaminating DNA. Probe-based, targeted sequencing has been used to enrich pathogens from complex host–pathogen DNA mixtures (21). For example, Keller et al. used probes to capture and sequence complete *Y. pestis* genomes from burial sites >1,500 years old (22). Enrichment is frequently achieved by designing a panel of probes to specifically target a handful of pathogens of interest (23,24). Similarly, commercial probe sets are available for many types of viruses and human pathogens (23–25). However, many of these probe sets are limited to specific pathogens that might not infect other host species.

Our goal was to develop a panel of biotinylated baits, or probes, to identify the eukaryotic and bacterial pathogens responsible for 32 major zoonoses (Table 1). We aimed to capture both known and related pathogens, using the fact that probes can capture sequences that are ≤10% divergent. To perform this

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capture, we used a modified version of the ultracon-
erved element (UCE) targeted sequencing technique
(26,27) to specifically enrich pathogen DNA. Biotiny-
ated baits are designed to target conserved genomic
regions among diverse groups of pathogens (Figure
1). The baits are hybridized to a library potentially
containing pathogen DNA. Bait-bound DNA frag-
ments are enriched during a magnetic bead purifica-
tion step before sequencing (Figure 2). The final li-
brary contains hundreds or thousands of orthologous
loci with single-nucleotide variants or indels from the
targeted pathogen groups that can then be used for
population or phylogenetic analyses.

Methods
We have compiled a detailed description of the meth-
ods used (Appendix 1, https://wwwnc.cdc.gov/
org/10.17504/ protocols.io.5jyl8znrg2w/v1). Code is
available on GitHub (https://www.github.com/neal-
org/10.17504/ protocols.io.5jyl8jnzrg2w/v1). A summary of our methods follows.

Panel Development
We developed a panel of baits for targeted sequenc-
ing of 32 zoonotic pathogens. To develop this pan-
el, we used the Phyluce version 1.7.1 (26,27) proto-
col to design baits for conserved loci within each
pathogen group. First, we simulated and mapped
reads from each species within a pathogen group
to a focal genome assembly (Table 1; Figure 1, panel A). We used the mapped reads to identify
putative orthologous loci that were >80% simi-
lar across the group and generated in silico baits
from the focal genome (Figure 1, panel B). These
baits were mapped back to each member (Figure
1, panel C) to identify single-copy orthologs within
the group. Next, we designed 2 overlapping 80-bp
baits from loci in each member of the group (Figure
1, panel D) and removed baits with >95% sequence
similarity (Figure 1, panel E). We repeated those
steps for each pathogen group (Figure 1, panel F). We compared the remaining baits with mam-
nalian genomes and replaced them to minimize

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<td>Genus</td>
<td>Listeria monocytogenes</td>
<td>Listeriosis</td>
</tr>
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<td>Genus</td>
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<tr>
<td>Nematodes (clade IVb)</td>
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</tr>
<tr>
<td>Nematodes (clade V)</td>
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<td>None</td>
</tr>
<tr>
<td>Onygenales</td>
<td>Order</td>
<td>Histoplasma capsulatum</td>
<td>Histoplasmosis</td>
</tr>
<tr>
<td>Pasteurella</td>
<td>Genus</td>
<td>Pasteurella multocida</td>
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<td>Genus</td>
<td>Rickettsia rickettsi</td>
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<td>Salmonella</td>
<td>Genus</td>
<td>Salmonella enterica</td>
<td>Salmonellosis</td>
</tr>
<tr>
<td>Streptobacillus</td>
<td>Genus</td>
<td>Streptobacillus moniliformis</td>
<td>Rat-bite fever</td>
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<tr>
<td>Trematoda</td>
<td>Class</td>
<td>Schistosoma mansoni</td>
<td>Schistosomiasis</td>
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<tr>
<td>Tremellales</td>
<td>Order</td>
<td>Cryptococcus neoformans</td>
<td>Cryptococcosis</td>
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<tr>
<td>Trypanosoma*</td>
<td>Genus</td>
<td>Trypanosoma cruzi</td>
<td>Sleeping sickness</td>
</tr>
<tr>
<td>Yersinia</td>
<td>Genus</td>
<td>Yersinia pestis</td>
<td>Plague</td>
</tr>
</tbody>
</table>

*Supplemented with additional probes/baits.
cross-reactivity with the host. Finally, we combined baits to capture 49 loci from each pathogen group into a panel that was synthesized by Daicel Arbor Biosciences (https://arborbiosci.com).

Museum-Archived and Control Samples
We extracted DNA from 38 museum samples by using the DNeasy Kit (QIAGEN, https://www.qiagen.com) (Table 2). We generated control samples...
by spiking naive mouse DNA with 1% microorganism DNA from *Mycobacterium bovis*, *M. tuberculosis*, *Plasmodium vivax*, *P. falciparum*, and *Schistosoma mansoni*. We then further diluted an aliquot of this 1% pathogen mixture into mouse DNA to create a 0.001% host-pathogen mixture. This range was designed to test the lower limits of detection but also represent a reasonable host-pathogen proportion. For example, *Theileria parva*, a tick-transmitted apicomplexan, is present in samples from 0.9% through 3% (28), and 1.5% of DNA sequence reads in clinical blood samples is from *P. vivax* (29).

**Library Preparation**

We generated standard DNA sequencing libraries from 500 ng of DNA per sample. We combined

<table>
<thead>
<tr>
<th>Table 2. Specimens examined using targeted sequencing in study of prospecting for zoonotic pathogens by using targeted DNA enrichment*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Museum accession no.</strong></td>
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</tr>
<tr>
<td>TK49674</td>
</tr>
<tr>
<td>TK49686</td>
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<td>TK57832</td>
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<td>TK70836</td>
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<tr>
<td>TK90542</td>
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<td>TK93223</td>
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<td>TK93289</td>
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<td>TK93402</td>
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<td>TK101275</td>
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</tr>
<tr>
<td>TK197046</td>
</tr>
<tr>
<td>TK199855</td>
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</table>

*ID, identification; SRA, National Center for Biotechnology Information Sequence Read Archive.*
individual libraries with similar DNA concentrations into pools of 4 samples and used the myBaits version 5 (Daicel Arbor Biosciences) high sensitivity protocol to enrich target loci. We used 2 rounds of enrichment (24 h at 65°C), washed away unbound DNA, and amplified the remainder for 15 cycles before pooling for sequencing.

Classifying Reads
First, we generated a dataset of target loci by mapping the probes to representative and reference genomes in RefSeq v212 with BBMap v38.96 (30). For each probe, we kept the 10 best sites that mapped with ≥85% sequence identity along with 1,000 bp upstream and downstream. These sequences were combined into a database to classify reads by using Kraken2 version 2.1.1 (31) (Figure 3, panel A). Next, we extracted pathogen reads with KrakenTools version 1.2 (https://github.com/jenniferlu717/KrakenTools). We assembled those reads (Figure 3, panel B) with the SPAdes genome assembler version 3.14.1 (32) and filtered them to remove low quality contigs (<100 bp and <10× median coverage). We removed samples that had <2 contigs from downstream analyses. During this time, we extracted target loci in available reference genomes (Figure 3, panel C). Next, we identified (Figure 3, panel D), aligned and trimmed (Figure 3, panel E) orthologs before concatenating them into a single alignment (Figure 3, panel F). Finally, we generated and bootstrapped a phylogenetic tree (Figure 3, panel G) by using RaxML-NG version 1.0.1 (33). We repeated those steps for each pathogen group (Figure 3, panel H).

Host Identification
There were sufficient mtDNA sequences from most samples to verify museum identifications by comparing reads to a Kraken2 version 2.1.2 (31) database of mammalian mitochondrial genomes. We filtered the classifications by removing samples with <50 classified reads and single-read, generic classifications.

Results
Panel Development
We used the ultraconserved element protocol developed by Faircloth et al. (26, 27) to develop a set of 39,893 biotinylated baits that target 32 pathogen groups responsible for 32 zoonoses. Each pathogen group is targeted at 49 loci with a few diverse taxa, *Bacillus cereus* and *Trypanosoma* species, targeted at 98 loci. We complied information on pathogen groups, focal taxa, genome accessions, and number of baits (Table 3).

Control Samples
We tested the efficacy of our bait set on laboratory-made host–pathogen mixtures containing DNA from...
Mycobacterium reads (tabase of target loci and found that 42.7% of all 1% or 0.001% pathogen DNA that was enriched or
We generated 4 control samples containing either
Schistosoma, Plasmodium falciparum, Mus musculus,
Borrelia, Campylobacter, Campylobacter jejuni
Cestoda, Chlamydia, Chlamydia trachomatis
Coxiella, Campylobacter, Coxielia burnetii

Prospecting Pathogens by Targeted DNA Enrichment

Table 3. Summary of probes developed for targeted capture of pathogen DNA in study of prospecting for zoonotic pathogens by using targeted DNA enrichment

<table>
<thead>
<tr>
<th>Pathogen group</th>
<th>Type</th>
<th>Probe count</th>
<th>Locus count</th>
<th>RefSeq genome count</th>
<th>Focal pathogen</th>
<th>GenBank accession no.</th>
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<tbody>
<tr>
<td>Anaplasma</td>
<td>Bacteria</td>
<td>368</td>
<td>49</td>
<td>57</td>
<td>Anaplasma phagocytophilum</td>
<td>GCF000013125</td>
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<tr>
<td>Apicomplexa</td>
<td>Eukaryote</td>
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<td>49</td>
<td>64</td>
<td>Plasmodium falciparum</td>
<td>GCA000002765</td>
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<tr>
<td>Bacillus cereus group*</td>
<td>Bacteria</td>
<td>833</td>
<td>98</td>
<td>134</td>
<td>Baclillus anthracis</td>
<td>GCA000008165</td>
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<td>Bartonella</td>
<td>Bacteria</td>
<td>1,812</td>
<td>49</td>
<td>31</td>
<td>Bartonella bacilliformis</td>
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<td>Borrelia</td>
<td>Bacteria</td>
<td>688</td>
<td>49</td>
<td>16</td>
<td>Borrellela burgdorferi</td>
<td>GCF00902145</td>
</tr>
<tr>
<td>Burkholderia</td>
<td>Bacteria</td>
<td>683</td>
<td>49</td>
<td>39</td>
<td>Burkholderia mallei</td>
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<td>Bacteria</td>
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<td>33</td>
<td>Campylobacter jejuni</td>
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<tr>
<td>Cestoda</td>
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<td>49</td>
<td>13</td>
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<td>49</td>
<td>25</td>
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<tr>
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<td>49</td>
<td>38</td>
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<td>49</td>
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<td>1,999</td>
<td>49</td>
<td>26</td>
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<td>617</td>
<td>97</td>
<td>10</td>
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<td>GCF000209065</td>
</tr>
<tr>
<td>Yersinia</td>
<td>Bacteria</td>
<td>225</td>
<td>49</td>
<td>22</td>
<td>Yersinia pestis</td>
<td>GCF000009065</td>
</tr>
</tbody>
</table>

*Supplemented.

Mus musculus, Mycobacterium tuberculosis, Plasmodium falciparum, P. vivax, and Schistosoma mansoni. We generated 4 control samples containing either 1% or 0.001% pathogen DNA that was enriched or not enriched. We classified reads against the database of target loci and found that 42.7% of all reads (Mycobacterium = 13.1%, Plasmodium = 28.1%, Schistosoma = 1.5%) were from control pathogens in the 1% enriched control sample. However, only 0.03% of the corresponding 1% unenriched control was from target loci. Aside from the raw percentages, we compared the coverage of each probed region in the 1% enriched and unenriched control samples (Figure 4, panels B–D) to understand how enrichment affected coverage at each locus. Mean coverage per Mycobacterium locus increased from 0.14× to 944.5× (6,746-fold enrichment), 0.53× to 1,527.4× for Plasmodium loci (2,882-fold enrichment), and 0.02× to 117.9× (5,895-fold enrichment) for schistosome loci. Because the sequencing library from the 0.001% unenriched sample did not work during the sequencing reaction, we do not have a baseline to examine enrichment in the 0.001% samples.

We extracted reads assigned to each pathogen group and assembled and aligned them with target loci extracted from reference genomes of closely related species by using tools from Phyluce version 1.7.1 (26,27). We were able to assemble 0–23 target loci per pathogen group in the control samples (Table 4). Assembled loci varied in size from 109 to 1,991 bp (median 636.5 bp). For each sample/group with >2 loci captured, we generated a phylogenetic tree along with other members of the taxonomic group (Figure 5). In each case, pathogen loci from the control samples were sister groups to the appropriate reference genome with strong bootstrap support. For example, the Schistosoma loci assembled from the 1% enriched control sample were sister to the S. mansoni genome (GCA000237925) in 100% of bootstrap replicates.

Museum Samples

Next, we tested our bait set on museum-archived tissues. We generated 649.3 million reads across all 38 samples (mean 17.1 million reads/sample). An initial classification showed that, on average, 4.3% of reads
were assignable to loci in the database. Those reads were designated to 93 genera. However, 78 of those genera were at low frequency (≤1,000 reads/sample) (Figure 4). Many of the low frequency hits are likely the result of bioinformatic noise. Bartonella and Plasmodium species were the most common genera; each was present in 36 of 38 museum samples. The distribution of Bartonella reads was strongly bimodal.

Figure 4. Identifying pathogen reads from controls and museum-archived tissue samples for study of prospecting for zoonotic pathogens by using targeted DNA enrichment. Control reads are indicated by the percentage of pathogen DNA 1% or 0.001%. A) Reads were compared with a database of target loci and assigned a taxonomic classification based on these results. Reads were assigned to 93 genera; of those, 17 (shown) were present in >1 sample, including controls, with ≥1,000 reads. A heatmap of those results shows the relative proportion of reads assigned to each genus. Details of samples are provided in Table 2. B–D) Coverage at each probed locus is shown across all control samples for Mycobacterium (B), Plasmodium (C), and Schistosoma (D). Each point in the chart is coverage calculated at a single target locus. Horizontal lines within boxes indicate medians, box tops and bottoms indicate lower and upper quartiles, and whiskers represent minimum and maximum values, excluding outliers. Each sample is indicated with a circle. E, enriched.
such that 18 samples had <12 reads and 18 samples had >1,000 reads (median 552 reads/sample). In 5 samples, the percentage of Bartonella reads was exceedingly high (>10%). In comparison, the median number of Plasmodium reads never exceeded 0.04% of reads from a single museum sample (mean 158.5 reads/sample).

We used phylogenetic analyses and rules of monophyly to identify putative pathogens to species or strain for each of the 15 genera with >1,000 reads (Figure 4, panel A). We were unable to assemble >1 target locus for any specimen in 13 genera. We were able to assemble 3–20 loci (mean 8 loci/sample) from 16 samples containing Bartonella (Figure 6), 3 loci from a sample containing Paraburkholderia reads (Figure 7), and 8 loci from a sample containing Ralstonia reads (Figure 8).

Host Identification
We compared reads from each sample to a database of mitochondrial genomes to identify the host. In general, reads from the mitochondria comprised a small proportion (<1%, mean 0.04%) of each sample (Figure 9). Despite the low number of mitochondrial reads, generic classifications from the mitochondrial database coincided with the museum identifications after filtering samples with ≤50 mitochondrial reads. For the remaining samples, the correct genus was identified by >85% (mean 98%) of reads from that sample. Classifying reads less than the generic level is limited by mitochondrial genome availability, but where possible, we were able to confirm museum identifications at the species level.

Discussion
We developed a set of 39,893 biotinylated baits for targeted sequencing of >32 zoonotic pathogens, and their relatives, from host DNA samples. To test the efficacy of the bait panel, we used 4 control samples that contained either 1% or 0.001% pathogen DNA and further subdivided into pools that were enriched and unenriched. Our results (Figure 4) showed a

### Table 4. Parasite reads identified in and loci assembled from control samples

<table>
<thead>
<tr>
<th>Enriched Pathogen concentration, %</th>
<th>Total reads</th>
<th>Schistosoma Reads</th>
<th>Plasmodium Reads</th>
<th>Mycobacterium Reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>True 0.001</td>
<td>509,672</td>
<td>3</td>
<td>168</td>
<td>556</td>
</tr>
<tr>
<td>True 1</td>
<td>398,469</td>
<td>587</td>
<td>52,274</td>
<td>112,141</td>
</tr>
<tr>
<td>False 1</td>
<td>375,786</td>
<td>15</td>
<td>17</td>
<td>83</td>
</tr>
</tbody>
</table>

Figure 5. Phylogenetic analysis of pathogens used in control samples for study of prospecting for zoonotic pathogens by using targeted DNA enrichment. A) Schistosoma; B) Plasmodium; C) Mycobacterium. Reads from each control pathogen (M. tuberculosis, P. falciparum, P. vivax, and S. mansoni) were extracted, assembled, aligned, and trimmed for maximum-likelihood phylogenetic analyses. The phylogenies were used to identify the species or strain of pathogen used in the controls. Blue indicates control samples. Bootstrap support values are indicated by colored diamonds at each available node. Branches with <50% bootstrap support were collapsed. Nodal support is indicated by color coded diamonds. Scale bars indicate nucleotide substitutions per site. Assembly accession numbers (e.g., GCA902374465) and tree files are available from https://doi.org/10.5281/zenodo.8014941.
large increase of pathogen DNA in the 1% enriched sample when compared with its unenriched counterpart. Specifically, enrichment increased the amount of pathogen DNA from 0.03% to 42.1%.

We were able to generate phylogenetically informative loci from *P. falciparum* in 0.001% enriched sample, which would hypothetically contain ≈39 genome copies. This finding implies that the bait set might be capable of identifying pathogens present in samples with only a few hundred genome copies. However, there are limitations to *Plasmodium* detection that should be considered.

In each sample, reads were detected from only a few loci rather than from the entire genome. For example, in the 1% enriched sample, 5,879 of the 398,469 reads came from 32 loci totaling 19.6 kb. Had the unenriched sample contained the same number of reads, randomly distributed across the genome, it would have amounted to 1 read every 62 kb. We found that enrichment increased coverage at probed loci from 0.23× to 863.3×, a 3,732.3-fold increase when averaged across all pathogens/loci (Figure 4). Those results show that although large amounts of host DNA might remain in a sample, the targeted loci are greatly enriched.

We tested the panel of baits on 38, museum-archived, small mammal samples without previous knowledge of infection history. Reads from these samples were initially designated to 93 different genera, but most of these genera contained a limited number of reads. For example, almost half of the 93 genera (n = 43) were identified on the basis of a single read across all 38 samples, most likely a bioinformatic artifact. We identified 15 genera in which 1 sample had >1,000 reads. For each of these 15 genera, we extracted any reads classified within the same family (e.g., genus *Bartonella*, family *Bartonellaceae*) and assembled, aligned, and trimmed them for phylogenetic analyses. In most cases, the reads failed the assembly step (n = 6), were filtered on the basis of locus size or coverage (n = 5), or assembled into multiple loci that were not targeted by our bait set (n = 2); we did not pursue those reads any further. However, we were able to generate phylogenies for specimens positive for *Bartonella*, *Ralstonia*, and *Paraburkholderia* species. *Bartonella* is a bacterial genus responsible for cat-scratch disease, Carrière’s disease, and trench fever (34). Transmission often occurs between humans and their pets or from infected fleas ticks, or other arthropod vectors (35). We were able to recover target loci for 14 of 36 specimens. A phylogeny of *Bartonella* species placed the museum samples in multiple clades (Figure 6). For example, 5 specimens formed a monophyletic clade sister to *B. mastomydis*. *B. mastomydis* recently was described from *Mastomys erythroleucus* mice collected in Senegal (36). Appropriately, the

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Figure 6. Phylogenetic analysis of *Bartonella* using museum archived samples in study of prospecting for zoonotic pathogens by using targeted DNA enrichment. Blue indicates museum archived samples; museum accession numbers are given (Table 1). Branches with <50% bootstrap support were collapsed. Nodal support is indicated by color coded diamonds. Scale bar indicates nucleotide substitutions per site. Assembly accession numbers (e.g., CA902374465) and tree files are available from https://doi.org/10.5281/zenodo.8014941.
samples we tested were collected from *M. natalensis* mice from Botswana (Table 2). Another clade contained *B. vinsonii* and a *Sigmodon* rat (TK90542) collected in Mexico. Zoonotic transmission of *B. vinsonii* has been implicated in neurologic disorders (37). Other museum samples probably contain novel *Bartonella* species/strains or at least represent species/strains without genomic references.

*Paraburkholderia* is a genus of bacteria commonly associated with soil microbiomes and plant tissues. We identified *Paraburkholderia* reads in 3 specimens and were able to place 1 of those in a phylogeny sister to a clade containing *P. fungorum* and *P. insulsa*. Because bootstrap values across the phylogeny were moderate in general, and weak in this particular region (Figure 7), placement of this sample is tenuous. *P. fungorum* is the sole member of *Paraburkholderia* believed to be capable of infecting humans, but it is only a rare, opportunistic, human pathogen (38–40).

*Ralstonia* is a bacterial genus closely related to the genus *Pseudomonas*. We identified *Ralstonia* reads in 5 samples and were able to place a specimen on a phylogeny. This sample is closely affiliated with *R. pickettii* (Figure 8). We are unaware of any examples of zoonotic transmission of *R. pickettii*. Rather, *R. pickettii* has been identified as a common contaminant in laboratory reagents (41), and outbreaks have been caused by contaminated medical supplies (42). We failed to identify nucleic acids in any of our negative controls during library preparation. Furthermore, if there were systemic contamination, we would expect to find *Ralstonia* species in all of our samples, rather than the 5 of 36 observed. Thus, because we cannot rule out reagent contamination, the presence of *Ralstonia* species in the museum samples should be interpreted with caution.

We were able to capture, sequence, and assemble loci from taxa that were not represented in the databases used to design the bait panel. This ability was possible for 2 reasons. First, the bait panel is highly redundant. The baits are sticky and able to capture
nucleic acid fragments that are ≤10%–12% divergent (43). We designed the panel with ≤5% sequence divergence between any pair of baits at a particular locus (Figure 10). Second, sampled loci within each pathogen group spanned a range of divergences. Conserved loci were more likely to catch more divergent species that might not have been present in our initial dataset. For example, we recovered multiple species of Bartonella that were not present in our probe set, for which related genomes were available. However, for Ralstonia and Paraburkholderia species, we identified these samples from reads targeted by probes for the genus Burkholderia, a pathogenic taxon in the same family (Burkholderacea). The ability to identify taxa at these distances is because of the more conserved loci targeted by the bait panel.

During the initial read classification stage, we identified low levels of Plasmodium species in all but 2 museum samples, which was unexpected. Museum samples contained ≤3,221 Plasmodium reads/sample (mean 428.3 reads/sample), but we were unable to assemble them into loci for phylogenetic analyses. This limitation effectively removed those samples from downstream analyses. The P. falciparum genome is extremely AT rich (82%, 44), which might result in bioinformatic false-positive results. We suspect that AT-rich, low-complexity regions of the host genome are misclassified as parasite reads. To test this hypothesis, we used fqtrim 0.9.7 (https://ccb.jhu.edu/software/fqtrim) to identify and remove low-complexity sequences within those reads. This filter by itself reduced the number of Plasmodium reads in the museum samples by 75.5% (maximum 298 reads, mean 57.2 reads). In comparison, only 8.2% of reads from 0.001% enriched control samples and 0.2% of reads from 1% enriched control samples were removed.

Several technical issues still need to be addressed. First, enrichment increases the targeted...
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loci coverage by 3 orders of magnitude. However, the amount of host DNA remaining in each sample is still high. Ideally, host DNA would be rare or absent. Second, the bait panel requires relatively large up-front costs. Third, although the bait panel is developed to target a wide range of taxa, it is not possible to know which species are missed. The best way to circumvent that issue is to use controls spiked with various pathogens of interest, similar to how mock communities are used in other metagenomic studies (45). Those mock controls are commercially available for bacterial communities (e.g., ZymoBIOMICS Microbial Community Standards; Zymo Research, http://www.zymoresearch.com), but we have been unable to find similar products that contain eukaryotic pathogens. Solutions to those problems will make targeted sequencing with bait panels a viable tool for pathogen surveillance. Fourth, the sensitivity of the probes will depend on the sequence divergence between the probes and pathogen DNA. The more diverged the 2 are, the less efficient the capture will be. This limitation indicates that pathogen groups that have biased or limited genomic data will be less likely to capture off-target species once divergence increases by >5%–10%. Finally, the current probe panel is capable of capturing and identifying pathogens if there are ≥3,000 genome copies in the sample. Sensitivity needs to be improved in future iterations of the panel. One method could be to target pathogen-specific, repetitive sequences (46). Because those sequences are already present in the genome hundreds to thousands of times, it should be possible to greatly increase the sensitivity of the probe panel.

Although further effort is required to resolve these issues, we believe that enrichment of pathogen DNA from museum tissue samples is a viable tool worth further development. In its current form, enrichment represents a coarse tool that can be used to scan for various pathogens from archived tissues. More refined tests, such as quantitative PCR and targeted sequencing, can be used to answer taxon-specific questions. Target enrichment will be necessary for maximizing the pathogen data that are available from the hundreds of thousands of museum-archived tissues and will play a critical role in understanding our susceptibility to future zoonotic outbreaks.

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References

Figure 10. Sequence identity between enriched reads and baits in the probe panel used for targeting zoonotic pathogens in study of prospecting for zoonotic pathogens by using targeted DNA enrichment. Reads from each sample were classified against a database of target loci. Sequence identity between pathogen-derived reads and the most similar bait in the bait panel for all pathogens excluding Bartonella species (A) and for only Bartonella species (B). Bartonella was the most common pathogen in our samples, and the number of reads was biased toward a few individuals.


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Public health surveillance of COVID-19 activity has expanded from monitoring of persons with laboratory-confirmed SARS-CoV-2 infection to including wastewater surveillance. Studies conducted in early 2020 provided proof of concept that wastewater surveillance of SARS-CoV-2 can be used to determine prevalence of COVID-19 in several countries (1–3). Other studies have since shown the viability of that surveillance indicator (4–7) with varying success when biomarkers such as pepper mild mottle virus (PMMoV) and crAssphage were used to normalize the measurements of SARS-CoV-2 in fecal matter in samples (4,8,9). Wastewater surveillance indicators become especially relevant when PCR testing eligibility changed or when clinical testing capacity was overwhelmed, resulting in an incomplete picture of local COVID-19 activity.

The Regional Municipality of Peel in Ontario, Canada (hereafter referred to as Peel) serves 1.5 million residents of the cities/towns of Brampton, Caledon, and Mississauga in Ontario, Canada (10). As of July 16, 2022, the COVID-19 incidence rate in Peel was one of the highest in Ontario; cumulative incidence was 12,098 laboratory-confirmed COVID-19 cases per 100,000 Peel residents, compared with 9,164/100,000 Ontario residents (11). Since April 2020, Peel has sampled untreated wastewater from its 2 wastewater treatment plants (WWTPs) and tested it for SARS-CoV-2. Peel’s WWTPs, serving ≈96% of the region’s residential postal codes, are Clarkson (population served 643,331) and G.E. Booth (population served 1,089,738).

On December 30, 2021, as the Omicron BA.1 variant surged in Ontario, the province restricted clinical PCR testing (which had previously been available to any symptomatic person or close contact of a COVID-19 case-patient) to groups at greatest risk, including hospitalized patients, patient-facing health-care workers, and those with occupational exposure. Surveillance data provided information for local decision-making and are a useful component of COVID-19 surveillance systems.

We determined correlations between SARS-CoV-2 load in untreated water and COVID-19 cases and patient hospitalizations before the Omicron variant (September 2020–November 2021) at 2 wastewater treatment plants in the Regional Municipality of Peel, Ontario, Canada. Using pre-Omicron correlations, we estimated incident COVID-19 cases during Omicron outbreaks (November 2021–June 2022). The strongest correlation between wastewater SARS-CoV-2 load and COVID-19 cases occurred 1 day after sampling (r = 0.911). The strongest correlation between wastewater load and COVID-19 patient hospitalizations occurred 4 days after sampling (r = 0.819). At the peak of the Omicron BA.2 outbreak in April 2022, reported COVID-19 cases were underestimated 19-fold because of changes in clinical testing. Wastewater data provided information for local decision-making and are a useful component of COVID-19 surveillance systems.

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care workers, and staff and residents in hospitals and congregate living settings (T2). This policy change, the only one to substantially affect the number of completed tests during our study period, resulted in a shift in Peel’s COVID-19 surveillance strategy. Wastewater surveillance of SARS-CoV-2 became a requisite tool for monitoring community-level COVID-19 activity and was used as a key indicator to provide information for local public health decision-making and communication (T3).

We report correlations between clinical COVID-19 indicators (reported cases and hospitalizations) and SARS-CoV-2 load in untreated wastewater at various lags across COVID-19 pandemic waves 2–6 in Peel during August 2020–June 2022. We also estimated the number of incident COVID-19 cases in Peel, on the basis of SARS-CoV-2 load in wastewater before the clinical PCR testing policy change, during the Omicron outbreaks when PCR testing was restricted. Last, we assessed the usefulness of normalizing SARS-CoV-2 concentrations to PMMoV.

**Methods**

**Wastewater Sampling and SARS-CoV-2 RNA Measurement**

We sampled wastewater from G.E. Booth and Clarkson WWTPs, located in Mississauga, 3–5 weekdays per week, according to the needs of Peel Public Health and the recommendations of the US Centers for Disease Control and Prevention (T4) (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/8/22-1580-App1.pdf). We obtained daily flow rates (m³/day) for each WWTP. In total, we included 356 samples from G.E. Booth and 359 samples from Clarkson in this study.

We collected 24-hour composite samples of untreated wastewater influent, before any screening or grit removal, by using Hach model AS950 automatic samplers (https://www.hach.com) and stored them in high-density polyethylene containers. We kept containers at 4°C and transported them to the University of Waterloo (Waterloo, ON, Canada) for extraction and quantification of SARS-CoV-2 RNA. Sample analysis and reporting occurred within 2 weeks of collection; most samples were analyzed within the same week.

We used a polyethylene glycol precipitation method (2) for each wastewater sample as follows: we added 40 mL of sample to a 50-mL centrifuge tube with polyethylene glycol (4 g) and NaCl (0.9 g) and spiked a surrogate (e.g., human coronavirus 229E or murine hepatitis virus) into the sample. The sample was shaken on ice for 2 h and left to settle at 4°C overnight. We then centrifuged the sample at 12,000 × g for 1.5 h to concentrate the virus into the solids with the supernatant discarded. We extracted SARS-CoV-2 RNA and purified it from the solids by using either TRIzol reagent (Invitrogen, https://www.thermofisher.com) or Power Microbiome Kit (QIAGEN, https://www.qiagen.com), following the manufacturer’s protocol, with up to 250 mg (wet weight) of the pellet resuspended in either TRIzol or TriZOL/PM1 solution. We eluted the RNA in 100 μL nuclease-free water. Extracted RNA then underwent 1-step quantitative reverse transcription PCR for SARS-CoV-2 (N1, N2 gene targets [T5]) and PMMoV (T6) (Appendix).

**COVID-19 Case-Patient, Hospitalization, and Testing Data**

We extracted nonnominal data for patients who met the provincial case definition of having confirmed or probable COVID-19 (T7). Data fields included residential postal code and episode date (earliest date of symptom onset, specimen collection, or date reported). Case-patient data were restricted to persons who had a permanent residential address in Peel at the time of COVID-19 diagnosis and who experienced episodes from August 30, 2020, through June 18, 2022 (n = 185,895). We classified case-patients by sewershed—G.E. Booth, Clarkson, septic system, or unknown—on the basis of residential postal code matched to the 2019 Postal Code Conversion File (https://www.canadapost-postescanada.ca/cpc/doc/en/marketing/postal-code-conversion-file-reference-guide.pdf), which we spatially joined with the Peel sewershed geographic boundary file. We validated postal codes that did not match with the Postal Code Conversion File by using the Canada Post Find a Postal Code web tool (https://www.canadapost-postescanada.ca/info/mc/personal/postalcode/fpc.jsf). We then aggregated case-patients by episode date and sewershed. Among 185,895 COVID-19 case-patients, 1.5% were not matched to either WWTP sewershed, 0.9% were associated with septic systems, and 0.6% were unable to be matched to the geographic boundary file.

Patient hospitalization information, obtained from COVID-19 case follow-up, is underreported in the Ontario Ministry of Health’s COVID-19 case registry, Case and Contact Management Solution. Therefore, we acquired aggregate COVID-19 patient hospitalization data from the Ontario Ministry of Health’s Daily Bed Census, for August 30, 2020, through June
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18, 2022 (https://data.ontario.ca/dataset/bed-census-summary-bcs). We extracted the daily number of acute care admissions among laboratory-confirmed COVID-19 patients, regardless of patient residence or reason for admission, to the 3 acute hospitals located in Peel (Trillium Health Partners [Credit Valley Hospital and Mississauga Hospital] and William Osler Health System [Brampton Civic Hospital]). To describe SARS-CoV-2 clinical testing trends during the study period, we extracted completed PCR tests for Peel residents from the Ontario Ministry of Health Ontario Laboratory Information System, by week of specimen collection.

Data Processing
We categorized data from August 30, 2020, through June 18, 2022, by epidemic wave, each characterized by the dominance of the wild-type or a variant of SARS-CoV-2. We reported SARS-CoV-2 N-gene values as the mean concentration (copies/mL) of the N1 and N2 gene targets for each sampling date and WWTP. We retained in the dataset mean concentration values below the limit of detection (0.5 copies/mL) or limit of quantification (3.5 copies/mL) as reported from the quantitative reverse transcription PCR analysis. Normalized data were presented as the mean concentration of N1 and N2 divided by the concentration of PMMoV. We calculated daily load per WWTP by multiplying the flow rate by the mean N-gene concentration or PMMoV-normalized data.

To visualize trends at each sewershed, we plotted daily wastewater loads and COVID-19 cases. We assessed wastewater load and case data for normality by visually inspecting quantile-quantile plots and histograms after applying various data transformations. The natural log transformation, after adding a constant of 1 (ln[x + 1]), resulted in approximately normally distributed data in both datasets (Appendix Figure 1). To assess the strength of linear associations between log-transformed SARS-CoV-2 loads in wastewater and log-transformed COVID-19 cases, we computed Pearson correlation coefficients (r) by using lags of 0–5 days between sampling date and case episode date. We repeated that analysis by using PMMoV-normalized, log-transformed wastewater load data. We considered correlation coefficients to be significant at p<0.05.

Because the number of COVID-19 patient hospitalizations at Peel hospitals were not specific to sewersheds, we summed the daily wastewater loads at both WWTPs, applied the ln(x + 1) transformation because it resulted in more normally distributed data, and calculated r between log-transformed total N-gene load and log-transformed hospitalizations. We computed correlation coefficients per wave at lags of 1–14 days between wastewater sampling date and hospitalization date. We repeated that analysis by using log-transformed PMMoV-normalized wastewater data.

Wastewater-to-Case Ratios, Linear Regression, and Case Estimations
After observing the strongest linear association between SARS-CoV-2 load in wastewater and reported COVID-19 cases at a 1-day lag, we computed the median daily wastewater-to-case ratio per wave. We compared these median wastewater-to-case ratios per wave by using the Kruskal-Wallis test, followed

Figure 1. Mean SARS-CoV-2 N-gene load (10^12 copies/d) in untreated wastewater at Clarkson Wastewater Treatment Plant and reported COVID-19 case-patients residing in the Clarkson sewershed, Regional Municipality of Peel, Ontario, Canada, September 1, 2020–June 18, 2022. A) Nonnormalized; B) pepper mild mottle virus normalized. Data are plotted on the logarithmic scale.
by the Dunn test for pairwise comparisons (18). Because clinical PCR testing eligibility was limited as of December 30, 2021, we hypothesized that laboratory-confirmed COVID-19 cases during the Omicron waves were underestimated. We therefore used wastewater load and reported case data in the pre-Omicron waves to fit a model to estimate reported case incidence during Omicron waves. We created a simple linear regression model by using the sum of the daily transformed N-gene load in wastewater at the 2 WWTPs and daily transformed COVID-19 case counts at the 2 WWTPs at a 1-day lag during the pre-Omicron waves (2–4), and we estimated the number of cases, with corresponding 95% prediction intervals, for the Omicron waves (5 and 6), on the basis of the measured wastewater SARS-CoV-2 load. For statistical analyses, we used Stata version MP17.0 (StataCorp LLC, https://www.stata.com).

Results

Trends in Wastewater SARS-CoV-2 Load and COVID-19 Clinical Indicators

During waves 2–4 (August 30, 2020–November 28, 2021, before Omicron emerged), the trends and magnitude of reported COVID-19 cases were temporally associated with both the non-normalized SARS-CoV-2 load and PMMoV-normalized load in wastewater (Figures 1, 2). The median daily SARS-CoV-2 load at G.E. Booth was double that of Clarkson, corresponding to the larger size of the G.E. Booth sewershed (Table 1). During waves 2 and 3, the numbers of average weekly clinical PCR tests conducted and overall percent positivity were similar (Table 1; Appendix Figure 1). Furthermore, trends in COVID-19 hospitalizations visually correlated with trends in wastewater load (Figure 3; Appendix Figure 3). During wave 4, dominated by the Delta variant, COVID-19 cases, hospitalizations, and clinical PCR tests were fewer; test positivity was lower; median N-gene loads in wastewater were less; and the proportion of wastewater samples with N-gene concentrations less than the limit of quantification was high (36%).

By December 2021, when Omicron BA.1 emerged (wave 5), the magnitude of reported cases no longer aligned with the magnitude of wastewater SARS-CoV-2 load (Figures 1, 2). SARS-CoV-2 load at both WWTPs reached a historic peak in January 2022. Of note, the largest number of daily COVID-19 case-patients ever reported in Peel, for both sewersheds, was on December 29, 2021. COVID-19 patient admissions at Peel hospitals also increased during wave 5; daily median was 12 and maximum was 63 admissions. Clinical PCR tests completed among Peel residents increased sharply in late December 2021 and dropped steeply in January 2022 after the change in PCR testing eligibility (Appendix Figure 1). Test positivity peaked at 31.4% during the week ending January 8, 2022.

COVID-19 wave 6, which occurred in Peel in spring 2022, was driven by Omicron BA.2. Daily median SARS-CoV-2 loads in wastewater were greater than in previous waves; however, loads did not exceed maximum daily values observed during the preceding Omicron BA.1 wave. Median daily COVID-19 patient hospitalizations remained similar to those in the previous wave.
wave. Weekly clinical SARS-CoV-2 PCR tests dropped by nearly 60% in wave 6, compared with waves 2–3.

**Association between SARS-CoV-2 Concentrations in Wastewater and Clinical Indicators**

For waves 2–6 combined, the strongest correlation between nonnormalized SARS-CoV-2 load in wastewater and reported COVID-19 cases occurred on the same day of sampling (G.E. Booth, $r = 0.6030$; Clarkson, $r = 0.6273$; both WWTPs, $r = 0.6296$; Table 2). Before Omicron, the strongest correlations occurred on the same day as sampling at G.E. Booth ($r = 0.8698$) and 1 day after sampling at Clarkson ($r = 0.8864$) and when load data were combined for both WWTPs ($r = 0.9106$). By wave, the strongest correlations occurred during the Alpha-dominant wave 3. Correlation coefficients were poorer as more time passed between the wastewater sample date and incident cases. PMMoV normalization, compared with no normalization, resulted in weaker correlations for both WWTPs before the Omicron waves and for G.E. Booth during the Omicron waves (Appendix Table 2).

Pearson correlation coefficients assessing the relationship between total wastewater SARS-CoV-2 load at the 2 WWTPs and total COVID-19 patient hospitalizations were highest during the Alpha-dominant wave 3 (Table 3; $r = 0.8679$ at a 4-day lag) and the Omicron BA.1-dominant wave 5 ($r = 0.9161$ at a 7-day lag). In the Delta-dominant wave 4, associations were weak and some were not

**Figure 3.** Mean combined SARS-CoV-2 N-gene loads ($10^{12}$ copies/d) in untreated wastewater at Clarkson and G.E. Booth Wastewater Treatment Plants and acute-care admissions of confirmed COVID-19 patients at Peel hospitals, Regional Municipality of Peel, Ontario, Canada, September 1, 2020–June 18, 2022. Data are plotted on the logarithmic scale. For data visualization purposes, daily hospitalization values of zero were converted to 0.1 and are shown along the x-axis.
Table 2. Pearson correlation coefficients ($r$) between $\ln(x + 1)$ transformed daily wastewater SARS-CoV-2 load and $\ln(x + 1)$ transformed incident COVID-19 cases, by wastewater treatment plant and epidemic wave, at various lags, Regional Municipality of Peel, Ontario, Canada*

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<td>0.7910†</td>
<td>0.6296†</td>
<td>0.9101</td>
<td>0.6601†</td>
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<td>0.7431</td>
<td>0.9438†</td>
<td>0.6266</td>
<td>0.7655</td>
<td>0.7357</td>
<td>0.6150</td>
<td>0.9106†</td>
<td>0.6341</td>
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<td>2</td>
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<td>0.9246</td>
<td>0.5295</td>
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<td>0.5774</td>
<td>0.8921</td>
<td>0.5830</td>
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<td>5</td>
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<td>0.5706</td>
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<td>0.7111</td>
<td>0.5678</td>
<td>0.8887</td>
<td>0.5115</td>
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</tbody>
</table>

*Colors indicate strength of association: gray, weak ($r<0.4$); yellow, moderate (0.45≤$r<0.7$); green, strong ($r≥0.7$). WWTP, wastewater treatment plant.†Largest $r$ per wave.

Statistically significant. Overall, across the study period, the greatest correlation occurred between wastewater load and hospitalizations 4 days after sampling ($r = 0.8189$). PMMoV normalization resulted in weaker correlation coefficients, and the greatest coefficient occurred 1 day after sampling ($r = 0.7883$; Appendix Table 3).

Estimation of COVID-19 Cases in Peel during the Omicron BA.1 and BA.2 Outbreaks

At each WWTP, the median wastewater-to-case ratios did not statistically differ from each other for waves 2–4. However, the ratios were significantly greater ($p<0.05$) during waves 5 and 6 (Appendix Table 4, Figure 4). Thus, starting in wave 5, each unit of SARS-CoV-2 load in wastewater was associated with fewer reported COVID-19 cases, as expected, resulting from reduced eligibility for clinical PCR testing.

To estimate the number of COVID-19 cases that would have been reported in waves 5 and 6 had testing eligibility not changed, we created a simple linear regression model by using the summed transformed wastewater load at both WWTPs and summed
COVID-19 case data, at a 1-day lag, from waves 2–4 (Figure 4). At the peak measured wastewater load of wave 5 on January 11, 2022, there were 1,160 reported COVID-19 cases, compared with a prediction of 7,515 cases (95% prediction interval 2,871–19,672), representing a 6.5-fold difference. At the peak of wave 6 (greatest measured wastewater load on April 21, 2022), there was an 18.7-fold difference between the estimated number of cases and reported COVID-19 cases (3,170 [95% prediction interval 1,224–8,211] vs. 170 reported cases).

Discussion
In December 2021, after the emergence of the Omicron variant, to conserve testing capacity the Ontario provincial government implemented changes to PCR test eligibility. As a result, we observed a rapid decrease in completed clinical PCR tests, high percentage positivity, and an increased wastewater-to-case ratio in Peel, indicating an underestimation of reported cases. Similar changes in wastewater-to-case ratios have been reported, resulting from changes in clinical testing strategies in other Canada municipalities (19). On the basis of the observed linear association between cases and SARS-CoV-2 load in wastewater in Peel, we estimated that the number of predicted cases was ≈6.5-fold greater than the number of reported cases at the peak of wave 5 (characterized by Omicron BA.1) and ≈18.7-fold greater than reported cases at the peak of wave 6 (Omicron BA.2). In January 2022 (wave 5), Peel Public Health provided near real-time incident case estimations based on wastewater concentrations to Peel hospitals that were experiencing considerable pressures for beds. Those data were, in turn, used to validate the hospitals’ short-term scenario planning and to predict further challenges to hospital occupancy and staffing.

This simple method of case estimation can be easily replicated; however, it relies on linear regression, frequent sampling, and a historical baseline. The linear associations observed in Peel might have been applicable because of sewage collection system characteristics specific to the region. For example, Peel’s storm water and sanitary sewage systems are separated. Furthermore, most of Peel’s wastewater comes from residential sources; during August 2020–June 2022, the source of 72% of wastewater effluent was residential and the sources of 28% were industrial, commercial, or institutional. Last, Peel’s 2 WWTPs serve >95% of Peel’s residents, representing high population-level coverage.

Our study used calculations of load, which require daily measurements of total flow. In earlier analyses, we found similar linear associations between SARS-CoV-2 N-gene concentrations (copies/mL) at the 2 Peel WWTPs and the COVID-19 cases and hospitalizations (data not shown). However, to provide information about Peel as a whole, we found it beneficial to calculate total load to combine data from 2 WWTPs.

Although reported COVID-19 cases during the Omicron waves were known to be an underestimation of true cases, overall trends in reported cases still correlated with trends of SARS-CoV-2 load in wastewater. This association was also observed between SARS-CoV-2 loads in wastewater and hospitalizations of COVID-19 patients during wave 5, which was dominated by Omicron BA.1. This finding might indicate that, within Peel, SARS-CoV-2 load in wastewater might be predictive of hospitalizations for COVID-19, independent of changes in testing uptake, although the optimal lags were variant dependent. The findings of wastewater signal being an indicator of community disease burden were also reported from another Ontario study (20). However, because of many complex factors, such as differing virulence of new variants, advancements in treatment, vaccine effectiveness, waning immunity, and outbreaks in hospitals, hospitalizations associated with COVID-19 can vary. Those factors may have explained the poor linear relationship.
between wastewater concentrations and hospitalizations in the Omicron BA.2-dominant wave 6.

In many wastewater surveillance systems, including the one for Peel, concentrations of SARS-CoV-2 in wastewater are normalized to concentrations of PMMoV, a fecal marker. In our study, PMMoV normalization generally did not improve the correlation between wastewater load and COVID-19 case counts, similar to findings in other studies (8,9,21), or COVID-19 patient hospitalizations, when compared with use of nonnormalized data. This lack of improved correlation might result from the low amount of inflow and infiltration as well as the source of wastewater being mainly residential (72%). Although normalization through PMMoV did not result in improved correlations in Peel, it might be useful in other systems (4). Furthermore, monitoring of fecal biomarkers may be used to assess sample quality (e.g., determining whether PMMoV concentrations are within an expected range for a given site).

Our results demonstrate the value of using population-based wastewater surveillance to detect increasing local COVID-19 activity and confirm declining case trends. Among the study limitations, data for numbers of COVID-19 cases included Peel residents and did not include patients who resided in neighboring jurisdictions but who would have contributed to SARS-CoV-2 in the G.E. Booth sewershed. However, we estimated that this contribution was relatively small, considering the large geographic area of the sewershed. Second, the COVID-19 hospitalization data used in our study were aggregated, and we were thus unable to discern whether patients were Peel residents. Third, our application of a historical, pre-Omicron, linear relationship to estimate COVID-19 cases during the Omicron waves was based on the assumption that fecal shedding patterns of SARS-CoV-2 remained the same regardless of SARS-CoV-2 variant, previous infection, and vaccination status. It has been reported that the amount of Omicron BA.2 on nasopharyngeal swabs was double that of Omicron BA.1 (22); but we are unaware of recent studies specific to virus in feces. If more fecal virus shedding is produced by Omicron than by other variants, our estimates of case underreporting would be overestimated. Fourth, we are unable to verify the number of true infections without population-level seroprevalence studies. Last, although linear regression is easily understood and accessible, interpretability of our case estimation results is limited as a result of autocorrelation. We found that residuals of the linear regression model exhibited positive autocorrelation, which may have resulted in less precise coefficient estimates and underestimated SEs and 95% prediction intervals. Further work is needed to determine modeling methods that might be more appropriate for analysis of wastewater time-series data (e.g., the SEIR [23] and PRESENS [24] models) and might also be easily implemented and interpretable by public health authorities. Despite this limitation, there was still value in using a simple method to approximate COVID-19 cases by using wastewater data. Furthermore, our predictions agreed with other estimates projected by hospital partners.

In summary, in the Regional Municipality of Peel, Canada, on the basis of strong historical linear associations between SARS-CoV-2 N-gene load in wastewater and reported COVID-19 cases, we estimated that reported COVID-19 cases were underestimated 19-fold at the peak of the Omicron BA.2 wave in April 2022. As a result of identifying SARS-CoV-2 wastewater load and population-level COVID-19 clinical outcomes, the monitoring of wastewater in Peel has provided critical information about community transmission of COVID-19 that is independent of clinical testing availability and uptake. Wastewater surveillance data can provide information for local decision-making and be a key metric in addition to traditional public health surveillance indicators, particularly for outcomes that are limited by availability of testing information and require triangulation of multiple data sources.

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The datasets (wastewater and clinical cases) are available from the corresponding author on reasonable request.
About the Author

Ms. Cheng is an epidemiologist at Peel Public Health, where she supports public health surveillance of communicable and infectious diseases.

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Address for correspondence: Monali Varia, Regional Municipality of Peel, 7120 Hurontario St., PO Box 630, RPO Streetsville, Mississauga, ON L5M 2C1, Canada; email: monali.varia@peelregion.ca
The COVID-19 pandemic has shown the need for accurate surveillance data. Incidence rate data, commonly collected as part of human surveillance, can only be interpreted with the understanding that local testing strategies vary over time. COVID-19 surveillance using wastewater testing, in which SARS-CoV-2 RNA fragments shed in the feces of infected persons are quantified, has been implemented in many countries (1–5). Wastewater data have been suggested as a complement to or even a substitute for human surveillance data, particularly in times of low human testing activity. The association between wastewater concentrations and incidence has been demonstrated in multiple settings, but few studies have succeeded in predicting incidence through wastewater surveillance, and the direct value of wastewater testing for epidemic control remains debatable (1).

In response to the pandemic, Denmark set up an extensive wastewater surveillance system, which was implemented in July 2021 and fully rolled out in October 2021. During the study period, the system included 201 wastewater treatment plant (WWTP) inlets, which were sampled 3 times a week and covered 85% of the population. Denmark has also had exceptionally high COVID-19 testing capacity, offering unlimited, free reverse transcription PCR (RT-PCR) testing through public testing stations (6,7; M.A. Gram et al., unpub. data, http://medrxiv.org/lookup/doi/10.1101/2023.02.06.23285556). The per capita testing rate has been among the highest in the world during some periods of the pandemic; the country tested up to 27% of the population per week in December 2021 and was capturing an estimated 70% of active COVID-19 cases at the start of 2022 (M.A. Gram et al., unpub. data). However, testing activity was scaled down in early 2022, to <1% per week by June 2022 (8,9).

Given the variation in testing rates, wastewater concentrations should not be directly compared with observed incidence. Instead, models should include information on changing testing rates over time.
Another strategy is to look at a subgroup of regularly tested persons, where the effect of fluctuations in testing patterns should be less pronounced. Such a subgroup exists in Denmark, where recommendations were made for regular screening tests for certain care personnel (10).

The association between wastewater data and incidence might be affected by the SARS-CoV-2 variants in circulation, because those variants could have different fecal shedding patterns. Viral load for oropharyngeal samples has been shown to be higher for Delta than previous variants (B. Li et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.07.07.21260122v2), but how fecal shedding differs among variants is not known (11). Other variables, such as temperature and traveling time of SARS-CoV-2 in sewers, dilution by precipitation or wastewater from industry, and inhibitors of laboratory analyses, might affect viral quantification, (12,13).

We used the results of wastewater surveillance to predict the observed incidence of SARS-CoV-2 infections in Denmark. We performed the analysis at the national and regional level and among a subgroup of healthcare personnel.

Methods

Overview
We conducted a time-series analysis, constructing a model to explain observed incidence by wastewater concentrations. Besides the main national-level analysis, we also tested the model at a regional level and on a subpopulation of healthcare personnel. We used the human testing rate as a covariate in our model and considered interactions between wastewater concentrations and the proportion of circulating Omicron versus Delta variants and between wastewater concentrations and wastewater temperature. The study period was September 27, 2021–June 26, 2022.

Data Sources

Wastewater
Throughout the study period, 24-hour composite samples were taken 3 times a week from 202 WWTP inlets across Denmark. Sampling started on Mondays, Tuesdays, and Thursdays. Where possible, the samples were flow-proportional, which enabled sampling of more water at times of heavy flow, providing a more representative sample of the 24-hour water flow. Otherwise, samples were time-proportional, sampling a fixed amount of water at fixed time intervals.

Samples were purified and analyzed using quantitative real-time RT-PCR at Eurofins Miljø, a central commercial laboratory in Vejen, Denmark. We measured cycle threshold (Ct) values for 2 SARS-CoV-2 genes (the N2 region of the nucleocapsid gene and the RNA-dependent RNA polymerase [RdRp]) and converted them to concentrations (copies/L). We calculated limits of detection (LOD) and limits of quantification (LOQ) for each gene in each sample. We imputed values <LOD as LOD/2, and <LOQ as (LOD + LOQ)/2. Starting in 2022, we also measured the concentrations of 2 indicators of fecal concentration: crAssphage and pepper mild mottle virus (14,15). For consistency with 2021 data, we used those measurements as data quality indicators but not to normalize SARS-CoV-2 concentrations.

For each sample, utility companies reported the volume of wastewater that entered WWTPs over the 24-hour sampling window and the temperature of wastewater upon entry. Utility companies also provided geographic information, which we used to calculate the resident population of each catchment area by linking to the Danish Civil Registration System (16).

Incidence
In-person PCR COVID-19 testing was available for free to all residents throughout our study period; results were collected centrally in the Danish Microbiology Database (17). Testing recommendations changed throughout the study period; the most substantial change occurred on March 10, 2022. After that date, tests for the general population were recommended only for symptomatic persons in groups at high risk (9). We extracted data on daily incidence of PCR-confirmed COVID-19 cases and weekly PCR testing rate from Denmark’s official COVID-19 statistics (18) for its 5 administrative regions.

Care Personnel
We used data on healthcare personnel, consisting of care home staff and in-home caretakers, for a secondary analysis. During September 4, 2021–April 28, 2022, weekly PCR tests were recommended for healthcare personnel for screening purposes. After this time and until the end of the study period, the recommendation was 1 test every 2 weeks (10). Because of those recommendations, we believed incidence in this group might be less affected by testing patterns and therefore a better measure of actual community incidence than observed incidence among the general population. Information on this group came from Denmark’s centrally collected data on COVID-19
we used log_{10}-transformed versions of those variables in our models, using the same transformation for all to ease interpretation. Exclusions

Because great fluctuations in the fecal load of wastewater are not expected, finding such a fluctuation in measurements of the fecal indicators pepper mild mottle virus and crAssphage indicated a likely failure in sampling or laboratory analysis. We therefore excluded samples with missing or extreme concentrations of these fecal indicators, defined as concentrations ≥3 SDs from the mean for each WWTP, on the log scale. We also excluded samples that the laboratory received on unexpected days of the week. Such samples might not have been comparable to others because more time had passed in which RNA content could have degraded during transit. Furthermore, we excluded samples with missing values for the volume of wastewater entering the WWTP over a 24-hour sampling period. Finally, we excluded samples from WWTPs where we had no geographic information defining the catchment area, which we needed to define the population served by each WWTP. We also discarded wastewater temperature data reported as <1°C or >30°C, but we did not exclude other data for those samples from the analysis.

Statistical Analysis

First, we plotted the national incidence and wastewater concentrations to compare patterns visually. Second, we fitted a model to see whether wastewater results were a predictor of national incidence. We split the data into a training and testing set. We used training data from before June 9, 2022, to select and estimate our models. We reserved data from June 9, 2022, onward (7 weekly datapoints) as an out-of-sample test dataset for model validation. We constructed an ARIMAX (autoregressive integrated moving average with exogenous variables) model, using incidence as the dependent variable and wastewater concentrations and testing rate as the explanatory variables. We tested including the interaction between wastewater concentrations and the circulating variant (expressed as proportion of Delta sequences) and the interaction between wastewater concentration and wastewater temperature. We only included temperature as an interaction with the wastewater concentration to restrict it to describe degradation of RNA and not the overall seasonal effect on incidence. Likewise, the proportion of Delta was included as an interaction with the wastewater concentration to adjust for different shedding patterns for the different variants. We selected which covariates to include based on the Akaike information criterion. For consistency, we used the terms selected for the national model in the secondary models as well. Third, we also estimated the model allowing for a time delay between wastewater results and incidence. We examined lag times of 0, 1, and 2 weeks in each direction, comparing the resulting models by...
the Akaike information criterion. Fourth, we validated the model on the out-of-sample dataset not used in the model estimation, using the root mean squared error. Fifth, we repeated the modeling steps in 2 secondary analyses: reestimating the main model independently for each region and for the subpopulation of care personnel only. For the care personnel model, we used both incidence and testing rate specific to care personnel. Sixth, we used the national model to predict the incidence that would have been observed had the testing rate remained stable throughout the study period by generating model predictions where we fixed the testing rate at a constant value. We used the highest recorded testing rate in the study period for this. We used R version 4.1.3 (The R Project for Statistical Computing, https://cran.r-project.org) for statistical analyses (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/22-1634-App1.pdf).

Results

Description of Data
We included 18,737 wastewater samples from 202 WWTPs in the study (Appendix Table 1). Our initial dataset consisted of 21,069 samples, but we excluded 2,361 samples (29 for extreme concentrations of fecal indicators, 301 from WWTPs with unknown populations, 81 that arrived on unexpected days of the week, 919 missing data on wastewater flow in the sampling period, and 1,031 with sampling method not listed as flow-proportional or time-proportional). Of the included samples, 15,801 were flow-proportional and 2,764 were time-proportional. After aggregating by week at national level, we had 39 wastewater data points. We included a median of 515 (interquartile range 480.5–532.5) weekly samples.

Wastewater concentrations and incidence followed similar patterns, increasing until early 2022, decreasing until late May, and then increasing again (Figure 1). Testing rates remained fairly stable during November 2021–February 2022, after which they decreased to low levels. Per person, on average 2.6 times as many weekly tests occurred among healthcare personnel as occurred among the general population.

Incidence followed a similar pattern in all regions (Appendix Figure 1), although slightly offset in time. Numbers throughout 2021 were slightly higher in the Capital region and neighboring Zealand region than in the other 3 regions. The pattern of testing rate over time also did not differ greatly by region (Appendix Figure 2).

Until late November 2021, nearly all human isolates sequenced were the Delta variant. Omicron quickly took over in December, reaching ≈50% halfway through the month and >95% in the first week of January 2022. After that, nearly all samples were Omicron (Appendix Figure 3).

Model Results
Our final models were based on wastewater concentration, testing rate, and the interaction of wastewater concentration with circulating variants (percentage Delta). They did not include the interaction between wastewater results and wastewater temperature, because it did not improve the model (Appendix). The model performed best with no lead or lag time between wastewater results and incidence.

The pattern of the model fit and validation estimates follows the pattern of observed incidence well (Figure 2; Appendix Figure 4). However, only 43% of validation points were covered by the 95% prediction intervals in the national model (Appendix Table 3).

The coefficients for wastewater results were generally 0.4–0.5 during Omicron; coefficients were lower during Delta by ≈0.15–0.20 (Table). The coefficient for wastewater was higher in the care personnel model.
Predicting COVID-19 Using Wastewater Surveillance

Predicted Incidence at Stable Testing
We used the national model to estimate the COVID-19 incidence that would have been observed if the testing rate had remained constant (Figure 3). We used the highest testing rate in our study period (270 weekly tests/1,000 persons, as in the week of January 17, 2022). The difference between the model predictions and the observed incidence can be used as a measure of underreporting. Given the estimate from serologic studies that 70% of actual cases were captured in early 2022, we estimate that ≈15% of actual cases were captured by the national PCR testing system from April 2022 on.

Discussion
We constructed a model to explain the observed incidence of COVID-19 in Denmark using wastewater data, information on the circulating variants, and the number of human tests performed as predictors. We found that we could accurately reconstruct the observed incidence curve. Results were consistent at a regional level and among the subgroup of frequently tested care personnel. Using data from a country with extensive wastewater and human testing systems, we demonstrated that predicting incidence based on wastewater surveillance is possible.

We used these results to predict the incidence that could have been observed in Denmark if testing activity had remained high. In Denmark, after mass-testing programs were rolled back in the spring of 2022, wastewater analysis became a key source of information for the healthcare system in its handling of the COVID-19 pandemic.

The steeper association between wastewater and incidence during the Omicron period than in the Delta period might indicate that shedding dynamics differ between variants. However, the transition from Delta to Omicron coincided with the peak of rollout of vaccine booster doses (22). Information is lacking on how vaccination affects fecal shedding, but nasopharyngeal viral loads appear lower among vaccinated persons (2). Further studies are needed on how
fecal shedding is affected by SARS-CoV-2 variants and vaccination status.

The model fit at the regional level was generally very similar to that for national results, although wastewater concentrations had less effect in the model for the Capital region. A likely factor is that the Capital region is dominated by 4 very large catchment areas, and correlations between wastewater concentration and incidence are poorer in larger catchment areas (1). Commuting across catchment areas, which is typical for the densely populated Capital region, might also have played a role.

We performed a subanalysis of healthcare personnel, who were consistently tested at a higher rate than the general population for screening purposes. We reasoned that the incidence of healthcare personnel might have been outweighed by other unmeasured factors that affect SARS-CoV-2 decay, such as retention time in the sewage system or other chemical components of wastewater. Unlike other studies (1), we did not find wastewater results to be a leading indicator of incidence. That similar model results, possibly because the overall testing rate in Denmark was so high.

We used the national model to estimate what observed incidence would have been if the testing rate had remained at its maximum. We estimated that the proportion of actual cases identified had fallen to ≤15% by April 2022. However, if tests became more targeted over time as recommendations for regular screening tests were relaxed, the percentage of cases identified could be higher.

SARS-CoV-2 is known to decay faster at higher temperatures (12,13), but including an interaction term between wastewater results and temperature did not improve our model fit. The effect of temperature might have been outweighed by other unmeasured factors that affect SARS-CoV-2 decay, such as retention time in the sewage system or other chemical components of wastewater.

Unlike other studies (1), we did not find wastewater results to be a leading indicator of incidence. That

\textbf{Table.} Estimated coefficients from the national model, care personnel model, and regional model in study using wastewater surveillance data to predict COVID-19 incidence, Denmark, October 2021–June 2022*

<table>
<thead>
<tr>
<th>Model</th>
<th>Term</th>
<th>Estimate (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>National</td>
<td>AR (1)</td>
<td>0.46 (0.16–0.76)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration</td>
<td>0.4 (0.34–0.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Testing rate</td>
<td>0.87 (0.81–0.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration × Delta (%)</td>
<td>−0.15 (−0.19 to −0.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>−0.14 (−0.32 to 0.04)</td>
<td>0.12</td>
</tr>
<tr>
<td>Care personnel</td>
<td>AR (1)</td>
<td>0.32 (−0.06 to 0.70)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration</td>
<td>0.52 (0.46–0.59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Testing rate (care personnel)</td>
<td>0.84 (0.73–0.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration × Delta (%)</td>
<td>−0.17 (−0.21 to −0.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>−2.45 (−2.83 to −2.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regional Capital Region</td>
<td>AR (1)</td>
<td>0.31 (−0.07 to 0.70)</td>
<td>0.12</td>
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<td></td>
<td>Wastewater concentration</td>
<td>0.31 (0.24–0.38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Testing rate</td>
<td>1.03 (0.94–1.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration × Delta (%)</td>
<td>−0.15 (−0.19 to −0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>−0.11 (−0.34 to 0.12)</td>
<td>0.3</td>
</tr>
<tr>
<td>Central Denmark</td>
<td>AR (1)</td>
<td>0.48 (0.45–0.52)</td>
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<td></td>
<td>Wastewater concentration</td>
<td>0.88 (0.84–0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Testing rate</td>
<td>0.14 (−0.16 to −0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration × Delta (%)</td>
<td>−0.14 (−0.60 to −0.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>−0.48 (−0.60 to −0.36)</td>
<td>&lt;0.001</td>
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<tr>
<td>North Denmark</td>
<td>AR (1)</td>
<td>0.47 (0.43–0.51)</td>
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</tr>
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<td>Wastewater concentration</td>
<td>0.93 (0.86–0.96)</td>
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<td>Testing rate</td>
<td>0.16 (−0.19 to −0.13)</td>
<td>&lt;0.001</td>
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<tr>
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<td>Wastewater concentration × Delta (%)</td>
<td>−0.49 (−0.64 to −0.33)</td>
<td>&lt;0.001</td>
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<td>Intercept</td>
<td>−0.49 (−0.64 to −0.33)</td>
<td>&lt;0.001</td>
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<tr>
<td>Southern Denmark</td>
<td>AR (1)</td>
<td>0.48 (0.44–0.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration</td>
<td>0.83 (0.78–0.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Testing rate</td>
<td>0.15 (−0.17 to −0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration × Delta (%)</td>
<td>−0.39 (−0.52 to −0.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>0.16 (−0.19 to −0.51)</td>
<td>0.4</td>
</tr>
<tr>
<td>Zealand</td>
<td>AR (1)</td>
<td>0.42 (0.37–0.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration</td>
<td>0.8 (0.73–0.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Testing rate</td>
<td>−0.14 (−0.17 to −0.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Wastewater concentration × Delta (%)</td>
<td>−0.11 (−0.26 to 0.04)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*The response variable incidence, wastewater concentration, and testing rate are included after log₁₀-transformation. AR (1) denotes the first-order autoregressive term.
Predicting COVID-19 Using Wastewater Surveillance

Figure 3. Predicted COVID-19 incidence at a constant testing rate (purple) based on the national model, compared with observed incidence (black), in study of wastewater surveillance data as a predictor of COVID-19 incidence, Denmark. The prediction is an estimate of the true incidence. The proportion of estimated true cases captured decreased from >80% to ≈20% during 2022.

difference might be because our analysis was based on weekly data, so we could only assess lag times in 7-day intervals. However, a lag of <1 week would likely have limited effects on public health action in practice. In addition, extensive human testing occurred during most of the study period, so infections might have been detected earlier than in other settings.

In our models, the coefficient for wastewater concentrations was <1. This result might seem surprising, because it means that a doubling in wastewater concentrations is not associated with a doubling in incidence (after adjusting for testing rate). Several explanations exist for this finding. First, the variability in the number of viral copies shed is best described by a log-normal distribution (23, 24). The cumulative number of copies shed by a population will therefore follow a highly skewed distribution (24, 25), which in itself is expected to lead to a coefficient <1, as seen in simulation models generating the relationship between the number of infected persons in the population and concentration of RNA in sewage (26). Second, observed incidence depends on the testing pattern; specifically, the probability that an infected person will be tested and have a positive result. The testing rate that we included in our models is an imperfect measure of this probability. Third, testing rate itself is influenced by incidence. Testing rates were highest, on average, when incidence was high. This factor might have increased the predictive power of testing rate in our models and therefore disadvantaged wastewater as a predictor. This interpretation is supported by the fact that the coefficient for testing rate in the national model (0.87 [95% CI 0.81–0.94]) is higher than the 0.7 that was found in Denmark’s method for estimating the reproduction number in the fall of 2020 (27). The secondary analysis of healthcare personnel provides further support. We expected observed incidence in this population to be a closer reflection of actual incidence (compared to national observed incidence numbers) because the testing rate was more stable. That expectation is consistent with our results: a larger coefficient for wastewater concentrations and marginally smaller (though with overlapping 95% CIs) coefficient for testing rate.

The first limitation of our model is that testing rate is influenced by incidence and changes in recommendations. However, this effect was likely smaller in our setting than in most others because there were high numbers of screening tests for asymptomatic people for much of the study period. Another limitation is that the performance of the model on the validation data was mixed. Validation model estimates clearly followed the same pattern as the observed incidence, but they were lower than the observed data; most of the 95% prediction intervals did not include the observed data. This discrepancy is likely because of the changes in recommendations in the first half of 2022, in which a gradual shift occurred toward less testing for screening purposes and a larger share of diagnostic testing of symptomatic persons. Finally, we could not incorporate the unknown effect of immunity (through vaccination or previous infection) on fecal shedding.

This study benefited from copious wastewater testing data because of the extensive surveillance system in Denmark. One remaining question is how well wastewater data perform in less developed surveillance systems. Denmark’s surveillance was scaled down after this study period to incorporate fewer WWTPs and fewer weekly samples. Repeating this analysis once enough data has been collected under the new system might help answer that question.

In conclusion, we performed a large-scale study of the association between wastewater results and observed incidence of COVID-19. Our relatively simple model makes it easy to specifically examine the
association between wastewater results and incidence. We found that wastewater testing results can be used to accurately model the observed incidence of COVID-19, in combination with data on human tests. This finding implies that wastewater testing can serve as a proxy for incidence in the context of little to no human testing. The link between wastewater concentrations and incidence has been stronger since Omicron has been dominant. We found no effect of temperature on the association. For a wastewater surveillance system as extensive as that of Denmark, we believe wastewater results are a trustworthy indicator of actual incidence, especially in a situation in which human testing rates continue to decline.

Acknowledgments
We gratefully thank our partners at the utility companies who collected the wastewater samples. We also thank our partners at Eurofins Miljø for performing the laboratory analyses.

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Data used for this study are available at https://github.com/olverorm/ww-incidence and https://zenodo.org/record/8060977.

About the Author
Mr. McManus is an epidemiologist at Denmark’s national public health institute, Statens Serum Institut, and a fellow of the European Programme for Intervention Epidemiology Training. His primary research interest is wastewater surveillance of COVID-19.

References


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Multidrug-Resistant Bacterial Colonization and Infections in Large Retrospective Cohort of Mechanically Ventilated COVID-19 Patients

Davide Mangioni, Liliane Chatenoud, Jacopo Colombo, Emanuele Palomba, Fernando A. Guerrero, Matteo Bolis, Nicola Bottino, Giuseppe Breda, Maria V. Chiaruttini, Gabriele Fior, Manuela Marotta, Giovanni Massobrio, Caterina Matinato, Antonio Muscatello, Paola Prevaldi, Sara Santambrogio, Francesca Tardini, Gianluca Zuglian, Giacomo Grasselli, Roberto Fumagalli, Andrea Gori, Nino Stocchetti, Gianpaola Monti, Alessandra Bandera, and the MDR in FIERA Study Group

Few data are available on incidence of multidrug-resistant organism (MDRO) colonization and infections in mechanically ventilated patients, particularly during the COVID-19 pandemic. We retrospectively evaluated all patients admitted to the COVID-19 intensive care unit (ICU) of Hub Hospital in Milan, Italy, during October 2020–May 2021. Microbiologic surveillance was standardized with active screening at admission and weekly during ICU stay. Of 435 patients, 88 (20.2%) had MDROs isolated ≤48 h after admission. Of the remaining patients, MDRO colonization was diagnosed in 173 (51.2%), MDRO infections in 95 (28.1%), and non-MDRO infections in 212 (62.7%).

Bacterial superinfections represent a major threat for patients in intensive care units (ICUs), severely affecting clinical course and length of hospital stay. The COVID-19 pandemic caused an unprecedented rate of ICU admissions and drastically changed ICU care itself, in terms of infection control measures and therapeutic usage of steroids and immunomodulating drugs. The percentages of hospital-acquired infections (HAIs) in COVID-19 patients vary widely, ranging from 7% to 13% in nonintensive hospital wards and up to 45% in ICUs (1–3).

Several studies have assessed the burden of multidrug-resistant organisms (MDROs) in COVID-19 patients admitted to ICUs, reporting heterogeneous results with prevalence ranging from 11% to 50% and incidence rate from 4.5 cases/1,000 patient-days to 30 cases/1,000 patient-days (4–21). However, studies published so far have relevant limitations, often not clearly discriminating between colonization and infection (8,9,11,12), and either including small...
populations or showing heterogeneity in clinical settings and microbiologic surveillance procedures when describing larger pool of persons, such as in multicentric studies (18–20).

Our study was conducted to address the need for further evidence on incidence and etiology of MDRO colonization and infections in mechanically ventilated COVID-19 patients. We analyzed clinical and microbiologic data systematically collected in a large ICU in northern Italy.

Methods

Study Design and Setting
We conducted a retrospective cohort study on routinely collected data of COVID-19 patients admitted to the Milano Fiera ICU during October 23, 2020–May 31, 2021. This ICU was a large COVID-19 ICU developed in Milan, Italy, to face the effect of the pandemic. It admitted patients who had SARS-CoV-2 infection requiring mechanical ventilation from different healthcare settings: emergency department, nonintensive hospital wards, and other ICUs. This ICU could accommodate up to 100 patients divided into distinct units (modules) managed by ICU staff from different hospitals. Microbiologic surveillance was standardized and consisted of perineal and nasal swab specimens for MDROs and endotracheal aspirate cultures obtained at ICU admission and then once (perineal and nasal swab specimens) or twice (endotracheal aspirate) a week. All modules referred to the IRCCS Ca’ Granda Ospedale Maggiore Policlinico Foundation for laboratory and microbiologic analyses and for infectious diseases specialist consultation.

Study Participants and Data Collection
All consecutive patients who had laboratory-confirmed SARS-CoV-2 infection and were admitted to the ICU were considered for inclusion. Exclusion criteria were age <18 years, length of mechanical ventilation <48 h, and lack of comprehensive clinical documentation. We collected demographic, clinical, laboratory, and outcome data from clinical records and microbiologic and therapeutic data from dedicated hospital databases (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/23-0115-App1.pdf). The study was registered by the Milan Area 2 Ethical Committee (#701_2021) and was conducted in accordance with standards of the Helsinki Declaration. Written informed consent was waived because of the retrospective nature of the analysis. The study was retrospectively registered at clinicaltrials.gov on March 24, 2022 (identifier: NCT05293418).

Microbiologic Data Processing
For each patient, we retrieved bacterial isolates from a microbiology database, which were independently reviewed by dedicated intensivists and infectious disease specialists and classified as contamination, colonization, or infection, according to international guidelines (Appendix) (22,23). In brief, infections were defined by the presence of a major bacterial load associated with clinical manifestations within the infection window period (±3 days from specimen collection) (22,23). Isolates were classified as colonization when no adverse clinical signs or symptoms were documented. We defined contamination as all microbiologic isolates that did not meet the criteria of infection or colonization and that were listed in the US Centers for Disease Control and Prevention National Healthcare Safety Network (https://www.cdc.gov/nhsn/index.html) list of common commensals. We retained only the first species-specific MDRO colonization of each patient for further analysis.

We distinguished new infectious episodes from persistent infections according to the European Centre for Disease Prevention and Control definitions (23). We stratified infection episodes as infection without sepsis, sepsis or septic shock according to Sepsis-3 criteria (24). We defined secondary bloodstream infections (BSIs) by using the secondary BSI attribution period according to the Centers for Diseases Control and Prevention National Healthcare Safety Network (22). We also defined isolates as MDROs when they were nonsusceptible to ≥1 agents in ≥3 antimicrobial drug categories (25) or when harboring specific antimicrobial drug resistance mechanisms (e.g., methicillin-resistant *Staphylococcus* spp., vancomycin-resistant *Enterococcus* spp., extended-spectrum β-lactamase/AmpC/carbapenemases-producing Enterobacteriales) by using rapid detection methods (4).

Statistical Analysis
We reported patient characteristics overall and for selected groups of interest, such as MDROs acquired before/after ICU admittance and MDRO infection/colonization. Medians (interquartile range [IQRs]) are reported for continuous variables and numbers (percentages) for categorical variables. We calculated crude incidence rates (IRs) per 1,000 patient-days and relative 95% CIs, considering for each patient any first species-specific MDRO colonization or each new MDRO/non-MDRO HAI (26). We used SAS version 9.4 software (SAS Institute, https://www.sas.com) for statistical analysis (Appendix).
Results

Population Description

A total of 451 patients from 46 different hospitals were admitted to ICUs during October 2020–May 2021. Of those, 435 were included in the analysis. We provide details of the patient selection process (Figure 1) and trends of patient admission by referring hospital per month (Appendix Figure 1).

Only 12/435 patients (2.7%) were reported to have MDRO colonization/infection before ICU admission. In 88/435 patients (20.2%), MDRO were isolated within 48 h upon entry to the ICU (MDR_{≤48h}), and those patients were similarly distributed between referring hospitals (Appendix Figure 2). This group was composed of 78 colonizations and 10 infections; 35/78 (44.9%) colonized patients subsequently had MDRO infections develop. Compared with the 347 patients who had no evidence of MDRO during the first 48 hours of ICU stay (no-MDR+MDR_{>48h}), the MDR_{≤48h} group was characterized by higher admittance from other ICUs and lower admittances from emergency departments (ICU 31/88 [35.2%] in MDR_{≤48h} vs. 86/347 [24.8%]) in no-MDR+MDR_{>48h}. 

Figure 1. Study flowchart showing patient selection process for multidrug-resistant bacterial colonization and infections in large retrospective cohort of COVID-19 mechanically ventilated patients admitted to ICU in Milan, Italy, October 2020–May 2021. ICU, intensive care unit; MDR, multidrug resistant. *Patients are grouped on the basis of the worst MDR event diagnosed in MDR colonization or MDR infection, irrespective of the presence of previous or later MDR colonization. †At ICU admission, there were 78 colonizations and 10 infections. During ICU stay, 35/78 (44.9%) colonized patients had MDR infections develop.
emergency department 15/88 [17.1%] in MDR$_{≤48h}$ vs. 102/347 [29.4%] in no-MDR+MDR$_{>48h}$. The MDR$_{≤48h}$ group showed slightly longer (although not significantly) length of stay in the ICU of origin than patients who developed MDRO events later during their stay and to no-MDR patients (medians 11.5, 9, and 7 days, respectively; $p = 0.09$). The MDR$_{>48h}$ group was also characterized by a larger amount of antimicrobial drug intake before ICU admission (no antimicrobial drug in 25/88 [28%] of MDR$_{≤48h}$ vs. 126/327 [36.3%] of no-MDR+MDR$_{>48h}$; $≥3$ classes of antimicrobial drugs in 12/88 [13.6%] of MDR$_{≤48h}$ vs. 23/347 [6.6%] of no-MDR+MDR$_{>48h}$). We compiled demographic and clinical characteristics by groups (Appendix Table 1) and duration between hospitalization and transfer to the ICU on the basis of patients’ setting of provenance (Appendix Table 2).

Of the 347 patients who had no MDRO isolates within the first 48 hours from ICU admission, 207 (67.5%) had $≥1$ MDRO event (MDR$_{≤48h}$: 107 [30.8%] patients had MDRO colonization only (MDR$_{COL>48h}$) and 100 [28.8%] had $≥1$ MDRO infection (MDR$_{INF>48h}$) (Figure 1). We compiled patient characteristics and outcomes (Table 1, https://wwwnc.cdc.gov/EID/article/29/8-203-0115-T1.htm) overall and for no-MDR and MDR$_{>48h}$ patients, further stratified as MDR$_{COL>48h}$ and MDR$_{INF>48h}$. Median age was 65 years (IQR 59–71 years); 95/347 (27.4%) patients were female. More than 80% of patients had $≥1$ concurrent condition, and hypertension was the most common (181/347, 52.2%). Patients who had ever smoked were more frequent in the MDR$_{INF>48h}$ group (26/100, 26%) than in the MDR$_{COL>48h}$ group (11/107, 10.3%; $p = 0.003$). Transfer to the ICU occurred mostly from nonintensive hospital wards (159/347, 45.8%), but relevant proportions were transferred directly from the emergency department (102/347, 29.4%) or from other ICUs (86/347, 24.8%). Patients were transferred to ICU early during hospitalization, a median time of 5 days from first hospital admittance.

Groups did not differ for steroid use or antimicrobial drug therapies received before ICU admission. According to clinical practice, steroids had been administered for SARS-CoV-2 infection management in 252/347 (72.6%) patients, mostly (228/347, 65.7%) with only a standard dose (dexamethasone 6 mg/d). Most patients (221/347, 63.7%) had previously received antimicrobial drugs before ICU admission. MDRO events before ICU admission were reported in only 4 patients (1.2%). During ICU stay, 118 patients (34%) died, but there were no significant differences between groups. When compared with no-MDR patients, we found that MDR$_{>48h}$ patients had a longer duration of mechanical ventilation (median 18 vs. 14 days; $p = 0.001$) and of ICU stay (median 25 vs. 15.5 days; $p = 0.001$). Those differences were largely caused by the MDR$_{INF>48h}$ group (Table 1).

**Bacterial Isolate Description and Incidence**

Complete microbiologic reports were available for 426/435 patients, including 338/347 patients (97.4%) with no MDRO isolates within the first 48 hours of ICU admission. We describe the selection process conducted to assess incidences of HAIIs and of MDRO events distinguishing between colonization and infection (Figure 2). We identified 801 bacterial isolates from 271 patients that correspond to first MDRO colonization (255 isolates in 173/338 patients, 51.2%) and new episodes of bacterial superinfections, either by MDRO (130 isolates in 95/338 patients, 28.1%) or antimicrobial drug–susceptible bacteria (non-MDRO, 416 isolates in 212/338 patients, 62.7%). A total of 73 (21.6%) patients had both MDRO colonization and MDRO infection develop during ICU stay, and infections were caused by the same colonizing bacterial species in nearly one third of them (24/73, 32.9%) (Appendix Table 3). Clinical interpretation of bacterial isolates as colonization/infection by attending physicians at the time of arrival of microbiologic results was found to be highly concordant with the retrospective evaluation conducted according to international guidelines (κ coefficient 0.902, 95% CI 0.890–0.913) (Appendix Table 4).

Overall, 546 bacterial HAIIs were recorded, 130 (23.8%) caused by MDRO. Gram-negative bacteria accounted for 59.7% (326/546) of all HAIIs and for 60% (78/130) of infections caused by MDROs. Bacterial species responsible for HAIIs varied by infection site and severity of infection (Appendix Tables 5, 6). Ventilator-associated lower respiratory tract infections (VALRTIs) represented most infectious episodes (359/546, 65.7%), followed by BSI (141, 25.8%) and urinary tract infections (40, 7.3%). Among BSIs, 31/141 (22%) were associated with a central line, 43 (30.5%) were secondary to VALRTI or urinary tract infections, and the remaining 67 (47.5%) were classified as primary BSI without a known bacteremic focus (Appendix Figure 3).

Among MDRO colonization, *Enterococcus faecium* (112/255 isolates, 43.9%) was the most frequent isolate, followed by *Klebsiella* spp. (34, 13.3%), *Escherichia coli* (26, 10.2%), *Staphylococcus aureus* (25, 9.8%), *Pseudomonas aeruginosa* (15, 5.9%) and *Acinetobacter baumannii* (13, 5.1%). We compiled the percentages of MDRO colonization, MDRO HAIIs, and non-MDRO
HAIs for the most frequently isolated bacteria of the World Health Organization priority pathogens list (27) (Appendix Figure 4).

First MDRO colonization occurred at a median time of 13 (IQR 8–12) days after ICU admission. HAIs caused by antimicrobial drug–susceptible bacteria occurred earlier than in those caused by MDROs at 6 (IQR 3–10) and 10 (IQR 6–17) days from admission (p<0.001) (Figure 3). The incidence rates for MDRO colonization was 29.97 cases/1,000 patient-days (95% CI 26.34–34.10), for MDRO infection was 14.99 cases/1,000 patient-days (95% CI 12.36–18.19), and for non-MDRO infection, was 50.12 cases/1,000 patient-days (95% CI 44.59–56.32). Infection rates varied substantially by infection site (Table 2).

**Association of Antimicrobial Drugs and Steroids to MDRO Events**

We investigated possible associations between MDRO events and previous steroid and antimicrobial drug therapies (Appendix Tables 7, 8). Because steroids were included in the management of COVID-19 pneumonia from the early stage of the disease, we evaluated their intake before and during ICU stay. Almost the entire population had received steroid therapy (313/338, 92.6%), without major differences...

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**Figure 2.** Study flowchart showing microbial isolates selection process for multidrug-resistant bacterial colonization and infections in large retrospective cohort of COVID-19 mechanically ventilated patients admitted to ICU in Milan, Italy, October 2020–May 2021. ETA, emergency treatment area; ICU, intensive care unit; MDR, multidrug resistant; MDRO, MDR organism. *Of 338 patients, 159 (47.0%) had either MDRO or non-MDRO infections; 74/338 (21.9%) had both MDRO and non-MDRO infections.
between no-MDR (132/140, 94.3%), MDR_{VOL>48h} (98/103, 95.1%) and MDR_{INF>48h} (83/95, 87.4%) (Appendix Table 7).

To assess possible association between MDRO events and previous antimicrobial drug use, we focused on therapies administered during the first 10 days of ICU stay. This timeline was set to balance observation time between no-MDR and MDR_{>48h} groups because three fourths of MDRO events occurred within this timeframe. Also, three fourths of patients in no-MDR group stayed in ICU >10 days (Appendix Table 8). Previous exposure to antimicrobial drugs was notably higher in patients who developed MDRO events than in patients who did not (116/197 [58.9%] in MDR_{>48h} vs 18/140 [12.9%] in no-MDR; p<0.001) (Appendix Table 8).

**Discussion**

We describe incidences and clinical characteristics of HAIs and MDRO events, distinguishing between colonization and infection, in a large cohort of ICU COVID-19 patients from a country with high prevalence of MDRO (28). Despite being composed of patients admitted from >45 different hospitals, our cohort is homogeneous for concurrent conditions and risk factors for MDRO acquisition, clinical severity of COVID-19, management of antimicrobial drug therapy, and infection prevention and control strategies within the ICU, including surveillance sampling.

Antimicrobial drug resistance represents a major challenge in the ICU. Its occurrence is the result of the influx of previously colonized patients and acquisition of MDROs during ICU stay, as a consequence of antimicrobial drug overexposure and interpatient transmission, as well as contact with colonized healthcare workers, fomites, or the environment. The incidence of MDROs is strongly influenced by pandemic periods, such as during COVID-19, when unprecedented patient loads in ICUs resulted in breaches in IPC, such as gaps in microbiologic surveillance, lack of communication between clinicians, and reduced attention to environmental measures and contact precautions among healthcare workers (29). In addition, ICU admissions caused by viral pandemics place a strain on ICU resources, requiring the reallocation of non-ICU beds, along with the use of non-ICU staff to meet the urgent demand. In this setting, strengthening measures, such as active surveillance with prompt recognition of outbreaks, staff training, increased environmental disinfection and cohorting, become essential to reducing MDRO circulation (30).

In the pre–COVID-19 pandemic era, the prevalence of infections caused by MDROs in ICU patients varied from a reported rate of 14.1% in VALRTIs acquired in ICUs in North America (31) to an average

| Table 2. Incidence rate of MDRO events, overall and divided by infection site, of COVID-19 patients admitted to ICU in Milan, Italy, October 2020–May 2021, who had no MDRO isolates within the first 48 h of admission* |
| Characteristic | Infections | VALRTIs | BSIs | UTIs | Total |
| MDRO events, first colonization plus new infections | | NA | NA | NA | 41.68 (36.98–46.99) |
| First MDRO colonization | | | | | |
| New MDRO infection | 9.44 (7.58–11.74) | 4.89 (3.55–6.75) | 0.47 (0.14–1.08) | 14.99 (12.36–18.19) |
| New non-MDRO infection | 33.25 (29.04–38.07) | 11.62 (9.23–14.64) | 4.19 (2.97–5.72) | 50.12 (44.59–56.32) |
| Overall new infections, MDRO plus non-MDRO | 42.41 (37.81–47.58) | 16.57 (13.51–20.31) | 5.15 (3.36–6.26) | 65.13 (58.76–72.2) |

*Values are IR/1,000 person-days (95% CIs). The time considered for IRs was set from ICU admission to discharge, except for VALRTI, where total intubation time was considered. BSIs, bloodstream infections; ICU, intensive care unit; IR, incidence rate; MDRO, multidrug-resistant organism; NA, not applicable (MDRO colonization refers to patients and not infection sites); UTIs, urinary tract infections; VALRTIs, ventilator-associated lower respiratory tract infections.
Several studies have been published on MDRO incidence, etiology and source of HAI in ICU COVID-19 patients (4–21) (Table 3, https://wwwnc.cdc.gov/EID/article/29/8-0115-T3.htm). Most of those studies evaluated overall MDRO infections or specific HAIs, such as BSI or VALRTIs (7,15–17,19,21), whereas colonization events were assessed in only a few studies (8–12,14). Incidence measures of MDRO events varied widely; cumulative incidence of the first MDRO event was 5%–57% (7,17) and incidence rate 2.6–31.48 cases/1,000 patient-days (11,16). The percentage of MDRO was 27%–100% for all recorded events (15,17). Compared with the amount of literature evaluating MDRO events during ICU stay, we found that few data are available on MDRO proportions among COVID-19 patients at ICU admission. In recent work of the multicenter HAI-ICU surveillance network in France, the percentage of MDR gram-negative bacteria among >4,000 COVID-19 patients admitted was 11.7% (34).

In our cohort, 20% of patients had MDRO isolation within the first 48 hours, indicating acquisition before ICU admittance. We found that patients who had MDROs isolated during the first 48 hours were more frequently transferred from other ICUs and exposed to a higher number of antimicrobial drugs before ICU admittance. Both of those factors are well known to be associated with development of infections by antimicrobial drug-resistant pathogens (6). Only 2.7% of our cohort had MDRO colonization/infection before ICU admission. The marked difference between expected and observed MDRO prevalence at ICU admission probably reflects the major issues in IPC during the emergency situation of the pandemic mentioned beforehand.

Considering patients without MDRO isolation within the first 48 hours, we observed no differences in demographic characteristics or in clinical severity at admission between patients who showed or not showed development of MDRO events during ICU stay, underlying consistency between groups at ICU admission. In our cohort, we did not find direct association between MDRO infection and in-ICU deaths. However, length of ICU stay and duration of mechanical ventilation were longer for patients with MDRO events and, among them, longer for patients who had infections than for colonized patients. No causative effect can be drawn from these results because occurrence of MDRO events could be either responsible for longer ICU stay or its direct consequence because of longer exposure time (35,36).

Active surveillance screening coupled with the evaluation of all microbial isolates enabled us to precisely identify patients who had with MDRO events. Two thirds of the cohort showed development of MDRO colonization or infection during ICU stay. Half of our patients were given diagnoses of MDRO colonization during ICU stay, compared with 21% observed in a recent study analyzing a smaller population (10). Our results can be, in part, explained by strict routine microbiologic surveillance, which enabled prompt and precise recognition of such cases. Data from previous studies on bacterial superinfections in COVID-19 ICU patients are heterogenous and describe MDRO HAIs in 11%–25% of the population (6,13). Our results confirm the substantial risk for mechanically ventilated COVID-19 patients to have MDRO infections develop; such infections affected almost 30% of our cohort during ICU stays. Also, more than twice as many patients had antimicrobial drug–susceptible HAIs.

We found high concordance between clinical diagnosis and retrospective evaluation of HAIs according to literature criteria. We believe this result well demonstrates how implementation of structured antimicrobial stewardship and IPC measures, with collaboration of infectious disease consultants and intensivists, can strongly effect management of critically ill patients, favoring accurate diagnosis and therapeutic choices, according to international guidelines.

Patients who had MDRO events had greater exposure to antimicrobial drugs the first 10 days of ICU stay than patients who had no MDRO findings. This observation is consistent with results of recent studies conducted on large population of patients, which showed major associations between exposure to specific antimicrobial drug classes and drug resistance, and a decreasing pattern over time (37,38). However, accurate analysis of the association between antimicrobial drug exposure and MDRO events was beyond the scope of this study because other variables, such as average intake time of each antimicrobial drug class and infections with antimicrobial drug-susceptible bacteria during the observation time, should be considered.

The first limitation of this study is that it was a retrospective monocentric cohort and, therefore, had intrinsic risks of limited accuracy and generalizability. However, interpretation of all microbiologic findings has been conducted ex post on the basis of standardized literature criteria and independent from the
physicians’ view. Also, even though the study was monocentric, patients were admitted from >45 hospitals and assisted by different hospital staff. Advantages to this study design derive from the standardized microbiologic surveillance, both in terms of timing and laboratory method, as well as from homogeneous antimicrobial stewardship and IPC strategies among ICU modules. This factor enabled us to provide precise and consistent data in terms of incidence of HAIs and MDRO events, not only infections but also colonization.

Second, this study was not conducted for evaluation of the effect of antimicrobial drugs on development of MDRO or the effect of MDRO events on ICU deaths and length of stay; the sample size was probably inadequate for these issues. Therefore, our findings on this issue should be interpreted with caution.

Third, patients’ data before ICU admission were retrieved from information registered at ICU entry and not from hospital databases of the single referring centers. Accuracy of previous MDRO events and steroids and antimicrobial drug treatments might be limited, although these factors play a major role in routine management of ICU patients, and we do not expect major gaps in data acquisition.

In conclusion, our in-depth analysis of incidence measures of HAIs and MDRO events contributes to increase knowledge of MDRO colonization and infections in ICU COVID-19 patients. These findings should be a priority in contributing toward IPC and antimicrobial stewardship policies for ensuring the best clinical care.

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Deidentified patient data used for the results reported in this article, including data in text, tables, figures, and appendixes, will be available to researchers who provide a methodologically sound proposal to achieve their aims. Proposals should be addressed to andrea.gori@unimi.it and davide.mangioni@policlinico.mi.it. To gain access, data applicants will need to sign a data access agreement.

D.M., J.C., G.Ma., and A.B. designed the study; L.C. and M.C.V. performed methods and formal analysis; D.M., J.C., E.P., F.A.G., M.B., B.B., M.C., G.F., M.M., G.Mo, P.P., S.S., F.T., and G.Z. performed investigations; D.M. and E.P. wrote the original draft of the paper, wrote, reviewed, and edited the paper, and performed a literature review; L.C., J.C., F.A.G., M.B., N.B., B.B., M.C., G.F., M.M., G.Ma., C.M., A.M., P.P., S.S., F.T., G.Z., G.G., R.F., A.G., N.S., G.Mo., and A.B. wrote, reviewed, and edited the paper; and N.B., N.S., G.Ma., and A.B. supervised the study. All authors have read and agreed to the published version of the manuscript.

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RESEARCH

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World TB Day

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- Yellow Fever Vaccine–Associated Viscerotropic Disease among Siblings, São Paulo State, Brazil
- Bartonella spp. Infections Identified by Molecular Methods, United States
- COVID-19 Test Allocation Strategy to Mitigate SARS-CoV-2 infections across School Districts
- Using Discarded Facial Tissues to Monitor and Diagnose Viral Respiratory Infections
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Economic Evaluation of Wastewater Surveillance Combined with Clinical COVID-19 Screening Tests, Japan

Byung-Kwang Yoo, Ryo Iwamoto, Ungil Chung, Tomoko Sasaki, Masaaki Kitajima

The COVID-19 pandemic has imposed substantial burdens on the global society. To find an optimal combination of wastewater surveillance and clinical testing for tracking COVID-19, we evaluated the economic efficiency of hypothetical screening options at a single facility in Japan. To conduct cost-benefit analyses, we developed standard decision models in which we assumed model parameters from literature and primary data, such as screening policies used at the Tokyo Olympic and Paralympic Village in 2021. We compared hypothetical 2-step screening options that used clinical PCR to diagnose COVID-19 after a positive result from primary screening using antigen tests (option 1) or wastewater surveillance (option 2). Our simulation results indicated that option 2 likely would be economically more justifiable than option 1, particularly at lower incidence levels. Our findings could help justify and promote the use of wastewater surveillance as a primary screening at a facility level for COVID-19 and other infectious diseases.

COVID-19, caused by SARS-CoV-2, has imposed substantial disease and social burdens on the global society; ≈6.85 million deaths were confirmed worldwide by February 2023 (1). To reduce disease burden, both clinical screening tests and epidemic surveillance systems are required and need to be efficiently implemented under tight budget constraints.

Although clinical PCR and antigen tests are essential for detecting individual cases, those tests have multiple limitations, such as testing avoidance behaviors, low detection rates among asymptomatic persons, and challenges when high demand for testing during epidemic peaks exceeds laboratory capacity. An additional limitation is the relatively high cost at a population level, which hinders frequent implementation even among high-risk subpopulations and essential workers. Because of those limitations, an epidemic surveillance system based on clinical tests tends to underestimate prevalence and have reduced representation because of insufficient sample sizes.

Wastewater surveillance is expected to address limitations of clinical tests (2). A sample of wastewater can be highly representative for all residents at a specific facility or for hundreds of thousands of residents in an area covered by a single wastewater treatment plant. Although wastewater surveillance is a risk measure of a community and not an individual resident, when compared as separate options, a simple cost comparison favors wastewater surveillance over clinical tests (3).

The appropriate sampling site can differ depending on the population level targeted by wastewater surveillance. When a large population is targeted, such as all residents within a citywide sewershed, sampling of influent wastewater at a wastewater treatment plant is most effective (4). When neighborhood-scale sewersheds are targeted, wastewater should be sampled from manholes or pumping stations (5). Finally, when a single facility is targeted, wastewater samples must be collected immediately after being discharged from the facility; in most cases, such samples can be collected from a manhole (6).

We aimed to find an optimal combination of wastewater surveillance and clinical testing that complement, rather than substitute for, each other. Therefore, we performed an economic evaluation to
estimate the return on investment (ROI) of hypothetical screening options at a single facility in Japan.

Methods
We conducted a cost-benefit analysis to estimate the economic efficiency of various hypothetical screening options for confirming SARS-CoV-2 infections among asymptomatic or presymptomatic persons at a single residential facility, as measured by ROI, an equivalent to benefit-to-cost ratio. If 1 option is cost-saving compared with its comparator, that option’s ROI is estimated to be >1. For example, an estimated ROI of 1.50 indicates that a $100 investment in 1 option will produce a net savings of $50. Our cost-benefit analyses adopted a societal perspective with a 1-month timeframe.

We compared 2 hypothetical 2-step screening options that used clinical PCR tests to diagnose individual COVID-19 cases after a positive result from a primary screening with antigen tests (option 1) or wastewater surveillance (option 2). Those screening options partly followed those used in the Tokyo Olympic and Paralympic Village in 2021 (6,7). We assumed antigen test results would be available in ≤1 hour, PCR test results would be available on the same day, and wastewater surveillance results would be available by the day after sampling.

More specifically, under option 1, the residents at a facility would all undergo antigen testing daily for 4 days as a primary screening. Any resident who tests positive would receive secondary screening on the same day with 2 PCR tests to confirm the diagnosis. Option 2 was to conduct wastewater surveillance at a facility as a primary screening for days 1–3. If a previous day’s wastewater surveillance indicated a positive result, all persons at the facility would undergo secondary screening with 2 consecutive PCR tests to clinically diagnose an infected case during days 2–4.

Option 1 and option 2 are substitutes only in terms of their primary screening, either antigen tests or facility-based wastewater surveillance. For both options, the primary screening (antigen tests or facility-based wastewater surveillance) and secondary screening (PCR for clinical diagnosis) are complementary.

We assumed model parameters on the basis of available literature and primary data and developed a standard decision model (Table 1; Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/8/22-1775-App1.pdf). Our base-case analysis with a deterministic model assumed a point estimate for each parameter. To address the uncertainties of model parameters, we also implemented a probabilistic analysis with Monte Carlo simulations by assigning distributions (Table 1). For instance, we assumed a triangular distribution for the parameter sensitivity of wastewater surveillance using a mode of 66% (range 46%–84%). That parameter sensitivity could be affected by various factors, including variability in viral shedding over the course of an infection and between different infected persons, dilution and decay of virus in the sewer, and analytical sensitivity of the method used for virus detection in wastewater. Monte Carlo simulations provided the mean and the 95% probabilistic confidence interval (PCI) values of the ROI estimates. We used TreeAge software (https://treeage.com) to perform analyses for decision models.

Because economic efficiency is highly sensitive to the disease incidence, our base-case analysis included the 3 scenarios: 10, 100, or 1,000 newly reported clinically positive cases per million residents per day (PMPD) in the area around the facility. In other words, our study did not assign a certain distribution for the incidence because of a very wide range of feasible values.

The 10 PMPD incidence value corresponds to the minimum level at which wastewater surveillance sampled at a wastewater treatment plant can detect SARS-CoV-2 (4). Our 1-way sensitivity analyses all assumed the incidence value of 100 PMPD, above which a correlation was observed in our primary data between SARS-CoV-2 RNA load in wastewater sampled at a wastewater treatment plant and the incidence based on clinical PCR tests in the area (21).

The 1,000 PMPD incidence value is equal to the ratio of 1 newly infected case among 1,000 residents in a hypothetical facility, and 1,000 was close to the smallest population of the sampling area in the Tokyo Olympic and Paralympic Village in 2021 (6). Our base-case analyses all assumed the facility had 100 residents, which we based on the average number of beds in long-term care facilities (LTCFs) in Japan (22).

Hypothetical study populations in our base-case analyses all were 100 residents at a LTCF who were expected to receive greater benefits from screening tests in terms of preventing COVID-19–related illness and death compared with the general population. For instance, LTCF residents in Japan have an average age of ≥86 years (29) and were estimated to be 19 times as likely to die after a clinical COVID-19 diagnosis than the general population in Japan (23).

Our study estimated the benefit of confirming 1 infected case by PCR under each screening option by using 2 components: the benefit of reducing hospitalization and death for a confirmed case, and
the benefit of preventing secondary infection. Because scant literature addressed the effectiveness of screening in reducing hospitalization and mortality rates among persons who test positive, we assumed effectiveness was equivalent to the clinical efficacy of antiviral agents among patients with COVID-19 at its early stage (e.g., <7 days after the onset of signs or symptoms), who are not hospitalized yet but could be subsequently hospitalized or die (28). We reduced the clinical efficacy by 30%, because 30% of infected persons never develop symptoms (30). Consequently, our screening effectiveness had a triangular distribution with a mode of 0.54 (range 0.23–0.62). We estimated the benefit of preventing secondary infection to be 0.57 under our base-case analyses, which was dependent on a reproduction number of 1.3 (27), an infectious period of 8.03 days (31), and other factors (30). In addition, our model accounted for the loss of an infected case that produced a second-generation infected case every day (Appendix).

To assign benefit values for reducing hospitalization and mortality rates, we estimated the related monetary value for 3 outcomes among confirmed cases: isolation (14–16), hospitalization (17–19), and death (20,24–26). All monetary values are expressed in 2022 US dollars (USD). We assigned a monetary value for 3 outcomes among confirmed cases: isolation ($16,212 + $64,394 x (proportion of severe cases among hospitalized cases)), hospitalization ($19,394 ($16,212–$25,227)), and other factors (28). In addition, our model accounted for the loss of an infected case that produced a second-generation infected case every day (Appendix).

### Table 1. Decision model parameters in an economic evaluation of wastewater surveillance combined with clinical COVID-19 screening tests, Japan*

<table>
<thead>
<tr>
<th>Parameters†</th>
<th>Point estimate (range)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wastewater surveillance</td>
<td>0.66 (0.46–0.84)</td>
<td>M. Kitajima, unpub. data</td>
</tr>
<tr>
<td>PCR‡</td>
<td>0.74 (0.64–0.83)</td>
<td>(8,9)</td>
</tr>
<tr>
<td>Ratio of antigen test against PCR test</td>
<td>0.76 (0.54–0.97)</td>
<td>(8–10)</td>
</tr>
<tr>
<td>PCR test after positive antigen test</td>
<td>0.99 (0.64–0.999)</td>
<td>(8,9)</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>0.974 (0.96–0.995)</td>
<td>(9,10)</td>
</tr>
<tr>
<td>Antigen test</td>
<td>0.99 (0.97–0.995)</td>
<td>(10)</td>
</tr>
<tr>
<td>Ratio of wastewater surveillance against PCR test</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>Cost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory cost of wastewater surveillance per facility per day</td>
<td>$379 ($189–$758)</td>
<td>(11,12)</td>
</tr>
<tr>
<td>Labor cost to sample at a facility per facility per day</td>
<td>$1,136 ($152–$2,045)</td>
<td>(13)</td>
</tr>
<tr>
<td>Antigen test§</td>
<td>$16 ($10–$23)</td>
<td>(14,15)</td>
</tr>
<tr>
<td>Clinical PCR§</td>
<td>$38 ($20–$53)</td>
<td>(14,15)</td>
</tr>
<tr>
<td>Isolation per test-positive case</td>
<td>$758 ($379–$1,515)</td>
<td>(16)</td>
</tr>
<tr>
<td>Hospitalization per case¶</td>
<td>$19,394 ($16,212–$25,227)</td>
<td>(17–19)</td>
</tr>
<tr>
<td>Value of QALY saved per case</td>
<td>$37,879</td>
<td>(20)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence per day per 1 million residents</td>
<td>100 (10–10,000)</td>
<td>(4,21)</td>
</tr>
<tr>
<td>No. residents at a facility</td>
<td>100 (50–200)</td>
<td>(6,22)</td>
</tr>
<tr>
<td>Mortality rate among persons who test positive#</td>
<td>0.0035 (0.0016–0.0104)</td>
<td>(23)</td>
</tr>
<tr>
<td>Ratio of mortality rate among persons ≥80 years of age vs. general population</td>
<td>19 (15–22)</td>
<td>(23)</td>
</tr>
<tr>
<td>Life-years saved by avoiding COVID-19</td>
<td>11.4 (11.1–11.7)</td>
<td>(24,25)</td>
</tr>
<tr>
<td>Ratio to convert life-years saved to QALYs saved</td>
<td>0.68 (0.64–0.71)</td>
<td>(24,26)</td>
</tr>
<tr>
<td>Hospitalization rate among persons who test positive</td>
<td>0.18 (0.04–0.40)</td>
<td>(17)</td>
</tr>
<tr>
<td>Proportion of severe cases among hospitalized cases</td>
<td>0.1 (0.05–0.19)</td>
<td>(17)</td>
</tr>
<tr>
<td>Effective reproduction number of infected cases</td>
<td>1.3 (0.9–2.0)</td>
<td>(27)</td>
</tr>
<tr>
<td>Screening effectiveness in reducing hospitalization and mortality rates</td>
<td>0.54 (0.23–0.62)</td>
<td>(28)</td>
</tr>
<tr>
<td>Ratio of loss value of missing an infected case compared with benefit value of finding an infected case</td>
<td>1 (0–2.0)</td>
<td></td>
</tr>
</tbody>
</table>

*All monetary values are expressed in 2022 US dollars. QALY, quality-adjusted life-years.
†All parameters with a minimum and a maximum value in this table are defined as a triangular distribution in the probabilistic analysis, detailed in the Appendix (https://wwwnc.cdc.gov/EID/article/29/8-22-1775-App1.pdf).
‡Because the second clinical PCR test was conducted immediately after the first clinical PCR test, the sensitivity of the second clinical PCR was assumed to be equal to the specificity of the PCR test in this table.
§Test cost plus labor cost for sampling; 30 min multiplied by minimum wage of $7 USD per hour (15).
¶Hospitalization cost was assumed as $16,212 + $64,394 x (proportion of severe cases among hospitalized cases – 0.05).
#The range was defined to range from the date before the vaccination period to the rate after the vaccination period (Appendix).
Results

When COVID-19 incidence was 10 PMPD, our deterministic base-case analysis indicated that option 1 alone, compared with doing nothing (comparator do-nothing), was not economically justifiable because its cost ($67.04) exceeded its benefit ($1.39) and the ROI of 0.021 ($1.39/$67.04) was <1.0 (Table 2). Although option 2 alone compared with do-nothing was not justifiable because of the low ROI (0.021), option 2 became justifiable when its comparator was changed from do-nothing to option 1. That is, compared with option 1, option 2 saved $13.44, which could be interpreted as relative benefit, and had a $0.25 lower benefit, which could be interpreted as relative cost. Thus, compared with option 1, the relative value of option 2 was a high ROI of 54 ($13.44/$0.25) (Table 2).

When COVID-19 incidence was 1,000 PMPD under our base-case analysis, we estimated the ROI of option 1 to be 2.10 and of option 2 to be 2.23 (Table 2). One-way sensitivity analysis of the deterministic model showed the threshold incidence values, above or below which an option’s ROI is >1. Those threshold values were 480 PMPD for option 1 alone, 450 PMPD for option 2 alone, and 630 PMPD for the relative value of option 2 (Table 3). One-way sensitivity analysis also showed that when incidence increased, the ROI of options 1 and 2 increased and that the relative value of option 2 declined (Figure).

Additional 1-way sensitivity analyses of the base-case analysis showed that within the feasible range of parameters, all 3 types of ROI estimates were sensitive to incidence and had values above and below 1.0. The ROI estimates of options 1 and 2 alone, compared with do-nothing, were robust to all parameters except incidence. The ROI estimates of options 1 and 2 alone had a negative association with test costs and a positive association with test sensitivity and specificity (Table 4).

The estimated range of the ROI for the relative value of option 2 includes negative values (Table 4). For the ratio of sensitivity of antigen tests against PCR, option 2 was always preferred over option 1; option 2 dominated option 1 when the ROI estimates were negative for that ratio. In other words, a simple linear relationship did not occur between the ratio of sensitivity of antigen tests against PCR and the ROI for the relative value of option 2. For instance, when that ratio increased from 0.64 to 0.97, the ROI estimate for the relative value of option 2 was always >1 (Appendix Table 8). When the ratio was 0.638, option 2’s benefit became equal to option 1’s benefit, which did not mathematically enable estimation of the ROI for the relative value of option 2. When the ratio increased from 0.54 to 0.63, option 2’s benefit exceeded option 1’s benefit; thus, option 2 dominated option 1.

The ROI estimates regarding the relative value of option 2 were sensitive to 3 cost-related parameters. In other words, an estimated threshold point existed, below or above which a preferred option changed. For instance, option 2 was preferred only when the labor cost to sample a facility was lower than the threshold point of $1,512. When labor cost exceeded that threshold point, option 1 was preferred. Likewise, when the cost of the antigen test was lower than the threshold point of $13.18, option 1 was preferred, but when it was greater than that threshold point, option 2 was preferred. Because the cost of wastewater surveillance per facility was fixed, the cost per facility resident could be substantially reduced by a larger number of facility residents. Therefore, when the number of residents was lower than the threshold point of 81, option 1 was preferred, but when it was greater than that threshold point, option 2 was preferred.

The probabilistic analyses showed that the base-case analyses with a deterministic model were robust, particularly for cost, benefit, and ROI estimates for option 1 alone or option 2 alone (Table 5). Although the estimated PCIs included a large negative value as a lower bound, option 2 was mostly preferred to option 1 when the incidence was 10 or 100

Table 2. Base-case analysis with a deterministic model in an economic evaluation of wastewater surveillance combined with clinical COVID-19 screening tests, Japan*

<table>
<thead>
<tr>
<th>Incidence†</th>
<th>Option 1</th>
<th></th>
<th>Option 2</th>
<th></th>
<th>Incremental cost§</th>
<th>Incremental benefit¶</th>
<th>Relative ROI#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cost</td>
<td>Benefit</td>
<td>ROI‡</td>
<td>Cost</td>
<td>Benefit</td>
<td>ROI¶</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>$67.04</td>
<td>$1.39</td>
<td>0.021</td>
<td>$53.60</td>
<td>$1.14</td>
<td>0.021</td>
<td>$13.44</td>
</tr>
<tr>
<td>100</td>
<td>$67.05</td>
<td>$14.09</td>
<td>0.21</td>
<td>$53.61</td>
<td>$11.94</td>
<td>0.22</td>
<td>$13.43</td>
</tr>
<tr>
<td>1,000</td>
<td>$67.12</td>
<td>$141.11</td>
<td>2.10</td>
<td>$53.75</td>
<td>$119.94</td>
<td>2.23</td>
<td>$13.37</td>
</tr>
</tbody>
</table>

*Option 1 is clinical testing only; option 2 is wastewater surveillance and clinical testing. If one option is cost-saving compared with its comparator, the option’s ROI is estimated to exceed 1. The comparator of options 1 and 2 is do-nothing. All monetary values are expressed in 2022 US dollars (USD).

†Disease incidence per day per 1 million residents in the area.

‡ROI is benefit divided by cost for each option.

§Incremental cost is the cost of option 2 minus cost of option 1. A negative value of incremental cost indicates that option 2 has a lower cost or is cost-saving, compared with option 1. This could be interpreted as option 2’s relative benefit.

¶Incremental benefit is the benefit of option 2 minus benefit of option 1. A negative value of incremental benefit indicates that option 2 has a lower benefit compared with option 1, which could be interpreted as option 2’s relative cost.

#Relative ROI is incremental cost divided by incremental benefit.
PMPD. More specifically, over 1,000 iterations, when incidence was 10 PMPD, option 2 was preferred in 84.7% of the time; when incidence was 100 PMPD, option 2 was preferred 80.8% of the time; and when incidence was 1,000 PMPD, option 2 was preferred 25.2% of the time. Thus, qualitative conclusions of probabilistic analyses were similar to those of deterministic analyses.

Discussion

Our simulation results indicate that a primary screening with wastewater surveillance (option 2) at a single facility was highly likely to be economically more justifiable than a primary screening with antigen tests (option 1), particularly at lower incidence levels (<630 PMPD). Option 2 tended to have a much lower cost (interpreted as relative benefit) and a slightly lower benefit (interpreted as relative cost) compared with option 1. Of note, when the comparator was do-nothing, option 1 alone and option 2 alone had low economic efficiency when the disease incidence was low; option 1 alone was economically justifiable only when the incidence was >480 PMPD and option 2 alone was economically justifiable only when the incidence was >450 PMPD. At incidence levels >1,000 PMPD, option 2 is economically less efficient than option 1 because clinical tests would not be implemented on day 1 under option 2, which would lead to more secondary infections and more costs for isolation or hospitalization. Our results appeared generally robust to the feasible range of model parameters, although some results were sensitive to parameters related to the disease incidence and cost of tests.

Our analytical models are expected to have high generalizability to and be robust for SARS-CoV-2 variants, unlike vaccination effectiveness, which can potentially be reduced by variants. In addition, our analytic approach would be readily applicable to other emerging infectious diseases.

The negative ROI estimates regarding the relative value of option 2 should be interpreted with caution because 2 opposite interpretations are possible. One interpretation prefers option 2, such as when option 2 detected many more infected cases than option 1 at a facility with <77 residents. On the contrary, the other interpretation prefers option 1, such as when fewer COVID-19 cases were missed by option 1 than option 2 and when the antigen test cost was <$12.64.

We expected the face validity of our simulation results to be achieved to some extent, partly because the assumptions of our hypothetical screening options mainly followed the screening policies used in the Tokyo Olympic and Paralympic Village (6,7). Also, the assumed range of the laboratory cost for wastewater surveillance ($189–$758) appeared reasonable, compared with costs reported by other studies (11,12,32).
In addition, we used conservative assumptions in our base-case analysis, such as relatively high costs for additional labor to sample wastewater at a facility for surveillance (13). Another set of conservative assumptions that reduced the benefit of confirming 1 infected case were the exclusion of COVID-19–related medical expenditure for outpatient care and the possible financial loss related to shutdown of a LTCF. We excluded those items from our analyses because cost-related data were absent in the literature.

One weakness of this study is the limited generalizability to other settings. We assumed the monetary value of finding 1 COVID-19 case at a facility depended partly on related medical expenditure and QALY saved. QALY varies in different countries; in Japan, the value is $37,879/QALY (20). Also, the monetary value of finding 1 case consisted of mortality rate in the population, hospitalization rate in the population, and medical expenditures per hospitalized case, all of which could vary substantially at the population level because of viral variants occurring over time and across regions within a country. In addition, mortality and hospitalization rates vary markedly among subpopulations defined by age and high-risk chronic conditions. Such uncertainties indicate the need to frequently update the simulation model to correspond to regional epidemics and target populations.

Because of the absence of literature, the validity of our ROI estimates was difficult to compare with estimates from previous studies. Although 1 study compared wastewater surveillance at a treatment plant and clinical PCR tests in its costs, that study compared cost per population screened without accounting for clinically confirmed cases after wastewater surveillance (3). Therefore, the estimates in that study were not appropriate comparisons for our ROI estimates. When the goal of screening is to identify and isolate an infected case, wastewater surveillance should be used as a primary screening, after which secondary screening should be performed by using clinical tests.

Major policy implications derived from this study’s findings are exemplified by the threshold levels to start or suspend a specific screening option. Compared with do-nothing, threshold incidence levels were 480 PMPD for option 1 alone and 450 PMPD for option 2 alone, but those thresholds are <1,000 PMPD. The 1,000 PMPD incidence is equivalent to 1 newly infected case at a single large facility with 1,000 residents. That is, before finding the first newly infected case at a single facility, options 1 and 2 should be started, ideally triggered when the incidence of the area around the facility, such as the city, town, or neighborhood, reaches the threshold levels we reported for each option.

The ROI estimates for the relative value of option 2 compared with option 1 tended to be high at a very low incidence, when the absolute benefit of option 2 is small compared with do-nothing. One practical incidence level to trigger option 2 is 10 PMPD, above which wastewater surveillance conducted by using a recently developed method can detect SARS-CoV-2 RNA at a treatment plant (4). Another trigger incidence is 100 PMPD, above which conventional wastewater surveillance methods can detect SARS-CoV-2 RNA (4). Regularly monitoring data from wastewater surveillance at a treatment plant could enable efficient triggers for option 1 and option 2 at a specific facility in the same area.

Because wastewater surveillance at a treatment plant covers a city-scale population, the additional cost per resident would be very small, even when...
focusing on an institutionalized population; for instance, increasing the per resident cost in our model by <1%. Although the central government of Japan implemented pilot projects of wastewater surveillance at both city and facility levels during fiscal year 2022 (33), government officials did not expand the scale of those projects, partly because of a lack of evidence regarding economic efficiency. Thus, our findings could help the central government of Japan justify the expansion of these projects.

Table 4. One-way sensitivity analyses of the base-case analysis in an economic evaluation of wastewater surveillance combined with clinical COVID-19 screening tests, Japan*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Parameter values</th>
<th>Return on investment†</th>
<th>Option 1</th>
<th>Option 2</th>
<th>Relative ROI‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wastewater surveillance</td>
<td>0.46</td>
<td>0.21</td>
<td>0.12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>PCr</td>
<td>0.84</td>
<td>0.21</td>
<td>0.26</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>0.64</td>
<td>0.18</td>
<td>0.22</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Ratio of antigen test against PCR test</td>
<td>0.83</td>
<td>0.23</td>
<td>0.22</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>PCR test after positive antigen test</td>
<td>0.54</td>
<td>0.14</td>
<td>0.22</td>
<td>-5.43</td>
<td></td>
</tr>
<tr>
<td>PCR test after positive antigen test</td>
<td>0.97</td>
<td>0.24</td>
<td>0.22</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>PCR test after positive antigen test</td>
<td>0.64</td>
<td>0.12</td>
<td>0.10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>PCR test after positive antigen test</td>
<td>0.999</td>
<td>0.21</td>
<td>0.22</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>0.96</td>
<td>0.21</td>
<td>0.21</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Antigen test</td>
<td>0.995</td>
<td>0.21</td>
<td>0.25</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Antigen test</td>
<td>0.97</td>
<td>0.19</td>
<td>0.22</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Antigen test</td>
<td>0.995</td>
<td>0.22</td>
<td>0.22</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wastewater surveillance cost per day per facility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory cost</td>
<td>$189</td>
<td>0.21</td>
<td>0.25</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Labor cost to sample</td>
<td>$758</td>
<td>0.21</td>
<td>0.18</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Labor cost to sample</td>
<td>$152</td>
<td>0.21</td>
<td>0.50</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Labor cost to sample</td>
<td>$2,045</td>
<td>0.21</td>
<td>0.15</td>
<td>-6.43</td>
<td></td>
</tr>
<tr>
<td>Antigen test</td>
<td>$10</td>
<td>0.33</td>
<td>0.22</td>
<td>-4.91</td>
<td></td>
</tr>
<tr>
<td>Antigen test</td>
<td>$23</td>
<td>0.15</td>
<td>0.22</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Clinical PCR</td>
<td>$20</td>
<td>0.215</td>
<td>0.24</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Clinical PCR</td>
<td>$53</td>
<td>0.207</td>
<td>0.21</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Isolation per test-positive case</td>
<td>$379</td>
<td>0.209</td>
<td>0.222</td>
<td>6.33</td>
<td></td>
</tr>
<tr>
<td>Isolation per test-positive case</td>
<td>$1,515</td>
<td>0.212</td>
<td>0.224</td>
<td>6.09</td>
<td></td>
</tr>
<tr>
<td>Hospitalization per case</td>
<td>$16,212</td>
<td>0.18</td>
<td>0.19</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Hospitalization per case</td>
<td>$25,227</td>
<td>0.26</td>
<td>0.27</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence per day per 1 million population</td>
<td>10</td>
<td>0.02</td>
<td>0.02</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Incidence per day per 1 million population</td>
<td>10,000</td>
<td>21</td>
<td>22</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>No. residents at a facility</td>
<td>50</td>
<td>0.21</td>
<td>0.12</td>
<td>-14.89</td>
<td></td>
</tr>
<tr>
<td>No. residents at a facility</td>
<td>1,000</td>
<td>0.21</td>
<td>0.94</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Mortality rate among persons who test positive</td>
<td>0.0018</td>
<td>0.19</td>
<td>0.20</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Mortality rate among persons who test positive</td>
<td>0.0104</td>
<td>0.30</td>
<td>0.32</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Mortality rate among persons who test positive</td>
<td>0.5</td>
<td>0.004</td>
<td>0.004</td>
<td>161</td>
<td></td>
</tr>
<tr>
<td>Mortality rate among persons who test positive</td>
<td>0.04</td>
<td>0.24</td>
<td>0.26</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Life-years saved by avoiding COVID-19</td>
<td>11.1</td>
<td>0.209</td>
<td>0.221</td>
<td>6.28</td>
<td></td>
</tr>
<tr>
<td>Life-years saved by avoiding COVID-19</td>
<td>11.7</td>
<td>0.211</td>
<td>0.224</td>
<td>6.21</td>
<td></td>
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<tr>
<td>Ratio to convert life-years saved to QALYs saved</td>
<td>0.64</td>
<td>0.207</td>
<td>0.220</td>
<td>6.33</td>
<td></td>
</tr>
<tr>
<td>Ratio to convert life-years saved to QALYs saved</td>
<td>0.71</td>
<td>0.212</td>
<td>0.225</td>
<td>6.19</td>
<td></td>
</tr>
<tr>
<td>Ratio to convert life-years saved to QALYs saved</td>
<td>0.64</td>
<td>0.207</td>
<td>0.220</td>
<td>6.33</td>
<td></td>
</tr>
<tr>
<td>Ratio to convert life-years saved to QALYs saved</td>
<td>0.71</td>
<td>0.212</td>
<td>0.225</td>
<td>6.19</td>
<td></td>
</tr>
<tr>
<td>Hospitalization rate among persons who test positive</td>
<td>0.04</td>
<td>0.08</td>
<td>0.08</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Hospitalization rate among persons who test positive</td>
<td>0.40</td>
<td>0.42</td>
<td>0.46</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Proportion of severe cases among hospitalized cases</td>
<td>0.05</td>
<td>0.18</td>
<td>0.19</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Proportion of severe cases among hospitalized cases</td>
<td>0.19</td>
<td>0.26</td>
<td>0.28</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Effective reproduction number of infected cases</td>
<td>0.9</td>
<td>0.28</td>
<td>0.31</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Effective reproduction number of infected cases</td>
<td>2.0</td>
<td>0.17</td>
<td>0.16</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Effective reproduction number of infected cases</td>
<td>0.23</td>
<td>0.09</td>
<td>0.10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Effective reproduction number of infected cases</td>
<td>0.62</td>
<td>0.24</td>
<td>0.26</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Effective reproduction number of infected cases</td>
<td>0.0</td>
<td>0.25</td>
<td>0.31</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>Effective reproduction number of infected cases</td>
<td>2.0</td>
<td>0.17</td>
<td>0.13</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*The lower and upper bounds of each parameter are shown to illustrate the association between a parameter and its ROI. Incidence was assumed to be 100 persons per day per 1 million residents in the area. All monetary values are expressed in 2022 US dollars. QALY, quality-adjusted life-years; ROI, return on investment.
†Option 1 is clinical tests only; option 2 is wastewater surveillance and clinical tests.
‡Relative ROI of option 2 compared with option 1.
Another major policy implication is the threshold level for the number of residents at a facility. Our base-case analyses used hypothetical study populations of 100 residents at an LTCF. Our sensitivity analyses showed that the ROIs for option 2 alone and option 2 combined with clinical COVID-19 screening tests, Japan*

<table>
<thead>
<tr>
<th>Incidence†</th>
<th>Option 1</th>
<th></th>
<th></th>
<th>Option 2</th>
<th></th>
<th></th>
<th>Relative value of option 2</th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cost</td>
<td>Benefit</td>
<td>ROI 1‡</td>
<td>Cost</td>
<td>Benefit</td>
<td>ROI 2‡</td>
<td>Inc. cost§</td>
<td>Inc. benefit¶</td>
<td>Rel. ROI#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>$67.04</td>
<td>$1.39</td>
<td>0.021</td>
<td>$53.60</td>
<td>$1.14</td>
<td>0.021</td>
<td>$13.44</td>
<td>$0.25</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean PA</td>
<td>$70.03</td>
<td>$1.43</td>
<td>0.021</td>
<td>$50.68</td>
<td>$0.97</td>
<td>0.021</td>
<td>$19.35</td>
<td>$0.46</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(95% PCI)</td>
<td>($49.85–$90.25)</td>
<td>($0.42–$2.85)</td>
<td>(0.006–0.043)</td>
<td>($25.27–$90.23)</td>
<td>($0.19–$2.04)</td>
<td>(0.004–0.051)</td>
<td>($54.48 to –$14.44)</td>
<td>($1.20 to –$194 to)</td>
<td>387</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>$67.05</td>
<td>$14.09</td>
<td>0.21</td>
<td>$53.61</td>
<td>$11.94</td>
<td>0.22</td>
<td>$13.43</td>
<td>$2.15</td>
<td>6.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean PA</td>
<td>$66.54</td>
<td>$14.75</td>
<td>0.22</td>
<td>$50.86</td>
<td>$10.37</td>
<td>0.23</td>
<td>$17.68</td>
<td>$4.38</td>
<td>5.74</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(95% PCI)</td>
<td>($48.77–$88.86)</td>
<td>($5.11–$28.35)</td>
<td>(0.07–0.45)</td>
<td>($24.95–$92.14)</td>
<td>($3.03–$20.71)</td>
<td>(0.05–0.60)</td>
<td>($52.33 to –$11.35 to)</td>
<td>($11 to –$24 to 37)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DA</td>
<td>$67.12</td>
<td>$141.11</td>
<td>2.10</td>
<td>$53.75</td>
<td>$119.94</td>
<td>2.23</td>
<td>$13.37</td>
<td>$21.16</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean PA</td>
<td>$69.50</td>
<td>$147.29</td>
<td>2.17</td>
<td>$50.61</td>
<td>$104.58</td>
<td>2.29</td>
<td>$18.89</td>
<td>$42.71</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(95% PCI)</td>
<td>($48.76–$89.54)</td>
<td>($52.37–$279.00)</td>
<td>(0.73–4.57)</td>
<td>($24.56–$89.89)</td>
<td>($30.91–$215.00)</td>
<td>(0.55–5.59)</td>
<td>($52.28 to –$110 to)</td>
<td>($21 to –$124 to)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A probabilistic model to compare clinical tests only (option 1) to wastewater surveillance combined with clinical tests (option 2). If one option is cost-saving compared with its comparator, the option’s ROI is estimated to exceed 1. The comparator of options 1 and 2 is do-nothing. DA, deterministic model analysis; inc., incremental; PA, probabilistic model analysis with Monte Carlo simulations; PCI, probabilistic confidence interval; rel., relative; ROI, return on investment.

†Disease incidence per day per 1 million residents in the area.

‡ROI is benefit divided by cost for each option.

§Incremental cost is the cost of option 2 minus cost of option 1. A negative value of incremental cost indicates that option 2 has a lower cost or is cost-saving, compared with option 1. This could be interpreted as option 2’s relative benefit.

¶Incremental benefit is the benefit of option 2 minus benefit of option 1. A negative value of incremental benefit indicates that option 2 has a lower benefit compared with option 1, which could be interpreted as option 2’s relative cost.

#Relative ROI is incremental cost divided by incremental benefit.

Although one of the general advantages of wastewater surveillance is fewer privacy and stigmatization concerns than possible with clinical surveillance (34), ethical issues could arise in 2 cases. First, targeting a specific facility or a small catchment could lead to social harm and financial burdens to the targeted population (34). Second, regardless of the target population size, ethical issues might arise when the wastewater surveillance is used for applying restrictive measures, such as group quarantine or business closure in the target area or facility (35). Researchers, policymakers, and regulators need to collaborate to account for ethical issues in implementing wastewater surveillance (36), which could enable wastewater surveillance to represent a new frontier in surveillance, monitoring, and screening.

In conclusion, our findings could help justify and promote the use of wastewater surveillance as a primary screening at a single facility when a set of quantified conditions estimated in our simulation are met.
Of note, regular wastewater surveillance at a treatment plant will help trigger the start of any screening tests at a specific facility. Because few economic evaluations of wastewater surveillance have yet been conducted, our findings can contribute to related academic fields and policy making.

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About the Author

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18. Global Health Consulting. The unit price of a COVID-19 patient—54,000 yen for mild cases, 80,000 yen for moderate cases, and 142,000 yen for severe cases—is not worth the investment cost, according to the president of Japan Municipal Hospital Association (JMHA) [in Japanese] [cited 2021 Nov 24]. https://gmmmed.gchc-j.com/?p=38100


etymologia revisited

**Trichinella spiralis**

[tri kuh neh’ luh spr a’ luhs]

*Trichinella* is derived from the Greek words *trichos* (hair) and *ella* (diminutive); *spiralis* means spiral. In 1835, Richard Owen (1804–1892) and James Paget (1814–1899) described a spiral worm (*Trichina spiralis*)–lined sandy diaphragm of a cadaver. In 1895, Alcide Raillet (1852–1930) renamed it as *Trichinella spiralis* because *Trichina* was attributed to an insect in 1830. In 1859, Rudolf Virchow (1821–1902) described the life cycle. The genus includes many distinct species, several genotypes, and encapsulated and nonencapsulated clades based on the presence/absence of a collagen capsule.

**References**


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In the past few decades, bacterial infections with limited therapeutic options have become a serious threat for medicine. This problem is primarily caused by antimicrobial resistance (AMR), which disseminates by clonal spread of resistant organisms and horizontal transmission of mobile genetic elements with AMR genes. Several taxa have been classified as main AMR pathogens, including Klebsiella pneumoniae and Enterobacter spp. of the order Enterobacterales (1), and carbapenemase-producing Enterobacterales (CPE) are among the most challenging multidrug-resistant organisms (2). Important carbapenemase types, metallo-β-lactamases (MBLs) of the families VIM and IMP, have been recorded in enterobacteria in Europe since 2001 (3), often in the Mediterranean region (4–10). The blaVIM/IMP gene cassettes have usually been located in class 1 integrons, either assembled in Pseudomonas spp. and then transferred to Enterobacterales (4–6) or typical for Enterobacterales (4,7–10). The integrons have been carried by diverse plasmids with various replicons (4,7,8,10,11).

In Poland, VIM-type enzymes were originally identified in 2006 in K. pneumoniae, followed soon by Enterobacter hormaechei (12). Molecular analysis of all 121 VIM/IMP CPE isolates from 2006–2012 revealed high prevalence of Enterobacter spp. (≈53%) and relatively low contribution of K. pneumoniae (≈9%). Enterobacter spp. was dominated by E. hormaechei sequence type (ST) 90 and ST89, mostly with In238-like integrons of Pseudomonas aeruginosa origin. We describe the genomic analysis of all VIM/IMP Enterobacter spp. isolates in Poland during 2006–2019, in the context of all VIM/IMP CPE from that period, and international Enterobacter spp. genomes from public databases.

Methods

Study Design, Bacterial Isolates, Whole-Genome Sequencing, and Species Identification

The National Reference Centre for Susceptibility Testing conducts CPE surveillance in Poland, collecting isolates with basic patient, hospital ward, and isolate data. We tested the isolates by using CarbaNP (13) and phenotypic tests (14), and used PCRs for blaNDM-, blaVIM-, blaIMP-, blaKPC-, and blaOXA-48-like genes (4). A collection of 934 isolates from 246 hospitals in 117 cities were all nonduplicate VIM/IMP CPE confirmed during 2006–2019. We sequenced all those isolates by using MiSeq (Illumina, https://www.illumina.com), with de novo assemblies as described (15), and subjected them to species identification on the basis of average nucleotide identities by using FastANI 1.32 with a ≥95%
cutoff (16). We further analyzed the largest group of 375 isolates of the genus Enterobacter from 145 hospitals in 76 towns. We also sequenced 9 selected isolates by using MinION (Oxford Nanopore Technologies, https://nanoporetech.com) (15). We performed hybrid assemblies by using Unicycler 0.4.8 (17).

**Molecular Typing and Comparative Genomic Analysis**

We performed multilocus sequence typing (MLST) of all 375 Enterobacter spp. isolates (18) in silico by using mlst (https://github.com/tseemann/mlst). We performed the in-sample clonality single-nucleotide polymorphism (SNP) analysis for individual sequence types (STs) by using BioNumerics 7.6.3 (Applied Maths, https://www.applied-maths.com) and using index (i.e., initial) isolates of the STs as references. For the SNP-based phylogenetic analysis in the international context, we downloaded all (nonfiltered) 3,244 Enterobacter spp. genomes available in RefSeq (https://www.ncbi.nlm.nih.gov/refseq) as of June 6, 2022, and subjected them to MLST. We included isolates of the major STs (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0199-T1.htm; Figure 1); 5 STs had >10 isolates each (258 [68.8%]): ST90 (117 [31.2%]) Enterobacter spp., ST89 (74 [19.7%]), ST121 (36 [9.2%]), ST66 (18 [4.8%]), and ST134 (13 [3.5%]). Isolates of closely related STs (single-locus variants) distinguished 56 STs (Table, https://wwwnc.cdc.gov/EID/article/29/8/23-0199-T1.htm; Figure 1); 5 STs had >10 isolates each (258 [68.8%]): ST90 (117 [31.2%]) Enterobacter spp., ST89 (74 [19.7%]), ST121 (36 [9.2%]), ST66 (18 [4.8%]), and ST134 (13 [3.5%]).

**Acquired AMR Genes, Integrons, and Plasmids or Genomic Islands Carrying bla\textsubscript{VIM/IMP} Genes**

We detected acquired AMR genes by using ABRicate and the ResFinder database with 99.5% identity criterion (19) and profiled replicon types with PlasmidFinder 2.1 (20). We performed structural analysis and annotation of MBL-encoding integrons, plasmids, and genomic islands manually in Geneious Prime 2022.0.1 (Biomatters, https://www.geneious.com) by using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We visualized plasmid and island structures by using BRIG (http://brig.sourceforge.net) and Easyfig 2.2.5. (http://mjsull.github.io/Easyfig).

**Nucleotide Sequence Accession Numbers**

We submitted genomic data for the Enterobacter spp. isolates to the US National Center for Biotechnology Information (BioProject no. PRJNA877430). Plasmid sequences are available under the following GenBank accession numbers: p743A, OQ111274; p5955A, OQ111275; p7753A, OQ111276; p4969H, OQ111277; p5435N, OQ111278; p5713F, OQ111279; p6234F, OQ111280. Sequences of genomic islands are available under the following GenBank accession numbers: EhlGI3, OQ116783; EhlGI4, OQ116782.

**Results**

**Taxonomic Distribution of VIM/IMP-Type CPE in Poland**

We collected 934 VIM/IMP CPE during 2006–2019 from 246 hospitals in 117 cities of all 16 regions of Poland (Appendix Figure 1, panel A). In annual numbers of cases, a gradual increase occurred, from a few cases during 2006–2008 up to 242 in 2019 (Appendix Table 2). We identified 9 genera, including Enterobacter (40.1%), Klebsiella (K. pneumoniae and K. oxytoca groups, 34.4%), Citrobacter (10.7%), Escherichia (9.2%), and Serratia (4.2%). The distribution of genera varied in time, including predominance of Enterobacter spp. and remarkable contribution of K. oxytoca during 2006–2013 (12) and still high prevalence of Enterobacter spp. but also a dynamic K. pneumoniae increase during 2014–2019 (Appendix Figure 1, panel B). Of note, annual numbers of Enterobacter spp. isolates grew at a roughly constant rate by the end of 2018, then escalating in 2019. VIM-type MBLs prevailed vastly (99.3%), whereas IMPs contributed marginally (0.7%). The 375 Enterobacter spp. isolates originated from 145 hospitals out of 76 towns and were recovered during various infections (64.3%), mainly of the urinary tract (31.5% of the infections) and wounds (28.6%), or from carriage (34.9%).

**Species and Clonality of Enterobacter spp.**

We identified 6 species among the 375 Enterobacter isolates, largely E. hormaechei (362 [96.5%]) with 5 sub-species: steigerwaltii (n = 244), xiangfangensis (n = 71), hoffmannii (n = 35), oharae (n = 11) and hormaechei (n = 1) (Appendix Table 2). The remaining species were E. rogenkampfii (8 [2.2%]), E. asburiae (2 [0.5%]), and E. kobei, E. ludwigii, and E. mori (1 [0.3%] each). We distinguished 56 STs (Table, https://wwwnc.cdc.gov/EID/article/29/8/23-0199-T1.htm; Figure 1); 5 STs had >10 isolates each (258 [68.8%]): ST90 (117 [31.2%]) of all Enterobacter spp., ST89 (74 [19.7%]), ST121 (36 [9.6%]), ST66 (18 [4.8%]), and ST134 (13 [3.5%]). Isolates of closely related STs (single-locus variants) represented clonal groups (CGs) or clonal complexes (CCs) (Table, Figure 1).

**bla\textsubscript{VIM} and bla\textsubscript{IMP} Genes and Their Integrons in Enterobacter spp.**

We found 5 bla\textsubscript{VIM} genes, primarily of the bla\textsubscript{VIM-1} group (91.5% of all MBLs in Enterobacter spp.); most were bla\textsubscript{VIM-4} (49.1%), bla\textsubscript{VIM-1} (40.6%), and bla\textsubscript{VIM-40} (1.9%) (Table). The bla\textsubscript{VIM-2} group included bla\textsubscript{VIM-2} (4.0%) and bla\textsubscript{VIM-32} (3.4%), whereas all bla\textsubscript{IMP} were bla\textsubscript{IMP-49} (1.1%). We characterized 16 integrons, including 4 new ones (Appendix Table 3). Elements of the In238 type
prevailed (190 [50.4%]; 30 STs), carrying bla\textsubscript{VIM-4} (In238/In238a), bla\textsubscript{VIM-40} (In1445), or bla\textsubscript{VIM-1} (In237a) genes. The second most prevalent In916 type (146 [38.7%]; 34 STs) had bla\textsubscript{VIM-1}. The bla\textsubscript{VIM-2}-like genes were located mostly in In1008-type integrons (26 [6.9%]; 5 STs), as bla\textsubscript{VIM-2} (In1008) or bla\textsubscript{VIM-20} (In1444). bla\textsubscript{IMP-19} was in a new element In2241. We noticed temporal changes in the integron distribution; the incidence of In238s grew from 2009 (n = 6) to 2014 (n = 24) and then stabilized, whereas that of In916 rapidly increased from the original identification in 2014 (n = 9) to 2019 (n = 57).

**Epidemiology of Major *E. hormaechei* Clones and Multiregional and Interregional Outbreaks**

The most widespread clone was *E. hormaechei* subsp. *steigerwaltii* ST90 (117 [31.2%]), recorded during 2009–2019 in 58 hospitals in 38 cities, mostly in southern regions (Figure 2; Appendix Table 4). Most of the 111 isolates with In238/In238a differed by 19–207 SNPs from the reference isolate (mean 71 SNPs) and formed a subclone (0–172 SNPs between closest relatives), likely resulting from multiregional expansion (outbreak I). We also classified 2 In238-carrying isolates of ST1762 (CC90) into this cluster (127–132 SNPs).

We observed *E. hormaechei* subsp. *steigerwaltii* ST89 (74 [19.7% of all isolates]) during 2006–2019 in 26 centers in 18 towns (Appendix Table 5, Figure 2). Most of the isolates (n = 67 [90.5% of ST89 isolates]) comprised 3 regional subclones with different integrons, representing outbreak II in Łódzkie (48 [0–75 SNPs between closest relatives]; In916), outbreak III in Wielkopolskie (12 [0–49 SNPs]; In1444), and outbreak IV in Kujawsko–Pomorskie (7 [4–12 SNPs]; In1445).
We identified *E. hormaechei* subsp. *xiangfangensis* CC121 isolates (ST121, 36 [9.6%]; ST1756, 3 [0.8%]) during 2014–2019 in 22 hospitals in 12 cities, mainly in the Mazowieckie and Łódzkie regions (Appendix Table 6, Figure 3). All those isolates were related to each other, with up to 84 SNPs with the reference (mean 46 SNPs); however, 2 outbreaks were distinguished based on the integron data: an interregional outbreak V (27 [0–46 SNPs between closest relatives]; In916) and a regional outbreak VI (6 [1–9 SNPs]; In238a).

Of the clones of lower incidence, ST66 and ST1754 (CG66; n = 19) were split into 2 genetically and geographically separated subclones (0–17 and 0–23 SNPs within the groups [404 SNPs between them]; both with In916), likely representing an interregional outbreak VII and a regional outbreak VIII (Appendix Table 7, Figure 4). ST134 (n = 13) showed variety as well, with a cluster of related organisms (9 [6–24 SNPs]; In238) arising from an apparent regional outbreak IX (Appendix Table 8, Figure 5).

**Phylogeny and International Context of Major *E. hormaechei* Clones**

The clonal analysis of all 3,244 *Enterobacter* spp. genomes in RefSeq (as of June 6, 2022) revealed 546 STs; 61 STs were represented by >10 records. Out of the major VIM-positive clones in Poland, only ST90 and ST66 were among the 10 most numerous STs. Otherwise, the prevalent RefSeq clones were either not present (e.g., ST171 and ST133) or marginal (e.g., ST78 and ST114). However, the RefSeq genomes were unfiltered, which could have affected some of the observations. The phylogenetic analysis of 46 international ST90 genomes revealed 2 main clades and most of the 117 isolates in Poland, including outbreak I, belonged to a branch with several carbapenemase-free isolates from the

![Figure 2. Geographic distribution and clonal analysis of *Enterobacter hormaechei* clonal complex 90 (ST90 and ST1762) in Poland, 2006–2019. A) Geographic distribution of the isolates; main administrative regions are labeled. Circles represent medical centers where the isolates were recorded. Sizes of the circles are proportional to numbers of cases of infection. B) SNP-based minimum-spanning tree of the isolates. Lengths of branches are related to numbers of SNPs between linked isolates. Numbers of SNPs are indicated above the branches or next to the dots. SNP, single nucleotide polymorphism; ST, sequence type.](Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 29, No. 8, August 2023)
United Kingdom, France, Portugal, and Brazil (Appendix Figure 6).

ST89 was represented in RefSeq only by 2 isolates in Germany (1 with GIM-1) and 24 NDM-1-positive isolates in Poland during 2017–2020, which we analyzed in a previous study (21). Therefore, the phylotree comprised 100 isolates, including 98 from Poland (Appendix Figure 7), and consisted of 2 major lineages, each split then into multiple branches, correlating with the regional distribution of the isolates, regardless of their MBL content. The first lineage contained all of the VIM outbreak II isolates in Łódzkie plus a cluster of related NDM isolates from a neighboring area. The second lineage was divided into 2 major branches, 1 of which comprised the VIM outbreak IV in Kujawsko-Pomorskie and a large NDM epidemic from the adjacent region of Mazowieckie. The other branch contained mainly isolates from western Poland, including the VIM outbreak III from Wielkopolskie. Consistently, the 2 isolates in Germany were also located on the latter branch.

Only 7 ST121 genomes were present in RefSeq; the 36 VIM isolates in Poland, including outbreaks V–VI, formed 1 of 2 main lineages together with isolates from Brazil, Uganda, Morocco, Germany, and Poland (NDM) (21) (Appendix Figure 8). A total of 51 international ST66 isolates formed 2 lineages; 8 isolates in Poland of the outbreak VII belonged, primarily, to the lineage with isolates from Spain, France, and Germany mainly, whereas 10 outbreak VIII isolates clustered within the second lineage of more global character (Appendix Figure 9). ST134 records were sporadic in RefSeq (n = 9), and the 13 isolates in Poland, including outbreak IX, were located within 1 lineage together with single isolates from the United States, Lebanon, and Iran (Appendix Figure 10).

Resistomes
The resistome analysis demonstrated a large number and a variety of acquired AMR genes (6–27 genes per isolate; mean 15.8) (Appendix Table 9), in addition to the natural Enterobacteriaceae cephalosporinase genes. Their exact numbers could be specified only for the 9 MinION-sequenced genomes because some genes were in multiple copies in individual isolates (Appendix Table 10). The diversity of resistomes (AMR gene types and numbers) was common across and within the epidemic subclones; for some of those, the only stable AMR genes (i.e., present in all isolates of a subclone) were those in the MBL integrons. For instance, the ST90 isolates of the outbreak I had 67 AMR gene profiles, and ST89 isolates of the outbreak II had 33 AMR gene profiles. Along with bla\textsubscript{VIM/IMPS}, most of the isolates had genes coding for extended-spectrum β-lactamases (\textit{bla\textsubscript{SHV}} and \textit{bla\textsubscript{CTX-M}} types, \textit{bla\textsubscript{GES-2}} and \textit{bla\textsubscript{PER-2}}) or acquired AmpC-like cephalosporinases (\textit{bla\textsubscript{CMY-2}}, \textit{bla\textsubscript{DHA-1}}, and \textit{bla\textsubscript{FOX-20}}). Along with various aminoglycoside-modifying enzyme genes, numerous isolates had the 16S rRNA methylase gene \textit{armA}, inactivating all aminoglycosides. Different variants of fluoroquinolone-resistance genes \textit{qnrA/B/E/S} were commonly represented; 65 isolates contained the \textit{mcr-9.1} colistin-resistance gene.

Plasmids Harborin \textit{bla\textsubscript{VIM}} Genes
We identified 44 plasmid replicon types with 1–8 replicons per organism. The most frequent replicons were IncHI2 (n = 237), IncHI2A (n = 232), IncA (n = 165), IncFII (n = 140), and IncFIA (n = 116). Replicon profiles remarkably varied both between and within the subclones (Appendix Table 11). Long-read sequencing revealed the plasmid content, and the replicon and AMR gene distribution between the plasmids in 7 isolates representing the main epidemic subclones: ST90–In238 (n = 2; outbreak I), ST89–In916 (outbreak II), ST121–In916 (outbreak V), ST121–In238a (outbreak VI), ST66–In916 (outbreak VII), and ST134–In238 (outbreak IX) (Appendix Table 10). We performed the structural analysis on the plasmids with \textit{bla\textsubscript{VIM}}-harboring integrons.

In the 4 isolates with In238/In238a, including the 2 ST90–In238 representatives, the integrons were on 4 different plasmids. In 1 of those (isolate 4969–09), In238 was on an IncHI2 plasmid (p4969H; ≈261 kb), related to numerous others from Enterobacteriales worldwide (91%–95% coverage; ≈100% identity), occasionally with \textit{bla\textsubscript{VIM/IMP}} genes (Appendix Figure 11). One such plasmid from the Czech Republic, p51929_MCR_VIM (93% coverage; ≈100% identity), also contained In238 (22). The second ST90–In238 isolate (6234–09) had that integron on a plasmid with unique FII and FIA replicons (p6234F; ≈91 kb); FII was of some similarity to pECL_A (≈83%) (23) and FIA to R27 (≈84%) (24). The IncFII+FIA scaffold matched 9 GenBank records well (>60% coverage, >98% identity) (Appendix Figure 12). Of note, in p54969H and p6234F, the In238 integron was located in novel, almost identical Tn21-like transposons Tn7536, similar to Tn1696 (25) (Appendix Figure 13).

The ST121–In238a isolate (5713–17) had In238a on an IncFIB-like plasmid (p5713F; ≈120 kb), with the replicon similar to pB171 (≈91%) (26), homologous to 8 \textit{bla\textsubscript{VIM}}-negative records (80%–90% coverage; ≈100% identity) (Appendix Figure 14). Last, in the ST134–In238 isolate (5435–13) the integron resided on an IncN3-like plasmid (p5435N; ≈46 kb), matching several records (89% coverage; ≈100% identity), including...
some with \( bla_{IMV/VM} \) genes (Appendix Figure 15). The In238-type integrons in p5713F and p5435N were not located in Tn21-like transposons.

In the 3 isolates with In916: ST89 (7753–18), ST121 (743–14) and ST66 (5955–16), the integron resided on IncA plasmids (p7753A, \( \approx 162 \) kb; p743A, \( \approx 170 \) kb; and p5955A, \( \approx 154 \) kb). Those isolates were highly related to each other and to 9 In916-carrying IncA plasmids (84%–96% coverage, \( \approx 100\% \) identity), including 5 from Italy (different Enterobacterales) and 1 from Poland (\( K. \ pneumoniae \)) (Appendix Figure 16). The AMR region containing an IS\( 26-{ bla_{IMV,12} }-{ In916 }-{ IS26 } \) module (\( \approx 37.8-\approx 51.8 \) kb). This region in p743A was almost identical to plasmids pGB\_VIM and pGA\_VIM from Italy (7) (Appendix Figure 17).

**Genomic Islands with \( bla_{VM} \) Genes**

An isolate representing the epidemic subclone ST89–In1445 (8770–11; outbreak IV) had a new genomic island \( EhGI3 \) with the \( bla_{VM,40} \) gene, and the isolate of the clone ST89–In1444 (2944–06; outbreak III) had another new genomic island with \( bla_{VM,30} \). \( EhGI3 \) (\( \approx 94.6 \) kb), inserted into the tRNA\(_{Gly} \) gene, was a \( clc \)-like integrative and conjugative element (ICE) (41% coverage and \( \approx 87\% \) identity with the \( clc \) reference [28]), similar to ICEs found mainly in pseudomonads (29) (Appendix Figure 18). \( EhGI4 \) (\( \approx 71.1 \) kb) was a mosaic region flanked by 2 IS26 copies with direct repeats, carrying In1444 and multiple AMR genes (e.g., \( armA \)).

**Discussion**

We describe VIM/IMP CPE in Poland, which markedly increased in recent years after a period of rather low prevalence. During 2017–2019, the annual VIM/IMP CPE numbers recorded by the National Reference Centre for Susceptibility Testing (n = 545) were comparable with KPC (n = 686) or OXA-48 (n = 383) producers but far behind NDM organisms (n>6,000 [https://www.kordz.nil.gov.pl]) (12,14,15,21,30). Among all carbapenemase-producing Enterobacter spp., the organisms with VIM/IMP-like enzymes were the predominant group (59.4%). The leading position of Enterobacter spp. among VIM/IMP CPE was maintained for all years of the study; however, the dynamic spread of \( K. \ pneumoniae \) in more recent years has notably changed the species composition. A substantial role of Enterobacter spp. among VIM CPE has been observed also in other countries of Europe (8,11).

The successful dissemination of VIM-producing Enterobacter spp. in Poland has depended largely on several epidemic subclones of \( E. \ hormaechei \) ST90, ST89, and ST121 lineages, responsible for multiregional and interregional outbreaks I–VI (\( \approx 63\% \) of all isolates). ST90 is a global clone, often reported with various carbapenemases (11). Its population in Poland has been dominated by the ST90–In238/In238a subclone, and since 2009 it has been spreading over a large territory (outbreak I). On the contrary, ST89 seems to be a local lineage, having been reported mostly in Poland with various VIMs, OXA-48, or NDM-1 so far. However, its repeated identification with GIM-1 in Germany indicates broader spread in central Europe (21,31,32).

The ST89 VIM-producing isolates in Poland were clustered into 3 regional subclones, ST89–In916, ST89–In1444, and ST89–In1445 (outbreaks II–IV), closely related to the previously described ST89 NDM-1 subclones from the same or neighboring areas (21). This finding indicates that ST89 has produced a series of regional sublineages, acquiring and then disseminating with different AMR genes. The epidemiology of ST121 has been unclear. According to RefSeq, it appears to be nonprevalent, although present broadly in the world. In Poland, it has spread extensively, acquiring several VIM integrons and causing major regional outbreaks (V–VI).

The second essential factor of the VIM-producing Enterobacter spp. expansion in Poland has been the horizontal transmission of 3 major VIM integron types. The In238 type with \( bla_{VM,1} \)-like genes and In1008 type with \( bla_{VM,2} \)-like genes formed 2 evolving families of elements, with individual variants differing by mutations in \( bla_{VM} \) cassettes, and by \( 3'\)-termini of these in the case of In238 (specific 169bp repeats in some variants) (12,33). Both types were found originally in \( P. \ aeruginosa \) in Poland in 1998 (In238) (33) and 2001 (In1008) (34) and most likely were transmitted to Enterobacterales during 2006–2009 (12). However, In238 variants have been observed more broadly in central and southern Europe (22,35–37). The third major integron type, Enterobacterales-specific In916, has been recorded since the early 2010s in Spain, Italy, and France (4,8,11,38), and in Poland it has spread since at least 2013 (R. Izdebski and M. Gniadkowski, unpub. data). All those integron types have been acquired by \( E. \ hormaechei \) at the beginning of their dissemination in Enterobacterales in Poland with various molecular platforms.

In our previous study, the 2006–2012 predominant In238-type integrons in \( E. \ hormaechei \) ST89 and ST90 were assigned to IncHI2, PCR-nontypeable (largely), or IncM plasmids (12). We long-read sequenced 2 ST90–In238 isolates, representing outbreak I, in this study and found them to have In238...
on the IncHI2+HI2A or IncFII+FIA (previously nontypeable) plasmids, suggesting exchange between them. Given that the integron was located within almost identical Tn21-like transposons (Tn7536) in both plasmids, those might have been responsible for the inter-plasmid transfer. However, the 2 remaining long-read sequenced ST121 and ST134 isolates with In238/In238a had these integrons on yet other plasmids, IncFIB (ST121) and IncN3 (ST134), and not in a transposonic context. This finding indicates that acquisition and circulation of the In238-like elements among 36 STs of Enterobacter spp. in Poland have been multifactorial and complex phenomena. Regarding acquisition, an interesting case was provided by the ST89 isolate with the In238-like integron In1445, located within the clc-type ICE EhG3. In238 variants have been frequent in VIM-producing P. aeruginosa (39) and P. putida in Poland (40), being usually chromosomal in those. EhG3 turned out to be almost identical to an ICE in 1 of the P. putida group isolates, indicating exchange of such elements between pseudomonads and Enterobacterales (P. Urbanowicz, M. Gniadkowski, unpub. data).

On the other hand, the proliferation of In916 seems to be relatively clear. In Europe, this integron has been associated with IncA, IncFIIK, IncHI2, IncN, or PCR-nontypeable plasmids (4,7,8), and in our study isolates, it has entirely correlated with the IncA plasmids. A close relatedness between the In916-carrying IncA plasmids in Poland and Italy was proved, which together with high conjugative potential (7) have explained their spread on a large geographic scale. As in Italy (7) and France (8), rapid dissemination of these plasmids in Poland since 2013–2014 has contributed to the increase in VIM-producing Enterobacterales and Enterobacter spp., making In916 the most prevalent integron in 2019 (≈63%). The In916-carrying IncA plasmids occurred in 30 Enterobacter STs, including ST89, CC121 and CG66 subclones of 4 regional outbreaks, revealing that both the horizontal and clonal spread contributed to their recent proliferation.

Our study has shown the epidemiology of VIM-producing Enterobacter spp. during 14 years of VIM CPE surveillance in Poland, substantially updating the previous report (12). The results enable the precise definition of several E. hormaechei subclones of a remarkable epidemic potential, responsible for a series of territorial outbreaks, and enable the characterization of the main molecular platforms transmitting integrons with bla_VIM genes in Enterobacter populations. The study revealed several factors specific for Poland or central Europe, namely the prominent role of apparently rare E. hormaechei clones (ST89 or ST121), peculiar integrons of pseudomonadal origins (In238 and In1008 types), and unique VIM-encoding plasmids (IncFII+FIA with In238). We have also demonstrated some cosmopolitan elements, such as the global status of the epidemic ST90 clone and pan-Europe dissemination of In916-carrying IncA-like plasmids. All these observations indicate that AMR VIM-producing E. hormaechei and the VIM-encoding plasmids create an epidemiologic danger for hospital environments throughout Europe that clinicians and infection control specialists should be aware of.

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References


etymologia revisited

Escherichia coli
[esh”ə-rık’e-ə co’lî]

A gram-negative, facultatively anaerobic rod, Escherichia coli was named for Theodor Escherich, a German-Austrian pediatrician. Escherich isolated a variety of bacteria from infant fecal samples by using his own anaerobic culture methods and Hans Christian Gram’s new staining technique. Escherich originally named the common colon bacillus Bacterium coli commune. Castellani and Chalmers proposed the name E. coli in 1919, but it was not officially recognized until 1958.

References:
The epidemiology of the *Streptococcus agalactiae* bacterium (group B *Streptococcus* [GBS]) in Southeast Asia differs from that traditionally seen in the literature. Investigations after the foodborne outbreak of GBS sepsis in 2015 in Singapore concluded that GBS sepsis is primarily a foodborne infection of adults in parts of Southeast Asia (1). The Singapore outbreak was associated with consumption of raw freshwater fish (2,3). The GBS were serotype III sequence type 283 (ST283) and behaved more aggressively in adults than other GBS; adults without comorbidities made up 22% of the ST283 bacteremia cases but only 2% of non-ST283 GBS cases (4). Subsequent studies showed that although ST283 is almost absent from the rest of the world, ST283 disease is widespread around Southeast Asia in humans and tilapia; human data from Laos and Thailand showed ST283 accounted for 76% (Laos) and 73% (Thailand) of invasive GBS collected during 2000–2017 (1,5). Aquaculture data from Malaysia, Thailand, and Vietnam show that ST283 accounted for 12%–100% of all GBS isolated from streptococcosis in farmed tilapia during 2003–2018 (1,6). Whole-genome analysis shows ST283 from humans and tilapia are 1 clone (1,7). Because consumption of raw freshwater fish is common in affected Southeast Asia countries (T. Barkham, unpub. data), raw tilapia could plausibly be the main source of human ST283, although this theory has not been studied.

This fishborne epidemiology is reflected in a report of 2 sisters in their 50s without comorbidities who returned to Laos after visiting friends and relatives in the United States. Both sisters became ill with ST283 bacteremia a day after a meal that included Mekong fish salad, traditionally made with raw freshwater fish (8). However, in case-control studies in Singapore in 2015, which reported statistically strong associations between eating raw freshwater fish and ST283 bacteremia, 21/40 participants in a retrospective study (3) and 2/9 participants in a prospective study (2) denied eating raw freshwater fish. This finding could be because of poor recollection or varying definitions of raw, as in the case of dishes prepared without heat but regarded as being no longer raw (e.g., raw fish fileted or ground and mixed with lemon juice or other sauces). Nevertheless, the possibility of interhuman transmission or that other foods are a source also deserves study. In addition, when authorities investigated a surge of 18 ST283 bacteremia cases in Singapore in July 2020, no affected persons admitted eating raw freshwater fish. Selling raw freshwater fish as a ready-to-eat food was illegal at that time in Singapore, so this denial was not surprising, but it might also suggest an alternative source or vehicle.

Studies of vaginal and rectal carriage in women in Southeast Asia have not reported sequence types, because their focus has been capsular serotypes for use in developing vaccines. Two small sequencing studies failed to find ST283; 1 looked at stool samples from 82 food handlers and fishmongers in Singapore during the 2015 outbreak (4), and the other looked at an opportunistic collection of 38 vaginal...
GBS samples isolated from women with colpitis in Hanoi, Vietnam, in 2016 (1).

Clinical evidence for carriage and human-to-human transmission of this bacterial strain is provided by several cases of ST283 neonatal early onset disease in Hong Kong (5) and Laos (1); at least 2 from Laos were isolated on the infants’ first day of life, which suggests maternal carriage. We sought to verify carriage. Because ST283 is known to account for 73% of invasive GBS in humans in parts of Thailand (1), we assessed ST283 carriage in a population in Thailand that consumes raw freshwater fish, as verified by the widespread occurrence of *Opisthorchis viverrini*, a liver fluke infection acquired by eating raw freshwater fish.

The Study
We collected samples in January 2019 from Nong Bua and Dong Mun subdistricts of Nong Kung Si District, Kalasin Province, Thailand. This area is within the Northeast region of Thailand, where the prevalence of liver fluke infection is high. Participants were a random sample of persons ≥15 years of age who had ever eaten uncooked freshwater fish, been infected by or treated for liver flukes, or knew of any family member who had had liver cancer. The study was approved by the Ethics Committee of Khon Kaen University, Thailand (approval no. HE601370). The first feces and urine samples of the morning were chilled and transported to the laboratory at Khon Kaen University. Each fecal sample was processed for parasite (fluke) examination by the formalin ethyl-acetate concentration technique, and aliquots were stored at −20°C and sent to Singapore for GBS detection. DNA was extracted with the QIA DNeasy PowerSoil Kit (QIAGEN, https://www.qiagen.com) with an additional wash step. We used PCR to detect serotype III GBS (9) and performed multilocus sequence typing (MLST) (10) on DNA extracts that were positive for serotype III. We included an internal control. We centrifuged each urine sample and kept the supernatant for *O. viverrini* antigen analysis (11).

We recruited 184 participants; 18 of the 184 stool samples were positive for serotype III GBS. MLST of these 18 samples found five ST283, three ST1, three ST651, one ST17, one ST862, and two with undefined profiles (with alleles 9,1,170,1,1,53,2, and 16,1,2,1,9,2,2); 3 had inadequate PCR products for sequencing. All samples were negative for PCR inhibition. Stool microscopy found fluke eggs in 4 (2.2%) samples. The *O. viverrini* urine-antigen test was positive in the same 4 samples and in 1 additional sample. We detected 1 serotype III GBS sequence type in each of those 5 stool samples: ST283, ST1, ST17, ST651, and 1 sequence without adequate PCR products for MLST.

Conclusions
This study demonstrates human carriage of GBS ST283 and establishes that humans might play a part in the transmission of ST283 or could even be the original source. Our finding supports the assumed transmission from mother to newborn described in Laos (1). Questions remain as to whether carriage is temporary, perhaps reflecting recent dietary exposure, or long lasting. The transmission of *Opisthorchis* flukes depends on human fecal waste being disposed of in fresh water and humans consuming raw fish from the same water, so each species consumes raw products from the other. The prevalence of those flukes in parts of Southeast Asia is a testament to the ongoing presence of these practices, which could explain the high rates of GBS ST283 in humans and tilapia in the same geographic areas. This hypothesized transmission cycle has not been studied for ST283.

The data reported here might not be representative of all the numerous different ethnic and cultural groups in and around Southeast Asia. We hope these data will stimulate and support further epidemiologic studies, including studies on patterns of consumption of raw freshwater fish in different cultural groups in Southeast Asia, as recommended in the ST283 risk profile published by the Food and Agriculture Organization of the United Nations (12).

In summary, we detected GBS ST283 in 2.7% of 184 stool samples collected in northeastern Thailand from a population known to consume raw freshwater fish. We remain uncertain of the dynamics of human carriage of GBS ST283 and its contribution to human-to-human transmission, human disease, and the contamination of aquaculture, but our findings indicate that human carriers might play a part in transmitting GBS ST283 or could be its original source.

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About the Author
Dr. Barkham studied medicine and microbiology in London, UK. He has been working in Singapore since 1999, and his interests include a mix of clinical service, research, and teaching.
References


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Diphtheria is a contagious, potentially fatal infection caused by toxin-producing bacteria of the *Corynebacterium diphtheriae* species complex, which includes *C. diphtheriae*, *C. ulcerans*, *C. pseudotuberculosis*, *C. rouxii*, *C. belfanti*, and *C. silvaticum*. Infection is localized principally in the upper respiratory tract, and production of diphtheria toxin (encoded by the *tox* gene) can cause systemic complications. Cutaneous diphtheria and diphtheria endocarditis can also act as sources of respiratory infections (1–4). Diphtheria surveillance has traditionally focused on respiratory illness caused by toxigenic *C. diphtheriae* but has been expanded in some countries to include all *C. diphtheriae* species complex infections irrespective of species, infection site, or toxigenicity, enabling broader disease monitoring. *C. diphtheriae* spreads via human-to-human contact; *C. ulcerans* and *C. pseudotuberculosis* are transmitted to humans primarily through animal contact.

Diphtheria was once a major cause of infant death, but global incidence has declined over the past century, largely because of mass vaccination. Consequently, diphtheria is now often considered a forgotten disease (5). Nevertheless, diphtheria reemergence has been reported in high-income countries and is closely related to patient travel history. Diphtheria is considered endemic in Madagascar, Comoros, and Mayotte in the southwest Indian Ocean, but few cases have been reported on other islands, including Réunion Island, an overseas department of France, where cases emerged in 2015 (6,7). Vaccination coverage is poorer in Mayotte (45% for 7- to 11-year-old children) than in Réunion Island (96% for children 11 months of age). Recent improvements in laboratory diagnostic capabilities, such as mass spectrometry use, have increased reports of *C. diphtheriae* species complex infections (8). However, knowledge of prevalence and origin of those infections is limited in this region. The aims of this study were to review the clinical, epidemiologic, and microbiologic characteristics of *C. diphtheriae* species complex infections on Réunion Island, France, 2015–2020.
Réunion Island during 2015–2020 and identify possible links with cases on other islands in the region.

The Study
We included all cases of *C. diphtheriae* species complex infections reported to the regional health agency and recorded at Réunion Island University Hospital during 2015–2020. We analyzed medical records and extracted age, sex, country of residence, recent travel, contact with animals, socioeconomic status, and diphtheria vaccination status for each case. We performed antimicrobial susceptibility testing; identified co-infecting strains; and determined *tox* gene presence, diphtheria toxin production, and biovar and sequence type (ST). We sent each isolate to the National Reference Center for Corynebacteria of the *diphtheriae* Complex (Institut Pasteur, Paris, France) to confirm species identity through multiplex PCR and biotyping as previously described (8–10). We detected the *tox* gene by using conventional PCR or, since 2019, by using multiplex real-time PCR (10). We assessed toxin production by using a modified Elek test (11). We determined antimicrobial drug susceptibility by using disk diffusion or by determining MICs (E-test; bioMérieux, https://www.biomerieux.com), in accordance with CASFM/EUCAST2021 (https://www.sfm-microbiologie.org/2021/04/23/casfm-avril-2021-v1-0) recommendations for benzylpenicillin, amoxicillin, cefotaxime, clindamycin, rifampin, and ciprofloxacin. We genotyped each isolate by using multilocus sequence typing (MLST) (12).

A total of 26 cases of *C. diphtheriae* species complex infections were recorded, from which 27 *C. diphtheriae* and 2 *C. ulcerans* isolates were cultured. Most (88.5%) infected patients were male; median age was 60 (interquartile range 32.5–67) years. Fourteen (50%) patients lived on Réunion Island, 3 (11.5%) in Mayotte, 4 (19.2%) in mainland France, 3 (11.5%) in Comoros, and 2 (7.8%) in Madagascar. Most (84.6%) patients had skin manifestations, and 16 patients were vaccinated (Table 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0106-T1.htm; Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/8/23-0106.pdf). Of 24 *C. diphtheriae* infections, 8 occurred in patients who had recently traveled to or originated from Madagascar, 4 who traveled to or originated from Mayotte, and 3 who traveled to or originated from Comoros. Since 2018, a total of 9 cases on Réunion Island have been considered locally acquired; all of those patients lived in poor socioeconomic conditions. *C. ulcerans* infections occurred in 2 patients living on Réunion Island who had not traveled recently but had contact with animals (Table 1; Figure). We performed a Spearman rank correlation to compare locally acquired strains isolated during 2015–2018 and 2019–2020; a 75% increase in locally acquired *C. diphtheriae* infections occurred in 2019–2020 (ρ = 0.8452; p = 0.0341).

Isolates were obtained from cutaneous lesion (n = 24), bone (n = 4), and respiratory (n = 1) samples. Eight of 27 *C. diphtheriae* isolates were toxigenic, yielding positive Elek test results. The 2 *C. ulcerans* isolates were nontoxigenic. *C. diphtheriae* isolates were characterized as biovars Mitis (n = 20) and Gravis (n = 7).

Patient isolates were co-infected most frequently with *Staphylococcus aureus* (n = 17) and *Streptococcus pyogenes* (n = 18). Benzylpenicillin resistance was observed in 80% of isolates according to CASFM/EUCAST2021 recommendations, but isolates were categorized as susceptible increased exposure according to EUCAST version 13.0 proposed breakpoints (https://www.eucast.org/clinical_breakpoints) (Appendix Table). One (3.5%) *C. diphtheriae* isolate was resistant to amoxicillin (CD8/FRC0402; MIC 1.5 mg/L).

![Figure](https://wwwnc.cdc.gov/EID/article/29/8/23-0106-F1.htm) **A** Number of cases diagnosed per year in study of emerging *Corynebacterium diphtheriae* species complex infections, Réunion Island, France, 2015–2020. Number of cases were classified according to geographic origin (A) or travel history of patients (B). Dotted lines indicate linear trends.
and 1 was resistant to rifampin. Both C. ulcerans isolates were resistant to clindamycin (100% natural low susceptibility), whereas clindamycin resistance was observed for only 1 C. diphtheriae isolate.

We identified 21 STs by MLST analysis, including ST88 for C. diphtheriae isolates from 4 patients and ST339 for both C. ulcerans isolates (Table 2). All C. diphtheriae STs had 2–5 mismatches, except ST87 and ST237, which had 1 mismatch between them. ST339 (C. ulcerans) had 7 mismatches with all C. diphtheriae STs.

Conclusions

We report increased prevalence of cutaneous C. diphtheriae species complex infections on Réunion Island during 2015–2020. Introduction of mass spectrometry analysis in hospital laboratories and increased clinician awareness might have led to increased case reporting. Our study confirms that C. diphtheriae species complex members are circulating and are likely underestimated in the southwest Indian Ocean (7,13). Moreover, we observed emergence of locally acquired cutaneous C. diphtheriae infections on Réunion Island since 2019. The number of imported cases in 2020 was probably limited because of the COVID-19 pandemic, which reduced travel. Indeed, all C. diphtheriae cases identified during 2015–2018 occurred in patients who had traveled from other islands in the Indian Ocean. In addition, cutaneous diphtheria appeared to be associated with poor socioeconomic living conditions, in which alcoholism, drug dependence, and homelessness are factors that increase risk for human-to-human transmission and virulence (14).

A total of 8 (30%) C. diphtheriae isolates were toxigenic and caused cutaneous infections. Nontoxigenic C. diphtheriae isolates (70%, n = 19) were obtained from cutaneous lesions, respiratory samples, and bone samples. Clinicians should be aware that nontoxigenic C. diphtheriae can potentially cause severe disease (1,14,15). Moreover, all isolates were co-infected with pyogenic bacteria, suggesting diphtheria infection should be considered under polymicrobial conditions.

MLST analysis identified 21 different STs; most were unrelated (≥2 mismatches) reflecting marked

**Table 2. Characteristics of isolates from 26 patients in study of emerging* Corynebacterium diphtheriae **species complex infections, Réunion Island, France, 2015–2020**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Isolate</th>
<th>Year</th>
<th>Isolation site</th>
<th>Species</th>
<th>Biovar</th>
<th>ftox gene</th>
<th>Elek test</th>
<th>ST†</th>
<th>Co-infections‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CD1/FRC0304</td>
<td>2015</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Negative</td>
<td>NA</td>
<td>102</td>
<td>S. pyogenes, S. aureus, A. haemolyticum</td>
</tr>
<tr>
<td>2</td>
<td>CD2/FRC0316</td>
<td>2015</td>
<td>Respiratory</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>95</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>3</td>
<td>CD3/FRC0314</td>
<td>2015</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Positive</td>
<td>Positive</td>
<td>421</td>
<td>S. aureus</td>
</tr>
<tr>
<td>4</td>
<td>CD4/FRC0376</td>
<td>2015</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Positive</td>
<td>Positive</td>
<td>388</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>5</td>
<td>CD5/FRC0383</td>
<td>2016</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>423</td>
<td>S. pyogenes, S. aureus</td>
</tr>
<tr>
<td>6</td>
<td>CD6/FRC0393</td>
<td>2016</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>423</td>
<td>S. pyogenes, S. aureus</td>
</tr>
<tr>
<td>7</td>
<td>CD7/FRC0385</td>
<td>2016</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Positive</td>
<td>Positive</td>
<td>91</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>8</td>
<td>CD8/FRC0391</td>
<td>2016</td>
<td>Cutaneous</td>
<td>C. ulcerans</td>
<td>NA</td>
<td>Negative</td>
<td>NA</td>
<td>339</td>
<td>S. dysgalactiae</td>
</tr>
<tr>
<td>9</td>
<td>CD9/FRC0391</td>
<td>2016</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>410</td>
<td>S. dysgalactiae</td>
</tr>
<tr>
<td>10</td>
<td>CD10/FRC0423</td>
<td>2016</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>415</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>11</td>
<td>CD11/FRC0477</td>
<td>2017</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Negative</td>
<td>NA</td>
<td>411</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>12</td>
<td>CD12/FRC0501</td>
<td>2017</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Positive</td>
<td>Positive</td>
<td>91</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>13</td>
<td>CD13/FRC0624</td>
<td>2018</td>
<td>Bone</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>237</td>
<td>S. aureus</td>
</tr>
<tr>
<td>14</td>
<td>CD14/FRC0630</td>
<td>2018</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Negative</td>
<td>NA</td>
<td>606</td>
<td>S. pyogenes, S. aureus</td>
</tr>
<tr>
<td>15</td>
<td>CD15/FRC0733</td>
<td>2019</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Negative</td>
<td>NA</td>
<td>351</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>16</td>
<td>CD16/FRC0782</td>
<td>2019</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Positive</td>
<td>Positive</td>
<td>688</td>
<td>S. pyogenes, S. aureus</td>
</tr>
<tr>
<td>17</td>
<td>CD17/FRC0809</td>
<td>2019</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Positive</td>
<td>Positive</td>
<td>688</td>
<td>S. pyogenes, S. aureus</td>
</tr>
<tr>
<td>18</td>
<td>CD18/FRC0819</td>
<td>2019</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Gravis</td>
<td>Positive</td>
<td>Positive</td>
<td>87</td>
<td>S. pyogenes, A. haemolyticum</td>
</tr>
<tr>
<td>19</td>
<td>CU1/FRC0391</td>
<td>2016</td>
<td>Cutaneous</td>
<td>C. ulcerans</td>
<td>NA</td>
<td>Negative</td>
<td>NA</td>
<td>339</td>
<td>S. aureus</td>
</tr>
<tr>
<td>20</td>
<td>CD19/FRC0849</td>
<td>2019</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Positive</td>
<td>Positive</td>
<td>426</td>
<td>S. pyogenes, S. aureus</td>
</tr>
<tr>
<td>21</td>
<td>CD20/FRC0865</td>
<td>2020</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>102</td>
<td>S. pyogenes, S. aureus</td>
</tr>
<tr>
<td>22</td>
<td>CD21/FRC0875</td>
<td>2020</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>707</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>23</td>
<td>CD22/FRC0893</td>
<td>2020</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>708</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>24</td>
<td>CD23/FRC0928</td>
<td>2020</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>88</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>25</td>
<td>CD24/FRC0970</td>
<td>2020</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>88</td>
<td>S. pyogenes, A. haemolyticum</td>
</tr>
<tr>
<td>26</td>
<td>CD25/FRC0975</td>
<td>2020</td>
<td>Cutaneous</td>
<td>C. diphtheriae</td>
<td>Mitis</td>
<td>Negative</td>
<td>NA</td>
<td>88</td>
<td>S. aureus</td>
</tr>
</tbody>
</table>

*CD, Corynebacterium diphtheriae; CU, C. ulcerans; NA, not applicable; ST, sequence type.
†Numbers in bold indicate a common ST shared among strains from different patients.
‡Co-infections with Arcanobacterium haemolyticum, Staphylococcus aureus, Streptococcus dysgalactiae, or Streptococcus pyogenes.
genetic diversity of isolates. ST88 was found in 4 patients living on Réunion Island who had not traveled recently, indicating probable local acquisition. ST88 had previously been reported only in patients from Mayotte. Therefore, our results show that multiple C. diphtheriae species complex clones are circulating in the southwest Indian Ocean (8). Both C. ulcersans strains belonged to ST339. The National Reference Center reported that ST339 is the predominant ST found in animals in France. Although considerable ST diversity was revealed, whole-genome sequencing will be required to further evaluate circulating C. diphtheriae clones in this region.

In conclusion, we describe emergence of locally acquired C. diphtheriae species complex infections on Réunion Island during 2019–2020. Local clinicians and microbiologists should remain aware of this neglected infection; improvements should be made in diagnostic methods and management of infected patients, such as maintaining availability of diphtheria antitoxin.

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About the Author
Dr. Garrigos is a research scientist in the microbiology department of Félix Guyon University Hospital of Réunion Island, France. His research interests focus on bacterial diseases, antimicrobial resistance, cystic fibrosis patients, and emerging infectious diseases.

References

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Chromosome-Borne CTX-M-65 Extended-Spectrum β-Lactamase–Producing Salmonella enterica Serovar Infantis, Taiwan

Ying-Shu Liao, Hsiao-Lun Wei, Hung-Chih Kuo, Bo-Han Chen, You-Wun Wang, Ru-Hsiou Teng, Yu-Ping Hong, Jui-Hsien Chang, Shiu-Yun Liang, Chi-Sen Tsao, Chien-Shun Chiou

A CTX-M-65–producing Salmonella enterica serovar Infantis clone, probably originating in Latin America and initially reported in the United States, has emerged in Taiwan. Chicken meat is the most likely primary carrier. Four of the 9 drug resistance genes have integrated into the chromosome: blaCTX-M-65, tet(A), sul1, and aadA1.

Salmonella enterica serovar Infantis is one of the most common Salmonella serotypes (1); it is frequently isolated from humans and animals, particularly from poultry (2). An increasing incidence of Salmonella Infantis infections has been reported in the United States (3), accompanied by emergence and spread of an extended-spectrum β-lactamase CTX-M-65–producing Salmonella Infantis clone in humans, food animals, and retail chicken (4,5). The clone probably originated in South America because it was initially discovered in persons who had traveled back from Peru, Bolivia, Ecuador, and Chile since 2012 (5). Domestically acquired infections were not identified in the United States until 2014 (5).

This clone is characterized by having a D87Y mutation in the gyrA gene and carrying multiple resistance genes, including aph(4)-la, aac(3)-Iva, aph(3’)-Ic, blaCTX-M-65, fosA3, floR, dfrA14, sul1, tet(A), and aadA1, located in 2 distinct regions of a pESI-like megaplasmid (4). The CTX-M-65–producing clone has been reported mostly in South America, North America, and some countries in Europe (4–12).

In Taiwan, Salmonella Infantis is not a common cause of human salmonellosis, accounting for only 0.61% (246/40,599) of all Salmonella isolates collected during 2004–2022. Salmonella Infantis isolates collected during 2004–2019 showed a low level of antimicrobial drug resistance (Appendix Table 1, https://www.cdc.gov/EID/article/29/8/23-0472-App1.pdf). However, in 2021, we identified that 7 of 14 Salmonella Infantis isolates from patients who had salmonellosis were multidrug-resistant (MDR), and in 2022, MDR strains accounted for 55% (21/38) of the Salmonella Infantis isolates recovered that year.

The 28 patients who contracted MDR Salmonella Infantis were from diverse age groups and geographic locations, and none of them had a history of international travel. During 2021 and 2022, the COVID-19 pandemic restricted travel abroad. We report a CTX-M-65–producing Salmonella Infantis clone in Taiwan.

The Study
We performed clustering analysis on pulsed-field gel electrophoresis (PFGE) patterns of Salmonella Infantis isolates, which showed that the MDR isolates recovered in 2021 and 2022 clustered closely together in a distinct group (Appendix Figure). Antimicrobial drug susceptibility testing showed that the MDR isolates had resistance to ampicillin, cefotaxime, ceftazidime, nalidixic acid, ciprofloxacin (intermediate susceptibility), gentamicin, chloramphenicol, sulfamethoxazole, trimethoprim, and tetracycline (Appendix Figure). The resistance profile closely resembled that of the widespread CTX-M-65–producing Salmonella Infantis clone (5).

We isolated Salmonella bacteria from retail raw chicken meat sold in 12 supermarket stores in Taichung City in 2022 to investigate the source of MDR Salmonella Infantis. All chicken meat samples were sourced from domestic farms. Salmonella bacteria were isolated from 191 (65.6%) of 291 chicken meat samples. A total of 379 Salmonella isolates were recovered from the 191 samples (1–2 isolates from each Salmonella-positive sample).

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Of the 379 isolates, 68.1% (258) were identified to be Salmonella Infantis, followed by Salmonella Kentucky (17.2%), Salmonella Brancaster (2.6%), Salmonella Goldcoast (2.6%), Salmonella Agona (2.4%), Salmonella Enteritidis (2.1%), and 6 other serovars (5.0%). Of the 191 samples, 11% were found to be contaminated with CTX-M-65–Producing Salmonella Infantis, Taiwan

Figure 1. Core genome multilocus sequence typing tree and relevant information for investigation of chromosome-borne CTX-M-65 extended-spectrum β-lactamase–producing Salmonella enterica serovar Infantis, Taiwan. The cluster highlighted in red consists of blaCTX-M-65–CARRYING strains. GenBank accession numbers are shown. ID, identification.
a mixture of *Salmonella* serovars. The 258 *Salmonella* Infantis isolates had 28 PFGE patterns, among which the 6 most common patterns were also observed in the MDR isolates from humans (Appendix Table 2). We performed a clustering analysis of PFGE profiles, which showed that the 258 *Salmonella* Infantis isolates from chicken meat, 28 MDR isolates from humans, and 1 isolate from a diseased pig recovered in 2022, were grouped in a common cluster (data not shown).

We conducted whole-genome sequencing of 51 *Salmonella* Infantis isolates from humans, chickens, and a pig by using the Illumina sequencing platform (https://www.illumina.com) to investigate drug resistance genetic determinants, plasmid incompatibility types, and their genetic relationships. Our analysis showed that all 51 *Salmonella* Infantis isolates belonged to sequence type 32, and 18 MDR *Salmonella* Infantis isolates recovered from humans, chickens, and a pig in 2021 and 2022 had a D87Y mutation in \(\text{gyrA}\), along with an IncFIB plasmid and 4 common resistance genes: \(\text{aadA1, bla}_{\text{CTX-M-65}}, \text{sul1, and tet(A)}\) (Appendix Table 3). In addition, 15 of the 18 \(\text{bla}_{\text{CTX-M-65}}\)–carrying isolates had 5 other drug resistance genes: \(\text{aac(3)-Iva, aph(3')-Ia, aph(4)-Ia, dfrA14, and floR}\). Two of the isolates had 4 of the 5 drug resistance genes, and 1 did not have any of the 5 genes.

We conducted clustering analysis of core genome multilocus sequence typing profiles, which showed that the \(\text{bla}_{\text{CTX-M-65}}\)–carrying isolates from Taiwan, when compared with non–\(\text{bla}_{\text{CTX-M-65}}\)–carrying strains, showed a closer genetic relationship with \(\text{bla}_{\text{CTX-M-65}}\)–carrying strains reported in North and South America, Europe, Australia, India, and Vietnam (Figure 1).

To investigate the location of drug resistance genes, we performed additional sequencing of 6 \(\text{bla}_{\text{CTX-M-65}}\)–carrying isolates and 1 pan-susceptible isolate by using the Oxford nanopore sequencing platform (https://nanoporetech.com). This approach provided long sequence reads, enabling us to assemble complete genome sequences. Our analysis showed that all 6 \(\text{bla}_{\text{CTX-M-65}}\)–carrying isolates from humans, chickens, and a pig in 2021 and 2022 had a D87Y mutation in \(\text{gyrA}\), along with an IncFIB plasmid and 4 common resistance genes: \(\text{aadA1, bla}_{\text{CTX-M-65}}, \text{sul1, and tet(A)}\) (Appendix Table 3). In contrast, \(\text{aadA1, bla}_{\text{CTX-M-65}}, \text{sul1, and tet(A)}\) were found in an \(\approx 126\)-kb DNA segment inserted within an ABC-F family ATPase gene in the chromosomes (Appendix Tables 4, 5).
Our investigation suggested that the 195-kb IncFIB plasmids and the 126-kb genomic islands found in the chromosome probably originated from a plasmid similar to pN16S097. This megaplasmid, which has a length of 318,524 bp, was initially detected in a *Salmonella* Infantis strain and has 9 of the mentioned drug resistance genes in 2 distinct regions (8).

We hypothesize that the 126-kb segment carrying *aadA1*, *bla*<sub>CTX-M-65</sub>, *sul1*, and *tet(A)* might have translocated from a pN16S097-like plasmid into a chromosome through IS26-mediated transposition, resulting in formation of an 8-bp (CCGGAAAG) tandem repeat at the insertion site. This process led to the loss of the megaplasmid, leaving a plasmid of ≈195 kb (Figure 2). Upon analyzing 5,253 genomes of *bla*<sub>CTX-M-65</sub>-carrying *Salmonella* Infantis strains available in GenBank, we did not observe a large DNA segment or a *bla*<sub>CTX-M-65</sub>-carrying segment inserted within an ABC-F family ATPase gene in the chromosomes.

**Conclusions**

The *bla*<sub>CTX-M-65</sub>-carrying *Salmonella* Infantis clone, previously identified in South and North America and some countries in Europe, has been detected in Taiwan. Chickens are suspected to be the primary source of *bla*<sub>CTX-M-65</sub>-carrying strains. Many PFGE genotypes have been found among the isolates from retail chicken meat, indicating that the *bla*<sub>CTX-M-65</sub>-carrying *Salmonella* Infantis strains have probably evolved and proliferated on chicken farms, rather than being contaminants from chicken processing plants. Integration of *bla*<sub>CTX-M-65</sub> into the chromosome suggests that this drug resistance gene might be more resiliently maintained within the strains.

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**About the Author**

Ms. Liao is a senior technical specialist at the Centers for Disease Control, Ministry of Health and Welfare, Taichung, Taiwan. Her primary research interests are molecular epidemiology and antimicrobial drug resistance of foodborne bacterial pathogens.

**References**


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The association between respiratory viruses and secondary invasive pulmonary bacterial disease is recognized, but the proportion of pulmonary invasive group A Streptococcus (PiGAS) infections after seasonal influenza is low compared with those for other bacterial pathogens (e.g., Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus) (1,2). However, PiGAS has been shown to complicate epidemics of measles and, notably, the 1918–1919 influenza pandemic (3).

Winter 2022–23 saw a marked increase in influenza and associated group A Streptococcus (GAS) infections in the United Kingdom as well as globally (https://www.gov.uk/government/publications/group-a-streptococcal-infections-activity-during-the-2022-to-2023-season/group-a-streptococcal-infections-second-update-on-seasonal-activity-in-england-2022-to-2023). In autumn 2022, an unusually high number of pediatric GAS pleural empyema cases associated with human metapneumovirus co-infection was described in Scotland (4). After similar cases were observed in adults, we aimed to characterize the burden of PiGAS in adults and contrast it to published and local historical data.

The Study
We identified patients with pulmonary samples or blood cultures found positive for S. pyogenes by the National Health Service (NHS) of Greater Glasgow and Clyde (GGC), which serves a population of 1.4 million, during December 1, 2017–November 31, 2022, through the Laboratory Information Management System. We identified the same specimens from NHS Lothian (population 850,000) and GGC during December 1, 2022–February 28, 2023 (https://www.nrscotland.gov.uk/statistics-and-data/statistics/statistics-by-theme/population/population-estimates/mid-year-population-estimates/mid-2021).

Samples assessed were sputum (inpatient), pleural fluid, endotracheal aspirate, bronchoalveolar lavage pulmonary tissue (postmortem), and blood cultures. We also identified S. pyogenes by molecular techniques (i.e., specific GAS PCR testing and 16s PCR). We defined cases as definite or probable PiGAS. A definite case required microbiologic criteria (e.g., S. pyogenes identified in blood or deep respiratory or pleural sample) and radiologic criteria (e.g., multifocal consolidation or pleural effusion or empyema or parenchymal necrosis) to be present. Probable cases were those with sputum samples in the microbiologic criteria and unifocal consolidation in the radiologic criteria, capturing patients meeting both criteria but not those of a definite case.

We extracted demographic, clinical, and laboratory data from electronic patient records. We derived Scottish Index of Multiple Deprivation (SIMD) scores by using postcodes. We also calculated Charlson comorbidity index scores. We referred S. pyogenes
isolates to the Scottish Microbiology Reference Laboratory for M-typing and Illumina short read sequencing (5). We used an annotated whole genome–core genome multilocus sequence typing approach to compare isolate sequence data with a publicly available core genome multilocus sequence typing scheme implemented in Ridom SeqSphere+ version 8.5.1 (http://www.ridom.de/seqsphere), enabling assignment to the M1 lineages described in the European Nucleotide Archive (5,6). Currently, >200 recorded M-types are used to identify outbreaks and determine cluster management; M1 is most commonly associated with invasive disease (7).

NHS GGC and Lothian provides public healthcare for ≈39% of Scotland’s population of 5.49 million (https://www.nrscotland.gov.uk/statistics-and-data/statistics/statistics-by-theme/population/population-estimates/mid-year-population-estimates/mid-2021). Like other countries, the United Kingdom is undergoing a resurgence of *S. pyogenes* infections that began in September 2022 (https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON429). We identified 38 patients with PiGAS (30 definite, 8 probable) in the 3-month study period (22 in GGC, 16 in Lothian). In the previous 5-year period in GGC, we identified 15 cases (12 definite, 3 probable, 1 metastatic) (Figure 1).

We observed no significant difference in the median age between the 2022–23 cohort and historical records (45 [interquartile range (IQR) 26] years) vs. 57 [IQR 23.5] years). Both groups were healthy at baseline, having a median Charlson comorbidity index score of 0. We noted no significant difference in chronic respiratory underlying conditions. The 2022–23 cohort was associated with a more deprived SIMD postcode (median SIMD score of 3 vs. 5 for historical records). Other demographic, biochemical, and hematologic characteristics were comparable (Table).

The PiGAS syndrome exhibits substantial leukopenia (median 0.53 [IQR 0.3–1] × 10⁹ cells/L) with a normal median white cell count (9.5 [IQR 4–18.7] × 10⁹ cells/L). Although respiratory symptoms predominated, diarrhea was reported in 18.4% (7/38) of cases in the 2022–23 outbreak. Microbiologically confirmed empyema was common, and a greater number of 2022–23 patients received an intercostal drain and exhibited radiologic evidence of empyema or pleural effusion (Table). Those characteristics, along with multifocal consolidation, were the radiologic hallmarks of PiGAS; cavitation also was common. A lower proportion of patients in the historical cohort had chest computed tomography results, precluding greater sensitivity (47% vs. 76%).

During 2017–2022, co-infecting respiratory viruses were varied, but in 2022–23, most patients tested positive for influenza A (Table; Figure 2). Only half of patients underwent an extended respiratory viral screen. Of typed *S. pyogenes* isolates from the current outbreak, 24/25 belonged to the *M1*UK lineage, contrasting with the historical cohort that involved a mixture of M types (M1, M12, M3.93, M44, and M5.23).

Conclusions
Europe is experiencing an increased incidence of invasive GAS disease (4). We report an unusually high incidence of severe PiGAS in adults from central Scotland. We also note an additional strong association with influenza A co-infection and the near-complete dominance of *M1*UK, contrasting with local and published precedent. M1 comprised 38% of adult and 58% of pediatric invasive GAS referrals in England during 2022–23, in contrast to the 96% we report (https://www.gov.uk/government/publications/group-a-streptococcal-infections-activity-during-the-2022-to-2023-season/group-a-streptococcal-infections-second-update-on-seasonal-activity-

![Figure 1. Monthly incidence of pulmonary invasive group A *Streptococcus* infections in adults ≥18 years of age, National Health Service Greater Glasgow and Clyde region, central Scotland, UK, December 2017–February 2023.](image-url)
The clinical phenotype of severe, often rapidly fatal PiGAS disease in young healthy adults parallels outbreaks described around World War I and in institutional facilities (3,8). The M1UK lineage is emerging as a dominant lineage worldwide and often associated with invasive GAS (9–12). The pathophysiology of PiGAS after a respiratory viral infection (influenza A in our cohort) is incompletely understood. In vitro and in vivo studies suggest prior influenza A infection increases both GAS adherence and internalization by binding to viral hyaluronic acid on the infected host cell surface, which is followed by increases in the abundance of, and access to, bacterial receptors and the GAS ligands fibrinogen and fibronectin (13). Influenza B is also implicated, both in the literature and locally during 2017–2018 (14).

M1 has an established association with severe disease (7). In particular, the M1uk strain appears to have an enhanced capability for transmission and virulence and is now the predominant strain in the

in-england-2022-to-2023). The clinical phenotype of severe, often rapidly fatal PiGAS disease in young healthy adults parallels outbreaks described around World War I and in institutional facilities (3,8). The M1uk lineage is emerging as a dominant lineage within M1 worldwide and often associated with invasive GAS (9–12).

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### Table

Characteristics associated with a historical cohort (2017–2022) and a recent epidemic of severe pulmonary infections in adults (2022–23) caused by *Streptococcus pyogenes*, central Scotland, UK*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>2017–2022, GGC only</th>
<th>2022–2023, GGC and Lothian</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. PiGAS cases</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>Median age, y (IQR)</td>
<td>57 (42.5–66)</td>
<td>45 (37–63)</td>
</tr>
<tr>
<td>Sex, no (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>13 (87)</td>
<td>20 (53)</td>
</tr>
<tr>
<td>F</td>
<td>2 (6)</td>
<td>18 (47)</td>
</tr>
<tr>
<td>Median SIMD score (IQR)</td>
<td>5 (3.5)</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td><strong>Blood parameters at admission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median C-reactive protein, mg/L (IQR)†</td>
<td>293 (198–360)</td>
<td>328 (172–410)</td>
</tr>
<tr>
<td>Median leukocyte count, × 10⁹ cells/L (IQR)‡</td>
<td>9.5 (3.1–13.7)</td>
<td>9.45 (4.1–22.1)</td>
</tr>
<tr>
<td>Median lymphocyte count, × 10⁹ cells/L (IQR)§</td>
<td>0.5 (0.3–0.7)</td>
<td>0.54 (0.31–1.35)</td>
</tr>
<tr>
<td><strong>Radiographic, no. (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>8 (53)</td>
<td>23 (61)</td>
</tr>
<tr>
<td>Focal consolidation</td>
<td>3 (20)</td>
<td>9 (24)</td>
</tr>
<tr>
<td>Multifocal consolidation</td>
<td>11 (73)</td>
<td>28 (74)</td>
</tr>
<tr>
<td>Cavitation or necrosis</td>
<td>2 (13)</td>
<td>7 (18)</td>
</tr>
<tr>
<td><strong>Background</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic respiratory disease, no (%)</td>
<td>7 (47)</td>
<td>9 (24)</td>
</tr>
<tr>
<td>Smoker, no (%)</td>
<td>2 (13)</td>
<td>10 (26)</td>
</tr>
<tr>
<td>No past medical history, no (%)</td>
<td>4 (26.7)</td>
<td>17 (44.7)</td>
</tr>
<tr>
<td>Median CCI score (IQR)</td>
<td>0 (0–3)</td>
<td>0 (0–3)</td>
</tr>
<tr>
<td><strong>Viral co-infection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. tested</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Influenza A</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Influenza B</td>
<td>3</td>
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<tr>
<td>Parainfluenza 1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Metapneumovirus</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>RSV</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>None detected</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU admission, no (%)</td>
<td>8 (53)</td>
<td>21 (55)</td>
</tr>
<tr>
<td>Median ICU length of stay, d (IQR)</td>
<td>5 (2–9.8)</td>
<td>15 (3.5–27)</td>
</tr>
<tr>
<td>Vasopressors, no. (%)</td>
<td>4 (26)</td>
<td>15 (39)</td>
</tr>
<tr>
<td>Invasive mechanical ventilation, no. (%)</td>
<td>7 (46)</td>
<td>16 (42)</td>
</tr>
<tr>
<td>Death, no (%)</td>
<td>3 (20)</td>
<td>6 (16)</td>
</tr>
<tr>
<td>Median days from admission to death (IQR)</td>
<td>1 (1–2)</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td><strong>M type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. typed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>12.0</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>3.93</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>44.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5.23</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Historical cohort comprises cases identified among the population served by NHS Greater Glasgow and Clyde; cases from the recent epidemic are those identified among populations served by NHS Greater Glasgow and Clyde and NHS Lothian. CCI, Charlson comorbidity index; ICU, intensive care unit; IQR, interquartile range; NHS, National Health Service; PiGAS, pulmonary invasive group A Streptococcus; RSV, respiratory syncytial virus; SIMD, Scottish Index of Multiple Deprivation.

†Reference range <5 mg/L.
‡Reference range 4–10 × 10⁹ cells/L.
§Reference range 1.1–5.0 × 10⁹ cells/L.
United Kingdom (6). This strain exhibits a hyper-virulent phenotype because of greater expression of streptococcal pyrogenic exotoxin A than global M1 strains. Case reports of severe rapidly fatal M1 Pi-GAS in young healthy patients echo outcomes seen in our cohort (15).

Modern molecular techniques have revolutionized our ability to investigate patterns of disease (e.g., widespread availability of rapid point-of-care tests for COVID-19 and influenza). We are experiencing a major outbreak of S. pyogenes infections with an unusual predilection for severe pulmonary disease in addition to the usual manifestations of disease by this pathogen, including distinctive viral and M-type associations in the winter and spring of 2022–23. The PiGAS phenotype we describe is similar to those from more sporadic reports identified from a review of published case series of severe pulmonary infections from S. pyogenes (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/23-0569-App1.pdf). Historical outbreaks probably underreported coexistent viral infections because of a lack of accessible point-of-care tests. Similarly, only half of patients had an extended viral respiratory screen, and we therefore risk underreporting metapneumovirus cases, an agent notable locally in GAS empyema in children immediately before December 2022 (4). Although our cohort is small, it is comparatively large compared with the few described in the literature and notable for the short timeframe of cases captured.

Our study highlights a new aggressive pattern of S. pyogenes infections linked to the dominant circulating M1,uk strain, manifesting as severe pulmonary disease and having a strong association with influenza A co-infection. Clinicians and public health officials need to be vigilant of such clinical manifestations while rates of iGAS remain high.

Acknowledgments
We thank Pota Kalima for searching our Laboratory Information Management System to identify S. pyogenes specimens. We also thank Jörg Rothgänger for his assistance in preparing the core genome multilocus sequence typing scheme for use in SeqSphere+.

About the Author
Dr. Davies is an infectious diseases and microbiology specialist trainee at the National Health Service Greater Glasgow and Clyde in Scotland. His primary research interests are GAS disease and microbiology diagnostic pathways.

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### June 2023

**Poxvirus Infections**

- Association of Persistent Symptoms after Lyme Neuroborreliosis and Increased Levels of Interferon-α in Blood
- Probable Transmission of SARS-CoV-2 from African Lion to Zoo Employees, Indiana, USA, 2021
- Epidemiologic Characteristics of Mpox among People Experiencing Homelessness, Los Angeles County, California, USA, 2022
- Case Studies and Literature Review of *Francisella tularensis*-Related Prosthetic Joint Infection
- Neurologic Complications of Babesiosis, United States, 2011–2021
- SARS-CoV-2 Seroprevalence Studies in Pets, Spain
- Similar Prevalence of *Plasmodium falciparum* and Non-*P. falciparum* Malaria Infections among Schoolchildren, Tanzania
- Early SARS-CoV-2 Reinfections Involving the Same or Different Genomic Lineages, Spain
- Risk for Infection in Humans after Exposure to Birds Infected with Highly Pathogenic Avian Influenza A(H5N1) Virus, United States, 2022
- SARS-CoV-2 Vaccine Effectiveness against Omicron Variant in Infection-Naive Population, Australia, 2022
- Increased Incidence of Legionellosis after Improved Diagnostic Methods, New Zealand, 2000–2020
- Risk Factors for Non-O157 Shiga Toxin–Producing *Escherichia coli* Infections, United States
- Evolution of Avian Influenza Virus (H3) with Spillover into Humans, China
- Detection of Novel Poxvirus from Gray Seal (*Halichoerus grypus*), Germany
- Tanapox, South Africa, 2022
- Replication of Novel Zoonotic-Like Influenza A(H3N8) Virus in Ex Vivo Human Bronchus and Lung
- Results of PCR Analysis of Mpox Clinical Samples, Sweden, 2022

To revisit the June 2023 issue, go to: [https://wwwnc.cdc.gov/eid/articles/issue/29/6/table-of-contents](https://wwwnc.cdc.gov/eid/articles/issue/29/6/table-of-contents)
Dengue Outbreak Response during COVID-19 Pandemic, Key Largo, Florida, USA, 2020

Devin Rowe, Catherine McDermott, Ysla Veliz, Alison Kerr, Mark Whiteside, Mikki Coss, Chad Huff, Andrea Leal, Edgar Kopp, Alexis LaCrue, Lea A. Heberlein, Laura E. Adams, Gilberto A. Santiago, Jorge L. Munoz-Jordan, Gabriela Paz-Bailey, Andrea M. Morrison; Florida Department of Health Dengue Investigation Team


DOI: https://doi.org/10.3201/eid2908.221856

We report a dengue outbreak in Key Largo, Florida, USA, from February through August 2020, during the COVID-19 pandemic. Successful community engagement resulted in 61% of case-patients self-reporting. We also describe COVID-19 pandemic effects on the dengue outbreak investigation and the need to increase clinician awareness of dengue testing recommendations.

Dengue, an arboviral disease caused by dengue viruses 1–4 (DENV-1–4), is transmitted by *Aedes aegypti* mosquitoes (1). Before 1935, dengue was endemic in Florida, USA (2); however, no locally acquired cases were reported until an outbreak in Key West during 2009–2010 (3). Since then, at least 1 locally transmitted DENV infection has been reported annually in Florida except for 2017 and 2021 (2). Because Florida is vulnerable to establishment of *Ae. aegypti*-vectored viruses such as dengue, chikungunya, and Zika (4,5), surveillance is crucial to detect pathogen introduction.

During the COVID-19 pandemic, detecting the cause for other febrile illnesses was challenging (6) and reluctance to seek medical care during the pandemic was reported (7). We report the response to a dengue outbreak in Florida during the COVID-19 pandemic in 2020.

The Study

On February 28, 2020, the Florida Department of Health (FDOH) was notified of a possible locally acquired dengue case in a non-Florida resident who was visiting Key Largo; the case-patient had symptom onset on February 18. After confirming DENV-1 infection, FDOH issued a countywide public health mosquitoborne illness advisory for Monroe County on March 9 (Figure 1). During that same month, the governor of Florida issued a statewide public health emergency declaration for the COVID-19 pandemic (8). By the end of March, public access to nonessential businesses and facilities was further restricted in Monroe County because of increased COVID-19 case numbers (9). Additional locally acquired dengue cases were not identified until June 16, when several concerned Key Largo residents called FDOH reporting suspected dengue illness. A mosquitoborne illness alert was subsequently issued for the county after 8 local dengue cases were confirmed.

FDOH notified the Florida Keys Mosquito Control District (FKMCD) of possible mosquito exposure locations for suspected cases during the 2-week incubation period through the potential 1-week viremic period after symptom onset. FKMCD enhanced aerial and truck spraying and canvassed neighborhoods to conduct vector surveillance, remove or treat mosquito larval habitats, and provide mosquito control education.

While also responding to COVID-19, FDOH fielded hotline calls for residents reporting dengue-like illness, interviewed suspected case-patients, conducted site visits, provided frequent healthcare
provider and community outreach, collected serum samples for DENV testing, and promptly provided updates to FKMCD and local media. Persons with suspected dengue were asked to provide contact information for other persons who shared mosquito exposure risks, such as persons from the same household, workplace, or outdoor events. FDOH reached out to contacts and offered DENV testing if they reported a recent unexplained febrile illness. Ethics approval was not required because the activities conducted were part of standard public health outbreak surveillance and response.

FDOH also conducted syndromic surveillance for chief complaint and discharge diagnosis records from local hospitals. FDOH reviewed all syndromic surveillance records in the primary hospital serving the outbreak area and countywide, prioritizing chief complaints and discharge diagnoses mentioning dengue or fever and any combination of thrombocytopenia, rash, or arthralgia. FDOH requested medical records for patient visits with no alternative diagnosis. If only dengue serology had been ordered, FDOH requested that specimens be forwarded to the state laboratory for reverse transcription PCR (RT-PCR) testing. If no alternative diagnosis had been made and no DENV testing previously ordered, FDOH offered testing for persons with suspected cases.

Consistent with Centers for Disease Control and Prevention (CDC) guidelines, FDOH tested acute specimens collected within 7 days after symptom onset by using DENV RT-PCR and IgM tests. We only routinely performed antibody testing on convalescent samples collected >7 days after symptom onset. Specimens with positive or equivocal DENV test results at commercial laboratories were forwarded to FDOH and similarly retested. CDC assisted with serologic confirmation, serotyping RT-PCR–positive samples, information for other persons who shared mosquito exposure risks, such as persons from the same household, workplace, or outdoor events. FDOH reached out to contacts and offered DENV testing if they reported a recent unexplained febrile illness. Ethics approval was not required because the activities conducted were part of standard public health outbreak surveillance and response.

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Figure 2. Phylogenetic reconstruction of dengue virus 1 from a dengue outbreak response during COVID-19 pandemic, Key Largo, Florida, USA, 2020. A) Central American lineage, 1986–2014; B) Caribbean lineage, 2008–2020. Maximum-likelihood tree of genotype V was inferred by using envelope gene sequences representing the Central American and Caribbean lineages. Red text indicates sequences obtained in this study. Sequence FL-Miami_human_2020 was obtained from a Miami-Dade County resident with recent travel history to Cuba. We obtained 2 sequences (GenBank accession nos. OM909246 and OM909247) from the National Reference Laboratory for Arboviruses, French Armed Forces Biomedical Research Institute, Bretigny-sur-Orge, France. Scale bar indicates nucleotide substitutions per site.
and provided RT-PCR testing for mosquito pools collected by FKMCD.

We identified 72 locally acquired dengue cases associated with Key Largo. Cases were primarily among female (51%) and non-Hispanic (83%) persons (Table). Self-reporting, including via contact outreach, drove initial case identification (61%), followed by commercial laboratory reporting (22%), and syndromic surveillance (7%); only 1 case was first identified through direct healthcare provider reporting. No case-patients had traveled outside the continental United States during the incubation period.

Overall, 31 cases were RT-PCR–positive and 41 were IgM-positive (Table). All RT-PCR–positive cases were DENV-1. Retrospective case finding and testing identified IgM-positive cases with reported symptom onset as early as January (Figure 1). Some persons identified through retrospective case finding reported a febrile illness several months prior. We presumed those febrile illnesses were dengue, but asymptomatic infections are common, and IgM is generally detectable for 3 months, making definitive confirmation of the timing of DENV infection difficult.

Among 96 *Ae. aegypti* mosquito pools collected during June 18–September 21, three tested positive for DENV-1 (Figure 1). We sequenced 15 positive samples, 12 from dengue cases and 3 from mosquito pools. Phylogenetic analysis showed grouping within the Caribbean lineage of DENV-1 genotype V (Figure 2). Sequences from mosquito pools and humans were almost identical. We published sequence data in GenBank (accession nos. OM831209–18, OM833055–59, and OM909246–47). Sequencing definitively differentiated this outbreak of Caribbean lineage DENV from the 2009–2010 Key West outbreak of Central American lineage.

Among case-patients, 43 (60%) visited a healthcare provider during the acute illness, within 1 week after symptom onset (Table). Providers considered COVID-19 as a potential diagnosis, which is evidenced by COVID-19 test orders for 38 (88%) of the acute dengue cases. Dengue was considered a potential diagnosis in only 16 acute cases, 13 of which had testing for both dengue and COVID-19. Providers primarily (75%) ordered dengue antibody testing when evaluating acute cases, which is inconsistent with CDC recommendations to use RT-PCR or DENV nonstructural protein 1 (NS1) test (10), an alternative to RT-PCR, in addition to IgM testing during the acute phase. No acute samples were tested using the DENV NS1 test. Among acute samples, 26 had comprehensive testing (both RT-PCR and IgM) performed at a reference laboratory at FDOH or CDC. Eight were positive for both assays, 14 were only DENV RT-PCR-positive, and 4 were only DENV IgM-positive. Ultimately, 54% of acute cases tested with only an IgM assay would have been missed if not for additional RT-PCR testing performed at a reference laboratory, compared with just 15% missed by using RT-PCR testing alone.

**Conclusions**

This investigation confirmed an ongoing dengue outbreak in the Key Largo area of Florida, USA, during January–August 2020. During that same timeframe, 1,692 COVID-19 cases were reported in Monroe County. We suspect the COVID-19 pandemic negatively affected dengue surveillance because of reluctance to seek medical care, competing demands on healthcare providers during a rapidly evolving pandemic, and similar clinical presentations between COVID-19 and dengue. The focus on COVID-19 was further evidenced by providers primarily ordering COVID-19 tests among patients with acute dengue seeking medical care. The use of multiple case-finding methods, including aggressive community engagement, helped mitigate some of those effects, as did pandemic-related travel restrictions in the county.

In conclusion, CDC recommends using either commercial DENV RT-PCR or NS1 tests in combination with serologic testing for samples collected during the acute phase of dengue illness (10). This outbreak highlights that those tests were underused. Improving clinician awareness of CDC recommendations could improve case detection in the future, especially for nonendemic areas at increased risk for DENV introduction.

Members of the Florida Department of Health Dengue Investigation Team: Angela Giaquinto, Brandie Peretz, Robert B. Eadie, Ian Stryker, Joseph Yglesias, Megan Ostl, Jazra Gibson, Jasmine Reiyce Boykin, and Danielle Stanek.

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We are grateful to the residents of Key Largo and Monroe County, Florida, for their cooperation during this outbreak investigation. We also thank Janeen Laven, Kelly Fitzpatrick, Amanda Panella, and Jason Velez for their assistance with serologic testing for cross-reactive flaviviruses and Gilda Gard and Guillaume André Durand for their assistance with sequencing samples from 2 international travelers linked to the outbreak.
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Plague

Plague (from the Latin plaga, “stroke” or “wound”) infections are believed to have been common since at least 3000 bce. Plague is caused by the ancestor of current *Yersinia* (named for Swiss bacteriologist Alexandre Yersin, who first isolated the bacterium) *pestis* strains. However, this ancestral *Y. pestis* lacked the critical *Yersinia* murine toxin (*ymt*) gene that enables vector-borne transmission. After acquiring this gene (sometime during 1600–950 bce), which encodes a phospholipase D that protects the bacterium inside the flea gut, *Y. pestis* evolved the ability to cause pandemics of bubonic plague. The first recorded of these, the Justinian Plague, began in 541 ace and eventually killed more than 25 million persons.

References:
SARS-CoV-2 Variants and Age-Dependent Infection Rates among Household and Nonhousehold Contacts

Reiko Miyahara, Kosuke Tamura, Tomoko Kato, Mineko Nakazaki, Kanako Otani, Yura K. Ko, Taro Kamigaki, Yuzo Arima, Hideki Tani, Kazunori Oishi, Motoi Suzuki

To determine the effects of age and variants of concern on transmission of SARS-CoV-2, we analyzed infection rates among close contacts over 4 periods in Toyama Prefecture, Japan. Among household contacts, odds of infection were 6.2 times higher during the period of the Omicron variant than during previous periods, particularly among children and adolescents.

SARS-CoV-2 has been spreading globally since 2019; new variants of concern (VOCs) caused several epidemic waves during 2020–2022. According to a meta-analysis, the overall household secondary attack rates were higher for the Omicron variant (42.7%) than for the Alpha (36.4%) and Delta (29.7%) variants (1). The transmissibility and age-dependent susceptibility for Omicron and Delta exhibited significant heterogeneity among studies (1,2), and children were identified as being more vulnerable than adults to new variants (2). Infection rates among close contacts, determined by SARS-CoV-2 diagnostic tests, can vary according to study design, site settings, nonpharmaceutical control measures, and contact patterns (3). Thus, assessing infection rates among household and nonhousehold contacts within the same geographic area and population by using consistent methods over time could provide more reliable and valid information about changes in the effects of age and VOCs on transmission risk. With this study, we aimed to analyze the effects of age and VOCs on SARS-CoV-2 transmission by using contact tracing data of index case-patients and household and nonhousehold contacts in a city in Toyama Prefecture, Japan.

The Study
We analyzed COVID-19 cases recorded in a city in Toyama Prefecture, Japan, over 4 periods, dominated by each of the 4 main virus variants: July 1–October 31, 2020 (pre-VOC period), April 1–30, 2021 (Alpha period), July 3–August 15, 2021 (Delta period), and January 3–23, 2022 (Omicron period) (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/8/22-1582-App1.pdf). Health center staff conducted telephone interviews with all COVID-19 case-patients, including those who were asymptomatic, to collect clinical information and recent activity history. According to the contact tracing guidelines of the National Institute of Infectious Diseases (Japan Ministry of Health, Labour and Welfare), we defined a close contact as someone who had contact with a COVID-19 case-patient during the period from 2 days before symptom onset until diagnosis (4). Close contacts were divided into household contacts (those who resided in the same household) and nonhousehold contacts (others who had contact with a confirmed COVID-19 case-patient for ≥15 minutes within a 1-meter distance without wearing any personal protective equipment). All contacts received SARS-CoV-2 PCR testing regardless of symptom status. If the PCR result for the first test was negative, contacts received PCR testing again if COVID-19–associated symptoms developed. We excluded from analysis close contacts with no PCR results.

All data management and analyses were conducted as part of the public health response in Toyama Prefecture and the National Institute of Infectious Diseases, and we used registered data collected according to the Infectious Diseases Law of Japan. Ethics approval was not required for this study.

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First, we determined the baseline characteristics of the index case-patients and close contacts for each of the 4 periods. Second, we calculated infection rates stratified by the characteristics of index case-patients (age, sex, history of contact with COVID-19 case-patients before diagnosis, and symptom status) and close contacts (age, sex, and interval between diagnosis of index case-patients and PCR results of contacts). To adjust for clustering effects, we calculated infection rates as the total number of positive contacts divided by the total number of close contacts (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering among contacts exposed to the same index case-patients, we analyzed odds ratios of the infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To adjust for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com). To account for clustering effects, we calculated infection rates (with 95% CIs) by using the svyset command in Stata (StataCorp LLC, https://www.stata.com).

We enrolled 1,057 patients and 3,820 contacts: 123 index case-patients and 530 close contacts, in the pre-VOC period; 246 index case-patients and 988 close contacts in the Alpha period; 304 index case-patients and 984 close contacts in the Delta period; and 384 index case-patients and 1,318 close contacts in the Omicron period (Appendix Table 1). We excluded close contacts without PCR results: 45 (8.5%) persons from the pre-VOC period, 29 (2.9%) from the Alpha period, 111 (11.3%) from the Delta period, and 173 (13.1%) from the Omicron period. Infection rates during the Omicron period were 35.0% (95% CI 28.3–42.2) for household contacts and 15.1% (95% CI 10.0–22.5) for nonhousehold contacts. After adjustment for age, symptoms, sex, contact history, interval from diagnosis of index case-patient to PCR test, and household size, the odds ratios for infection were 6.22 times higher among household contacts and 3.55 times higher among nonhousehold contacts during the Omicron period than during the pre-VOC period (Table; Appendix Table 2). The risk for infection among household contacts 0–19 years of age increased significantly, from 3% in the pre-VOC period to 38% during the Omicron period (Appendix Figure 2). In contrast, during the study period, infection rates for nonhousehold contacts in this age group were

<table>
<thead>
<tr>
<th>Variable</th>
<th>Household contacts</th>
<th>Nonhousehold contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>No. PCR positive</td>
</tr>
<tr>
<td>Period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-VOC</td>
<td>1,144</td>
<td>294</td>
</tr>
<tr>
<td>Alpha</td>
<td>155</td>
<td>20</td>
</tr>
<tr>
<td>Delta</td>
<td>251</td>
<td>48</td>
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<tr>
<td>Omicron</td>
<td>329</td>
<td>83</td>
</tr>
<tr>
<td>Index case-patient age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–19</td>
<td>214</td>
<td>54</td>
</tr>
<tr>
<td>20–39</td>
<td>493</td>
<td>111</td>
</tr>
<tr>
<td>40–59</td>
<td>309</td>
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<td>≥60</td>
<td>129</td>
<td>45</td>
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<tr>
<td>Close contact age, y</td>
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<td></td>
</tr>
<tr>
<td>0–19</td>
<td>295</td>
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<td>20–39</td>
<td>259</td>
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</tr>
<tr>
<td>40–59</td>
<td>359</td>
<td>84</td>
</tr>
<tr>
<td>≥60</td>
<td>227</td>
<td>51</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Odds ratios were adjusted for age, sex, symptoms of index case-patients at the time of diagnosis, contact history, interval from diagnosis of index case-patient to PCR tests, and number of persons in the same household. NA, not applicable; VOC, variant of concern.
lower, despite a higher number of contacts compared with nonhousehold contacts in other age groups (Appendix Figure 3). Infection rates among household contacts ≥60 years of age decreased during the Delta period (12%) but increased again during the Omicron period (29%). Regarding infectivity throughout all time periods, the risk for infection from index case-patients ≥60 years of age was higher than that from index case-patients of other ages (Appendix Figure 2).

Conclusions
Our study showed that odds of infection were 6.2 times higher for household contacts during the Omicron period than during the pre-VOC period and that children and adolescents were particularly vulnerable (2). Despite increased nonhousehold contact among persons 0–19 years of age, nonphysical contact (5) and nonpharmacological control measures (6) in school and daycare centers may have led to lower infection rates and fewer large outbreaks in schools.

In addition, infection rates for contacts ≥60 years of age decreased during the Delta period but increased again during the Omicron period, potentially because of waning immunity associated with SARS-CoV-2 vaccination and the attenuated effect on the Omicron variant (7), even with high vaccination rates (93%) among persons >65 years of age during the Omicron period (Appendix Figure 1). In addition, the infectivity of elderly persons tended to be higher than that of persons in other age groups even after vaccine introduction (8), possibly because of close contact, such as caregiving and nursing care. The value of protecting those who care for elderly case-patients should thus be emphasized.

A limitation of this study was the varied timing and frequency of PCR testing. As the number of days from symptom onset to diagnosis decreased over time, infection rates were associated with the timing of testing and symptoms at the time of testing. We might have missed asymptomatic infections and potentially overcounted infected case-patients among contacts who might have been exposed to other places or infected persons.

Our finding of increased odds of infection among household contacts during the period of the Omicron variant, particularly among children and adolescents, highlights the need for periodic surveys to investigate comparative infectivity by epidemic strain as well as susceptibility and trends by age group over time in the same area and population. Such studies would account for variations in local conditions such as control regulation, contract tracing strategy, population age structure, and vaccination coverage.

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References

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Uniting for Ukraine Tuberculosis Screening Experience, San Francisco, California, USA

Janice K. Louie, Rocio Agraz-Lara, Laura Romo, Cristy Deiterich, Cathleen Xing, Susannah Graves

Ukraine surveillance data suggest high tuberculosis (TB) incidence, including multidrug resistance. Of 299 newcomers from Ukraine screened in San Francisco, California, USA, by using an interferon-γ-release-assay (IGRA) and chest radiograph, 7.4% were IGRA positive and 1 had laboratory-confirmed pansusceptible TB. Screening with IGRA and chest radiograph can help characterize TB risk.

World Health Organization surveillance data estimate that Ukraine has the fourth highest tuberculosis (TB) incidence in the European Region, at 71 cases/100,000 population in 2021 (1–3). Ukraine is believed to have a high burden of rifampin- and multidrug-resistant TB, accounting for ≈31% of culture-confirmed cases in 2021 (2,3). In addition, 22% of persons from Ukraine who have TB are infected with HIV; TB is the leading cause of death in this population (2,3).

In April 2022, the US Department of Homeland Security announced the Uniting for Ukraine (U4U) program to provide a pathway for citizens from Ukraine to enter the United States under humanitarian parole (4). U4U requires that parolees ≥2 years of age submit an attestation to the US Citizenship and Immigration Services confirming that TB screening with symptom review and an interferon-γ-release assay (IGRA) are performed within 90 days of US entry (5). In response, the San Francisco Department of Public Health TB Clinic partnered with the SFDPH Newcomers Health Program, a county Refugee Health Assessment Program, to reach out to community, professional, and faith-based groups to encourage expedited, no-cost TB screening to promptly identify and treat any U4U parolees who had latent or active TB (6,7).

The Study
To meet attestation requirements, U4U parolees who had a San Francisco address were screened for TB symptoms (fever, cough, night sweats, weight loss, fatigue, or hemoptysis) and tested by using the IGRA QuantiFERONTB Gold In-Tube Test (https://www.quantiferon.com). HIV testing was offered to all persons ≥2 years of age, and a chest radiograph was offered to persons ≥15 years of age to aid early identification of pulmonary TB. Parolees who had a positive IGRA result but unremarkable chest radiograph were offered latent TB infection (LTBI) treatment according to US Centers for Disease Control and Prevention recommendations (8).

Patients who had chest radiograph abnormalities suggestive of TB, regardless of IGRA result, underwent further evaluation by collection of 3 sputum samples for acid-fast bacilli (AFB) smear and culture and 1 sputum sample for nucleic acid amplification testing (NAAT) with the GeneXpert MTB/RIF assay (Cepheid, https://www.cephied.com), which tests for TB and rifampin resistance. If sputum cultures were negative, LTBI treatment was recommended (8). Patients who had positive results for TB by NAAT or AFB culture were given treatment for active TB according to national recommendations (9).

During May 10, 2022–April 14, 2023, a total of 299 U4U parolees underwent TB screening (Table). Median age was 33 years (range 8 months–84 years); 116 (38.8%) were male. All patients denied previous active or latent TB. Of 298 patients ≥2 years of age, 274 (91.9%) agreed to HIV testing; all showed negative results. None of the 299 patients screened reported alcohol or substance use or previous incarceration. Three (1.0%) patients reported having a medical TB risk factor; all 3 had diabetes. All patients denied TB symptoms except for 1 (described later in this report).
Of the 299 patients, 22 (7.4%) had positive IGRA results; median age was 51.5 (range 17–81) years. Of 245 patients ≥15 years of age, 240 (98.0%) received a chest radiograph. Seven (2.9%) patients had abnormal chest radiograph results, consistent with possible TB, including 4 patients who had negative IGRA results; median age was 43 (range 36–63) years.

One parolee had laboratory confirmation of active TB. The patient reported productive cough and rhinorrhea for 10 days but no other TB symptoms. The patient had no epidemiologic or medical risk factors; HIV test result was negative, IGRA test result was positive, and chest radiograph identified upper lobe nodules. Sputum samples tested showed few AFB smear-positive, NAAT-positive results without rifampin resistance and grew *Mycobacterium tuberculosis* that was pansusceptible to isoniazid, rifampin, ethambutol, and pyrazinamide. The patient received TB therapy; all household contacts, including a child <5 years of age, tested negative by IGRA at baseline and 8–10 weeks later.

Conclusions

Despite surveillance data reporting high TB incidence (including drug-resistant TB) in Ukraine, only 7.4% of parolees in this investigation received diagnoses of LTBI, and only 1 had laboratory-confirmed, pansusceptible, active pulmonary disease (1–3). Most parolees were female, possibly reflecting that many men have remained in Ukraine during wartime. All parolees with LTBI were ≥18 years of age, consistent with reports that TB is uncommon in children from Ukraine (1–3). Most parolees reported no concurrent medical condition, and none tested were HIV positive. The percentages of U4U parolees testing positive by IGRA was low compared with other San Francisco immigrant populations; in the past 5 years, of clients undergoing screening for homeless shelter housing, 20.2% who originated from Mexico and 27.5% from Central America (including Belize, El Salvador, Guatemala, Honduras, and Nicaragua) have tested IGRA positive (San Francisco Department of Public Health, unpub. data).

U4U parolees might not be representative of populations from Ukraine most likely to be given a diagnosis of TB. For example, data for Ukraine for 2021 suggest that HIV, alcohol use, malnutrition, and diabetes are major TB risk factors; those factors were uncommon or absent in the San Francisco U4U population (1–3). Our numbers are reflective of the San Francisco U4U program only and might not be generalizable to other jurisdictions. Nevertheless, vigilance in the U4U population remains warranted because armed conflict and mass displacements have historically been associated with increases in TB incidence, drug-resistant TB, and TB deaths, possibly caused by disruptions in healthcare services, malnutrition, and need for temporary housing with associated crowding and poor hygiene (11).

The Centers for Disease Control and Prevention Division of Global Migration and Quarantine has established requirements for overseas screening
of new refugees before entry into the United States, which include medical history, physical examination, and TB screening (12). For persons originating from countries that have a TB incidence of ≥20 cases/100,000 persons, the overseas screening requirement for persons ≥15 years of age includes a chest radiograph (IGRA optional); for those 2–14 years of age, only IGRA is necessary (12). Within 90 days of US arrival, a domestic screening, including history, physical examination, and review of overseas screening results, is recommended; depending on the person, refugees might undergo further evaluation for LTBI (if overseas IGRA was not performed or the result is >6 months old) or active TB (if new symptoms or physical examination abnormalities have developed since overseas screening) (13).

In humanitarian situations through which newcomers enter the United States emergently from high-incidence countries without previous overseas evaluation, domestic TB screening with IGRA and chest radiograph (in persons ≥15 years of age) might be merited to match existing overseas refugee screening recommendations. Because IGRA can show false-negative results for >20% of persons who have active TB, addition of a chest radiograph can help enable rapid and sensitive detection of pulmonary TB, ensure prompt treatment, and prevent local transmission (14). Our inclusion of chest radiographs also provides reassuring data suggesting that infectious pulmonary TB is not being missed in U4U parolees entering San Francisco, despite the high incidence reported in surveillance data for Ukraine.

In late 2022 and early 2023, the Department of Homeland Security implemented programs similar to U4U for new parolees from Venezuela, Nicaragua, Cuba, and Haiti (15). Those parolees have not been screened overseas, have the same TB attestation requirements as U4U, and might have entered the United States under circumstances that convey higher TB risk (e.g., extreme poverty, expolitical prisoners, or long and crowded land journeys) (15). The establishment of U4U screening has enabled SFDPH to assess parolee populations from high-incidence countries.

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We thank Gloria Perez and Sammi Truong for generously working with the U4U population and the SFDPH TB clinic staff for providing dedicated care to our TB patients.

About the Author
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**April 2023 Vectorborne Infections**

- Challenges in Forecasting Antimicrobial Resistance
- Pediatric Invasive Meningococcal Disease, Auckland, New Zealand (Aotearoa), 2004–2020
- Bacterial Agents Detected in 418 Ticks Removed from Humans during 2014–2021, France
- Association of Scrub Typhus in Children with Acute Encephalitis Syndrome and Meningoencephalitis, Southern India
- *Nocardiopsis pseudobrasiliensis* Co-infection in SARS-CoV-2 Patients
- Monitoring Temporal Changes in SARS-CoV-2 Spike Antibody Levels and Variant-Specific Risk for Infection, Dominican Republic, March 2021–August 2022
- Extensive Spread of SARS-CoV-2 Delta Variant among Vaccinated Persons during 7-Day River Cruise, the Netherlands
- Mapping Global Bushmeat Activities to Improve Zoonotic Spillover Surveillance by Using Geospatial Modeling
- Adeno-Associated Virus 2 and Human Adenovirus F41 in Wastewater during Outbreak of Severe Acute Hepatitis in Children, Ireland
- Outbreaks of SARS-CoV-2 Infections in Nursing Homes during Periods of Delta and Omicron Predominance, United States, July 2021–March 2022
- Yezo Virus Infection in Tick-Bitten Patient and Ticks, Northeastern China
- Effectiveness of BNT162b2 Vaccine against Omicron Variant Infection among Children 5–11 Years of Age, Israel
- Monkeypox Virus Infection in 2 Female Travelers Returning to Vietnam from Dubai, United Arab Emirates, 2022
- Experimental Infection and Transmission of SARS-CoV-2 Delta and Omicron Variants among Beagle Dogs
- Highly Pathogenic Avian Influenza A(H5N1) Virus Outbreak in New England Seals, United States
- Emergence and Persistent Dominance of SARS-CoV-2 Omicron BA.2.3.7 Variant, Taiwan
- *Rickettsia conorii* Subspecies *israelensis* in Captive Baboons
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- Two-Year Cohort Study of SARS-CoV-2, Verona, Italy, 2020–2022
- Chikungunya Outbreak in Country with Multiple Vectorborne Diseases, Djibouti, 2019–2020
- Blackwater Fever Treated with Steroids in Nonimmune Patient, Italy
- *Helicobacter aiurogastricus* in Patient with Multiple Refractory Gastric Ulcers, Japan
- Harbor Porpoise Deaths Associated with Erysipelothrix rhusiopathiae, the Netherlands, 2021
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- Human Metapneumovirus Infections during COVID-19 Pandemic, Spain
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**Mycobacterium abscessus**

Meningitis Associated with Stem Cell Treatment During Medical Tourism


Mycobacterium abscessus infections have been reported as adverse events related to medical tourism. We report *M. abscessus* meningitis in a patient who traveled from Colorado, USA, to Mexico to receive intrathecal stem cell injections as treatment for multiple sclerosis. We also review the management of this challenging central nervous system infection.

*Mycobacterium abscessus* is a rapidly growing nontuberculous mycobacterium (NTM) commonly found in soil and water (1). Pulmonary, skin, and soft tissue infections are common nosocomial infections that are often associated with inadequate sterilization of water and reagents (2). Rarely, *M. abscessus* can infect the central nervous system (CNS), causing chronic meningitis or abscess, often in the setting of trauma, surgery, or dissemination in immunocompromised hosts (3). CNS infections typically manifest as subacute headache, fever, meningismus, or some combination, along with focal neurologic signs if there is an abscess (3). *M. abscessus* infection has been reported as a complication of medical tourism (i.e., when patients travel abroad for medical treatment or cosmetic surgeries) (4,5). *M. abscessus* infections are challenging to treat, requiring prolonged multidrug regimens or surgical intervention (6). We present a patient with *M. abscessus* meningitis associated with intrathecal stem cell injections during medical tourism.

The Case

This report centers on an immunocompetent woman in her 30s who had been diagnosed with multiple sclerosis (MS) and met 2017 McDonald criteria 3 years before our initial encounter. Her initial MS symptoms were episodic left arm and left leg numbness, and she had multifocal brain, cervical, and thoracic spine demyelinating lesions identified by magnetic resonance imaging. She had never received disease-modifying therapies or other immunosuppressive medications. Results of her baseline neurologic examination was unremarkable.

In October 2022, the patient traveled to a commercial clinic in Baja California, Mexico. During a 4-day visit, she underwent 2 lumbar punctures for intrathecal injection of donor umbilical cord stem cells programmed to treat MS. She pursued treatment at this clinic after reviewing its associated website as part of her research on stem cell treatments for MS.

The day after the second intrathecal injection, she visited an emergency department in the United States for positional headache and received an epidural blood patch for presumed postlumbar puncture cerebrospinal fluid (CSF) leak. She reported nocturnal fevers, but vital signs, neurologic examination, complete blood counts, and computed tomography of the head were unremarkable. She was discharged after her headache improved but subsequently received 2 blood patches in the outpatient setting for recurrent headaches.

Because of persistent fevers, the patient was admitted to an outside hospital 5 days after receiving the third blood patch. At admission, she was febrile (101.3°F), but vital signs and complete blood counts were otherwise within reference ranges and HIV serology results were negative. Neurologic examination...
remained unremarkable. Sampling of her CSF revealed 74 nucleated cells/µL (76% neutrophils, 20% lymphocytes, 2% monocytes), 64 red blood cells/µL, 84 mg/dL of protein, and 29 mg/dL of glucose (serum glucose 96 mg/dL). Results of herpes simplex virus PCR and enterovirus real-time reverse transcription PCR tests were negative. There was no growth on aerobic or anaerobic bacterial cultures. She received vancomycin and 1 dose of cefepime before changing over to meropenem because rash developed during cefepime infusion. She transitioned to imipenem for a 10-day total course; fever resolved, and headache improved.

Days after completing antibiotics, the patient experienced worsening headache and recurrence of fevers, prompting her admission to our institution. We resampled her CSF and found it contained 104 nucleated cells/µL (50% neutrophils, 42% lymphocytes, 8% monocytes), 3 red blood cells/µL, 47 mg/dL of protein, and 31 mg/dL of glucose (serum glucose 85 mg/dL). We identified 13 CSF-specific oligoclonal bands (reference <2) and noted her IgG index was 1.21 (reference <0.6); those values were consistent with MS but also a potential indicator of CNS infection. We obtained magnetic resonance images of the patient’s brain and cervical, thoracic, and lumbar spine, with and without gadolinium, and found no evidence of active demyelination or infection. We prescribed a course of vancomycin and ceftriaxone as empiric meningitis coverage, and the patient noted improvement of fever and headaches. The patient’s empiric meningitis coverage, and the patient noted improvement of fever and headaches. The patient’s empiric meningitis coverage, and the patient noted improvement of fever and headaches. The patient’s empiric meningitis coverage, and the patient noted improvement of fever and headaches. We prescribed the patient a treatment course that included azithromycin (500 mg intravenously [IV] 1×/d), imipenem (500 mg IV 4×/d), and trimethoprim/sulfamethoxazole (5 mg/kg IV 3×/d), tedizolid (200 mg orally 1×/d), and ciprofloxacin (400 mg orally 3×/d), some of which led to intolerable gastrointestinal symptoms. After identifying the NTM as *M. abscessus*, we adjusted the patient’s treatment course on the basis of drug susceptibilities using Clinical and Laboratory Standards Institute cutpoints for resistance (Table 1) (7). We stopped trimethoprim/sulfamethoxazole and ciprofloxacin and instituted eravacycline (80 mg IV 1×/d) due to favorable CNS penetration. We initiated ceftaroline (600 mg IV 3×/d) due to synergistic activity with imipenem (8). The final treatment regimen (Table 2) brought improvement in headaches and resolution of fevers. In total, it took 8 weeks of evaluations after her initial visit to the outside emergency department to identify and treat *M. abscessus*. Repeat CSF culture after 3 weeks of treatment revealed no growth. The patient has now completed >3 months of treatment without recurrent symptoms.

**Conclusions**

This patient’s case highlights a serious complication associated with medical tourism. Promising preclinical studies of stem cell treatments has led to stem cell tourism (9). A purported ability of stem cell treatments to repair disabling CNS damage has encouraged patients to frequently pursue such treatments, despite the lack of demonstrated efficacy. Without regulatory approval, stem cell treatments are frequently marketed online, with scientific messaging and patient testimonials to project an aura of legitimacy. Such treatments have been linked to serious complications, leading to warnings from the US Centers for Disease Control and Prevention and the US Food and Drug Administration (10–12).

Peripheral stem cell treatments have been associated with a range of adverse events, including...
infections and neoplasms (10). There is little to ensure the integrity of the manufacturing or administration for such unregulated treatments, and there is no postmarketing surveillance. Inadequate use of sterile technique or use of contaminated water during manufacture or administration of stem cell products may lead to infections. The largest reported incident involved 20 culture-confirmed bacterial infections secondary to donor umbilical cord blood products proposed as treatment for orthopedic conditions (13). In addition to infections, neoplastic and inflammatory lesions have been associated with intrathecal stem cell treatments (9,10).

Diagnosis of M. abscessus infection requires isolation of NTM and use of molecular techniques to identify species and subspecies (1). M. abscessus is difficult to treat because of its in vitro antimicrobial resistance and biofilm formation (1). The species is generally resistant to typical antituberculous drugs, and those used for prolonged multidrug regimens are often poorly tolerated (14,15). Drug efficacy is dramatically reduced by the presence in M. abscessus of an erythromycin ribosome methylase gene, erm(41), which induces macrolide resistance, or mutant resistance via the 23S ribosomal RNA gene. As such, macrolide companion drugs must be carefully selected (6). Regimens to treat CNS M. abscessus infection are based on limited evidence because of the rarity of cases and unknown CSF penetration of many drugs (3,15). Surgical debridement of abscesses may be necessary.

The literature describes cases of M. abscessus infection involving contributory immunosuppression, trauma, or neurosurgery, making our patient’s case rather unique. She has completed >3 months of treatment without recurrent symptoms. In addition to the direct injury from M. abscessus meningitis, patients like the one we report face an increased risk of neurologic disability because chronic CNS infection precludes use of immunosuppressive therapies for MS. Therefore, counseling MS patients on the risks of stem cell tourism is fundamental. There is no proven benefit to intrathecal stem cell treatments, and such treatments should be offered only through registered clinical trials. Clinicians should be aware of potential harms of stem cell tourism and report adverse events to public health agencies.

Acknowledgments
We thank the patient discussed in this case report, those involved in her clinical care, and the Colorado Department of Public Health and Environment. The patient verbally consented to her deidentified case being reported and signed our institution’s standard consent form.

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**January 2023**

**Vectorborne Infections**

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- Multicenter Case-Control Study of COVID-19–Associated Mucormycosis Outbreak, India
- Role of Seaports and Imported Rats in Seoul Hantavirus Circulation, Africa
- Risk for Severe Illness and Death among Pediatric Patients with Down Syndrome Hospitalized for COVID-19, Brazil
- Molecular Tools for Early Detection of Invasive Malaria Vector Anopheles stephensi Mosquitoes
- Integrating Citizen Scientist Data into the Surveillance System for Avian Influenza Virus, Taiwan
- Widespread Exposure to Mosquitoborne California Serogroup Viruses in Caribou, Arctic Fox, Red Fox, and Polar Bears, Canada
- Seroepidemiology and Carriage of Diphtheria in Epidemic-Prone Area and Implications for Vaccination Policy, Vietnam
- Akkermansia muciniphila Associated with Improved Linear Growth among Young Children, Democratic Republic of the Congo
- High SARS-CoV-2 Seroprevalence after Second COVID-19 Wave (October 2020–April 2021), Democratic Republic of the Congo
- Human Immunity and Susceptibility to Influenza A(H3) Viruses of Avian, Equine, and Swine Origin
- Genomic Epidemiology Linking Nonendemic Coccidioidomycosis to Travel
- Risk for Severe COVID-19 Outcomes among Persons with Intellectual Disabilities, the Netherlands
- Effects of Second Dose of SARS-CoV-2 Vaccination on Household Transmission, England
- COVID-19 Booster Dose Vaccination Coverage and Factors Associated with Booster Vaccination among Adults, United States, March 2022
- Pathologic and Immunohistochemical Evidence of Possible Francisellaceae among Aborted Ovine Fetuses, Uruguay
- Bourbon Virus Transmission, New York, USA
- Genomic Microevolution of Vibrio cholerae O1, Lake Tanganyika Basin, Africa
- Genomic Confirmation of Barrelia garinii, United States

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**Plasmodium falciparum pfhrp2 and pfhrp3 Gene Deletions in Malaria-Hyperendemic Region, South Sudan**

**Burden of Postinfectious Symptoms after Acute Dengue, Vietnam**

**Survey of West Nile and Banzi Viruses in Mosquitoes, South Africa, 2011–2018**

**Detection of Clade 2.3.4.4b Avian Influenza A(HSN8) Virus in Cambodia, 2021**

**Using Serum Specimens for Real-Time PCR-Based Diagnosis of Human Granulocytic Anaplasmosis, Canada**

**Photobacterium damselae subspecies damselae Pneumonia in Dead, Stranded Bottlenose Dolphin, Eastern Mediterranean Sea**

**Early Warning Surveillance for SARS-CoV-2 Omicron Variants, United Kingdom, November 2021–September 2022**

**Efficient Inactivation of Monkeypox Virus by World Health Organization–Recommended Hand Rub Formulations and Alcohols**

**Detection of Monkeypox Virus DNA in Airport Wastewater, Rome, Italy**

**Successful Treatment of Balanomithia mandrillaris Granulomatous Amebic Encephalitis with Nitroxoline**

**Clinical Forms of Japanese Spotted Fever from Case-Series Study, Zigiui County, Hubei Province, China, 2021**

**COVID-19 Symptoms by Variant Period in the North Carolina COVID-19 Community Research Partnership, North Carolina, USA**

**Increased Seroprevalence of Typhus Group Rickettsiosis, Galveston County, Texas, USA**

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Candidatus Neoehrlichia mikurensis Infection in Patient with Antecedent Hematologic Neoplasm, Spain

Paola González-Carmona,2,3 Aránzazu Portillo,2 Cristina Cervera-Acedo, Daniel González-Fernández, José A. Oteo

We report a confirmed case of Candidatus Neoehrlichia mikurensis infection in a woman in Spain who had a previous hematologic malignancy. Candidatus N. mikurensis infections should be especially suspected in immunocompromised patients who exhibit persistent fever and venous thrombosis, particularly if they live in environments where ticks are prevalent.

Candidatus Neoehrlichia mikurensis is an α1-proteobacterium (family Anaplasmataceae) transmitted by Ixodes spp. ticks. Although previously described in ticks and mammals in Europe and Asia, the species name was derived from a report in 2004 from Mikura Island, Japan, where the bacterium was found in endothelial cells from rat (Rattus norvegicus) spleens and in Ixodes ovatus ticks (1). In 2010, Candidatus N. mikurensis was identified as a human pathogen in Sweden (2). Since then, several case series and individual cases of patients with Candidatus N. mikurensis infections have been described, mainly in persons who were immunosuppressed because of hematologic neoplasms, splenectomies, or immunosuppressive drug treatment (3–9). However, Candidatus N. mikurensis can cause disease (neoehrlichiosis) in immunocompetent persons or cause asymptomatic infections (10,11). In 2019, Candidatus N. mikurensis was cultured in tick cell lines and infection was transferred to human endothelial cells derived from skin microvasculature and pulmonary arteries, demonstrating endothelial cell tropism. Tropism partly explains the clinical spectrum caused by the bacterium, usually consisting of persistent and recurrent fever and thrombosis and vasculitis with or without erysipelas-like skin lesions (12). In Spain, Candidatus N. mikurensis was found in Ixodes ricinus ticks removed from cows in 2013, but the bacterium was not detected in humans (13). We describe a case of Candidatus N. mikurensis infection in an immunocompromised patient from Asturias in northern Spain.

The Study
In September 2020, stage IV-B germinal center diffuse large B-cell lymphoma was diagnosed in a splenectomy specimen from a 68-year-old woman. She completed first-line treatment with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone and achieved complete remission. On June 21, 2021 (≈5 months after lymphoma treatment had ended), she experienced arthromyalgia, anorexia, night sweats, and vespertine fever. Her family physician began treatment with metamizole and cefuroxime at usual doses because of urine sediment alterations. Several days later, deep vein thrombosis developed in her right leg. Because of her previous malignancy and treatment, she was attended at her hospital’s hematology service. She was slightly anemic (hemoglobin 11.7 g/dL, reference range 12–16 g/dL) and had leukopenia (2.28 × 10³ leukocytes/µL, reference range 4–14 × 10³ leukocytes/µL) and a low neutrophil count (0.4 × 10³ neutrophils/µL, reference range 1.8–8.5 × 10³ neutrophils/µL). C-reactive protein level was elevated (62 mg/L, reference range <10 mg/L), hyponatremia was...
Present (133 mmol Na/L, reference range 135–145 mmol Na/L), and high levels of ferritin (536 μg/L, reference range 20–200 μg/L) and β2 microglobulin (8.50 mg/L, reference range 0.8–2.4 mg/L) were observed. Other measured hematologic and biochemical parameters, including procalcitonin, were within reference ranges. Other analyses, such as antinuclear antibody testing, blood and urine cultures, and serologic assays against Coxiella burnetii, herpes virus, cytomegalovirus, and Epstein-Barr virus, did not indicate acute infection. A chest radiograph and computed tomography scan and an abdominal ultrasound did not reveal pertinent abnormalities. Recurrence of lymphoma was suspected, and a positron emission tomography/computed tomography scan showed diffuse and homogeneous bone marrow hypermetabolism without evidence of neoplastic activity at other levels.

Empirical treatment was begun with piperacillin/tazobactam and granulocyte colony stimulating factor at conventional doses; 1 week later, the patient had recovered from leukopenia, but fever persisted. A bone marrow biopsy, which did not show neoplastic infiltration or alterations in hematopoietic cells, was performed and processed for different microbiologic tests. A possible tick-related infection was suspected because the patient lived in an area endemic for Lyme disease and other tick-related infection was suspected because the patient recalled having suffered a tick bite 20 days before onset of symptoms. A bone marrow DNA extract and serum sample collected during the acute infection phase (August 2021) were sent to the Special Pathogens Laboratory, Center for Rickettsioses and Arthropod-Borne Diseases, at San Pedro University Hospital–Center for Biomedical Research of La Rioja in Logroño, Spain, to screen forCandidateatus N. mikurensis infection associated with neutropenia has been attributed to the intake of metamizole for symptom control. However, another case of doxycycline-treated Candidatus N. mikurensis infection associated with neutropenia has been reported (8). EDTA-anticoagulated blood and serum specimens were collected 4

We performed PCR targeting the panbacterial 16S rRNA gene, fragments of 16S rRNA gene from Anaplasmataceae (designated as 16S rRNA-EHR), groEL from Candidatus N. mikurensis, and msp2 from A. phagocytophilum (Table 1). We detected PCR amplicons of the expected sizes for groEL and panbacteria and family-specific 16S rRNA in bone marrow and acute phase serum samples; nucleotide sequences corresponded to Candidatus N. mikurensis. The groEL amplicon (1,232 bp) showed the highest (99.3%) sequence similarity with that of Candidatus N. mikurensis from a wild rodent (Microtus agrestis) from Siberia in Russia (GenBank accession no. MN701626) but differed from other highly conserved sequences from Siberia and the Far East; the sequence was 98.8% identical to Candidatus N. mikurensis found in Ixodes ricinus ticks from Spain (13) (Table 2). We constructed a phylogenetic tree for groEL sequences by using the maximum likelihood method (Figure). We found no differences for the 16S rRNA-EHR sequence (306 bp). The panbacteria 16S rRNA sequence (available upon request from the authors) showed 3–27 mismatches with the 16S rRNA from Candidatus N. mikurensis. We did not detect A. phagocytophilum by PCR in the acute samples. We deposited nucleotide sequences of groEL and 16S rRNA genes generated in this study in GenBank under accession nos. OQ579033 (groEL) and OQ581737 (16S rRNA).

On the basis of PCR results, the patient was treated with doxycycline (100 mg 2×/d for 3 wk), and fever disappeared after 72 hours. Neutropenia was attributed to the intake of metamizole for symptom control. However, another case of doxycycline-treated Candidatus N. mikurensis infection associated with neutropenia has been reported (8). EDTA-anticoagulated blood and serum specimens were collected 4
(December 2021) and 6 (February 2022) months after onset of the acute infection phase, and we screened for Candidatus N. mikurensis at the Center for Rickettsioses and Arthropod-Borne Diseases, as previously described. We detected Candidatus N. mikurensis DNA in blood collected at 4 months but not in serum. The patient was healthy and blood test results did not show abnormalities at that time. Follow-up PCR of specimens collected at 6 months yielded negative results (Table 2). We did not detect IgG against A. phagocytophilum.

**Conclusions**

We report a confirmed case of Candidatus N. mikurensis infection in Spain, detected in human bone marrow aspirate, serum, and EDTA-blood samples, that was no longer detected months after completing antimicrobial drug treatment. A broad clinical spectrum of tickborne
diseases is found in Spain. Human cases of Lyme borreliosis, Mediterranean spotted fever, and other tickborne rickettsioses have been described, including *Dermacentor* tick-borne necrosis erythema lymphadenopathy, *Rickettsia sibirica mongolitimonae* infection, *R. massiliae* infection, *R. aeschlimannii* infection, babesiosis, human anaplasmosis, tularemia, *Borrelia hispanica* relapsing fever, tick paralysis, Crimean-Congo hemorrhagic fever, and α-gal syndrome or other allergic reactions (14). Since we discovered *Candidatus N. mikurensis* in *I. ricinus* ticks in Spain (13), we have conducted surveillance of this bacterium. *Candidatus N. mikurensis* should be considered a potential cause of persistent fever and venous thrombosis in patients with hematologic malignancies who live in environments where ticks are prevalent. *Candidatus N. mikurensis* infections should be particularly suspected in patients who are immunosuppressed but also should be considered in patients with other vascular conditions who are not immunocompromised (15).

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We identified 2 fatal cases of persons infected with hantavirus in Arizona, USA, 2020; 1 person was co-infected with SARS-CoV-2. Delayed identification of the cause of death led to a public health investigation that lasted ≈9 months after their deaths, which complicated the identification of a vector or exposure.

The COVID-19 pandemic has affected public health investigation and response activities for other illnesses; COVID-19 has particularly challenged the diagnosis of respiratory illnesses because of similar clinical manifestations. Hantavirus pulmonary syndrome is a rare disease transmitted predominantly by infected rodents shedding the virus through saliva, urine, and feces. Sin Nombre virus is the strain of hantavirus identified in 1993 in deer mice (*Peromyscus maniculatus*) in the Four Corners region of the southwestern United States; in total, 81 human cases of hantavirus have been documented throughout Arizona through 2019 (1–3).

In March 2020, deaths of a mother and son living both on and around the White Mountain Apache Reservation in Arizona, USA, just outside of the Four Corners region, were reported to the Arizona Department of Health Services (ADHS). On September 15, 2020, the Centers for Disease Control and Prevention (CDC) notified ADHS that the mother tested positive for hantavirus, and the son was confirmed to be co-infected with both hantavirus and SARS-CoV-2.

The Study

Patient 1 (P1) was a 25-year-old Native American woman with an unremarkable medical history who lived at her primary residence (residence A), a four-plex apartment on the White Mountain Apache Reservation, until her death in March 2020. She often visited her extended family at residence B, a single-family home 120 miles away, in eastern Arizona. P1 reported progressive shortness of breath beginning on March 12 (Figure 1). She stayed at a casino during March 13–17 and cleaned her apartment during March 17–18.

On March 18, P1 was transported to the hospital by emergency medical services (EMS) reporting shortness of breath, abdominal pain, and hemoptysis. In the emergency department (ED), she was febrile (temperature 101°F), tachypneic, and hypoxic; she was later intubated. Asphyxiation was initially suspected because of mixing cleaning chemicals. A chest radiograph showed diffuse bilateral infiltrates and an acute respiratory distress syndrome pattern. The ED physician documented that the radiograph looked suspicious for hantavirus, COVID-19, or diffuse bacterial pneumonia. ED physicians also diagnosed multiorgan system failure, metabolic acidosis, and metabolic encephalopathy. P1 was transferred to another hospital for a higher level of care; she was placed on extracorporeal membrane oxygenation. She died on...
March 19. Antemortem nasopharyngeal swab real-time reverse transcription PCR (rRT-PCR) testing for SARS-CoV-2 and respiratory viral panel testing for influenza A/B were both negative (4).

Patient 2 (P2) was the 11-year-old Native American male child of P1 and had an unremarkable medical history. He split time between residence A (second half of February 2020) and residence B (March 2020); he visited residence A at least 1 time in March.

On the morning of March 20, P2 was reportedly feeling unwell for 2 days and was warm but afebrile, for which he was given aspirin (dose unknown). He vomited later that day and had difficulty sleeping that evening, for which he was given 2 diphenhydramine/acetaminophen tablets (dose unknown). He awoke during the night because of difficulty breathing and collapsed out of his bed. He became unresponsive. EMS subsequently transported him to the ED, where he was pronounced dead on March 21.

The county medical examiner performed an autopsy on P2 on March 23. Postmortem nasopharyngeal swab testing for SARS-CoV-2 was rRT-PCR negative. During the autopsy, the examiner suspected an underlying pulmonary process contributing to his cause of death. Multiple tissues were sent to CDC for analysis; samples were received on May 15. On July 1,
samples of P2’s lung and trachea tissues tested positive for SARS-CoV-2 by rRT-PCR (5), despite negative immunohistochemical results.

Because of the epidemiologic link between the 2 cases and unknown etiology of death for P1, tissues from P1 were submitted to CDC and received on July 28. Pathologic findings for both cases were similar; however, P1 did not have evidence of SARS-CoV-2. The pathologist observed findings that resembled hantavirus infection, which were later confirmed by positive IHC assay on liver and kidney tissues on September 15 (5). Because of resemblance between tissues of the 2 case-patients, P2’s tissues were reexamined, and hantavirus IHC results were positive on lung and kidney tissues. The county medical examiner later determined hantavirus to be the major

Figure 2. Phylogenetic tree for Orthohantavirus short (S) segment of samples from 2 patients who died of hantavirus infection, Arizona, USA. We inferred the phylogenetic history of full-length Sin Nombre virus S segment using maximum-likelihood estimation. Non–Sin Nombre virus species, Black Creek Canal virus, and Bayou virus are included as outgroups. Bold indicates isolates from this study; squares indicate those from patient 1 and circles those from patient 2. Numbers at nodes indicate bootstrap support >70% after 1,000 iterations. Phylogenetic trees were made using a nucleotide alignment of Orthohantavirus S segments. GenBank accession numbers are provided. Scale bar indicates nucleotide substitutions per site. Additional phylogenetic trees for Orthohantavirus medium and large segments of Sin Nombre virus are in the Appendix (https://wwwnc.cdc.gov/EID/article/29/8/22-1808-App1.pdf).
contributing factor to P1 and P2’s deaths. Hantavirus genomes from P1 and P2 were closely related, indicating a common source of exposure (Figure 2). We submitted hantavirus sequences from the cases to GenBank (accession nos. ON571574–93).

On September 15, CDC alerted ADHS of the 2 positive hantavirus results. The next day, a call was held with federal, state, and local partners to coordinate a collaborative case investigation. On October 5, the Indian Health Service (IHS) conducted an environmental investigation of residence A and collected 16 human blood samples from 17 residents living in the fourplex, including household members of P1 and P2. Hantavirus serology assays for 16 human samples all tested negative for hantavirus IgM and IgG (6). IHS provided hantavirus prevention public service announcements to local health officials; the announcements were later disseminated to the community through newspaper and radio.

We conducted an environmental investigation because both residence A and residence B displayed potential for deer mice habitat. Trapping conducted in residence A confirmed the presence of rodents by identifying house mice (Mus musculus) in 4 of 6 snap traps; we did not test the mice because that species is not a known reservoir for hantavirus. Unfortunately, we were not able to conduct trapping at residence B. All partners involved mutually decided to end the investigation.

The time interval from symptom onset to diagnosis was ≈6 months. Despite the local ED physician suspecting hantavirus in P1, medical records showed no evidence of hantavirus testing ordered at either hospital. Local health and medical staff were focused on the response to initial cases of SARS-CoV-2 in the region. The time between postmortem tissue submission and subsequent sample testing contributed to the delay. Testing delays might have resulted from CDC requirement of confirmatory diagnostic testing on all confirmed or suspected COVID-19 deaths (5). Hantavirus was identified 6 weeks after P2’s tissue examination; P1’s lung tissue resembled that of P2 but tested negative for SARS-CoV-2. Delays in case identification caused the environmental investigation to be conducted 7 months after disease onset. The rodent population might have changed during that period, preventing identification of the vector and exposure.

Conclusions
In the cases we report, hantavirus infection was not promptly detected when patients sought medical care or during postmortem examination. To aid in the diagnosis of hantavirus, we recommend adoption of the 5-point hantavirus screening tool for areas outside the Four Corners region (7,8). The COVID-19 pandemic likely played a role in delayed detection of hantavirus for these cases because of its effects on aspects of healthcare and public health (9–12). Hantavirus education should continue to be a priority in healthcare facilities in disease-endemic regions, including on tribal lands. Community education can help to minimize the impact of hantavirus cases by offering tools to prevent exposure and encourage seeking prompt medical care.

During pandemic response, public health partners should continue to monitor and respond to other pathogens. Medical providers should consider both alternative and concurrent diagnoses in the presence of COVID-19–like illness, including rare pathogens such as hantavirus. Timely investigations of high-consequence illnesses will enable public health organizations to take prompt action.

Acknowledgments
We acknowledge the 2 patients, their families, and the White Mountain Apache Tribe. We also thank the many tribal health partners whose efforts were essential to this investigation. Last, we acknowledge the late Dr. Sherif Zaki and his team at the CDC Infectious Diseases Pathology Branch (National Center for Emerging and Zoonotic Infectious Diseases, Division of High-Consequence Pathogens and Pathology) for their collaborative efforts in this investigation.

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References


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etymologia revisited

Streptomycin

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

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

References:

Multidrug-Resistant *Shigella sonnei* Bacteremia among Persons Experiencing Homelessness, Vancouver, British Columbia, Canada


Increased invasive bloodstream infections caused by multidrug resistant *Shigella sonnei* were noted in Vancouver, British Columbia, Canada, during 2021–2023. Whole-genome sequencing revealed clonal transmission of genotype 3.6.1.1.2 (CipR.MSM5) among persons experiencing homelessness. Improvements in identifying *Shigella* species, expanding treatment options for multidrug resistant infections, and developing public health partnerships are needed.

*Shigellosis* manifestations range from mild gastrointestinal infection to severe illness with dysentery and sepsis (1). In high-income countries, *Shigella sonnei* is the most common species, causing infections typically among men who have sex with men (MSM) and travelers (1,2). Transmission occurs through sexual contact in MSM or the fecal–oral route from contaminated water, food, or fomites (3). Although clinical manifestations range broadly, *S. sonnei* rarely causes invasive bloodstream infections. Only a few published case reports describe bacteremia (4–6), mostly among malnourished children, MSM, or adults with HIV, diabetes, cirrhosis, or immunosuppression (4,6,7). We describe the epidemiology, genotyping, and resistance determinants of clonal multidrug-resistant (MDR) *S. sonnei* bacteremia in Vancouver, British Columbia, Canada, and discuss challenges in diagnosing *Shigella* bacteremia in the microbiology laboratory. The University of British Columbia/Providence Health Care Research Ethics Board approved our study (H22-02183).

The Study

The microbiology laboratory at St. Paul’s Hospital (Vancouver, BC, Canada) serves acute-care hospitals and the surrounding community in downtown Vancouver. We searched the laboratory database for *S. sonnei* found in feces and blood samples during January 2010–January 2023, separated into 2010–2020 (historical) and 2021–2023 (recent) periods. We reviewed medical records of patients with bacteremia and recorded demographics, symptoms, housing, sexual orientation, travel, substance use, coexisting conditions, hospitalization, antimicrobial susceptibility testing (AST), treatment, and mortality.

We processed positive blood cultures detected by BacT/Alert system (bioMérieux, https://www.biomerieux.com) using VitekMS+ (bioMérieux) or FilmArray BCID (BioFire Diagnostics; https://www.biofiredx.com) with established microbiology protocols and identified pathogens. We identified *Shigella* in feces using benchtop biochemical methods, Vitek2 ID (bioMérieux) and Polyvalent Agglutination Sera (Remel, http://www.remel.com). If we suspected *Shigella* in blood samples, we used Vitek2 ID and polyvalent serology. No changes in laboratory testing protocols occurred during 2010–2023. We performed AST for ampicillin, trimethoprim/sulfamethoxazole, ciprofloxacin, ceftriaxone, and azithromycin according to Clinical and Laboratory Standards Institute M100 standards (https://clsi.org/
We performed whole-genome sequencing (WGS) on isolates incubated in Mueller-Hinton broth, extracted on MagNA Pure 24 (Roche; https://diagnostics.roche.com), and processed on GridION R10.4 flowcells (Nanopore, https://nanoporetech.com). We basecalled runs with Guppy version 6.3.9 (https://github.com/nanoporetech/rerio) and uploaded to BugSeq (https://bugseq.com) for automated analysis.

We identified 11 cases of *S. sonnei* bacteremia that occurred within the historical (n = 2) or recent (n = 9) periods during 2010–2023. We also observed a recent increase in fecal isolates with *S. sonnei* (Figure). Differences in the proportion of bacteremic among all shigellosis cases occurring during the recent compared with the historical period were not statistically significant (7.7% vs. 2.9%; p = 0.21 by Fisher exact test). Among recent cases, 89% (8/9) of patients were male (median 45 years of age, interquartile range 35–54 years) (Table). All but 1 were underhoused or experiencing homelessness and had polysubstance use disorder. Most (89%) inhabited Vancouver’s downtown eastside, the neighborhood with the highest density of Vancouver’s urban poor population. Unlike the historical case-patients, none were MSM or had travel histories. Most (89%) were not severely immunocompromised. Case-patient A had multiple myeloma but stable housing. Five (55%) patients presented with sepsis and 6 (67%) were hospitalized; the remaining patients declined recommended hospital admission.

Among the 2021–2023 cases, AST profiles were identical except in case A. The isolates were resistant to ampicillin, trimethoprim/sulfamethoxazole, ciprofloxacin, and azithromycin and susceptible to ceftriaxone. An isolate from case-patient A displayed ceftriaxone resistance and azithromycin susceptibility. *Shigella* was initially misidentified as *E. coli* in all 9 cases (8 by VitekMS+, 1 by FilmArray BCID). All isolates were indole negative, non–lactose fermenting (NLF) colonies, subsequently identified correctly as *S. sonnei* by Vitek2 ID and confirmed by polyvalent serology.

We performed WGS on all 2021–2023 *S. sonnei* isolates from blood samples. Using a new genotypic framework (8), we identified 8 of the 2021–2023 isolates as 3.6.1.1.2 (CipR.MSM5); the isolate from case-patient A genotyped as 3.6.3 (Central Asia III). The strain from the 2016 case genotyped as 3.7.18 (Global III); the 2013 strain did not undergo WGS. We identified mutations *gyrA* S83L, *gyrA* D87G, and *parC* S80I encoding ciprofloxacin resistance and plasmid AA336-borne *mphA* and *ermB* encoding azithromycin resistance in all isolates from persons experiencing homelessness (PEH). The 3.6.3 strain carried *gyrS1*, *gyrA* D87Y, *gyrA* S83L and *parC* S80L, which confers ciprofloxacin resistance, and *blaCTX-M-15* which confers ceftriaxone resistance.

**Conclusions**

The recent increase in *S. sonnei* bacteremia might reflect the overall increase in shigellosis, including *S. sonnei* isolated from feces. The increased proportion of bacteremia cases in the past 2 years compared with the 11-year historical period was not statistically significant. However, the 7.7% prevalence of bacteremia is still very high compared with a range of rates, 0.4%–7.3%, reported in the literature (9). Historically, shigellosis has occurred in British Columbia predominantly as a sexually transmitted enteric infection among MSM (10). In our study, invasive shigellosis among PEH was probably transmitted.
fecal-orally through contaminated environment and hands. An outbreak among PEH in Oregon was similarly believed to have resulted from inadequate access to hygiene and sanitation (11). WHO warns of outbreak risk from *S. sonnei* being introduced into areas with suboptimal water, sanitation, and hygiene standards (12). Because patient immunosuppression fails to explain increased bacteremia, other factors, such as drug use, malnutrition, and high inoculum dose, should be considered.

A study in Seattle, Washington, USA described a contemporary increase in MDR *S. sonnei* cases among PEH (13). Although the report did not comment on bacteremia, it highlighted the circulation of extensively-drug resistant *S. sonnei* carrying the extended-spectrum β-lactamase CTX-M-27. Although extensively-drug resistant *S. sonnei* was rare in our review, increased hospitalization and the need for parenteral therapy because of MDR *S. sonnei* bacteremia still substantially affected the healthcare system. One third of our patients did not complete treatment, potentially leading to ongoing transmission and illness. In addition to finding and treating cases, essential community control measures include working with housing providers to promote handwashing and sanitation practices, providing advice on recognizing and controlling infectious diarrhea, and developing pathways to reengage those who refuse hospitalization to complete parenteral antimicrobial treatment as outpatients.

Isolates from 8/9 PEH were genotype 3.6.1.1.2 (CipR.MSM5/BAPS3), epidemiologically distinct from the single isolate typed as 3.6.3 (Central Asia III). The isolate from the 2016 case had a different genotype, 3.7.18, matching that from an outbreak reported in California, USA (14). Despite its geographic proximity to Vancouver, the recent *S. sonnei* outbreak among PEH in Seattle involved yet another different genotype, 3.7.29.1.4.1 (global III VN2.KH1.Aus) (14).

The 3.6.1.1.2 strain predominant in our study has previously been described in Australia, England, and the United States (8).

Our study included analysis of data only from patients seeking treatment at a hospital where blood

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Table. Characteristics and outcomes of cases of *Shigella sonnei* bacteremia during recent and historical time periods, Vancouver, British Columbia, Canada*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases of <em>Shigella sonnei</em> bacteremia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recent, 2021–Jan 2023, n = 9</td>
</tr>
<tr>
<td></td>
<td>Historical, 2010–2020, n = 2</td>
</tr>
<tr>
<td>Age, y</td>
<td>Median 45, range 27–69</td>
</tr>
<tr>
<td>Sex</td>
<td>Median 62, range 56–68</td>
</tr>
<tr>
<td></td>
<td><strong>8 (99%) male</strong></td>
</tr>
<tr>
<td></td>
<td><strong>2 (100%) male</strong></td>
</tr>
<tr>
<td>Housing</td>
<td></td>
</tr>
<tr>
<td>Private residence</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Single room occupancy hotel/shelter</td>
<td>5 (56)</td>
</tr>
<tr>
<td>No fixed address</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Rehabilitation center</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Men who have sex with men</td>
<td>0</td>
</tr>
<tr>
<td>Travel history</td>
<td>0</td>
</tr>
<tr>
<td>Substance use</td>
<td>8 (89)</td>
</tr>
<tr>
<td>Coexisting conditions</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Immunocompromising conditions†</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Liver disease</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Chronic renal disease</td>
<td>0</td>
</tr>
<tr>
<td>Recurrence</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Hospitalization</td>
<td></td>
</tr>
<tr>
<td>Admitted to hospital ward</td>
<td>6 (67)</td>
</tr>
<tr>
<td>Declined hospital admission</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Antimicrobial test results</td>
<td></td>
</tr>
<tr>
<td>Ampicillin resistant</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole resistant</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Ciprofloxacin resistant</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Azithromycin resistant</td>
<td>8 (89)</td>
</tr>
<tr>
<td>Ceftriaxone resistant</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>7–14 d of effective oral antimicrobial agent</td>
<td>0</td>
</tr>
<tr>
<td>7–14 d of effective IV antimicrobial agent</td>
<td>6 (67)</td>
</tr>
<tr>
<td>Incomplete</td>
<td>3 (33)</td>
</tr>
<tr>
<td>30-d all-cause mortality</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Values are no. (%) except as indicated. IV, intravenous.</strong></td>
<td></td>
</tr>
</tbody>
</table>
| †Organ transplantation, malignancy receiving chemotherapy, asplenia, uncontrolled HIV (CD4 count <200 cells/μL), and immunosuppressive medications.
cultures were collected, possibly representing the sickest cohort of patients, leading to underestimation of actual infections. Not all cases with S. sonnei isolated from feces have yet been analyzed to determine wider epidemiologic and antimicrobial resistance trends. Further studies are needed to elucidate whether the high rate of bacteremia reflects increased virulence of this strain, higher inoculum size, or host determinants.

Because current laboratory methods can misidentify Shigella as E. coli in bloodstream infections, laboratories must scrutinize diagnoses of E. coli bacteremia in high-risk patients with infectious diarrhea and sepsis. Clinicians and public health officials should be made aware of MDR S. sonnei bacteremia as a cause of increased illness among PEH and the need for parenteral therapy in the event of resistance to first- and second-line antimicrobial agents.

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References

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We estimated SARS-CoV-2 seroprevalence in children in Oregon, USA, at 6 time points. Seroprevalence increased linearly during November 2020–December 2021 and peaked in February 2022 at 38.8% (95% CI 32.8%-46.5%). We observed no increase in the seroprevalence trend after widespread school reopening. Seroprevalence estimates complement case-based cumulative incidence.

Through June 30, 2022, a total of 140,820 pediatric cases of COVID-19 had been reported in Oregon, USA, representing ≈17.3% of all reported COVID-19 cases in the state. However, understanding the true burden of pediatric COVID-19 infection poses a challenge. Children are more likely to have asymptomatic or mild disease, and pediatric infections are, therefore, less likely to be reported to public health authorities (1,2). Clarifying pediatric SARS-CoV-2 prevalence is important because it is well established that children can transmit SARS-CoV-2 to other children and adults (3,4). In addition, children are at risk for severe complications, including postinfectious multisystem inflammatory syndrome in children (MIS-C) (5). Seroprevalence provides additional insight into the true cumulative incidence of COVID-19 in children.

The Study
To estimate the seroprevalence of COVID-19 infection in children in Oregon, blood was collected in 6 phases during November 1, 2020–June 30, 2022, from a cross-sectional convenience sample and tested for SARS-CoV-2 nucleocapsid IgG, in alignment with the World Health Organization seroepidemiologic investigation protocol (6). We recruited Oregon healthcare facilities with ≥6 inpatient pediatric hospital beds to participate in this study and asked them to provide ≤100 specimens per phase; 5 facilities agreed to participate. We asked facilities to submit random samples of deidentified residual serum samples from patients ≤17 years of age visiting any ambulatory, emergency, or inpatient healthcare setting and to include specimen collection date and patient’s date of birth. The initial round of sampling was November 1–December 31, 2020. We extended the project timeline and collected additional samples bimonthly during October 1, 2021–June 30, 2022.

Specimens were stored according to instructions provided by the test manufacturer and transported to the Oregon State Public Health Laboratory (Hillsboro, Oregon, USA). We tested the specimens with a SARS-CoV-2 IgG assay (Abbott Laboratories, https://www.abbott.com), which detects antibodies to the nucleocapsid protein of SARS-CoV-2. Nucleocapsid IgG immunoassays detect antibodies produced after infection and do not detect antibodies produced after vaccination with vaccines licensed for use in the United States. The manufacturer reports test sensitivity (Sn) of 100% (95% CI 95.9%-100%) at ≥14 days past symptom onset and specificity (Sp) of 99.6% (95% CI 99.1%-99.9%). We calculated unadjusted seroprevalence estimates for each collection period as the percentage of all specimens that tested positive. We adjusted seroprevalence estimates for test performance as observed prevalence + (specificity – 1) divided by sensitivity + (specificity – 1) (7).

We obtained adjusted 95% CI with parametric bootstrapping (8). Because SARS-CoV-2 antibody detection is dependent on test timing and assay, we also performed sensitivity analysis with seroprevalence estimates adjusted for declining assay sensitivity over 130 days of convalescence (9,10) (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/23-0471-App1.pdf) We used R version 4.1.2 (The R Foundation for
Pediatric SARS-CoV-2 Seroprevalence, Oregon

We collected 1,869 specimens from 5 facilities during 6 phases. The mean number of specimens collected during each phase was 312 (range 215–438). Overall, we observed a strong linear trend (p = 0.001) for adjusted seroprevalence estimates during November 1, 2020—December 31, 2021; seroprevalence increased by ≈0.7% per 4-week period during that period (Figure). After the Omicron surge, adjusted seroprevalence increased sharply from 13.4% (95% CI 9.8%–18.4%) in December 2021 to 38.8% (95% CI 32.8%–46.5%) in February 2022. Adjusted seroprevalence estimates then decreased but did not return to pre–February 2022 levels (Table). Adjusting for declining assay sensitivity over time led to estimates that were larger and less stable over convalescence (Appendix).

Conclusions

Our repeated cross-sectional study estimated pediatric seroprevalence in Oregon at 6 points across 20 months of pandemic response. During that period, K–12 public schools reopened statewide, vaccines were rolled out in 2 phases to children 12–15 and 5–11 years of age, and universal indoor masking mandates remained in place during the school year until March 12, 2022 (Figure). After widespread school reopening in early 2021, with a masking mandate in place, pediatric seroprevalence in October 2021 was 11.8%, and no increase in trend was observed. The only sudden increase in pediatric seroprevalence followed the Omicron surge in early 2022. Seroprevalence began to decline after its February 2022 peak but did not return to pre-Omicron levels.

Seroprevalence can provide more accurate estimates of the true cumulative incidence of SARS-CoV-2 infection than case reporting to public health entities does, because seroprevalence data capture evidence of previous infection in persons who are not tested through the traditional healthcare system because of asymptomatic or mild disease, lack of testing access, refusal to test, or self-testing at home (11). We estimated 1.7–2.8 times the number of infections in children from seroprevalence than the reported cumulative incidence in Oregon (Figure). This total is a lower degree of underascertainment than had been reported by the Oregon Health Authority since the beginning of the pandemic.

Table. Seroprevalence of SARS-CoV-2 nucleocapsid antibodies in children, Oregon, USA, November 1, 2020–June 30, 2022*

<table>
<thead>
<tr>
<th>Collection dates</th>
<th>Sample size</th>
<th>Unadjusted seroprevalence (95% CI)</th>
<th>Adjusted seroprevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020 Nov 1—Dec 31</td>
<td>438</td>
<td>0.032 (0.015–0.048)</td>
<td>0.033 (0.002–0.052)</td>
</tr>
<tr>
<td>2021 Oct 1—Oct 3</td>
<td>370</td>
<td>0.122 (0.090–0.159)</td>
<td>0.118 (0.090–0.161)</td>
</tr>
<tr>
<td>2021 Dec 1—Dec 31</td>
<td>278</td>
<td>0.137 (0.099–0.183)</td>
<td>0.134 (0.098–0.184)</td>
</tr>
<tr>
<td>2022 Feb 1—Feb 28</td>
<td>215</td>
<td>0.391 (0.325–0.459)</td>
<td>0.388 (0.328–0.465)</td>
</tr>
<tr>
<td>2022 Apr 1—Apr 30</td>
<td>279</td>
<td>0.272 (0.221–0.329)</td>
<td>0.269 (0.222–0.333)</td>
</tr>
<tr>
<td>2022 Jun 1—Jun 30</td>
<td>289</td>
<td>0.301 (0.249–0.358)</td>
<td>0.298 (0.250–0.362)</td>
</tr>
</tbody>
</table>

*We calculated adjusted prevalence as observed prevalence + (specificity – 1) divided by sensitivity + (specificity – 1). Adjusted 95% CI obtained with parametric bootstrapping.
reported in seroprevalence studies of children during May–July 2021 in the United States (1).

Because seroprevalence studies measure circulating antibodies at the time of testing and SARS-CoV-2 antibodies wane over time, seroprevalence is limited in its ability to estimate cumulative incidence as the pandemic progresses (12). In addition, time to seroreversion is dependent on the target antigen and the assay used (9,10,13). One study found a mean time of seroreversion of 19 weeks with use of the Abbott IgG immunoassay, compared with 91 weeks using the Roche pan-Ig immunoassay (13). In our study, waning immunity was apparent as estimated seroprevalence decreased following its Omicron-related peak in February 2022. A sensitivity analysis, adjusting for declining sensitivity of the Abbott immunoassay, partially accounted for this decline; more complex models have been published to correct seroprevalence estimates for assay performance (13). However, now that essentially all persons in the United States have been infected with SARS-CoV-2, the use of an assay with waning sensitivity to remote infections may permit continued examination of more granular temporal changes in seroprevalence in the context of changing policy and variant predominance.

The Centers for Disease Control and Prevention began collecting pediatric seroprevalence data for the Multistate Assessment of SARS-CoV-2 Seroprevalence in Commercial Labs (MASS-C) in July 2020 (14). MASS-C estimates of pediatric seroprevalence in Oregon are consistently higher than our estimates (15). Although MASS-C similarly derives its estimates from a convenience sample of serum specimens, testing is performed with the Roche antinucleocapsid total antibody assay (14). In addition, MASS-C estimates are weighted for age and sex, and the demographics of that pediatric population may demographically differ from our study population.

We obtained our convenience sample from children who received care from large pediatric healthcare facilities throughout the state; findings are not necessarily generalizable to the entire state pediatric population. Limitations of seroprevalence testing include lack of antibody development by some infected persons (including immunocompromised persons) and, in others, waning of antibodies to undetectable levels, such that seroprevalence becomes a less reliable proxy for cumulative incidence as the duration of the pandemic increases (12).

Traditional public health case-based reporting substantially underestimates the burden of COVID-19. In this study, seroprevalence estimates made using an assay with waning sensitivity to remote infections provided evidence that the widespread reopening of schools with a masking mandate in place did not increase the rate of pediatric SARS-CoV-2 infections. Case-based cumulative incidence estimates failed to capture the magnitude of the Omicron variant’s effect on Oregon’s pediatric population. Serosurveillance of SARS-CoV-2 antibodies in Oregon’s pediatric population complements case-based surveillance and can inform future public health interventions and policy decisions.

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Detection of Orientia spp.
Bacteria in Field-Collected Free-Living Eutrombicula Chigger Mites, United States

Kaiying Chen, Nicholas V. Travanty, Reuben Garshong, Dac Crossley, Gideon Wasserberg, Charles S. Apperson, R. Michael Roe, Loganathan Ponnusamy

Scrub typhus, a rickettsial disease caused by Orientia spp., is transmitted by infected larval trombiculid mites (chiggers). We report the molecular detection of Orientia species in free-living Eutrombicula chiggers collected in an area in North Carolina, USA, to which spotted fever group rickettsiae infections are endemic.

Rickettsioses are distributed worldwide, caused by bacteria in the family Rickettsiaceae, genera Orientia and Rickettsia (1). The pathogens are transmitted by host-feeding arthropods, including ticks, mites, fleas, and lice (2). Among those arthropods are trombiculid mites, which have a widespread global distribution and high species diversity. Among the different stages of the lifecycle of trombiculid mites, only the larvae are ectoparasites, commonly known as chiggers (3). Some species are vectors of intracellular bacterial pathogens in the genus Orientia that causes a potentially lethal human febrile disease, scrub typhus (4,5). Scrub typhus results in considerable illness and death; it causes >1 million cases of illness each year (5). A recent review (6) concluded that trombiculid mites might be widespread vectors of other zoonotic agents not yet recognized. Spotted fever group rickettsioses diseases, including Rocky Mountain spotted fever, have many of the same symptoms as scrub typhus. A recent review of the literature from 1997–2017 estimated >60% of the rickettsial diseases outside the United States were misdiagnosed (7).

Until recently, scrub typhus was exclusively reported from the so-called tsutsugamushi triangle, stretching from Pakistan in the west to far-eastern Russia in the east to northern Australia in the south. However, scrub typhus was reported recently in the Middle East, southern Chile, and Africa (5,8). The occurrence of scrub typhus pathogens in chiggers in the United States was not investigated. We report the identification of Orientia species in free-living chiggers collected at recreational parks in North Carolina, USA.

The Study
In 2022, we collected free-living chiggers using the tile method (9) in different locations in North Carolina: we placed tiles on the ground and then visually inspected for the presence of chiggers after ≈1 minute. When chiggers were present, we collected them with forceps or a small paintbrush and transferred them into vials with 95% ethanol. We identified subsamples from each collection location based on morphological characteristics using published taxonomic keys (10). We identified chiggers as Eutrombicula on the basis of their morphology. In addition, we obtained chigger images of Eutrombicula using scanning electron microscopy at the Analytical Instrumentation Facility at North Carolina State University (Raleigh, North Carolina, USA) (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0528-App1.pdf).

We surface-sterilized individual free-living chiggers and extracted total nucleic acids using the DNeasy Blood & Tissue Kit (QIAGEN, https://www.qiagen.com) (11). In total, 95 chiggers from 10 different locations (10 chiggers/location; 1 location had 5 chiggers) were subjected to microbiome analyses (Figure 1). We randomly selected 8 chiggers for molecular identification using previously described 18S ribosomal RNA gene primers and PCR (12). Amplicons were Sanger
Orientia spp. in Chigger Mites, United States

The sequences (GenBank accession nos. OQ789321–5) were submitted for BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis and showed 99–100% identity with homologous sequences of Eutrombicula spp. (accession no. KY922159).

To determine the total bacteria present in the chiggers, we constructed a 16S rRNA sequence library of individual chiggers using the Illumina 16S rRNA (V3-V4 region) metagenomics sequencing library preparation protocol (Illumina, https://www.illumina.com) (13). Sequencing was performed at the University of North Carolina Microbiome Core Facility (Chapel Hill, North Carolina, USA). The Illumina FASTQ files were processed using Quantitative Insights into Microbial Ecology 2 (Appendix). An analysis of amplicon sequence variants (ASVs) revealed that chigger mites contained reads of a bacterial sequence classified as Orientia tsutsugamushi. Orientia tsutsugamushi-positive chigger sequence reads were found in 5/10 sampling sites as follows: 3 sites in the Piedmont region (Falls Lake State Recreation Area [1 positive/10 chiggers], Kerr Lake State Recreation Area [8 positive/10 chiggers], and Morrow Mountain State Park [1 positive/10 chiggers]); and 2 sites in the Coastal Plains region (Lumber River State Park [9 positive/10 chiggers] and Merchant Millpond State Park [1 positive/5 chiggers]) (Figure 1). By using the Greengenes 16S rRNA database, we found 13 ASVs to O. tsutsugamushi. To further confirm those results, we extracted representative sequences from the sequencing data and conducted BLASTn searches against the National Center for Biotechnology Information (NCBI) databases. We found 12 ASVs showed a high nucleotide identity (99.5%–100%) to the O. tsutsugamushi strain Kato (D38624), isolated from a human scrub typhus case in Kurosawa village, Japan (https://u.osu.edu/scrubtyphus/the-kato-strain). One ASV exhibited 89.46% homology to an O. tsutsugamushi sequence that was excluded from the phylogenetic analysis. We then examined the approximate phylogenetic relationships for 12 of the O. tsutsugamushi sequence variants from our study sites with other O. tsutsugamushi sequences obtained from the NCBI database using BLASTn (accessed January 18, 2023). We performed multiple alignments with the ClustalW program and maximum-likelihood method tree with the Kimura 2-parameter method from the MEGA X (https://www.megasoftware.net) software package. The phylogenetic analysis revealed that all 12 of the O. tsutsugamushi ASVs clustered closely to O. tsutsugamushi strains from Asia (Figure 2).
To further verify the identity of *O. tsutsugamushi* detected in our free-living chigger samples, we amplified a 47-kDa *htrA* (high-temperature requirement A) gene in the 20 chigger samples that were positive for the *O. tsutsugamushi* 16S rRNA gene. The primers for the first round of PCR were Ot-145F and Ot-1780R, and for the second round, Ot-263F and Ot-1133R (15) (Appendix). The 47-kDa gene amplification products were Sanger sequenced at Eton Bioscience. Four samples (FC28, 36, 38, 109) were 92.6%–97.29% identical to the *O. tsutsugamushi* HN82 strain (GenBank accession no. LC431268) and 92%–97% identical to the *O. tsutsugamushi* Kato strain (accession no. LS398550) after trimming low-quality sequences. Sixteen samples yielded ambiguous sequences, suggesting the presence of multiple *Orientia* species or primer binding sites caused by high variation in the 47 kDa gene among *Orientia* species in our samples. Jiang et al. (15) studied the genetic variation of

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**Figure 2.** Phylogenetic tree of *Orientia tsutsugamushi* 16S rRNA gene sequences (≈399 bp) from free-living chiggers collected in North Carolina, USA, and their reference sequences in GenBank. The tree was constructed using the maximum-likelihood method. Bold text indicates study sequences. *Rickettsia parkeri* was used as an outgroup. We conducted bootstrap analyses with 1,000 iterations to evaluate the strength of the tree topologies. GenBank accession numbers are in parentheses. Scale bar represents 0.01 substitutions per nucleotide position.
Orientia in this region of the genome and reported percent identity of 17 isolates of Orientia as 82.2%–83.3% (15). Among our 4 Orientia 47 kDa sequences from North Carolina chiggers, identity was 93.97%–98%. Phylogenetic analysis revealed that all 4 of those O. tsutsugamushi sequences clustered to O. tsutsugamushi strains from Asia (Appendix Figure 2).

Conclusions

This study identified Orientia species within the United States in free-living Eutrombicula chiggers that were collected in North Carolina. This result is epidemiologically significant because it indicates vertical circulation of Orientia species in chiggers collected within the continental United States. The presence of Orientia species in free-living larvae suggests that the bacteria are maintained through transovarial transmission. Further studies are needed to complete sequencing of the 47-kDa htrA gene (htrA) in our samples, determine how widely distributed Orientia spp.-infected free-living and host-attached chiggers are in the United States, and ascertain whether wild animals that serve as hosts for chiggers become infected and infectious and develop symptoms of illness. Clinicians in this region should be alert for possible human cases of illness resulting from Orientia spp. infection.

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Aneurysm Infection Caused by *Desulfovibrio desulfuricans*

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*Desulfovibrio* species are gram-negative, sulfate-reducing, nonfermenting, anaerobic bacteria found in the environment, including in soil, water, and sewage, as well as in the digestive tracts of humans and animals (1). They rarely cause human infections, and their clinical prevalence and features are unclear (2,3). We describe a case of an infected aneurysm caused by *Desulfovibrio desulfuricans* MB that was treated successfully with artificial vessel replacement and appropriate antimicrobial therapy. Clinicians should suspect *Desulfovibrio* spp. infection in similar cases.

An 84-year-old man in Japan who had undergone endovascular aortic repair 9 years earlier had an infected aneurysm develop. We detected *Desulfovibrio desulfuricans* MB at the site. The patient recovered after surgical debridement, artificial vessel replacement, and appropriate antimicrobial therapy. Clinicians should suspect *Desulfovibrio* spp. infection in similar cases.

*Desulfovibrio* species are gram-negative, sulfate-reducing, nonfermenting, anaerobic bacteria found in the environment, including in soil, water, and sewage, as well as in the digestive tracts of humans and animals (1). They rarely cause human infections, and their clinical prevalence and features are unclear (2,3). We describe a case of an infected aneurysm caused by *Desulfovibrio desulfuricans* MB that was treated successfully with artificial vessel replacement and antimicrobial therapy after identification of the causative pathogen.

An 84-year-old man who had undergone endovascular aortic repair (EVAR) 9 years earlier was referred for suspected mycotic thoracoabdominal aortic aneurysm. His medical history included hypertension, type 2 diabetes mellitus, and chronic kidney disease. Six weeks before referral, he had eaten grilled fish; 2 weeks later, he had experienced fever with transient chills, followed by persistent abdominal pain for 2 weeks. Computed tomography revealed a fishbone lodged in the ileocecal tract, with a hyper-absorptive zone in the arterial wall; the post-EVAR abdominal aortic aneurysm was larger than it had been 4 months before. He had received antimicrobial therapy 5 days before referral. Blood cultures after treatment were negative.

At admission, the patient did not appear distressed. Blood pressure was 112/80 mm Hg, pulse rate 60 beats/min, body temperature 36.4°C, respiratory rate 18 breaths/min, and Glasgow coma scale score 15. On physical examination, chest and cardiovascular findings were unremarkable; abdominal tenderness was noted on palpation. Laboratory tests indicated that leukocyte count was 11.05 × 10^3 cells/L, C-reactive protein 12.12 mg/dL, serum creatinine 1.39 mg/dL, and hemoglobin A1c level 9.9.

Results of additional blood culture performed under treatment with ampicillin/sulbactam (3 g/6 h) was also negative.

On day 5 of admission, surgical debridement was performed, followed by partial removal of the EVAR graft and in situ Y-graft placement with revascularization, including the bilateral renal and superior mesenteric arteries. Intraoperative findings showed partial abscess formation in the abdominal artery wall. Gram stain of the abscess pus showed gram-negative rods (Figure, panel A), and subsequent anaerobic intraoperative pus cultures were observed (Figure, panel B). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry using MALDI Biotyper library version 9 (Bruker Daltonics, https://www.bruker.com) did not reliably identify the pathogens (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/23-0403-App1.pdf). We therefore conducted 16S ribosomal RNA

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**Figure.** Detection and colonization of *Desulfovibrio desulfuricans* in an 84-year-old man in Japan who had undergone endovascular aortic repair 9 years earlier. A) Gram stain of pus. *D. desulfuricans* MB has a gram-negative spiral rod appearance (arrow). Original magnification ×1,000.

B) Colonies of *D. desulfuricans* MB on ABHK agar. Biochemical properties showed positive results for catalase and negative for indole and urease. In vitro susceptibility testing revealed that it had the following MICs: meropenem, ≤2 µg/mL; cefotaxime, ≤2 µg/mL; ampicillin/sulbactam, ≤4 µg/mL; piperacillin/tazobactam, ≤16 µg/mL; and clindamycin, >8 µg/mL.
(16S rRNA) sequencing of the isolates by using the BLAST sequence homology search program (EzBiocloud, https://www.ezbiocloud.net) for analysis. Sequencing showed 99.86% homology with *D. desulfuricans* MB ATCC27774 (GenBank accession no. CP001358.1) and 1,470/1,472 bp nucleotide matches. The desulfoviridin test was positive, and the biochemical properties of the isolates were consistent with those of *D. desulfuricans* MB. On day 6, we performed thoracic endovascular aortic repair, coil embolization of the celiac and inferior mesenteric artery, and colonoscopic fishbone resection.

The postsurgical course and antibiotic treatment were uneventful. The patient received 4 weeks of ampicillin/sulbactam (3 g/6 h) treatment after surgical debridement. He was discharged with continued oral administration of amoxicillin/clavulanate. Because the infected EVAR graft was retained, ongoing antibiotic treatment was recommended.

*D. desulfuricans*, which has 2 genotypes (Essex and MB), is the most pathogenic among *Desulfovibrio* spp., and bacteremia occurs when there is bacterial translocation from the intestinal tract (3–5). *Desulfovibrio* spp. can also cause appendicitis, abdominal abscesses, and septic arthritis; however, an infected aneurysm is extremely rare, and only 2 cases, including 1 suspected case, have been previously reported (3,6). One of the reasons for the rarity is that *Desulfovibrio* spp. require a long incubation period (3–7 days) for detection in blood culture, and they cannot be identified by using biochemical reaction tests (3,7). Except in 2 instances, previous cases have also required identification using 16S rRNA. *Desulfovibrio* spp. were not included in many system databases until recently, possibly contributing to the inability to identify them using MALDI-TOF mass spectrometry (2). However, even though *Desulfovibrio* spp. are included in the MALDI-TOF mass spectrometry library we used, we were unable to identify the pathogen. Factors such as the absolute number of strains registered in the *Desulfovibrio* spp. library may make identification difficult, and the accuracy of identification may still be problematic. Difficulty in identification may therefore result in underdiagnosis.

In our patient, the fishbone perforated the ileocecal region, enabling hematogenous bacteremia to enter and cause the infection. Uncontrolled type 2 diabetes may also have played a role. No pathogens were detected in the blood cultures, possibly because of previous administration of antimicrobial therapy. However, after consultation with the infectious disease specialist and microbiologist, we performed 16S rRNA sequencing, which led to detection of *D. desulfuricans* MB and appropriate antimicrobial administration. When gram-negative bacilli are detected in anaerobic cultures of infected aneurysms, *Desulfovibrio* spp. infection should be suspected, especially when gastrointestinal disease is present.

In general, *Desulfovibrio* spp. are susceptible to chloramphenicol and metronidazole; most are susceptible to imipenem and clindamycin, but the optimal treatment for *Desulfovibrio* spp. infection has not been established (3,7). Our report suggests that ampicillin/sulbactam and amoxicillin/clavulanate may also be effective therapeutic options for *Desulfovibrio* spp. infections.

References


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Rapid Serologic Test for Diagnosis of Yaws in Patients with Suspicious Skin Ulcers

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Yaws is a neglected tropical disease caused by *Treponema pallidum* subsp. *pertenue* (TPE) that causes cutaneous ulcers. It predominantly affects children living in remote communities. The World Health Organization designated 2020 as the year that yaws would be eradicated. That year, 87,877 clinically suspected cases were reported, but only 346 (from 7 countries, primarily western Pacific countries) were confirmed as yaws (1). Thus, confirming a yaws diagnosis on the basis of ulcerative lesions remains challenging for yaws eradication (2). Standard tests for yaws diagnosis require sample processing in a laboratory, which is often unavailable in rural health centers where yaws is endemic (3). A mainstay for achieving yaws eradication is integration of point-of-care tests into surveillance strategies.

The Chembio DPP (Dual Path Platform) Syphilis Screen & Confirm kit (https://chembio.com) is a rapid serologic test that can be used to diagnose yaws. We evaluated its capacity to detect patients with ulcers that tested PCR positive for *Treponema pallidum* subsp. *pertenue*. DPP detected 84% of ulcers that were positive by PCR.

We assessed the ability of qualitative and quantitative measurements of DPP to identify active TPE in tropical ulcers. We used data from a community trial of patients with skin ulcers suggestive of yaws, conducted in Namatanai, Papua New Guinea, during 2018–2019 (7). We compared ulcer PCR results for TPE with serologic results of the DPP test T line, NT line, or both, read by the naked eye or by using the quantitative reader. The study protocol was approved by the Medical Research Advisory Committee of the Papua New Guinea National Department of Health. Participants provided written informed consent for collection of biological samples.

We tested samples from 995 suspicious skin ulcers by using DPP and PCR. The mean age of participants was 15.9 (SD ±14.1) years, and 454 (46.5%) were female. Median ulcer duration was 4 (interquartile range 2–8) weeks; median size was 2.0 (interquartile range 1.5–2.5) cm. For 745 (78.1%) case-patients, the ulcer was a first episode, and 662 (72.4%) had only 1 ulcer at the time of examination. Overall, 287 (28.8%) had a positive TPE PCR result. Ulcers positive by PCR were more frequently found in younger persons with only 1 ulcer, which was long-lasting and a first episode (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0608-App1.pdf). DPP reader results were available for 828 (83.2%) of the ulcers, of which PCR results were positive for 247 (29.8%).

Sensitivity of DPP detection of TPE PCR-positive cases with the naked eye was highest when we used the NT line, and specificity was highest when we used a combination of T and NT lines (Table). Using the values from the reader, we evaluated the optimal combination of cutoff values for the DPP T and NT lines, which maximized the sum of sensitivity and specificity to distinguish lesions that were positive and negative for TPE by PCR (Figure). That combination (T ≥1 and NT ≥28) provided sensitivity of 75.7% and specificity of 77.6% (Table). The subanalysis of DPP performance according to participants’ characteristics showed higher specificity for children <7
The proportion of cutaneous ulcers in yaws-endemic regions that were TPE positive declined from 44% to 8% after 1 round of azithromycin mass drug administration (delivery to all consenting members of target community, regardless of diagnosis) (7,8). In that context, the combined T and NT lines, recommended for surveillance, would be sufficient to partially detect ongoing yaws transmission, but PCR confirmation would be required to discern TPE ulcers from latent cases or false-positive results (Appendix Table 4).

**Table.** Performance of DPP Syphilis Screen & Confirm kit in study of rapid serologic test for diagnosis of yaws in patients with suspicious skin ulcers

<table>
<thead>
<tr>
<th>Detection technique</th>
<th>PCR negative</th>
<th>PCR positive</th>
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<tbody>
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<td>Detected, no.</td>
<td>Not detected, no.</td>
</tr>
<tr>
<td>Naked eye</td>
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<td></td>
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<tr>
<td>T line</td>
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<td>351</td>
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<tr>
<td>NT line</td>
<td>278</td>
<td>430</td>
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<td>T and NT line</td>
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<td>486</td>
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<tr>
<td>Reader</td>
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</tr>
<tr>
<td>T &gt;1 and NT reader</td>
<td>130</td>
<td>451</td>
</tr>
</tbody>
</table>

*DPP (Dual Path Platform) Syphilis Screen & Confirm kit, Chembio (https://chembio.com). NPV, negative predictive value; NT line, antibodies against non–Treponema pallidum antigen; PPV, positive predictive value; T line, antibodies against T. pallidum antigen.†Values used as cutoff with the DPP reader were calculated to maximize the sum of sensitivity and specificity (Figure).

The proportion of cutaneous ulcers in yaws-endemic regions that were TPE positive declined from 44% to 8% after 1 round of azithromycin mass drug administration (delivery to all consenting members of target community, regardless of diagnosis) (7,8). In that context, the combined T and NT lines, recommended for surveillance, would be sufficient to partially detect ongoing yaws transmission, but PCR confirmation would be required to discern TPE ulcers from latent cases or false-positive results (Appendix Table 4).
The DPP test can provide up to 84% sensitivity for detecting TPE PCR-positive ulcers with the naked eye when using the NT line, although the specificity of this strategy is low (61%). The automatic reader did not increase sensitivity. Our results should be interpreted by bearing in mind that the reference and index tests provide information regarding different features or manifestations of yaws: skin ulcers with TPE DNA and serologic activity of the host. Therefore, different disease phases such as incubation period or latency, or other confounders such as syphilis infections, may contribute to conflicting PCR and DPP results.

Overall, the DPP test showed a reasonably high capacity to identify yaws in persons with TPE PCR-confirmed ulcers. That level of performance is suitable for qualitatively identifying ongoing transmission of yaws in the community during the late phases of eradication. However, for individual diagnoses, PCR confirmation of suspicious ulcers remains necessary; new point-of-care tests with higher sensitivity and specificity would be valuable.

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**Soft Tissue Infection of Immunocompetent Man with Cat-Derived Globicatella Species**

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Cats are major reservoirs of zoonotic infections. Their long, sharp teeth predispose to deep-tissue bite injuries, and direct inoculation of feline saliva gives high risk for secondary infection. Infecting pathogens usually reflect colonizing oral microbiota; *Pasteurella* and *Streptococcus* species are the most common (1). Bacteria of the genus *Globicatella* are small, gram-positive cocci that resemble viridans-group streptococci. *Globicatella sanguinis* is the only known species to cause human infection, having been implicated in small numbers of bloodstream, heart, central nervous system and urinary tract infections (2). *G. sulfidifaciens* is the only other known *Globicatella* species, but human infection has not been described (3). We report a novel *Globicatella* species causing extensive soft tissue infection and tenosynovitis in an immunocompetent man after cat bite injuries.

A 48-year-old obese man came to the emergency department in 2020 because of painful bilateral hand swelling, 8 hours after sustaining several bites from a single feral cat. He had multiple puncture wounds and abrasions, without evidence of surrounding cellulitis. His wounds were bathed in povidone–iodine solution and dressed, and a booster dose of tetanus vaccine was administered. He was discharged and given oral doxycycline, ciprofloxacin, and metronidazole treatment because of history of penicillin allergy. He returned to the emergency department 24 hours later because of evolving flexor sheath infection in his left little and right middle fingers and cellulitis of both forearms (Figure). He was given intravenous vancomycin, ciprofloxacin, and metronidazole, then underwent debridement and washout. He was given 5 days of oral doxycycline and metronidazole postoperatively and made a full recovery. The patient provided fully informed, written consent for this case to be published, with accompanying clinical photographs.

We obtained tissue samples from debridement sites and a swab specimen from the right middle finger for microbiological analysis with Gram stain and bacterial culture on blood, chocolate, cystine-lactose-electrolyte—deficient, and fastidious anaerobe agars. No organisms were seen on Gram stain. A scant growth of *Staphylococcus epidermidis* was isolated from the right middle finger tissue sample, as well as a *Streptococcus*-like organism that grew best on chocolate agar. Culture yield may have been affected by previous antimicrobial drug treatment.

We report a novel *Globicatella* species causing extensive soft tissue infection in a man bitten by a stray domestic cat in the United Kingdom. We identified this bacterium by 16S rRNA gene sequencing, whole-genome sequencing, and biochemical profiling and determined antimicrobial drug susceptibility.

**Figure.** Clinically apparent areas of infection with *Globicatella* species in patient with soft tissue ianfection after cat bite, United Kingdom: A) left little finger, B) right forearm, C) right middle finger, and D) right hand.
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker, https://www.bruker.com) of the *Streptococcus*-like organism gave no reliable identification (score 1.31). We referred this isolate (designated G1610988) to the UK Health Security Agency Reference Laboratory for further characterization.

We obtained partial 16S rRNA gene sequence data after block-based PCR as described (4). Those data did not match any named species in the GenBank database. The closest sequence database match was to *Globicatella* sp. feline oral taxon 122 (99%–100% identity) (5).

API Rapid ID 32 Strep analysis (bioMérieux, https://www.biomerieux.com) gave an organism identification of *Erysipelothrix rhusiopathiae* (98.7%). The isolate was negative for pyrrolidonyl aminopeptidase and leucine aminopeptidase and positive for bile aesculin (Diatabs; Rosco Diagnostic, https://www.rosco.dk) diagnostic tablets for bacterial identification. Repeat matrix-assisted laser desorption/ionization time-of-flight mass spectrometry at the reference laboratory gave no reliable identification (score 1.41). Comparison with biochemical profiling of *G. sanguinis* and *G. sulfidifaciens* type strains showed notable differences (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/22-1770-App1.pdf). The conditions required for culture were not different between *Globicatella* species.

We conducted antimicrobial drug susceptibility testing by using MIC gradient strips (Liofilchem, https://www.liofilchem.com) and PK/PD and non–species-related breakpoints (6). Gentamicin was identified as an unsuitable treatment option, MIC 1.0 mg/L (breakpoint 0.5 mg/L). Treatments suitable for use with caution were ampicillin, MIC ≤0.016 mg/L (2.0 mg/L); cefotaxime, MIC 0.004 mg/L (1.0 mg/L); penicillin, MIC ≤0.016 mg/L (0.25 mg/L); linezolid, MIC 1.0 mg/L (2.0 mg/L); ciprofloxacin, MIC 0.032 mg/L (0.25 mg/L); and moxifloxacin, MIC 0.016 mg/L (0.25 mg/L). No PK/PD non–species-related breakpoints were available for teicoplanin, MIC 0.032 mg/L; vancomycin, MIC 0.25 mg/L; clindamycin, MIC 1.0 mg/L; erythromycin, MIC 0.032 mg/L; tetracycline, MIC 0.064 mg/L; chloramphenicol, MIC 2.0 mg/L; or rifampin, MIC 0.004 mg/L.

To corroborate the 16S rRNA gene sequence results, we conducted whole-genome sequencing on a HiSeq 2500 platform (Illumina, https://www.illumina.com) at the UK Health Security Agency Central Sequencing Laboratory by using its standard paired-end 101-bp sequencing protocol. We extracted genomic DNA from lysate by using the QIAsymphony DSP DNA Mini Kit and automated QIAsymphony SP/AS Instruments (QIAGEN, https://www.qiagen.com). We trimmed and filtered sequencing reads by using Trimmomatic (7) for quality control, then assembled by using SPAdes version 3.15 (8). Comparison with published *Globicatella* genomes by using FastANI (9) showed an average nucleotide divergence of 20.29% to its most closely related cluster (*G. sulfidifaciens*), suggesting a distinct and previously undescribed species (Appendix Figure).

Genomic sequences of isolate G1610988 have been deposited in the European Nucleotide Archive (Biosample accession no. SAMEA110751862). Partial sequence of the 16S rRNA gene has been deposited in GenBank (accession no. MW242777).

In conclusion, cat bites are common sources of zoonotic infection. This report highlights the role of cats as reservoirs of as yet undiscovered bacterial species that have human pathogenic potential. Currently recommended antimicrobial drug regimens for treating cat bites can be expected to include the *Globicatella* species described.

This study was conducted as part of our routine clinical and laboratory work. N.K.J. is a Cambridge Clinical Research Fellow, funded by Addenbrooke’s Charitable Trust (Company no. 10469089, Charity no. 1170103, Grant no. G112768A) and the National Institute for Health and Care Research Cambridge Biomedical Research Centre training scheme. Y.W. is an Institutional Strategic Support Fund Springboard Fellow, funded by the Wellcome Trust and Imperial College London. K.L.H., B.P., and Y.W. are affiliated with the National Institute for Health Research Health Protection Unit in Healthcare Associated Infections and Antimicrobial Resistance at Imperial College London in partnership with the UK Health Security Agency (previously Public Health England), in collaboration with Imperial Healthcare Partners, the University of Cambridge, and the University of Warwick.

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Imported Cholera Cases, South Africa, 2023

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Since February 2022, Malawi has experienced a cholera outbreak of >54,000 cases. We investigated 6 cases in South Africa and found that isolates linked to the outbreak were Vibrio cholerae O1 serotype Ogawa from seventh pandemic El Tor sublineage AFR15, indicating a new introduction of cholera into Africa from south Asia.

The seventh cholera pandemic arrived in Africa during 1970, and the related cholera strain, Vibrio cholerae O1 biotype El Tor (7PET), has since become endemic in many countries in Africa (1–3). As of March 20, 2023, at least 24 countries globally reported ongoing cholera cases. Several countries in southeastern Africa, in particular Malawi and Mozambique, were experiencing outbreaks. In addition, outbreaks were spreading regionally, including to Tanzania, Zambia, Zimbabwe, and South Africa. The largest active cholera outbreak on the continent was in Malawi: 54,841 cases and 1,684 deaths reported during February 28, 2022–March 20, 2023 (4).

South Africa is not considered endemic for cholera; previous outbreaks have typically been associated with importation events. However, cholera remains under active surveillance in South Africa. The National Institute for Communicable Diseases is notified of all suspected cases. All V. cholerae isolates are submitted to the Centre for Enteric Diseases, which provides further laboratory investigation, including phenotypic and genotypic characterization (Appendix 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0750-App1.pdf) (5). Ethics approval was obtained from the Human Research Ethics Committee, University of the Witwatersrand, Johannesburg, South Africa (protocol reference no. M210752).
As of February 28, 2023, a total of 6 cholera cases in South Africa had been laboratory confirmed by the Centre for Enteric Diseases; fecal samples were collected from patients February 1–23, 2023. All cases occurred in Gauteng Province (Table): 3 case-patients were female (19–44 years of age) and 3 male (23–41 years of age). Cases 1–3 were imported or import-related cases. Case-patients 1 and 2 (sisters) left Johan-

### Table. Characteristics of cholera cases and classification of *Vibrio cholerae* O1 serotype Ogawa sequence type 69 isolates from patient fecal samples, Gauteng Province, South Africa, 2023

<table>
<thead>
<tr>
<th>Case</th>
<th>Date sample collected</th>
<th>Cholera case classification</th>
<th>Comment on case classification</th>
<th>Patient age, y/sex</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2023 Feb 1</td>
<td>Imported case</td>
<td>Infected in Malawi</td>
<td>37/F</td>
<td>Acute diarrhea and dehydration</td>
</tr>
<tr>
<td>2</td>
<td>2023 Feb 2</td>
<td>Imported case</td>
<td>Infected in Malawi</td>
<td>44/F</td>
<td>Mild diarrhea</td>
</tr>
<tr>
<td>3</td>
<td>2023 Feb 5</td>
<td>Related to imported case</td>
<td>Close household contact of case-patient 1 (direct link to imported case)</td>
<td>41/M</td>
<td>Acute diarrhea and dehydration</td>
</tr>
<tr>
<td>4</td>
<td>2023 Feb 16</td>
<td>Locally acquired indigenous case</td>
<td>No travel history; no evidence of direct link to an imported case</td>
<td>27/M</td>
<td>Acute diarrhea and dehydration</td>
</tr>
<tr>
<td>5</td>
<td>2023 Feb 12</td>
<td>Locally acquired indigenous case</td>
<td>No travel history; no evidence of direct link to an imported case</td>
<td>23/M</td>
<td>Mild diarrhea</td>
</tr>
<tr>
<td>6</td>
<td>2023 Feb 23</td>
<td>Locally acquired indigenous case</td>
<td>No travel history; no evidence of direct link to an imported case</td>
<td>19/F</td>
<td>Mild diarrhea</td>
</tr>
</tbody>
</table>

Figure. Maximum-likelihood phylogeny of *Vibrio cholerae* O1 El Tor isolates collected in South Africa, 2023, compared with 1,443 reference seventh pandemic *V. cholerae* El Tor (7PET) genomic sequences. A6 was used as the outgroup. The genomic waves and acquisition of the ctxB7 allele are indicated. Color coding indicates the geographic origins of the isolates; sublineages previously introduced into Africa (AFR1–AFR14) are shown at right. A magnification of the clade containing the 6 isolates from South Africa (red text) is shown at right. For each genome, name (or accession number), country where contamination occurred, and year of sample collection are shown at the tips of the tree. The 6 isolates collected in South Africa belong to a new 7PET wave 3 sublineage called AFR15. Blue dots indicate bootstrap values ≥90%. Scale bars indicate number of nucleotide substitutions per variable site.
nesburg on January 15, 2023, and traveled together to Chinsapo, Lilongwe, Malawi, in one of the districts reporting active outbreaks, where they stayed until their departure on January 29, 2023. Both women reported onset of symptoms within 12 hours of departure during the bus trip back to Johannesburg. Case-patient 3 was a close household contact of case-patient 1. Case-patients 4–6 acquired infection locally and were classified as indigenous cases; none had travelled or had any link to the imported or import-related cases or to one another. We identified isolates associated with all 6 cases as *V. cholerae* O1 serotype Ogawa and all were PCR-positive for the cholera toxin–producing gene.

We used whole-genome sequencing, comparative genomics, and phylogenetic analysis to further characterize the isolates (Appendix 2 Tables 1, 2, https://wwwnc.cdc.gov/EID/article/29/8/23-0750-App1.xlsx). The 6 *V. cholerae* O1 isolates had similar genomic features, including the toxin-coregulated pilus gene subunit A gene variant, *tcpA*^{CIRSh1}, a deletion (ΔVC_0495-VC_0512) within the vibrio seventh pandemic island II (VSP-II), and an SXT/R391 integrating conjugating element called ICEVchln5, encoding resistance to streptomycin (*strAB*), sulfonamides (*sul2*), trimethoprim (*dfrA1*), and trimethoprim/sulfamethoxazole (*dfrA1* and *sul2*) and resistance or intermediate resistance to chloramphenicol (*floR*). The isolates also had mutations of *VC_0715* (resulting in the D89N substitution), conferring susceptibility to polymyxins (Appendix 6). The isolates had a specific nonsynonymous single-nucleotide variant (SNV) in the *vcrA* gene (*VC_A120*) (resulting in the D89N substitution), conferring susceptibility to polymyxins (6).

To place these 6 isolates into a global phylogenetic context, we constructed a maximum-likelihood phylogeny of 1,443 genomes (Appendix 2 Table 3) with 10,679 SNVs evenly distributed over the nonrepetitive, nonrecombinant core genome. All isolates from South Africa clustered together (median pairwise distance of 4 [range 0–8] core-genome SNVs) in the 7PET lineage wave 3 clade, containing isolates carrying the *ctxB7* allele (Figure) (6). However, those isolates did not belong to any of the sublineages previously found in Africa (AFR1 and AFR3–AFR 14) (Figure) (3,6,7); instead, they tightly grouped with genomes of South Asia variants, suggesting that the 2022–2023 cholera outbreak in Malawi and cases in South Africa in our study were associated with a newly imported 7PET strain, sublineage AFR15, from south Asia. All but 1 of the closest genomes were either collected locally and identified in Pakistan during June–December 2022 or detected within the framework of cholera surveillance in the United States or Australia (8).

In conclusion, we show that isolates from cases in South Africa, which have been linked to the 2022–2023 cholera outbreak in Malawi, belong to the seventh pandemic El Tor sublineage AFR15. Those cases did not result from resurgence of a strain previously circulating in any region of Africa but were caused by a cholera agent newly introduced into Africa from south Asia. This finding offers valuable information to all public health authorities in Africa. Genomic microbial surveillance and cross-border collaborations have a key role to play in identifying new cholera introductions, areas prone to cholera importation, and the main routes of cholera circulation. All of these elements are key to better understanding cholera epidemiology in Africa.

**Acknowledgement**

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**References**

Asymptomatic Healthcare Worker PCR Screening during SARS-CoV-2 Omicron Surge, Germany, 2022

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During 2022, a total of 9,515 asymptomatic healthcare workers of a large hospital in Germany underwent SARS-CoV-2 PCR screening twice weekly. Of 398,784 saliva samples, 3,555 (0.89%) were PCR positive (median cycle threshold value 30). Early identification of infected healthcare workers can help reduce SARS-CoV-2 transmission in the hospital environment.

COVID-19, caused by the SARS-CoV-2 virus, results in acute pulmonary and extrapulmonary manifestations and frequently causes long-term sequelae (1). In Germany, ≈38.5 million SARS-CoV-2 infections and ≈174,000 COVID-19 deaths had been reported through May 2023 (2). Among those, ≈30.2 million infections and ≈47,000 deaths occurred during 2022, when SARS-CoV-2 Omicron variant dominance was accompanied by a mean hospitalization incidence of 5.87 (2). SARS-CoV-2 infection rates among hospitalized patients were reported to be ≤10%–15% (3). Healthcare workers (HCWs) also were exposed to an elevated risk of acquiring and shedding SARS-CoV-2 infections (4). Regular SARS-CoV-2 testing of asymptomatic HCWs has been found to reduce viral transmission to patients and coworkers (5). We report data from a systematic SARS-CoV-2 PCR screening program comprising >9,500 HCWs in a large hospital in Germany during 2022.

Klinikum Nürnberg is a tertiary care hospital with 2,233 beds at 2 sites in Nuremberg, Germany, and cares for ≈100,000 inpatients and ≈170,000 outpatients per year. During January–November 2022, all 9,515 hospital staff were instructed to participate in a government-mandated regular SARS-CoV-2 PCR screening program. According to federal law in Germany, participation was mandatory irrespective of the level of working exposure risk or vaccination status (Table; Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0156-App1.pdf).

Asymptomatic HCWs collected saliva samples twice weekly via self-sampling using a reliable gaging method (6); part-time workers collected samples less frequently. Samples were subjected to PCR testing by an external provider, and turnaround time between sampling and electronic reporting was 24–38 h. However, staff with acute COVID-19 symptoms were immediately PCR tested in house. Persons with PCR-verified
infection were quarantined for 5–7 days and excluded from the testing program for the next 10 weeks. In November 2022, hospital staff who had no direct patient contact discontinued the screening program.

A total of 398,784 PCR tests were performed, among which 3,555 (0.89%) were positive; 2,782 persons tested positive ≥1. The cumulative infection rate of all tested asymptomatic HCWs was 29.2%. We observed a minimum positivity rate (0.25%) during January 2022 and the highest numbers of positive tests in March (1.89%) and October (1.69%) 2022 (Figure, panel A). The median cycle threshold (Ct) value of all positive PCR tests was 30 (interquartile range [IQR] 27–32), suggesting that SARS-CoV-2-positive staff were detected at an early phase of infection. Asymptomatic HCWs who tested SARS-CoV-2-positive frequently had symptoms develop a few days after detection, accompanied by lower Ct values (data not shown).

We categorized hospital staff into 5 groups: physicians, nurses, facility services, administration, and miscellaneous. Physicians constituted 13.4% of hospital staff and showed an infection rate of 13.6%. According to contingency table analysis, that rate results in a relative risk (RR) for infection of 1.02 (95% CI 0.91–1.14; p = 0.77 by Fisher exact test). Nurses accounted for 37.2% of hospital staff and exhibited an infection rate of 37.4% resulting in an RR for infection of 1.01 (95% CI 0.95–1.07; p = 0.81 by Fisher exact test). Thus, despite having the most intense contact with patients, neither of the 2 groups was significantly overrepresented in infection events (Figure, panel B; Appendix Table 1).

This 12-month SARS-CoV-2 PCR screening surveillance program of asymptomatic HCWs resulted in an average positivity rate of 0.89%. A meta-analysis of data from January–August 2020 collected by hospitals worldwide reported an average of 1.9% of asymptomatic HCWs tested PCR-positive for SARS-CoV-2 (7). We detected 3,555 COVID-19 cases among 2,782 (29.2%) HCWs infected ≥1 time. That number corresponds to results from another 12-month study in South Africa encompassing medical laboratory staff that had an overall cumulative infection rate of 25.7% (8). Comparisons warrant caution because of...
different spatiotemporal dominance of SARS-CoV-2 variants; vaccination status of HCWs, considering lower efficacy of vaccines against Omicron (9); and different infection control measures applied among hospitals (10). Furthermore, the surveillance study we report was not a randomized controlled trial, does not provide data on asymptomatic courses or rates of false positive PCR results, nor does it provide detailed information regarding seroprevalence or symptoms that developed.

The finding that physicians and nurses who were at the frontline of the COVID-19 outbreak response at Klinikum Nürnberg were not overrepresented in infection numbers speaks in favor of an efficient hygiene regimen. Besides measures such as compulsory patient screening, high-quality protective equipment, or regular ventilation, we believe that effective identification of asymptomatic HCWs in a preinfectious status might be one cornerstone of SARS-CoV-2 infection prevention in hospitals.

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References

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Six Extensively Drug-Resistant Bacteria in an Injured Soldier, Ukraine

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Blood and surveillance cultures from an injured service member from Ukraine grew *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterococcus faecium*, and 3 distinct *Pseudomonas aeruginosa* strains. Isolates were nonsusceptible to most antibiotics and carried an array of antibiotic resistant genes, including carbapenemases (*bla*<sub>NDM-1</sub>, *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>OXA-72</sub>) and 16S methyltransferases (*armA* and *rmtB4*).

The ongoing conflict in Ukraine has placed extraordinary pressure on medical infrastructure and health delivery services in the region (1). Previous reports from Eastern Ukraine have noted the emergence of multidrug-resistant (MDR) *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriales* infections during hospitalization (2). Those strains encompassed a variety of clonal lineages, with many carrying carbapenemases, extended-spectrum β-lactamases (ESBLs), and 16S methyltransferases (2,3). We describe the isolation of 6 extensively drug-resistant (XDR) organisms from a single soldier from Ukraine.

A man in his mid-50s suffered multiple traumatic injuries after a vehicle fire, including full-thickness burns covering 60% of his total body surface. He was initially treated in a medical facility near Dnipro, Ukraine, before being transferred to a hospital in Kyiv, Ukraine, where healthcare practitioners performed burn wound debridement and escharotomies. Thereafter, the patient was transported to a US military hospital in Germany, where doctors obtained blood, urine, respiratory, and peri-rectal surveillance cultures. Surveillance cultures grew *A. baumannii*, *Enterococcus faecium*, *Klebsiella pneumoniae*, and 2 distinct morphologies of *P. aeruginosa*. Blood cultures grew a third *P. aeruginosa* (Table). By using the Vitek 2 automated system (bioMérieux, https://www.biomerieux.com), the gram-negative organisms were found to be nonsusceptible to almost every antibiotic tested (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/29/8/23-0567-App1.pdf), with the exception of *A. baumannii*, which was susceptible to tetracycline (MIC 2 μg/mL). The *E. faecium* was nonsusceptible to vancomycin. Researchers used a customized Sensititer panel (Thermo Scientific, https://www.thermofisher.com) to test the gram-negative organisms against colistin, eravacycline, imipenem/relebactam, meropenem/vaborbactam, omadacycline, and plazomicin; they used disk diffusion (Hardy Diagnostics, https://hardydiagnostics.com) to test against ceferodol (Appendix Table 1). Researchers performed whole-genome sequencing of all isolates by using an Illumina MiSeq and the MiSeq Reagent Kit version 3 (600 cycles, 2 × 300 bp) (Illumina, https://www.illumina.com).

The *K. pneumoniae* isolate, designated MRSN 110821, was nonsusceptible to every antibiotic tested (Appendix Table 1). Testing identified 24 antimicrobial resistance genes, including the carbapenemases *bla*<sup>NDM-1</sup> and *bla*<sup>OXA-48</sup>, the RMTase *armA*, and the ESBL *bla*<sup>CTX-M-15</sup> (Table). Five plasmid replicons were identified (Appendix Table 2), but long-read sequencing is underway to better understand the plasmid structure (data not shown). Colistin resistance likely resulted from a previously characterized E82K mutation in the 2-component transcriptional regulator PhoP (4). Ceferodol resistance could be linked to mutations in the outer membrane protein OmpK36 combined with NDM (5). The isolate also carried several hypervirulence genetic markers, including *yhi16* (yersiniabactin siderophore), *iuc1* (aerobactin), and *rmpADC/rmpA2* (mucoviscosity and capsule).

The isolate belonged to clade B1 of the clonal lineage sequence type (ST) 395 (6) and was K-antigen capsular biosynthesis loci, K39, and O-antigen.

<table>
<thead>
<tr>
<th>Table. Characteristics of 6 isolates cultured from an injured service member from Ukraine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSN ID</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>110819</td>
</tr>
<tr>
<td>110818</td>
</tr>
<tr>
<td>110817</td>
</tr>
<tr>
<td>110606§</td>
</tr>
<tr>
<td>110821</td>
</tr>
<tr>
<td>110820</td>
</tr>
</tbody>
</table>

*Bold indicates high-impact genes. MRSN. The Multidrug-Resistant Organism Repository and Surveillance Network; ID, identification; ST, sequence type.
†In silico–derived multilocus STs.
§Blood culture isolate.

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type O2 variant 1 (O2v1). ST395 was first described in France in 2010, and carbapenemase-producing strains are increasingly being reported across Europe (6). We downloaded all clade B1 ST395 isolates from Pathogenwatch (https://pathogen.watch) and constructed a phylogenetic tree (Figure). We included 3 ST395 genomes identified by Sandfort et al, which they cultured from patients from Ukraine who were hospitalized in Germany (7). Of note, MRSN 110821 was separated by just 20 single nucleotide polymorphisms from NRZ-78043a from the Sandfort study and by just 19 from NRZ-78056b from that same study (Figure). Those 3 isolates clustered more broadly with isolates from Russia and Finland (Figure), but have since acquired armA, blaNDM-1, and the mucoviscosity and capsule loci rmpADC, further increasing their antibiotic resistance profile and virulence potential.

We found A. baumannii MRSN 110819 to be resistant to all antibiotics except cefiderocol, colistin, eravacycline, and omadacycline (Appendix Table 1). The isolate was assigned to ST78, a clonal group known as the Italian clone because it emerged in Italy in the mid-2000s (8). This clonal group has also been identified in war wounds of service members from Ukraine during the earlier conflict in Eastern Ukraine (2).

The 3 P. aeruginosa isolates belonged to 3 distinct strains (Table). All 3 isolates had high MICs to 20 of the 23 antibiotics tested (Appendix Table 1). Only colistin and cefiderocol appeared effective in vitro, although MRSN 110818 was susceptible to imipenem/relebactam using US Food and Drug Administration breakpoints (MIC 2 mg/L). All 3 carried carbapenemases, ESBLs, and 16S methyltransferases (Table). MRSN 110818 and 110817 belonged to well-known (ST357) and emerging (ST773) epidemic, high-risk clones that are increasingly associated with horizontally acquired β-lactamases (9). The single blood isolate was assigned to ST1047.

E. faecium MRSN 110820 carried 8 AMR genes, including the vanA operon (Table). The strain was assigned to ST117, a member of clonal complex 78.

Gaps in such services as infection control, caused by limited resources and personnel, are...
exacerbating the transmission of MDR organisms in Ukraine. As a result, healthcare networks in Europe now consider prior hospitalization in Ukraine to be a critical risk factor for colonization of MDR organisms (7,10). Healthcare practitioners treating citizens of Ukraine need to be cognizant of the increased risk for MDR organism transmission and infection imposed by the conflict in Ukraine and implement appropriate infection control measures to mitigate their spread.

Isolates for this study were collected under the auspices of routine public health surveillance. Sequences have been deposited into GenBank (BioProject nos. PRJNA950448, PRJNA950449, PRJNA950450, and PRJNA950451). The Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) is a department within Walter Reed Army Institute of Research’s Bacterial Diseases Branch, a unique entity that serves as the primary surveillance organization for antibiotic-resistant bacteria across the Army, Navy, and Air Force.

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Dr. Mc Gann is a microbiologist and deputy director of the Multidrug-Resistant Organism Repository and Surveillance Network in Silver Spring, Maryland, USA. His primary research interests are the emergence and spread of antibiotic resistance and using whole-genome sequencing techniques to unravel bacterial epidemiology.

References

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Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Virus in Domestic Cat, France, 2022

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On December 27, 2022, the avian influenza National Reference Laboratory of the Agency for Food, Environmental and Occupational Health & Safety in France confirmed a case of highly pathogenic avian influenza (HPAI) A(H5N1) clade 2.3.4.4b virus in a domestic cat that lived near a duck farm infected by a closely related virus in France during December 2022. Enhanced surveillance of symptomatic domestic carnivores in contact with infected birds is recommended to prevent further spread to mammals and humans.

We detected highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus in a domestic cat that lived near a duck farm infected by a closely related virus in France during December 2022. Enhanced surveillance of symptomatic domestic carnivores in contact with infected birds is recommended to prevent further spread to mammals and humans.

On December 27, 2022, the avian influenza National Reference Laboratory of the Agency for Food, Environmental and Occupational Health & Safety in France confirmed a case of highly pathogenic avian influenza (HPAI) A(H5N1) clade 2.3.4.4b virus in a domestic cat. The cat lived with a human family next to a breeding duck farm, which had notified the animal health services of possible HPAI on December 9, 2022, after observing a 20% drop in egg production. After HPAI H5N1 clade 2.3.4.4b virus was confirmed at the farm, 8,375 ducks were culled on December 14. On December 20, the cat displayed disturbances in general condition, including apathy and mild hyperthermia, and was seen by a veterinary surgeon. The cat’s condition worsened; pronounced neurologic and respiratory (dyspnea) symptoms appeared, resulting in compassionate euthanasia on December 24. Veterinarians collected sinonasal, tracheal, and anal swab samples after death, and a screening laboratory performed real-time reverse transcription PCR (RT-PCR) targeting the matrix protein and hemagglutinin (H5) genes. The laboratory sent H5–positive tracheal and sinonasal swab samples to the National Reference Laboratory, which confirmed HPAI H5N1 virus by using specific real-time RT-PCR for H5 clade 2.3.4.4b and neuraminidase (N1) genes (Table).

We compared the complete sequence of the HPAI H5N1 virus found in the cat (A/cat/France/22P026544/2022) with other HPAI H5N1 virus sequences circulating in France in the same area, including the virus found in the neighboring duck farm (A/duck/France/22P025647/2022). Phylogenetic analyses of HPAI H5N1 genomes indicated that the virus from the cat belonged to the A/duck/Sarato/29-02/2021–like genotype, which has been the predominant virus genotype circulating in France and Europe since September 2022. The cat virus sequence was directly related to virus sequences identified in the same area in December 2022 (Figure). Furthermore, the virus isolated from the neighboring duck farm (Figure) had only 2 nt differences (out of 13,507 total nts) compared with the cat virus, resulting in an E627K mutation in polymerase basic protein 2 and E26G mutation in nonstructural protein 2 in the cat virus. The E627K mutation has been described as a major marker of influenza virus adaptation to mammalian hosts (1). The E26G mutation has a possible role in virus adaptation to temperature changes (2). Since September 2021, a total of 90 sequences of HPAI H5N1 clade 2.3.4.4b viruses detected in mammals have been available in the GISAID EpiFlu database (https://www.gisaid.org), 20 of which have the E627K mutation, most probably indicating a rapid selection of this mutation in mammalian hosts (3).

This virulence marker is in addition to those already observed in circulating HPAI H5N1 viruses detected in birds in Europe, such as the PB1-F2 N66S mutation (4). Since winter 2021–22, the number of reported cases of HPAI H5N1 clade 2.3.4.4b infections in mammals has increased (5,6), likely caused by several factors. First, a higher prevalence of HPAI H5 viruses in wild and domestic birds might increase the probability of interactions between infected birds and mammals (scavenging, shared habitat). Second, increased surveillance of avian influenza in wildlife might lead to more detection in mammals. Third, currently circulating viruses might infect mammalian hosts more easily. HPAI H5N1 virus detection in mammals is often linked to clinical signs, such as neurologic symp-
Few indications of intermammal HPAI H5N1 clade 2.3.4.4b contamination exist with the exception of massive infections in seal colonies in the United States (6) and a mink farm in Spain in 2022 (6,7). In our case report, negative results from serologic and real-time RT-PCR analyses of samples from the dog and other cat in the same household indicate a lack of intermammal transmission.

In conclusion, we show that HPAI H5N1 clade 2.3.4.4b can infect cats; HPAI H5N1 clade 1 and clade 2.2 have been sporadically detected in cats since 2004 (8). The close interactions and proximity of domestic cats and humans and rapid selection of mutations (after 1 passage from bird to mammal) could result in a virus with potential for interhuman transmission, indicating a considerable public health threat. Given that HPAI H5N1 circulates at high levels in wild and domestic birds, and virus was detected in a domestic cat,

<table>
<thead>
<tr>
<th>Sample</th>
<th>Matrix protein</th>
<th>H5 hemagglutinin</th>
<th>H5 hemagglutinin 2.3.4.4b</th>
<th>N1 neuraminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal swab</td>
<td>25.5</td>
<td>25.5</td>
<td>26</td>
<td>29.4</td>
</tr>
<tr>
<td>Sinonasal swab</td>
<td>33.3</td>
<td>31.5</td>
<td>32.9</td>
<td>36.7</td>
</tr>
<tr>
<td>Anal swab</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are PCR cycle thresholds. NA, not applicable; ND, not detected.

Figure. Phylogenetic analysis of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus detected in domestic cat, France, 2022. Tree was created by using MEGA 7 software (https://megasoftware.net) and the neighbor-joining method with 1,000 bootstrap replicates for complete concatenated HPAI H5N1 virus segments. All sequences belong to the A/duck/Saratov/29-02V/2021–like genotype. Red solid circle indicates virus sequence from cat; black solid circle indicates sequence from a nearby duck farm. Both sequences are available in the GISAID database (https://www.gisaid.org) under accession nos. EPI_ISL_16395206 (cat) and EPI_ISL_16740903 (duck). Red bracket indicates closely related sequences detected during the same period and area in France from domestic bird farms. Scale bar indicates nucleotide substitutions per site.
we recommend enhanced surveillance of symptomatic domestic carnivores in contact with infected birds to rapidly identify potential transmission events to other domestic animals and prevent further spread to humans. Our report also indicates that adequate protective equipment and barrier measures should be provided to avoid direct transmission of HPAI to persons exposed to infected birds (6, 9).

Acknowledgments
We thank all originating laboratories, where specimens were first obtained, and submitting laboratories, where sequence data used for the phylogenetic analyses were generated and submitted to the GISAID EpiFlu database (Appendix, https://wwwnc.cdc.gov/EID/article/29/8-23-0188-App1.xlsx); and Benjamin Houillé, Pascale Massin, Martine Cherbonnel-Pansart, Claire Martenot, Rachel Busson, Angelina Oroso, Katell Louboutin, Carole Guillemot, Yannick Blanchard, Alice Herteau, Anne Bernadou, Christine Castor, Laurent Filleul, and Karim Tararbit for aiding in the avian influenza diagnostic analyses and further investigations.

About the Author
Mr. Briand is a scientist at the National Reference Laboratory for avian influenza and Newcastle disease in France. His research interests focus on phylogeny, virology, and molecular epidemiology, especially for avian influenza and Newcastle disease viruses.

References

Case Report of Leprosy in Central Florida, USA, 2022

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Florida, USA, has witnessed an increased incidence of leprosy cases lacking traditional risk factors. Those trends, in addition to decreasing diagnoses in foreign-born persons, contribute to rising evidence that leprosy has become endemic in the southeastern United States. Travel to Florida should be considered when conducting leprosy contact tracing in any state.

Leprosy, or Hansen disease, is a chronic infectious disease caused by the acid-fast rod Mycobacterium leprae. Leprosy primarily affects the skin and peripheral nervous system, and disease course is largely dependent on individual susceptibility to M. leprae (I). Leprosy has been historically uncommon in the United States; incidence peaked around 1983, and a drastic
reduction in the annual number of documented cases occurred from the 1980s through 2000 (2). However, since then, reports demonstrate a gradual increase in the incidence of leprosy in the United States. The number of reported cases has more than doubled in the southeastern states over the last decade (2). According to the National Hansen’s Disease Program, 159 new cases were reported in the United States in 2020; Florida was among the top reporting states (2).

Central Florida, in particular, accounted for 81% of cases reported in Florida and almost one fifth of nationally reported cases (3). Whereas leprosy in the United States previously affected persons who had immigrated from leprosy-endemic areas, ≈34% of new case-patients during 2015–2020 appeared to have locally acquired the disease (4). Several cases in central Florida demonstrate no clear evidence of zoonotic exposure or traditionally known risk factors. We report a case of lepromatous leprosy in central Florida in a man without risk factors for known transmission routes. We also review the mounting epidemiologic evidence supporting leprosy as an endemic process in the southeastern United States.

A 54-year-old man sought treatment at a dermatology clinic for a painful and progressive erythematous rash (Figure). The lesions began on his distal extensor extremities and progressed to involve his trunk and face. He denied any domestic or foreign travel, exposure to armadillos, prolonged contact with immigrants from leprosy-endemic countries, or connections with someone known to have leprosy. He has resided in central Florida his entire life, works in landscaping, and spends long periods of time outdoors. Biopsies of multiple sites demonstrated a diffuse dermal infiltrate composed of disorganized aggregates of foamy histiocytes and lymphocytes. Fite stains revealed acid-fast bacilli within histiocytes and cutaneous nerve twigs, a pathognomonic finding of leprosy. He was referred to an infectious disease specialist who, under the direction of the National Hansen’s Disease Program, prescribed triple therapy with dapsone, rifampin, and clofazimine.

Transmission of leprosy has not been fully elucidated. Prolonged person-to-person contact through respiratory droplets is the most widely recognized route of transmission (1). A high percentage of unrelated leprosy cases in the southern United States were found to carry the same unique strain of *M. leprae* as nine-banded armadillos in the region, suggesting a...
strong likelihood of zoonotic transmission (4). A recent systematic review analyzing studies conducted during 1945–2019 supports an increasing role of anthropogenic and zoonotic transmission of leprosy (5). However, Rendini et al. demonstrated that many cases reported in eastern United States, including Georgia and central Florida, lacked zoonotic exposure or recent residence outside of the United States (6).

Given those reports, there is some support for the theory that international migration of persons with leprosy is a potential source of autochthonous transmission. Reports from Spain linked an increase in migration from other countries to an increase in autochthonous leprosy (7). The number of international migrants in North America increased from 27.6 million persons in 1990 to 58.7 million in 2020 (8), so a link to migration may account for the increase in incidence of leprosy in historically nonendemic areas. Further, reports from the Centers for Disease Control and Prevention show that, although the incidence of leprosy has been increasing, the rates of new diagnoses in persons born outside of the United States has been declining since 2002 (Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/8/22-0367-App1.pdf) (9). This information suggests that leprosy has become an endemic disease process in Florida, warranting further research into other methods of autochthonous transmission.

Leprosy is a reportable condition in the state of Florida and is monitored primarily through passive surveillance. According to the Florida Department of Health, practitioners are required to report leprosy in Florida by the next business day (10). Contact tracing is critical to identifying sources and reducing transmission. In our case, contact tracing was done by the National Hansen’s Disease Program and revealed no associated risk factors, including travel, zoonotic exposure, occupational association, or personal contacts. The absence of traditional risk factors in many recent cases of leprosy in Florida, coupled with the high proportion of residents, like our patient, who spend a great deal of time outdoors, supports the investigation into environmental reservoirs as a potential source of transmission.

In summary, our case adds to the growing body of literature suggesting that central Florida represents an endemic location for leprosy. Travel to this area, even in the absence of other risk factors, should prompt consideration of leprosy in the appropriate clinical context. By increasing local physician efforts to report incidence and supporting further research to assess routes of transmission, a congruent effort can be made to identify and reduce spread of the disease. The patient described in the case report reviewed the text and photographs in the report and gave written consent to publish them. A copy of the consent form is on file with the authors. This report was deemed exempt from Institutional Review Board approval by Kansas City University.

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Dr. Bhukhan is an upcoming transitional year resident at the University of Central Florida/HCA Osceola Hospital and upon completion will join the Kansas City University-Graduate Medical Education/Advanced Dermatology and Cosmetic Surgery Dermatology Residency program in Orlando. Her primary research interests include dermatology, infectious disease, and global health.

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Advanced Age and Increased Risk for Severe Outcomes of Dengue Infection, Taiwan, 2014–2015

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Dengue, a mosquito–borne flavivirus infection, is increasingly a disease of older adults who are more likely to have chronic diseases that confer risk for severe outcomes of dengue infection. In a population-based study in Taiwan, adjusted risks for dengue-related hospitalization, intensive care unit admission, and death increased progressively with age.

Dengue is an Aedes aegypti mosquito–borne flavivirus infection; a secular trend for dengue is a shift in the age of case-patients and deaths from children to adults and older adults (1,2). We report a population-based study of age-specific dengue risks for hospitalization, intensive care unit admission, and death in Taiwan.

Dengue is not endemic in Taiwan but is introduced frequently; population immunity is low. In 2014–2015, large outbreaks led to 51,344 cases confirmed by the Taiwan Centers for Disease Control (Taiwan CDC) (3). In those years, suspected cases were tested at the Taiwan CDC by reverse transcription PCR, IgM/IgG, and paired IgM/IgG for seroconversion to confirm dengue infection; point-of-care NS1 antigen detection assays were introduced in September 2015. The database of reported cases was linked to the national administrative health database to report patient outcomes and to establish age-specific rates. Because reporting is required for reimbursement, the National Health Insurance Research Database provides near-complete ascertainment of all medical encounters for >99% of the island’s population. We adjusted for sex, socioeconomic status, and presence of underlying conditions (2). We did not review individual patients’ medical records in this linked-database analysis.

Age-specific attack rates during the outbreak were similar across age groups (Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/8/23-0014-App1.pdf), consistent with low population immunity rates. Lower attack rates in children <10 years of age, in whom infections are more likely to be asymptomatic or mildly symptomatic, likely reflected a lower frequency of medical consultation and laboratory confirmation. We examined records of 51,344 case-patients, 15,847 of whom were hospitalized, in an analysis of healthcare utilization and deaths. Adjusted rates for hospitalization, ICU admission, and death within 30 days of confirmation of dengue illness increased sharply with advancing age, compared with rates among young adults (Figure). In contrast, case rates by age group were relatively flat.

The epidemiologic shift of dengue to adults and older adults has increased recognition that advanced age is a risk factor for severe and fatal outcome after dengue infection (2,3), adding dengue to the list of other flavivirus infections for which advanced age is well established to be a cardinal risk factor for severe disease. Age-specific curves by age for clinically severe dengue, seasonal influenza, and COVID-19 are similar; however, mortality rates and case-fatality ratios for dengue are considerably lower (4,5). Case-fatality ratios for dengue in Pan American Health Organization countries are <0.05%; in southern Brazil, mortality rates for dengue are 10-fold to 100-fold lower than for seasonal influenza (4–6).

Adults and older adults comprise an increasing proportion of dengue cases in many countries, even as those populations reach ages where the prevalence of chronic diseases increases (7). Because chronic diseases contribute to more severe dengue outcomes, the overall effects of dengue attributed to those underlying conditions are increasing in turn. In adults, underlying diseases, specifically diabetes, chronic renal disease, and heart disease, are associated with higher relative odds for progression to severe disease than secondary infection (8). However, in administrative database studies in Mexico, Brazil, and Colombia, comorbidities were shown to confer a higher risk for death in hospitalized patients across the age spectrum (8,9). Our understanding of pathophysiological mechanisms associated with increased dengue severity in patients with comorbidities and advanced age is limited, as it is for patients with influenza and COVID-19.

1Current affiliate: Medigen Vaccines Biologics Corporation, Taipei, Taiwan.
Since 1990, dengue cases and deaths have increased disproportionately among adults >50 years of age compared with children <15 years of age, in whom the number and proportion of cases and, especially, deaths, have declined (1). As of May 2023, Asia accounts for most global dengue cases; it is also the fastest-aging region, containing more than half of the world’s population >65 years of age. Projections suggest that 10% of the total global population by 2060 will be persons ≥65 years of age living in Asia (10). Ongoing demographic trends and the 2 risk factors, advanced age and chronic disease, for developing severe to fatal dengue underscore the need to improve protection from dengue for older adults and persons with chronic diseases in all regions in which dengue is endemic, particularly in Asia.

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About the Author

Dr. Huang is a professor at the Institute of Hospital and Health Care Administration of the College of Medicine at National Yang Ming Chiao Tung University, Taipei. Dr. Huang’s primary research interest has been assessing how individual, provider, and system characteristics may influence provision and quality of care for communicable and noncommunicable diseases.

References


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Figure. Dengue outcome event rates by age group in Taiwan. A) Hospitalizations; B) ICU admissions; C) deaths. ICU, intensive care unit.
Fatal Meningitis from Shiga Toxin–Producing Escherichia coli in 2 Full-Term Neonates, France

Guillaume Geslain, Aurélie Cointe, Philippe Bidet, Céline Courroux, Soumeth Abasse, Patricia Mariani, Stéphane Bonacorsi

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DOI: https://doi.org/10.3201/eid2908.230169

We report fatal meningitis in 2 neonates in France caused by Shiga toxin 1–producing Escherichia coli. Virulence factors capsular K1 antigen and salmochelin were present in both strains, potentially representing a new hybrid pathotype. Clinicians should remain aware of emerging pathotypes and design therapeutic strategies for neonatal Escherichia coli infections.

Escherichia coli can acquire virulence factors associated with increased pathogenicity, causing intestinal or extraintestinal infections. Shiga toxin (Stx)–producing E. coli (STEC) cause intestinal infections and hemolytic uremic syndrome (HUS). E. coli virulence factors K1 antigen and salmochelin are associated with neonatal E. coli meningitis (1). Hybrid pathotypes have been described, such as Stx-producing O80:H2, which causes HUS and bacteremia because of extraintestinal virulence–associated plasmid pS88 (2). We report 2 cases of full-term neonates who died of meningitis caused by STEC serotypes O117:H7 and O156:H7.

Patient 1, an 8-day-old full-term boy born in the Paris, France, area, was seen at the emergency department because of a 48-hour history of abdominal pain and rectorrhagia without fever and drowsiness since that morning. Septic shock with colitis developed rapidly, requiring admission to the pediatric intensive care unit (PICU). Hemolysis was not observed, and initial acute kidney failure resolved promptly. Blood culture results were positive for E. coli K1, urine culture results were negative, and 3 stool cultures yielded STEC. Cerebral magnetic resonance imaging on PICU day 7 showed severe diffuse lesions indicating meningoencephalitis and subdural empyema. Cerebrospinal fluid (CSF) obtained by lumbar puncture 2 days later contained 2,270 leukocytes/μL, showed negative Gram stain and culture, and was PCR-positive for K1 and β-glucuronidase genes, indicating E. coli meningitis. Prompted by clinical and laboratory features potentially associated with HUS, PCR on blood and CSF DNA were performed and identified stx1. The patient died on day 28.

Table. Genes encoding confirmed or putative virulence factors found in Shiga toxin–producing Escherichia coli ST504 strains that caused fatal meningitis in 2 full-term neonates, France*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene function</th>
<th>E. coli O117:H7</th>
<th>E. coli O156:H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>iucC</td>
<td>Aerobactin biosynthesis</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>iutA</td>
<td>Aerobactin receptor</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>fyuA</td>
<td>Yersiniabactin receptor</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>ip2</td>
<td>Yersiniabactin biosynthesis</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>iroN</td>
<td>Salmochelin receptor</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>iroD</td>
<td>Salmochelin biosynthesis</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>chuA</td>
<td>Hemin uptake</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>cvaA</td>
<td>Colicin V</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>iss</td>
<td>Increased serum survival protein</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>sitA</td>
<td>Iron transport protein</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>ibeA</td>
<td>Invasin</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>vat</td>
<td>Vacuolating autotransporter toxin</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>iha-like</td>
<td>Putative Iha-like adhesin</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>mchB</td>
<td>Microcin</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>mchC</td>
<td>Microcin</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>mchF</td>
<td>Microcin</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>mcmA</td>
<td>Microcin</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>gad</td>
<td>Glutamate decarboxylase</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>iha</td>
<td>Adherence protein</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>capU</td>
<td>Hexosyltransferase homologue protein</td>
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<td>Positive</td>
</tr>
<tr>
<td>sigA</td>
<td>Serine protease autotransporter</td>
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</tr>
<tr>
<td>stx1A-a</td>
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<td>Positive</td>
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<td>stx1B-a</td>
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</tr>
<tr>
<td>kpsF</td>
<td>Polysialic acid group 2 capsule expression protein</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>kpsD</td>
<td>Translocation of capsule of groups 2 and 3</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>neuC</td>
<td>K1 capsular antigen</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*E. coli serotypes were O117:H7 (case 1, 8-day-old boy) and O156:H7 (case 2, 4-day-old boy). ST, sequence type.
Patient 2, a full-term boy born in an overseas department of France, was transferred to a PICU at 4 days of age because of fever, drowsiness, and failure to feed. Septic shock without colitis developed rapidly, without hemolysis or initial thrombocytopenia. On PICU day 4, blood culture results were positive and urine culture results negative for E. coli; CSF contained 900 leukocytes/mm³ and showed E. coli growth in culture; Stx was not initially evaluated. The patient died on PICU day 9.

Whole-genome sequencing of blood and stool isolates (patient 1) and CSF isolate (patient 2) indicated the E. coli strains belonged to sequence type (ST)504 and phylogroup B2; serotypes were O117:H7 for patient 1 and O156:H7 for patient 2. Both strains harbored the gene encoding Stx1a protein but not eae (intimin) or ehxA (enterohemolysin) genes (3,4). Genetic determinants for extraintestinal virulence factors K1 capsular antigen, yersiniabactin, and salmochelin were present in both strains and the aerobactin operon in the O117:H7 strain. Screening for confirmed or putative virulence factors as previously described (5) (Table) showed neither strain harbored an pSS88-like plasmid (6).

Both strains differed substantially from E. coli K1 strains usually reported as causes of meningitis. K1 strains mainly belong to the ST95 complex (7) and most common serogroups are O18, O1, O7, O83, and O45 S88 (6,8). STEC O117:H7 ST504 strains were described in 2005 in 20 adults with persistent traveler’s diarrhea (3). Those strains and STEC strains described in another study of traveler’s diarrhea were atypical because they did not express lysine decarboxylase, β-galactosidase, intimin, or enterohemolysin (4). No invasive infections were reported before the 2 cases we describe, although some strains expressed extraintestinal virulence factors. However, because PCR for Stx is not routinely performed on neonatal invasive E. coli strains, STEC O117:H7 might be underestimated. Whole-genome sequencing of neonatal E. coli meningitis strains would help determine the role of STEC in fatal neonatal meningitis. In our patients, simultaneous presence of K1 antigen and salmochelin might explain isolate invasiveness (1). Moreover, STEC O156:H7 harbored the invasive IbeA, which promotes blood–brain barrier translocation.

When we analyzed the EnteroBase database (9) in late 2022, we identified only 4 O156:H7 and 39 O117:H7 strains distributed among 4 STs (ST504, n = 17; ST5292, n = 18; ST6880, n = 3; and ST9996, n = 1), all belonging to the ST504 complex. BLAST (https://blast.ncbi.nlm.nih.gov) analysis of STEC O117:H7 ST504 complex sequences from EnteroBase consistently identified extraintestinal virulence factors yersiniabactin and aerobactin (5); salmochelin (12 of 39 sequences) and K1 antigen (20 of 39 sequences) were inconsistently present. Since routine STEC sequencing began in 2017 in France, 3 other STEC O117:H7 ST504 complex strains have been identified (2 from patients with nonhemorrhagic diarrhea and 1 from an asymptomatic carrier).

Neither neonate described in this report had typical HUS, possibly because the E. coli strains lacked genes encoding Stx2, intimin, and enterohemolysin. However, Stx might have promoted intestinal translocation of the bacteria. Furthermore, antimicrobial drug therapy might have induced intracerebral Stx production, thereby contributing to fatal outcomes (10).

ST504 complex STEC strains exhibit only moderate intestinal virulence. However, we show that those strains can translocate into blood and CSF in neonates, especially if they produce K1 antigen and salmochelin. ST504-complex STEC expressing K1 antigen and salmochelin might be new hybrid pathotypes in neonates, even for those born at full term, with both extraintestinal pathogenic and neonatal meningitis virulence factors. Clinicians should remain aware of emerging pathotypes and new preventive and therapeutic strategies for E. coli infections in neonates.

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Dr. Geslain is a pediatrician in the pediatric intensive care unit at Robert Debré University Hospital in Paris and a PhD student in the infection, antimicrobials, modelling, evolution laboratory at the Université Paris Cité INSERM. His research interests focus on infectious diseases.

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Rio Negro Virus Infection, Bolivia, 2021

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In May 2021, an agricultural worker originally from Tren-メンタル, Argentina, sought treatment for febrile illness in Tarija, Bolivia, where he resided at the time of illness on- set. The patient tested negative for hantavirus RNA, but next-generation sequencing of a serum sample yielded a complete genome for Rio Negro virus.

Rio Negro virus (RNV; family Togaviridae, genus Alphavirus), a Venezuelan equine encephalitis virus (VEEV) antigenic subtype VI virus, was first reported in 1987 after being isolated from mos- qui toes collected in Chaco, Argentina (1). The virus has since been isolated or molecularly detected in mosquitoes and rodents in Argentina and bats in Uruguay (2–6). Although RNV was serologically associated with an outbreak of undifferentiated febrile illness in Argentina, molecular evidence of RNV infection in humans is lacking (4,7,8). High RNV seroprevalence among horses in Uruguay suggests the virus likely circulates throughout the region (9). Dengue viruses 1–4 are leading causes of acute febrile illnesses in Latin America, but confirmatory testing is often not performed. Surveillance is also not routinely performed for other viral etiologies of acute febrile illnesses (e.g., arenaviruses, hantaviruses, other arboviruses). In regions of Bolivia where hantaviruses are known to circu- late, a national surveillance program collects blood samples, along with clinical and epidemiologic information, including risk factors associated with hantavirus infection (e.g., agricultural work) from patients manifesting nonspecific signs and symptoms (e.g., fever, headache, nausea, myalgia, ar-

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Figure. Inferred phylogenetic relationships of Rio Negro viruses collected from mosquitoes, bats, and rodents in South America and the human-derived RNV sequence described in this report from Tarija, Bolivia (bold text). A) Phylogenetic tree made with an alignment of partial nsP4 sequences of Rio Negro viruses. Mossa das Pedras virus is included as an outgroup. B) Phylogenetic tree made with an alignment of partial E3/GP2 sequences of Rio Negro viruses. Mossa das Pedras virus is included as an outgroup. GenBank accession numbers are provided. Scale bars indicate substitutions per site.
A 21-year-old man, a migrant agricultural worker originally from Tremental, Argentina, with no related medical history, sought treatment on May 31, 2021, in Padcaya Municipality, Tarija Department, Bolivia, where he resided at the time of illness onset (Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/8/22-1885-App1.pdf). On arrival, he reported a 1-day history of fever, chills, headache, nausea, arthralgia, myalgia, thoracic pain, back pain, and hyperemia; his temperature at the time, 37.6°C, was the maximum during his hospitalization. Physical examination revealed bilateral crackles but observations were otherwise unremarkable. He was admitted to hospital with suspected SARS-CoV-2 or hantavirus infection, but other infectious etiologies, such as dengue or a bacterial urinary tract infection, were also considered. The antimicrobial levofloxacin and corticosteroids dexamethasone and betamethasone were empirically prescribed. Initial clinical testing included complete blood count, basic metabolic panel, and urinalysis; all results were unremarkable. Results of a SARS-CoV-2 rapid test was negative. A blood sample was sent to CENETROP for hantavirus testing. The patient ultimately made a full recovery and was discharged after 5 days on June 5, 2021.

In December 2021, archived samples collected for hantavirus surveillance were inactivated in CENETROP’s Biosafety Level 3 laboratory and tested for hantavirus antibodies and RNA using CDC ELISA and real-time reverse transcription PCR (RT-PCR) (Appendix). RNA from RT-PCR-positive and -negative samples was sent to CDC for NGS. The specimen collected from the patient was negative for hantavirus RNA. A near-complete genome (excluding 40 of >11K nt) was obtained using NGS and identified by genomic analysis as RNV (Figure 1), a result supported by alphavirus RT-PCR followed by sequencing. The RT-PCR amplicon had the highest identity, 97.8% (441/451 nt), to GenBank RNV reference strain NC_038674, suggesting the amplicon was not a product of laboratory contamination. RNV was not identified in a subset of hantavirus RNA-positive (n = 7) and -negative (n = 4) specimens collected from the same area or in negative controls.

VVEV is a substantial human and animal pathogen considered a persistent zoonosis in Latin America; RNV is a VVEV subtype VI arbovirus, closely related to subtype I viruses, which have resulted in large-scale human outbreaks of >100,000 cases (10). RNV has been isolated from Culex spp. mosquitoes and rodents in Argentina (2–5). Evidence of RNV infection in humans has been limited to serologic studies (4,7,8). We report molecular evidence of human infection with RNV in a patient who sought treatment with signs and symptoms of a nonspecific febrile illness. No serum specimen from the patient was available for serologic testing for RNV. No other complete or nearly complete pathogen genomes (>50% coverage) were generated by de novo analysis in this patient sample, and RNV RNA was not detected in other tested specimens or the negative sequencing control.

Because the patient sought treatment in a rural area of Bolivia, follow-up has been challenging, and limited information is available on the patient’s epidemiologic history. Additional information is lacking on the patient’s travel history and potential exposures to mosquitoes, rodents, bats, and horses that could further characterize the potential distribution and risk factors for RNV infection in the region. The true burden of RNV as a cause of human disease in Bolivia and the region is unknown; however, because initial manifestation consists of nonspecific signs and symptoms, RNV infections could be overlooked or misdiagnosed. To bolster surveillance and diagnostic capacity for RNV and other emerging viruses, it is critical for healthcare sectors in Latin America to look beyond dengue and other common causes of acute febrile illnesses.

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Ms. Loayza Mafayle, a pharmaceutical biochemist and MSc in clinical microbiology, is head of the molecular biology laboratory at Centro Nacional de Enfermedades Tropicales in Santa Cruz de La Sierra, Bolivia. Her areas of expertise include laboratory surveillance of a variety of viruses affecting the health of Bolivian citizens, including arboviruses, hantavirus, New World arenavirus, influenza, SARS-CoV-2, and mpox.

References

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Case of Extensively Drug-Resistant Shigella sonnei Infection, United States

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We report extensively drug-resistant (XDR) Shigella sonnei infection in an immunocompromised patient in Texas, USA. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry failed to identify XDR Shigella, but whole-genome sequencing accurately characterized the strain. First-line antimicrobials are not effective against emerging XDR Shigella. Fosfomycin, carbapenems, and tigecycline are potential alternatives.

Shigella, the causative agent of shigellosis, can invade human gut mucosa and cause acute bacterial diarrhea. In the United States, antimicrobial resistant Shigella infections are frequently associated with men who have sex with men, persons experiencing homelessness, international travelers, immunocompromised persons, and persons living with HIV (1). The Infectious Diseases Society of America (https://www.idsociety.org) recommends ciprofloxacin, azithromycin, and ceftiraxone as first-line antimicrobials for shigellosis and trimethoprim/sulfamethoxazole and ampicillin as alternatives. Recently, extensively drug-resistant (XDR) Shigella species resistant to all 5 of those recommended agents have rapidly increased. XDR Shigella now accounts for 5% of all Shigella isolates in the United States (1). We describe possible challenges associated with accurately diagnosing a new, emerging strain, XDR S. sonnei, because traditional microbiologic tools may fail to identify this pathogen.

In January 2023, a man, 33 years of age, sought treatment at an emergency department (ED) for acute onset of loose stools and abdominal pain. The patient reported previous history of recurrent small bowel obstructions because of adhesions from an appendectomy. He first tested positive for HIV in January 2022 and was taking bicitracin/eritromicina/tenofovir alafenamide. His HIV viral load was undetectable, and CD4 count was 828 cells/µL at the time of admission.

In the ED, we initially treated the patient with 1 dose each of intravenous ciprofloxacin (400 mg) and oral metronidazole (500 mg), along with fluid resuscitation. Upon patient admission, we started him on piperacillin/tazobactam (4.5 mg IV every 6 h [5 doses total]) and oral vancomycin (125 mg 4×/d [4 doses total]). After PCR was negative for Clostridium difficile (Cepheid Xpert C. difficile; https://www.cepheid.com), we discontinued oral vancomycin. Enteric bacterial molecular panel (BD MAX Extended Enteric Bacterial Panel; Fisher Scientific, https://www.fishersci.com) was positive for Shigella spp. On day 2 of his hospital stay, the patient voluntarily discharged against medical advice with 7-day prescriptions for oral doxycycline and oral ciprofloxacin. Antimicrobial and biochemical susceptibility identification results (VITEK Solutions; bioMérieux; https://www.biomerieux.com) were available 1 day after discharge. During follow-up with his primary care physician 2 weeks after being hospitalized, the patient reported that all symptoms of abdominal pain and diarrhea had resolved despite ineffective antimicrobial therapy.

We isolated a non–lactose fermenter colony forming unit from the cultured fecal sample. Although MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) mass spectrometry using VITEK MS (bioMérieux) misidentified the isolate as Escherichia coli, a VITEK biochemical panel correctly identified the isolate as S. sonnei. Using bioMérieux API50 CH strips, we biochemically characterized the isolate, which we classified as S. sonnei biotype g (ONPG +, rhamnose –, xylose –) (2). Phenotypic antimicrobial susceptibility testing showed the strain was resistant to all 5 antimicrobial drugs recommended for Shigella infection (Table). The isolate was resistant to ampicillin/sulbactam, 1st generation cephalosporins, cefuroxime, cefuroxime/axetil, cefpodoxime, cefazidime, and cefepime, as well as all quinolones and tetracycline. However, that strain of XDR Shigella is susceptible to fosfomycin, carbapenems, and tigecycline, which can be used as therapeutic alternatives (Table). In spite of in vitro susceptibilities of the strain to some other antimicrobial drugs—cephalosporins, aminoglycosides, and nitrofurans—they do not penetrate the intestinal mucosa well and so are not recommended for treatment (1).

Whole-genome sequencing average nucleotide identity analysis determined the isolate was S. sonnei (98.56% identity) (3). Other closely related species had lower average nucleotide identity values: S. flexneri (98.37%), S. dysenteriae (97.94%), and E. coli NC_011601.1 (96.86%). The closest bacterial genome identified using KmerFinder was S. sonnei NZ_CP053751.1 (4). The isolate was MLST sequence type 152, the predominant S. sonnei isolate (5,6); cgMLST type was 194163 (7).

ResFinder identified putative antimicrobial resistance genes from the genome (Table (8)). Extended-spectrum β-lactamase blalam-blale5-252 was the putative resistance gene against penicillin and cephalosporins. Chromosomal mutation gyrA (p.S83L) and plasmid-encoded grrB19 were the ciprofloxacin-resistant genes of the isolate. Mph(A) was responsible for azithromycin resistance. Sul1, sul2, dfrA1, and...
dfmA17 were the putative resistance genes potentially responsible for trimethoprim/sulfamethoxazole resistance. We found virulence genes using VirulenceFinder (http://cge.cbs.dtu.dk/services/VirulenceFinder) (9). SigA in the SHI-1 pathogenicity island and iucC, iutA, shiA, and shiB in the SHI-2 pathogenicity island were present in the genome (5). Other virulence genes in the genome were anr, cia, colE7, csgA, hlyE, lpfA, nlpl, senB, sitA, terC, traT, yehA, yehB, yehC, and yehD. Whole-genome shotgun sequencing and antibiogram results and other information on this isolate are available from the National Center for Biotechnology Information BioSample database (no. SAMN34030354).

In our study, we found Shigella sonnei causing abdominal pain and diarrhea in a patient; MALDI-TOF mass spectrometry initially misidentified the pathogen as E. coli, but biochemical testing, confirmed by whole-genome sequencing, correctly identified S. sonnei. Clinicians and laboratories should be vigilant for this emerging XDR strain predominantly circulating among HIV-infected MSM (10) and aware of its resistance to all commonly recommended empiric and alternative antimicrobial drugs.

### Table. Antimicrobial MICs and putative resistance genes of Shigella sonnei strain MB23166 from a case of XDR S. sonnei infection, United States†

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC</th>
<th>Interpretation</th>
<th>Putative resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-line antimicrobial treatment†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥4</td>
<td>R</td>
<td>qnrB19, gyrA (p.S83L)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≥64</td>
<td>R</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>≥256</td>
<td>R</td>
<td>mph(A)</td>
</tr>
<tr>
<td><strong>Alternative antimicrobial treatment†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≥32</td>
<td>R</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>≥320</td>
<td>R</td>
<td>sul1, sul2, dfra1, dfra17</td>
</tr>
<tr>
<td><strong>Other antimicrobials used for the patient before identification of XDR Shigella</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole</td>
<td>≥256</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>64</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>24</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td><strong>Potential antimicrobials for XDR Shigella</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>1.5</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤0.5</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤0.25</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤0.25</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.5</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>≤16</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>4</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>4</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Cefotetan</td>
<td>≤4</td>
<td>S‡</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>≤4</td>
<td>S‡</td>
<td></td>
</tr>
<tr>
<td>Cefotizoxime</td>
<td>≤1</td>
<td>S‡</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>6</td>
<td>S‡</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤1</td>
<td>S‡</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>2</td>
<td>S‡</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>≤16</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>4</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/subactam</td>
<td>≥32</td>
<td>R</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>≥128</td>
<td>R</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>≥128</td>
<td>R</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>≥64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td>≥64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>≥64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime/axetil</td>
<td>≥64</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>≥8</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>16</td>
<td>I</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>≥64</td>
<td>R</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Cefpime</td>
<td>≥64</td>
<td>R</td>
<td>blacTX-M-27</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>≥32</td>
<td>R</td>
<td>gyrA (p.D87G), gyrA (p.S83L)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≥8</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>≥8</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>≥16</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≥16</td>
<td>R</td>
<td>tet(A)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>16</td>
<td>I</td>
<td></td>
</tr>
</tbody>
</table>

†Although susceptible in vitro, not effective clinically for Shigella species according to Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, 32nd edition (https://clsi.org).

*‡According to 2017 Infectious Diseases Society of America guidelines (https://www.idsociety.org).*
References


Online search activity has previously been shown to have some predictive power for other diseases (2). Multiple symptoms are associated with COVID-19, but “new loss of smell or taste” is highly specific (odds ratio ≈10) (3). Loss of taste is confounded because flavor occurs partly through retronasal olfaction, and most persons do not differentiate between changes in taste versus flavor. In psychophysical smell and taste tests of persons with acute COVID-19, 72% had an olfactory defect and 19% had a gustatory defect (4). Early studies in the pandemic noted a correlation between Google Trends searches for loss of smell and taste and COVID-19 cases (5,6). This correlation occurred even before anosmia was publicly recognized as a COVID-19 symptom (6), underscoring the possibility that olfactory and gustatory symptoms are useful indicators for COVID-19 surveillance.

SARS-CoV-2–induced olfactory dysfunction has been studied at the cellular level and in human trials (7). Nasal sustentacular epithelial cells adjacent to olfactory neurons have high angiotensin-converting enzyme 2 receptor levels and are a key site of virus replication. SARS-CoV-2 enters cells either by fusing at the cell surface or in endosomes (7). Those 2 pathways vary between cell and tissue types; respiratory and olfactory epithelial cells use endosomal and cell surface pathways, and cell surface pathways require activation by cell surface proteases (e.g., TMPRSS2) (7). Mutations associated with Omicron caused it to be TMPRSS2-resistant (8) and display enhanced replication in the upper respiratory tract, consistent with less severe lung disease, lower mortality rates (9), and less frequent self-reported olfactory dysfunction (10). A hypothetical correlate is that olfactory dysfunction might be a proxy for general risk for infection of lung cells at the population level. Given this potential link, we examined whether Internet searches for “loss of smell” and “loss of taste” correlate with waves of COVID-19 deaths with a lead-lag relationship, and if so, whether that correlation is maintained across different waves of COVID-19 variants.

To robustly test for a potential association, we analyzed Google Trends searches for “loss of smell” and “loss of taste” across 5 different English-speaking countries and 3 different years (2020, 2021, and 2022) and examined the correlation to reported COVID-19 hospitalizations and deaths (Figure). We retrieved weekly query frequencies for “loss of smell” (or “anosmia”) and “loss of taste” (or “ageusia”) from the Google Extended Trends API for Australia, Canada, South Africa, the United Kingdom, and the United States. Using public sources, we computed weekly COVID-19–associated mortality and hospitalization

Figure. Longitudinal association of COVID-19 hospitalization and death with online search for loss of smell or taste. A) Weekly COVID-19–associated deaths (per 100,000 population), hospitalizations (per 100,000 population), and Google search trends for ‘loss of smell’ and ‘loss of taste’ (per 10 million search sessions) in the United States during March 2020–September 2022. Vertical broken lines delimit calendar years. B) Cross-correlation between Google trends of the 2 search queries, and the 2 outcomes in 5 countries (columns) over the entire COVID-19 pandemic period of March 2020–September 2022 (top row) and disaggregated by calendar year. Statistically significant correlations (p<0.01) are indicated by a data point. Lag between paired search trend and outcome is shown in weeks.
rates for February 2020–August 2022. For each country, we computed cross-correlation between paired search trend and outcome for each week between −6 (lead) and 6 (lag) for the study period and each calendar year (Appendix, https://wwwnc.cdc.gov/EID/article/29/8/23-0071-App1.pdf).

We observed a strong correlation in the United States between deaths, hospitalization, and searches for loss of smell or taste with surprisingly similar amplitudes for all major waves (Figure, panel A), including those associated with Omicron in December 2021. Cross-correlation was high (0.68–0.85) and significant (p<0.01) across all 5 countries; the peak trend for loss of smell or taste preceded hospitalization and deaths by 2–3 weeks (Figure, panel B). This correlation was seen across all years combined and was evident for most country-year combinations. The association appeared weak in years when outcome rates were low (e.g., Australia in 2020). The analysis indicates the correlation is robust over 3 years and multiple variant waves and that loss of smell or taste might give officials a useful lead indicator of the risk for COVID-19–associated hospitalizations and deaths. However, if this finding is to be used predictively, the persistence of this association would need to be closely tracked and monitored.

Strengths of this investigation are the long-duration longitudinal analysis across multiple countries, the use of simple search criteria and variable search terms, and analysis of the temporal lead-lag relationship. Limitations include potential for bias on the basis of media news cycles, the population scale of the analysis, and socioeconomic selection bias related to internet access. Future correlations will need to be monitored. Search activity might be a more useful indicator of infection levels than COVID-19–associated deaths. Despite these caveats, this accessible metric should be considered as a public health predictor.

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D.T. declares competing interests as a founder of olfactory test company (u-Smell-it LLC) and for European Union patent (DM/212486) and pending US patents (29,743,100 and 29,750,313). J.S. and Columbia University disclose partial ownership of SK Analytics. J.S. discloses consulting for BNI.

About the Author

Dr. Toomre is a professor in the department of cell biology at Yale University School of Medicine. His research focus is the quantitative studies of exocytic traffic in living cells, including primary cilia, which are specialized cellular antennae that play roles in vision and smell.

References

Contemporary American artist Alexis Rockman was born and raised in New York City and studied animation at the Rhode Island School of Design and fine arts at the School of Visual Arts in Manhattan. His meticulously detailed paintings, which often depict ecosystems transformed by climate change, invasive species, and human activity, are found in public and private collections around the world. The Princeton University Art Museum states: “The artist’s vivid series of large canvases and intimate watercolors points to how an increasingly interconnected world has generated profound ecological change. Rockman is among the most accomplished contemporary eco-artists, having for several decades examined issues at the nexus of natural history, climate change, and biodiversity.”

About the Cover

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Those same issues mirror in large degree the central thesis of One Health, which stresses interconnections among the health of humans, animals, plants, and their shared environment. One Health issues include emerging and reemerging zoonotic diseases, neglected tropical diseases, vectorborne diseases, antimicrobial resistance, and environmental contamination. According to a 2022 analysis published in *Nature Climate Change* by Camilo Mora and other environmental scientists, humans are now more likely to come in contact with a broader range of infectious agents than ever before. Sometimes infectious diseases are spread by new or unexpected routes, including environmental sources, medical tourism, contaminated food and water from sources thought to be safe, or expansion of disease vectors into new areas.

Rockwell’s painting *Ark*, featured on this month’s cover, shows the aftermath following the capsizing of a cargo vessel laden with animals and provides a visual touchstone for contemplating the jarring impact of unexpected calamity. This disaster may have been triggered by instability from the ship’s being overloaded, collision with underwater wreckage, or rough waters spawned by a cyclone. The unnatural reddish glare of the sky contrasts with the lurid green polluted water teeming with mutated creatures, and together with a glimpse of land crusted with plastic and other debris reveal a ravished environment. Many of the animals being ferried to safer havens have been thrown into the water. Perhaps the elephant will make it to land, but others such as the moose and camels bobbing on the surface will likely drown or fall prey to the aquatic predators. Still clinging to the ship are a rhinoceros, leopard, panda, polar bear, and various other animals, alive but trapped.

*Ark* serves as a potent reminder of best laid plans gone awry and countermeasures thwarted. Its inherent metaphor is perhaps analogous to the realm of infectious diseases. Despite having improved diagnostics and treatments, effective and safe vaccines, and interventions to mitigate health threats, it is not smooth sailing for public health professionals challenged by known issues such as an underfunded infrastructure and by an unexpected rise in the spread and acceptance of misinformation as fact. Continued support for laboratory and epidemiology resources needed for ongoing surveillance of emerging and reemerging infectious disease threats of the future remain crucial to maintain strong, stable public health systems.

Note: EID has previously featured artwork by Alexis Rockman on its April 2006 and May 2009 covers.

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- Participatory Mathematical Modeling Approach for Policymaking during the First Year of the COVID-19 Crisis, Jordan
- Compliance Trajectory and Patterns of COVID-19 Preventive Measures, Japan, 2020–2022
- Interspecies Transmission of Swine Influenza A Viruses and Human Seasonal Vaccine-Mediated Protection Investigated in Ferret Model
- Genomic Characteristics of Emerging Intraerythrocytic Anaplasma capra and High Prevalence in Goats, China
- COVID-19 Epidemiology in Delta Variant Dominance Period in 45 High-Income Countries, 2020–2021
- Pharyngeal Co-Infections with Monkeypox Virus and Group A *Streptococcus*, United States, 2022
- Highly Pathogenic Avian Influenza H5N1 Clade 2.3.4.4b Virus in Wild Birds, Chile
- Rat Hepatitis E Virus in Norway Rats, Ontario, Canada, 2018–2021
- Emergence of GI.4 Sydney[P16]-like Norovirus-Associated Gastroenteritis, China, 2020–2022
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- Reoccurring *Escherichia coli* O157:H7 Strain Linked to Leafy Greens–Associated Outbreaks, 2016–2019
- Patients Characteristics During Early Transmission of SARS-CoV-2, Palau, January 13–February 24, 2022

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Article Title
Clinical Characteristics of Corynebacterium ulcerans Infection, Japan

CME Questions

1. Your patient is a 64-year-old man with clinical symptoms of diphtheria. On the basis of the case series of 34 patients in Japan from 2001 to 2020 by Yamamoto and colleagues, which one of the following statements about the demographic and clinical characteristics of patients with Corynebacterium ulcerans infection is correct?
   A. Incidence rate remained stable during the study period
   B. Mortality rate was 2%
   C. About two-thirds of C. ulcerans disease was respiratory and one-third nonrespiratory
   D. Nonrespiratory cases were primarily bloodstream infections

2. On the basis of the case series of 34 patients in Japan from 2001 to 2020 by Yamamoto and colleagues, which one of the following statements about clinical characteristics compared between patients with respiratory vs nonrespiratory symptoms of C. ulcerans infection, and among the 3 severity subgroups of patients with respiratory symptoms, is correct?
   A. Patients in the respiratory symptom group were significantly younger than those in the nonrespiratory group
   B. Cases in the respiratory symptom group were less severe than those in the nonrespiratory group
   C. The 3 respiratory subgroups did not differ significantly in C-reactive protein (CRP) levels
   D. 4 of 10 severe cases, but no mild (n=5) or moderate (n=6) respiratory cases, received diphtheria antitoxin

3. According to the case series of 34 patients in Japan from 2001 to 2020 by Yamamoto and colleagues, which one of the following statements about clinical and treatment implications of clinical characteristics, treatment-related factors, and outcomes of C. ulcerans infection is correct?
   A. To prevent spread of C. ulcerans and Corynebacterium diphtheriae, adults should be vaccinated with diphtheria toxoid vaccine
   B. All patients with moderate or severe C. ulcerans infection should receive diphtheria antitoxin
   C. C. ulcerans infection in the UK, Belgium, and Japan is primarily transmitted via livestock farming
   D. C. ulcerans infections do not need to be reported to public health agencies
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Article Title
Healthcare-Associated Infections Caused by Mycolicibacterium neoaurum

CME Questions

1. You are advising a large health maintenance organization about health care-related Mycolicibacterium neoaurum infection. On the basis of the new case report and case series of 36 previously reported episodes of M. neoaurum infection by Shapiro and colleagues, which one of the following statements about demographic and clinical characteristics of M. neoaurum infection is correct?
   A. Infections were most common in children
   B. 36 of 37 patients had serious chronic comorbidities
   C. One quarter of infections involved medical devices such as central venous catheters (CVCs)
   D. The most common infection was pneumonia

2. On the basis of the new case report and case series of 36 previously reported episodes of M. neoaurum infection by Shapiro and colleagues, which one of the following statements about diagnosis and management of M. neoaurum infection is correct?
   A. Most M. neoaurum infections were identified promptly
   B. M. neoaurum strains were resistant to multiple antimicrobial drugs
   C. One quarter of patients died
   D. 16 of 19 patients with CVC-related bacteremia had the CVC removed, with relative risk for relapse 0.083

3. According to the new case report and case series of 36 previously reported episodes of M. neoaurum infection by Shapiro and colleagues, which one of the following statements about clinical implications of demographic and clinical characteristics, diagnosis, and management of M. neoaurum infection is correct?
   A. M. neoaurum infections primarily affect immunocompromised persons
   B. Compared with most other RGM, M. neoaurum is more virulent
   C. As the population of persons with chronic medical morbidities increases, it is likely that more M. neoaurum infections will be recognized
   D. The study proved that M. neoaurum infections should be treated with combination antimicrobial therapy