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Joaquin Sorolla y Bastida (1863–1923). The Wounded Foot, 1909. Oil on canvas 43 × 39 in. /109.2 × 99.1 cm. Digital image courtesy of the Getty's Open Content Program, The J. Paul Getty Museum, Los Angeles, CA

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EMERGING INFECTIOUS DISEASES

June 2015

On the Cover

Joaquin Sorolla y Bastida 1863–1923
***The Wounded Foot*, 1909**

Oil on canvas 126.4 x 116.5 x 7.6 cm (49 3/4 x 45 7/8 x 3 in.)

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About the Cover p. 1094

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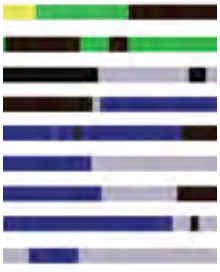
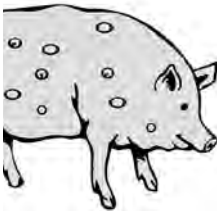


p. 930

p. 970



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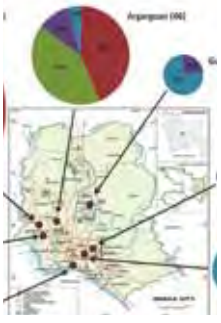

p. 1028

p. 1032

Letters

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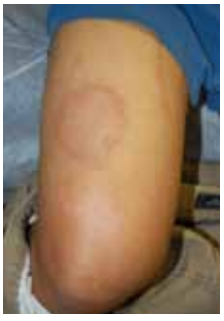

p. 1077

Book Review

About the Cover

Etymologia

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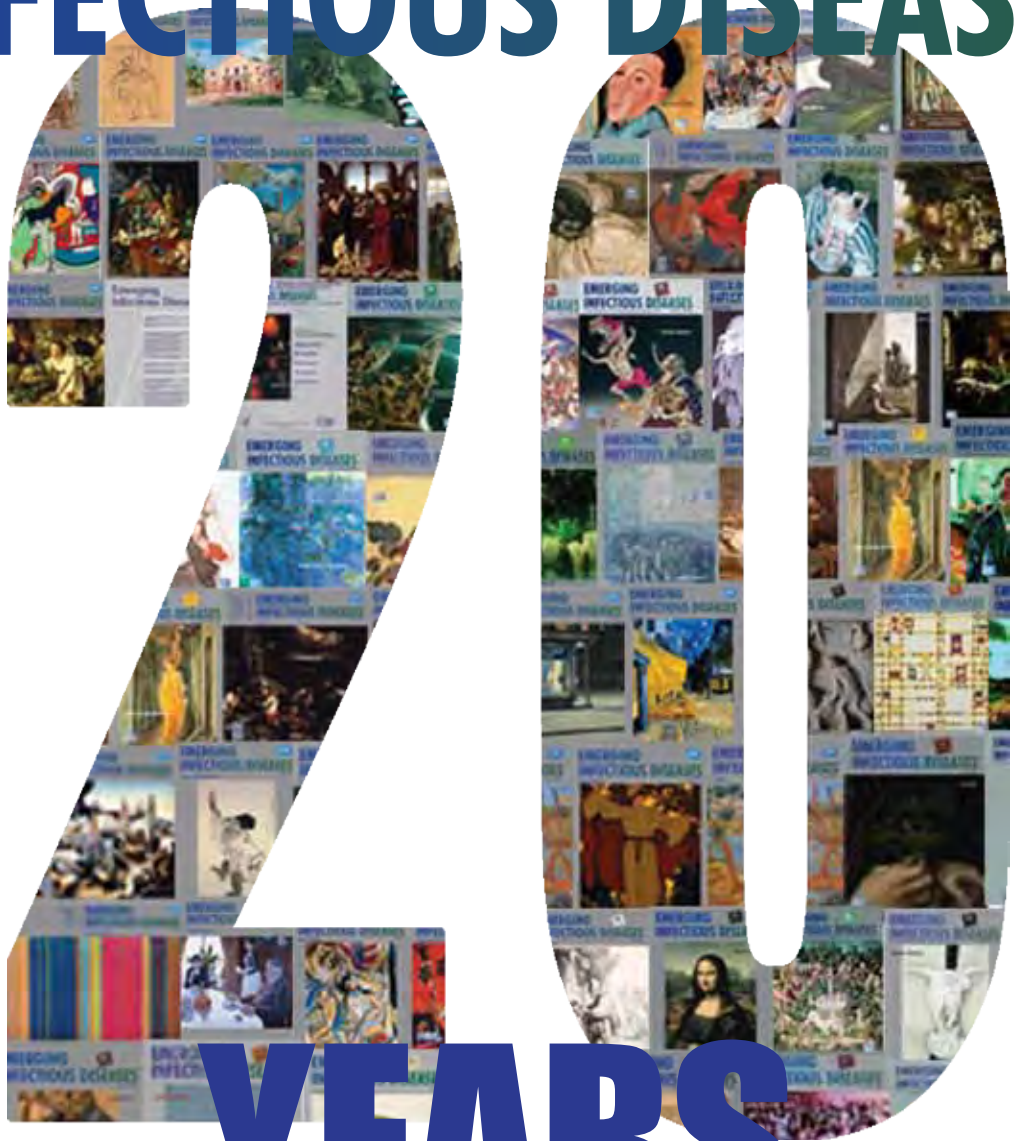

20 YEARS

Presenting the ongoing challenges that emerging microbial threats pose to global health

Sequence Type 4821 Clonal Complex Serogroup B *Neisseria meningitidis* in China, 1978–2013

Bingqing Zhu, Zheng Xu, Pengcheng Du, Li Xu, Xiaofang Sun, Yuan Gao, Zhujun Shao

Serogroup B *Neisseria meningitidis* strains belonging to sequence type 4821 clonal complex (CC4821), a hyperinvasive lineage first identified for serogroup C in 2003, have been increasingly isolated in China. We characterized the outer membrane protein genes of 48 serogroup B and 214 serogroup C strains belonging to CC4821 and analyzed the genomic sequences of 22 strains. Four serogroup B strains had porin A (i.e., PorA), PorB, and ferric enterobactin transport (i.e., FetA) genotypes identical to those for serogroup C. Phylogenetic analysis of the genomic sequences showed that the 22 CC4821 strains from patients and healthy carriers were unevenly clustered into 2 closely related groups; each group contained serogroup B and C strains. Serogroup B strains appeared variable at the capsule locus, and several recombination events had occurred at uncertain breakpoints. These findings suggest that CC4821 serogroup C *N. meningitidis* is the probable origin of highly pathogenic CC4821 serogroup B strains.

Neisseria meningitidis bacteria are a leading cause of bacterial meningitis and other serious invasive bacterial infections. Among the 12 identified serogroups, A, B, C, Y, W, and X are responsible for most invasive meningococcal diseases. The geographic distribution and epidemic capabilities of *N. meningitidis* differ according to serogroup (*1*). On the basis of the epidemiology of *N. meningitidis*, many countries have included different formulations of the meningococcal vaccine in their routine immunization programs (*2*–*4*). These vaccines have significantly reduced the incidence of meningococcal diseases (*5*,*6*). However, in several countries, the introduction of vaccines targeting specific serogroups may have led to the replacement of vaccine serogroups by other, nonvaccine, serogroups (*7*–*11*). Serogroup replacement can occur as a result of capsule switching (*3*,*8*,*12*–*19*) or as a result of importation of a serogroup meningococcus from other regions (*20*).

In the past century in China, most meningococcal epidemics were caused by strains of *N. meningitidis* that belonged to sequence type 1 (ST-1) and ST-5 clonal complexes (CC1 and CC5, respectively) (*21*). Therefore, beginning in the early 1980s, a polysaccharide vaccine against serogroup A was incorporated into the routine immunization program. Use of this vaccine led to a significant decrease in the incidence of meningococcal diseases (*22*). However, in 2003, a serogroup C outbreak caused by a CC4821 strain was reported in China; this clonal lineage had not been detected in other countries (*23*). CC4821 corresponding to serogroup C has subsequently become one of the dominant lineages in China (*21*). To combat this serogroup replacement, several vaccines were developed against serogroup C or serogroups A and C. During 2005–2010, subsequent to the time when serogroup C and A *N. meningitidis* infections had been prevalent, cases caused by serogroup W strains belonging to CC11 began increasing in China (*24*,*25*). In addition to these 3 serogroups, serogroup B strains have been isolated from patients and healthy carriers. Serogroup B strains showed high genetic diversity and were usually associated with sporadic infections (*21*). Some of the prevalent clonal lineages that are common in many countries (e.g., CC32 and CC41/44) (*26*) are rarely isolated in China (*21*). However, CC4821 became a dominant lineage among serogroup B strains since they were first identified in 2005. When we retrospectively studied the strains in our collection, CC4821 strains were isolated as early as in 1978, and the lineage included serogroup B and C strains (*27*). Nevertheless, few CC4821 strains were isolated during 1970–1980, and no CC4821-related outbreaks were identified during that time (Z. Shao, unpub. data).

Capsular switching between *N. meningitidis* serogroups B and C is frequently observed (*3*,*7*,*8*,*12*,*16*–*19*); therefore, serogroups B and C most likely have similar DNA sequences in the capsule locus, leading to increased horizontal DNA transfer between these serogroups (*16*). We propose that capsular switching occurred between the

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CC4821 serogroup B and C *N. meningitidis* strains. To elucidate the relationship between them, we investigated the epidemiology of CC4821 serogroup B strains, characterized the outer membrane protein (OMP) genes of these strains, and analyzed the genome sequences and capsule locus sequences of specific strains.

Materials and Methods

Meningococcal Meningitis Surveillance in China

A population-based surveillance system for meningococcal meningitis exists throughout China. Provincial Center for Disease Control and Prevention (CDC) staff routinely collect strains suspected to be *N. meningitidis* on the basis of morphologic and biochemical characteristics, and they periodically conduct surveys of *N. meningitidis* carriers for outbreak investigation, surveillance, and research purposes. If no strain is isolated, clinical specimens are collected by the provincial CDC. The strains and specimens are sent to the China CDC national reference laboratory for identification, or they are tested at the provincial CDC, and results are sent to China CDC. Our laboratory identifies strains and performs multilocus sequence typing (MLST) on confirmed *N. meningitidis* strains.

Meningococcal Strains and DNA Preparation

Forty-eight serogroup B and 214 serogroup C *N. meningitidis* strains previously assigned to CC4821 were included in this study. These strains were collected from 20 provinces in China during 1978–2013. Among the 48 serogroup B strains, 9 were from patients and 39 were from asymptomatic carriers. Among the 214 serogroup C strains, 91 were from patients and 123 were from asymptomatic carriers. One strain was identified as serogroup B by PCR; serogroups for the other strains were determined by slide agglutination with specific rabbit antisera (Remel Europe Ltd, Kent, UK) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/6/14-0687-Techapp1.pdf).

The selected strains were propagated on single plates containing Columbia agar in a 5% CO_2 atmosphere at 37°C for 18 h. Genomic DNA was extracted by using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Sequencing of OMP Genes

The porin A (*porA*), *porB*, and ferric enterobactin transport (*fetA*) genes were amplified from freshly prepared DNA. The PCR and sequencing were performed as previously described (*28*–*30*).

Genome Sequences of Meningococcal Strains

Eight serogroup B and 14 serogroup C *N. meningitidis* CC4821 strains were sequenced by constructing 2 paired-end libraries with average insert lengths of 500 bp. The sequences were generated by using an Illumina HiSeq 2000 sequencing platform (Illumina, San Diego, CA, USA) and assembled into contigs and scaffolds by using SOAP*denovo*, release 1.04 (http://soap.genomics.org.cn/soapdenovo.html). Genes were predicted by using Glimmer (*31*) with default parameters and then annotated by sequence comparisons with nucleotide and nonredundant protein sequence databases and the SwissProt (http://web.expasy.org/docs/swiss-prot_guideline.html) database by using BLAST (http://blast.ncbi.nlm.nih.gov) with an e-value of 1e–5. The genome sequence data obtained in this study were submitted to GenBank under the accession numbers JMBH00000000, JMCN00000000–JMCZ00000000, JMDA00000000–JMDH00000000. The complete reference genome sequences of multiple *N. meningitidis* strains and 1 *N. lactamica* strain were downloaded from the Completed Genomic Sequence section of the publicly available Entrez Genome database (http://www.ncbi.nlm.nih.gov/genomes/static/EG_T.html).

Phylogenetic Analysis of *N. meningitidis* Genome Sequences

All 22 CC4821 genomes and 14 reference *N. meningitidis* genomes were used to construct a phylogenetic tree, and the genome of *N. lactamica* strain 020-06 was used as the outgroup. We identified the core genes in these 37 genomes in 2 steps. First, we used OrthoMCL (http://orthomcl.org/orthomcl/) to cluster all genes into orthologous groups and then selected the groups that were shared by all 37 genomes. Second, we removed the orthologous groups associated with known mobile genetic elements, such as genomic islands, phages, and transposons. The remaining orthologous groups were considered to be core genes. For all 37 genomes, the amino acid sequences of the core genes were concatenated, and multiple sequence alignments were performed by using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/). We then constructed a phylogenetic tree using the neighbor-joining method with MEGA4 (http://www.megasoftware.net/).

Identification and Analysis of Capsule Locus

The contigs containing capsule locus sequences were compared with the *N. meningitidis* complete reference genome sequences by using blastn (http://blast.ncbi.nlm.nih.gov) to identify orthologous sequences and determine their levels of similarity. For contigs with a gap within the UDP-glucose 4-epimerase (*galE*) gene, PCR and Sanger sequencing were performed to close the gap. The capsule locus between genes transcriptional accessory protein (*tex*) and *galE* of CC4821 serogroup B strains were then aligned with the corresponding sequences from all serogroup B complete genomes by using MUSCLE version 3.6. On the

basis of the alignment results, we used the genome with the highest level of similarity with CC4821 serogroup B strains as the reference sequence in subsequent analyses. To reveal the relationship between CC4821 serogroups B and C, we aligned the capsule locus genes from all study strains with those of serogroup C isolate 053442 (ST-4821) and the selected serogroup B reference strain.

Identification of Recombination Breakpoints

We compared the capsule locus between genes *tex* and *galE* from study and reference strains by using MUSCLE. We analyzed recombination events within the capsule locus sequences by using different methods in RDP (Recombination Detection Program) version 4 beta 27 (http://web.cbio.uct.ac.za/~darren/rdp.html) with default parameters.

Results

Epidemiology of CC4821 Serogroup B Strains

During the 1970s and 1980s, a total of 12 CC4821 serogroup B and C strains (6 from each serogroup) were collected from 6 provinces in China: Hebei, Henan, Jiangxi, Liaoning, Shanxi, and Shanghai. During March 2005–March 2013, *N. meningitidis* strains belonging to CC4821 serogroup B were isolated from the cerebrospinal fluid or blood samples of meningococcal patients in 10 provinces and from pharyngeal swab specimens from healthy carriers in 9 other provinces in China. These 19 provinces represent diverse geographic and climate conditions, and the cases were not related. (Figure 1)

OMP Genotype Profiles

The *porA* gene was sequenced for all studied strains. The genotype profiles revealed a high degree of diversity even among strains with identical STs. Serogroup B *N. meningitidis* strains had 26 PorA genotypes, and serogroup C strains had 16. Among these, 8 genotypes were detected among both serogroup B and C strains, representing 35.4% of serogroup B and 77.6% of serogroup C strains. P1.7-2, 14 was the predominant genotype in serogroups C (55.6%) and B (12.5%). Two combinations of ST and PorA genotype were observed in serogroup C and B strains: ST-4821: P1.7-2, 14 and ST-4821: P1.20, 23-1. In total, 4 serogroup B strains had combination genotypes that were the same as those for serogroup C strains (online Technical Appendix Table 2). To elucidate the relationship between the serogroup B and C strains, we sequenced the *porB* and *fetA* genes for strains with the combination genotype ST-4821: P1.7-2, 14 or ST-4821: P1.20, 23-1. The sequencing analysis showed that all 4 serogroup B strains had identical PorB and FetA genotypes (3-48 and F3-3, respectively), which were also the main PorB and FetA genotypes in the serogroup C strains.

Phylogenetic Analysis of Genome Sequences

A total of 1,200 core genes (385,358 aa, corresponding to 50.9% of the genome of serogroup B *N. meningitidis* strain MC58) were selected for the phylogenetic analysis as described in Materials and Methods. The neighbor-joining phylogenetic tree reconstructed from the concatenated amino acid sequences of these genes showed that the 22 CC4821 strains were clustered into 2 closely related groups (groups I and II), which were distantly related to the *N. meningitidis* reference strains belonging to other CCs (Figure 2). Both groups contained serogroup B and serogroup C strains, although there were more serogroup C strains in group I and more serogroup B strains in group II. Each group consisted of 11 strains, but there were more invasive strains in group I (n = 9) than in group II (n = 4).

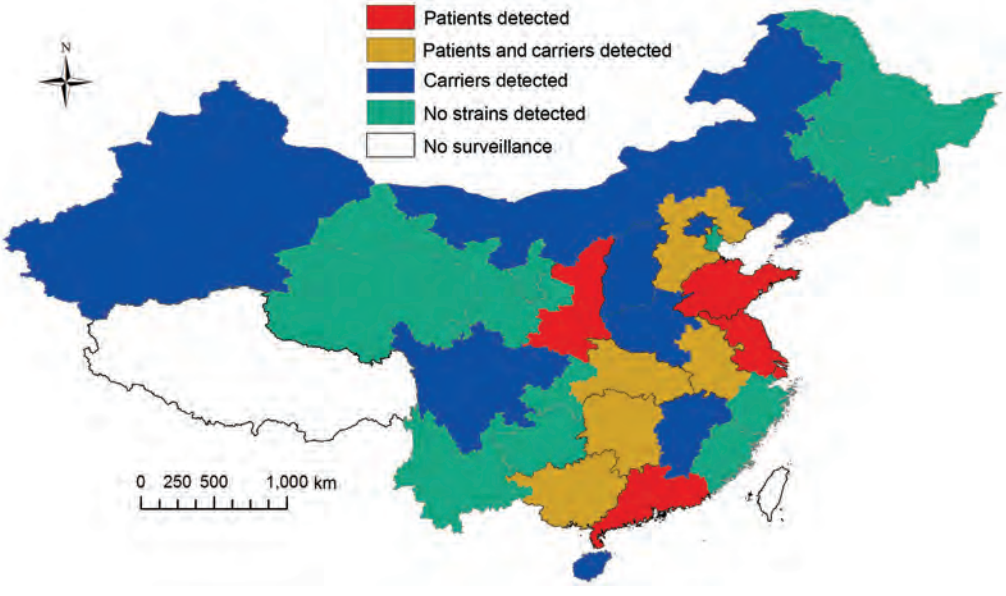

Figure 1. Distribution of *Neisseria meningitidis* sequence type 4821 clonal complex (CC4821) serogroup B strains in China, 1978–2013. Invasive strains were detected in 5 provinces (red), carriage strains were detected in 9 provinces (blue), and invasive and carriage strains were detected in 5 provinces (gold). Regions where CC4821 strains were not found or where surveillance is not conducted are also shown.

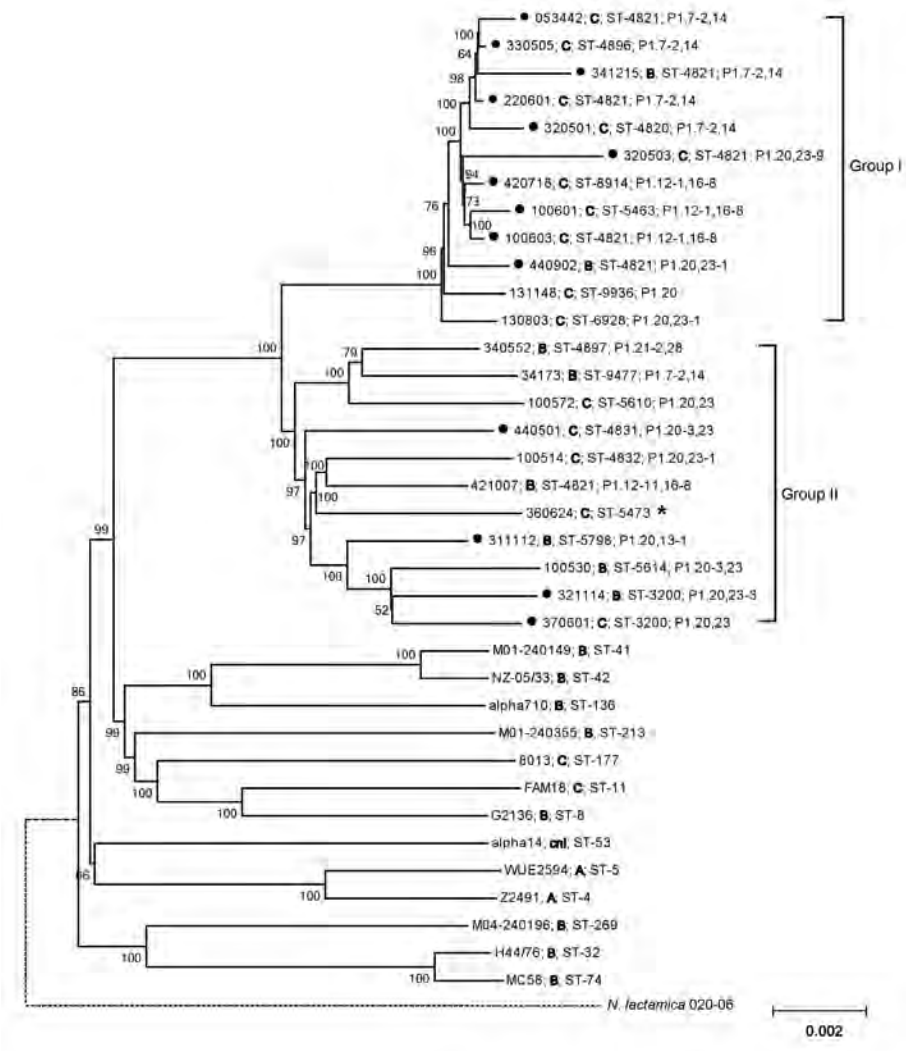

Figure 2. Phylogenetic analysis of genome sequences for *Neisseria meningitidis* strains. With the exception of reference strain 053442 (serogroup C, sequence type 4821), all strains in groups I and II were sequenced in this study. The strain identification number, serogroup (in boldface), sequence type, and porin A type are shown for each sequence. Bootstrap values are listed at nodes. Black dots preceding identification numbers indicate strains isolated from patients. The dotted line between *N. lactamica* 020-06 and *N. meningitidis* represents a distance not to scale. The star indicates that the porin A gene was not detected by PCR or genome sequencing. Scale bar indicates amino acid substitutions per site.

The reference strain 053442, which was isolated from a patient and belonged to CC4821, was clustered with group I. Considering the genetic distances between the strains, the strains in group II were less closely related to each other than those in group I.

Analysis of Capsular Locus

The capsule locus sequences between genes *tex* and *galE* were retained from 20 sequenced CC4821 genomes (12 serogroup C and 8 serogroup B). For 2 strains, there were gaps within *galE* or *ctrA* gene.

The DNA sequences of the capsular locus were compared with the homologous gene clusters of the reference strains (053442, serogroup C, ST-4821; H44-76, serogroup B, ST-32) (Figure 3). All capsular locus genes of 9 serogroup C strains (group I) were 99.9%–100% identical to that for reference strain 053442. The other 3 serogroup C strains (group II) were less similar to 053442 at the genes upstream of polysialic acid capsule export outer-membrane lipoprotein (*ctrA*) and downstream of sialic acid synthase (*siaC*). All serogroup B strains had polysialyltransferase (*siaD*) genes that were distinct from that for reference strain 053442 and did not contain the O-acetyltransferase (*oatC*) gene between genes *siaD* and open-reading frame 2. With the exception of 1 serogroup B strain (341215, in group I), which shared high similarity (99.8%–100%) with 053442 at the genes upstream of *siaD*, the serogroup B strains had low similarity at *ctrC* and preceding genes. Serogroup B and C strains shared <99% similarity with strain H44-76 at genes *tex*, *ctrD*, *ctrC*, *siaA*, *open-reading frame 2*, and *galE*. However, *siaD* genes of the serogroup B strains and H44-76 shared ≥99.7% similarity.

We used RDP, GENECONV (http://www.math.wustl.edu/~sawyer/geneconv/), BootScan (*32*), and the 3seq method (*33*) to analyze the recombination events within the capsule locus, but failed to obtain consistent results about the breakpoint. Nevertheless, all the results indicated that different and multiple events had occurred at the

capsule locus within and among strains (Figure 4). Compared with strains belonging to group II, those belonging to group I (genome-based phylogenetic tree) had fewer recombination events, and most of the events were within the same serogroup.

Discussion

Phylogenetic analysis of the *N. meningitidis* core genome amino acid sequences showed that all 22 sequenced CC4821 strains were closely related, irrespective of serogroup, ST, and PorA type, which indicated capsular switching between serogroups C and B. However, serogroups B and C were detected in both phylogenetic groups, so the direction of capsule switching remains to be determined (*34*). The multiple recombination events may have occurred within the capsule locus, which explains why we could not define the recombination breakpoints at the capsule locus. The present evidence is not sufficient to confirm whether capsular switching occurred from serogroup C to B or vice versa. Further study is required to examine how the capsular switching occurred.

In previous studies of *N. meningitidis* capsule switching, MLST and OMP genotyping were used to characterize the relationship between the new variants and the candidate

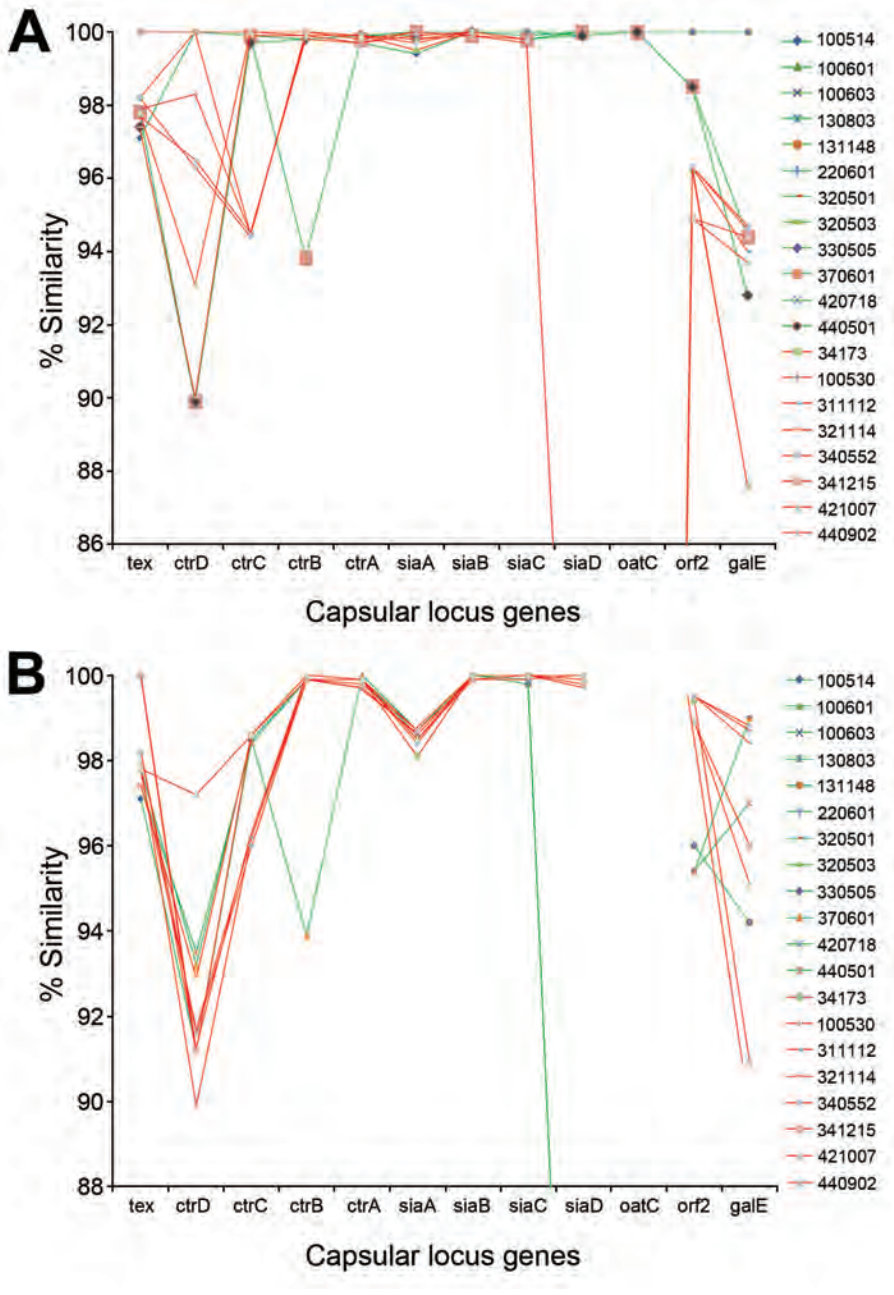

Figure 3. Analysis of capsular locus sequences from *Neisseria meningitidis* strains belonging to the sequence type 4821 clonal complex (indicated on right). A) Similarity between the capsular locus genes of study strains and those from reference strain 053442 (serogroup C). B) Similarity between the capsular locus genes of study strains and those from reference strain H44-76 (serogroup B). Green lines indicate serogroup C strains; red lines indicate serogroup B strains.

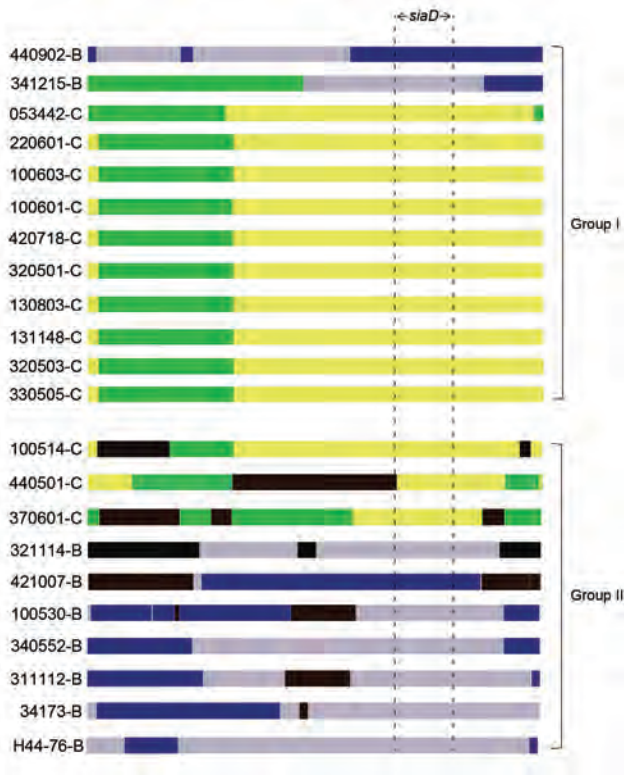

Figure 4. Analysis of the recombination events *Neisseria meningitidis* strains belonging to the sequence type 4821 clonal complex (strain numbers and serogroup are shown on the left). The result was from analysis using the 3seq (*33*) method in RDP (http://web.cbio.uct.ac.za/~darren/rdp.html). Group I and group II refer to the groups in Figure 2. Green regions represent serogroup C–specific sequences; yellow regions represent the recombination within serogroup C; gray regions represent serogroup B–specific sequences; blue regions represent the recombination within serogroup B; black regions represent the recombination between serogroup B and C. Location of the polysialyltransferase gene (*siaD*) is shown.

parental organisms (*3*,*7*,*8*,*16*,*18*,*19*). If the molecular profiles (e.g., ST, PorA, and FetA) are identical or highly similar between 2 different serogroups, capsule switching is inferred. In our study, only 4 ST-4821 serogroup B *N. meningitidis* strains were observed to share the same STs and OMP gene types (PorA, PorB, and FetA) with serogroup C *N. meningitidis*. However, analysis of the core genome sequencing results showed that all serogroup B strains, even those with specific profiles, are closely related to serogroup C strains. Horizontal DNA transfer occurs frequently between *N. meningitidis* strains. Recombination events involving the MLST locus and the *porA*, *porB*, and *fetA* genes would impede the analysis of capsule switching and might lead to false conclusions (*15*). An analysis based on genomic sequence can reveal the relationships among strains more accurately, so additional genome analysis is required to resolve the observed discrepancies.

CC4821 serogroup C *N. meningitidis* had been the dominant lineage in China for a decade (2003–2014), although the incidence of invasive disease remained at a moderate level (<0.1 case/100,000 population; Z. Shao, unpub. data) because of mass vaccination. The emergence and circulation of CC4821 serogroup B *N. meningitidis* might increase the incidence of invasive meningococcal diseases and even cause epidemics and outbreaks in China. This potential risk can be attributed to the CC4821 lineage itself and to the particularity of vaccine against serogroup B *N. meningitidis*. Since the first outbreak occurred in 2003, the CC4821 serogroup C *N. meningitidis* epidemic has rapidly involved most provinces of China. This epidemic indicates that CC4821 *N. meningitidis* had substantial ability to spread extensively and cause invasive disease. The core genome–based phylogenetic analysis showed that CC4821 strains from patients and healthy carriers were unevenly clustered into 2 groups, suggesting a difference in pathogenicity between these 2 groups of strains. Furthermore, group I, which possessed more patient-derived strains, was the most recent clade on the tree, and the strains in this group were more closely related to each other than those in group II, indicating that group I was a highly invasive sublineage of CC4821. Because of the emergence of serogroup B strains belonging to this highly invasive *N. meningitidis* CC4821 sublineage, we must remain alert for a potential epidemic. An effective protective vaccine specifically against the serogroup B capsule polysaccharide does not exist (*35*). Although several vaccines consisting of specific proteins have been licensed for use against serogroup B infection (*36*), their effectiveness varies by clonal lineage, and their effectiveness has not been studied in China. This critical public health concern highlights the need for further epidemiologic surveillance to monitor changes in the incidence of meningococcal disease caused by *N. meningitidis* CC4821 serogroup B and for improved public health disease control strategies in the future.

Circulation of the CC4821 clonal lineage has not been observed in other regions, even though it is hyperendemic in China. The reason for this limited distribution is not readily apparent, which highlights the need for continued surveillance. The virulence and pathogenic mechanisms of this newly identified hyperinvasive lineage are not well understood (*23*). Comparative genome analysis within CC4821 strains and those from other CCs may help to identify potential additional virulence factors of *N. meningitidis*. In China, mass vaccination against meningococcal disease targets only *N. meningitidis* serogroups A and C. Thus, monitoring the appearance and spread of CC4821 in other serogroups is important because capsule switching among *N. meningitidis* serogroups C, B, W, and Y has been observed in several countries (*14*,*16*).

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Dr. Zhu is a research scientist at the State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. Her primary research interest is the variation and prevalence of *N. meningitidis* bacteria.

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Estimated Deaths and Illnesses Averted During Fungal Meningitis Outbreak Associated with Contaminated Steroid Injections, United States, 2012–2013

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Learning Objectives

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

- Describe the 2012–2013 outbreak of fungal meningitis linked with contaminated steroid injections and the effects of the public health response on preventing contaminated injection administration, cases, and deaths, based on a report by the US Centers for Disease Control and Prevention
- Discuss the nature of the public health response to the 2012–2013 outbreak of fungal meningitis linked with contaminated steroid injections
- Identify factors associated with meningitis and stroke during the 2012–2013 outbreak of fungal meningitis linked with contaminated steroid injections

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During 2012–2013, the US Centers for Disease Control and Prevention and partners responded to a multistate outbreak of fungal infections linked to methylprednisolone acetate (MPA) injections produced by a compounding pharmacy. We evaluated the effects of public health actions on the scope of this outbreak. A comparison of 60-day case-fatality rates and clinical characteristics of patients given a diagnosis on or before October 4, the date the outbreak was widely publicized, with those of patients given a diagnosis after October 4 showed that an estimated 3,150 MPA injections, 153 cases of meningitis or stroke, and 124 deaths were averted. Compared with diagnosis after October 4, diagnosis on or before October 4 was significantly associated with a higher 60-day case-fatality rate (28% vs. 5%; p<0.0001). Aggressive public health action resulted in a substantially reduced estimated number of persons affected by this outbreak and improved survival of affected patients.

In September 2012, the US Centers for Disease Control and Prevention (CDC), in collaboration with state and local health departments, initiated a multistate investigation into an outbreak of fungal infections linked to injections of preservative-free methylprednisolone acetate (MPA) produced at a single compounding pharmacy (New England Compounding Center [NECC], Framingham, MA, USA) (*1*,*2*). Three lots of MPA produced by NECC were implicated in this outbreak: 05212012@68, 06292012@26, and 08102012@51 (hereafter called lots 05, 06, and 08, respectively) (*2*). The Mycotic Diseases Branch Laboratory at CDC confirmed the presence of *Exserohilum rostratum,* an environmental mold and the primary pathogen in this outbreak, in lots 08 and 06 (*3*); detection of *E. rostratum* DNA in lot 05 was reported to CDC (*3*). This contamination resulted in one of the largest outbreaks of health care–associated infections and the largest outbreak of fungal meningitis documented in the United States. Thousands of public health officials at federal, state, and local levels, along with clinicians and administrative staff, worked over a period of many months to respond to this unprecedented outbreak.

CDC and partners quickly took action for several reasons: the high mortality rate seen in previous outbreaks of fungal meningitis (*4*,*5*), concern that subacute clinical signs and symptoms would not prompt exposed persons to seek health care evaluation until they had severe disease, and the large number of persons potentially exposed to contaminated MPA. Several key public health actions took place during the 10-day period of September 25–October 4, 2012. On September 25, NECC was informed that 3 MPA lots from its pharmacy appeared to be implicated in this outbreak. The next day, NECC issued a voluntary recall of the 3 lots. On September 28, CDC and state partners initiated efforts to notify all 13,534 persons potentially exposed to the implicated MPA to provide information on exposure and indications for seeking medical care.

CDC and partners developed diagnostic and treatment guidelines on the basis of expert opinion and incoming laboratory and patient data. These guidelines and subsequent updates were posted on CDC's outbreak website (*6*) and disseminated through Health Advisory notices. A Health Advisory notice containing the first diagnostic and treatment guidance for this new clinical entity was distributed on October 3 (*7*) and was posted online on October 4. On that day, a joint Food and Drug Administration and CDC telebriefing (by telephone) publicized the outbreak and confirmed the presence of fungal contamination in unopened vials from lot 08 MPA (*8*). We conducted analyses to estimate the effect of these public health actions on the size and severity of this outbreak as measured by changes in the numbers of case-patients and deaths.

Methods

Overview of Modeling Used to Assess Effects of Public Health Actions

We restricted our analyses to patients with meningitis, including those with stroke caused by presumed meningitis. Cases of parameningeal (e.g., epidural abscess) or peripheral joint infection were not included in this analysis. To evaluate the effect of public health actions on the size of this outbreak, we sought to determine numbers of 1) MPA injections averted, 2) cases of meningitis or stroke averted, and 3) deaths caused by meningitis or stroke averted. Information about volume of MPA recalled was unavailable, so we estimated the recalled volume from data in NECC MPA shipping records. To estimate cases averted, we calculated attack rates in 2 steps. First, we modeled attack rates for patients with single injections. For patients with multiple injections, we conducted a Monte Carlo simulation that assigned a single responsible injection for each patient in each simulation. This simulation also provided estimates of uncertainty. To estimate deaths averted, we applied the case-fatality rate (CFR) for case-patients before most public health actions had occurred for the case-population at risk (i.e., number of case-patients diagnosed after October 4 and still alive 60 days later and estimated number of case-patients predicted to have occurred after October 4 if no recall had occurred).

Case Definition

Case definitions developed during the outbreak response were used in this analysis (*9*). A case-patient was defined as a person who, after May 21, 2012, received an epidural or paraspinal MPA injection from an implicated lot and subsequently had either meningitis of unknown etiology or posterior circulation stroke without cardioembolic source

and without a documented normal cerebrospinal fluid (CSF) profile. Meningitis was defined as >5 leukocytes/mm^3 in CSF from a person with compatible symptoms (e.g., headache, stiff neck). Date of diagnosis was defined as the date when an initial lumbar puncture yielded CSF that met the meningitis case definition or the reported date of stroke diagnosis for stroke cases. Cases were defined as laboratory confirmed if histopathologic, microscopic, culture, or molecular evidence of a fungal pathogen was present and associated with the clinical syndrome. Inclusion in this analysis was limited to cases reported on or before the final case count published on October 23, 2013.

MPA Injections Averted

NECC shipping records provided information on date of shipment, volume, and lot number of MPA shipped to each of the 75 injection facilities that received contaminated lots of MPA. To estimate volume of steroid recalled, we modeled the MPA use rate at each injection facility. For the 52 facilities that received >1 shipment of implicated MPA, we estimated a per-facility MPA use rate on the basis of reorder frequency and shipment volumes. This approach could not be used for 23 facilities that had a single shipment (i.e., they placed no reorders). For those facilities, we developed a simple linear regression model to estimate use rate with average volume of MPA as the predictor (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/6/14-1558-Techapp1.pdf). We assumed that use of NECC MPA at each injection facility began on the first business day after a shipment date, that use occurred at the facility only on business days, and that use ceased completely on September 27, 2012. These methods enabled us to estimate weekly volume of NECC MPA used across all 75 clinics.

To estimate the volume recalled, we subtracted the total volume of estimated use before September 27 from the total volume shipped. To estimate the number of MPA exposures potentially averted, we divided the total volume of MPA recalled by 1 mL/injection volume (i.e., amount of MPA used per injection for 80% of patients).

Cases of Meningitis or Stroke Averted

Previously published data suggested that attack rates varied by lot number and age of the vial, with age being measured as length of time from vial production to injection date (*1*). Consequently, we anticipated that we would need to estimate separate attack rates for each of the 3 implicated MPA lots to account for vial age. To account for changes in attack rates by vial age, we calculated the attack rate for each calendar week during which injections took place and produced separate estimates for each lot of MPA. For case-patients with 1 injection, weekly lot-specific attack rates were calculated as follows: number of case-patients receiving an injection of lot X during a given week divided by estimated volume of lot X used that week (denominator was obtained from the MPA use rate analysis). A Poisson distribution was then fitted to the observed data to estimate the attack rate for each week during which injections were given (online Technical Appendix). The model's covariates were week of injection, lot number, and interaction between *week* and *lot* variables, selected because these variables showed evidence of modifying results.

Next, for case-patients with >1 injection, we developed a probabilistic model that used a Monte Carlo simulation to randomly assign a single injection as responsible for each case-patient's infection (online Technical Appendix). A total of 100 simulations were performed; for each simulation, the probability of injection assignment was derived from the predicted weekly lot-specific attack rates among case-patients with only 1 injection. A Poisson regression model, using the same covariates as those used in the model for case-patients with 1 injection, was then fit to the 100 simulated datasets to produce a distribution of predicted number of cases (online Technical Appendix). To capture additional uncertainty, 100 additional simulations of the predicted number of cases were generated so that 10,000 estimates of the predicted number of cases were generated for each week and lot combination.

From this distribution, we then summed the median number of cases predicted for each week and lot combination after the recall date to obtain an estimated total number of cases averted. Finally, 95% CIs were calculated to capture uncertainty in our estimates (online Technical Appendix). Extrapolation of weekly attack rates for lots 05 and 06 were continued until the model predicted that the volume of MPA in those lots had been completely used (Figure 1). However, lot 08 was in use for only 5 weeks before the recall on September 26, 2012. Thus, for this lot, we limited the extrapolation of attack rates and cases averted to 3 weeks after the recall (until October 14, 2012).

Deaths and Strokes Due to Meningitis Averted

To estimate the number of deaths averted, we first calculated the 60-day CFR for patients given a diagnosis early in the outbreak (on or before October 4), before widespread publicity and posting of clinical treatment guidelines. This 60-day CFR was then applied to 2 groups of case-patients to estimate deaths averted: 1) patients given a diagnosis after October 4 who were still alive 60 days after diagnosis and 2) averted cases predicted to have occurred after October 4 without a recall.

Evaluating Changes in Number of Deaths During the Outbreak

The 60-day CFR was plotted by week of diagnosis to examine mortality trends during the course of the outbreak.

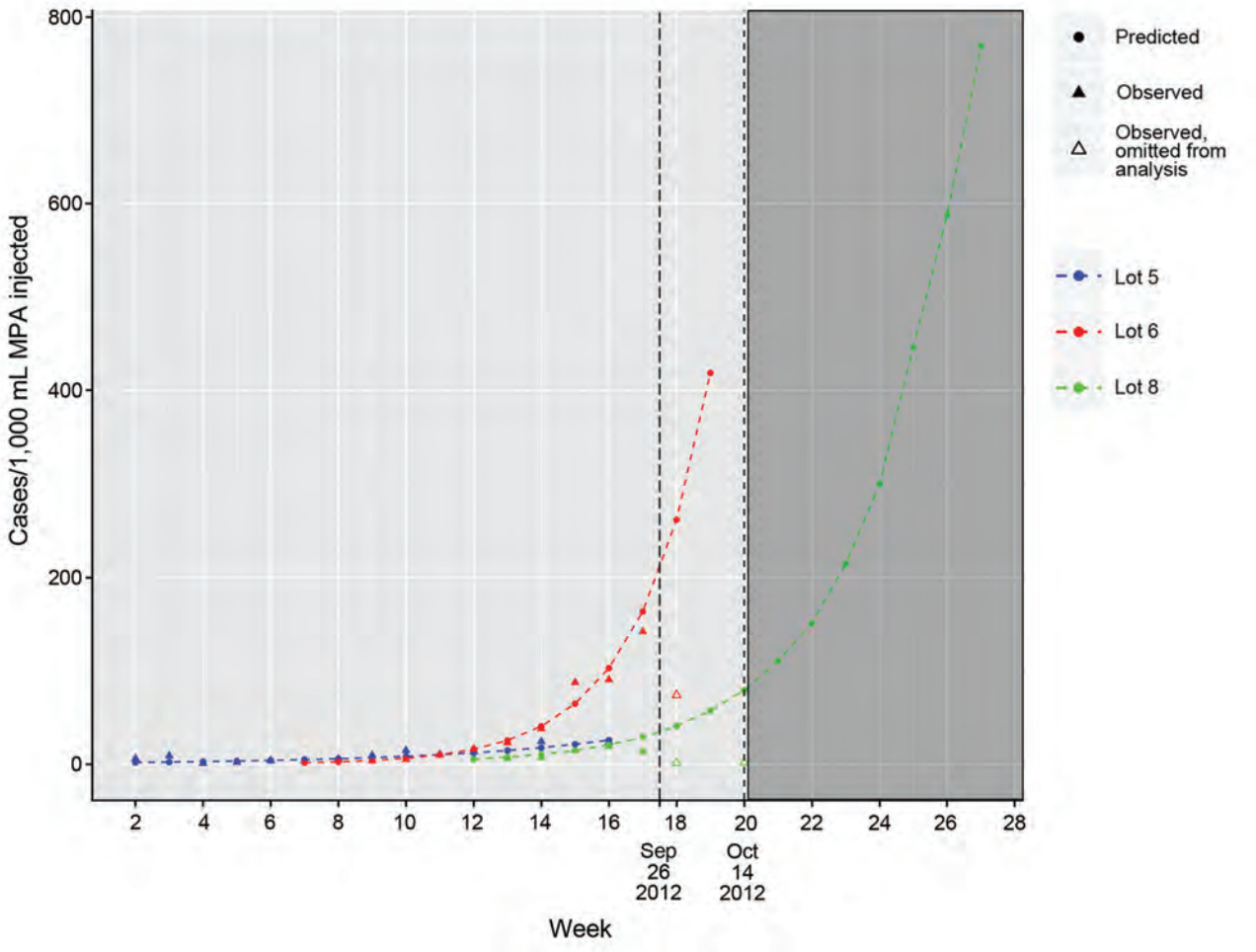

Figure 1. Weekly lot-specific observed and predicted attack rates (cases/1,000 mL injected) for illness among persons injected from 3 lots of methylprednisolone acetate (MPA) contaminated with the fungus *Exserohilum rostratum*, United States, 2012. Observed rates obtained by combining single injection data and 1 representative multiple injection simulated dataset (observed rates for multiple injections varied by simulation). Observations after September 23, 2012, were excluded in the prediction model. Long-dashed line indicates the date of the recall of the 3 lots of MPA (September 26, 2012); short-dashed line indicates the boundary of reliable estimates of attack rates in our model (October 14, 2012).

To understand whether these mortality trends were associated with changes in case-patient clinical characteristics, we compared measures of disease severity and treatment rapidity for case-patients given a diagnosis on or before October 4, 2012 to those given a diagnosis after that date. A χ^2 test (dichotomous variables) and the Wilcoxon rank-sum (continuous variables) test were used. We estimated the survival function of time from diagnosis to death by using a Kaplan-Meier estimator. The log-rank test was used to compare survival of the 2 groups.

Data on case-patient clinical characteristics were collected from a standardized case report form created for this outbreak response. States reported case-patient deaths and dates of death routinely to CDC; thus, deaths occurring after completion of a case report form were captured systematically. The collection of patient-level data was deemed a public health emergency and was not subject to ethics review or informed consent procedures.

Results

We identified 391 patients with meningitis or stroke caused by presumed meningitis in 19 states (*10*). Detailed data were available for analyses for 389 (99%) of these patients.

Exposures Averted

NECC records indicated that 17,675 vials comprising 29,641 mL of the implicated MPA lots were distributed to 75 facilities in 23 states. Of the 11,773 mL of lot 05 shipped, we estimated that none was recalled; of the 10,847 mL of lot 06 shipped, we estimated that 132 mL (1%) was recalled; and of the 7,021 mL of lot 08 shipped, we estimated that 3,018 mL (43%) was recalled (Table 1) (On-

Table 1. Methylprednisolone acetate (MPA) injections shipped, used, and recalled by lot*

Lot no.	Volume MPA shipped, mL	Volume MPA used, mL	Volume MPA recalled, mL
05	11,773	11,773	0
06	10,847	10,715	132
08	7,021	4,003	3,018
Total	29,641	26,491	3,150

*Volume of MPA shipped was derived from New England Compounding Center's shipping records. Volume used and recalled was estimated by using the assumption that all shipped MPA was available for use at each clinic.

line Technical Appendix). A total estimated 3,150 mL of MPA was shipped but not administered because of the recall. Using an estimated per-injection volume of 1 mL, an additional 3,150 injections with the implicated lots of MPA could have occurred if all 3 lots of contaminated MPA had not been promptly recalled (Table 1).

Weekly Lot-Specific Attack Rates and Cases Averted

Of the 391 case-patients, 370 (95%) had injection data available for calculating attack rates. Of the 370 patients, 221 (60%) had 1 injection, 116 (31%) had 2 injections, 32 (9%) had 3 injections, and 1 (<1%) had 4 injections. Figure 1 shows observed and estimated weekly lot-specific attack rates in patients for each of the 3 contaminated lots of MPA.

For lot 05, predicted weekly attack rates increased from 1.9 (95% CI 0.8–4.7) cases/1,000 mL MPA administered during the first week of injections to 25.8 (95% CI 7.7–81.3) cases/1,000 mL MPA administered during the last week of injections; for these predictions, we assumed that injections continued until all vials were used. Lot 06 had predicted weekly attack rates that increased from 1.6 (95% CI 0.8–3.1) cases/1,000 mL MPA administered during the first week to 418.6 (95% CI 226.8–768.1) cases/1,000 mL MPA administered during the final week of injections (with the assumption that injections continued until all the vials were used). Lot 08 had predicted weekly attack rates that increased from 5.5 (95% CI 1.2–23.0) cases/1,000 mL MPA administered during the first week to 57.3 (95% CI 10.7–302.8) cases/1,000 mL MPA administered during the third week (October 14, 2012) after the recall notice. For all 3 lots, predicted attack rates rose as time from the medication production date increased (Figure 1). We estimate that without the recall of the 3 lots of MPA, 153 (95% CI 61–467) additional cases of meningitis would likely have occurred in persons exposed between September 26, 2012, and October 14, 2012 (Table 2).

Meningitis Deaths Averted

Of the 389 patients with available detailed data, 40 died ≤60 days of diagnosis (60-day CFR 10%). Of the 82 patients given a diagnosis on or before October, 23 (28%) died ≤60 days of diagnosis compared with 17 (6%) of the 307 patients given a diagnosis after October 4 (p<0.0001), an absolute risk reduction of 22% (Table 3). Of the 110 patients with laboratory-confirmed infection, 19 died ≤60 days of diagnosis (60-day CFR 17%). Of the 33 patients given a diagnosis on or before October 4, 13 (39%) died ≤60 days of diagnosis, compared with 6 (8%) of 77 who had a diagnosis after October 4 (p<0.0001) (Table 3).

Kaplan-Meier analysis and the log-rank tests showed that patients given a diagnosis after October 4 had improved overall survival compared with those given a diagnosis on or before October 4 (p<0.0001) (Figure 2). Patients given a diagnosis after October 4 also had lower CSF median leukocyte count (29 cells vs. 1,064 cells; p<0.0001); lower median CSF protein (71 g/dL vs. 117 g/dL; p<0.0001); higher median CSF glucose (55 mg/dL vs. 38 mg/dL; p<0.0001); and fewer symptoms when care was sought (median 4 vs. 5; p<0.0001) (Table 3). For the 240 patients with documented receipt of antifungal treatment, patients given a diagnosis after October 4 were more likely to receive antifungal drugs <48 hours of diagnosis (84% vs. 59%; p = 0.0006) (Table 3). The 60-day CFR by week of diagnosis was 50%–100% from August 27 through September 30; the CFR dropped substantially during the first week of October, to 3%–7% (Figure 3).

We applied the 60-day CFR (28%) of the 82 patients given a diagnosis of meningitis on or before October 4 to that of the 290 patients whose meningitis was diagnosed after October 4 and who were alive at 60 days (Table 3); we also applied that CFR to the 153 (95% CI 61–467) cases estimated to have been averted. With these calculations, we estimated that, without public health actions and early diagnosis and intervention by clinicians, 124 (range 98–211) additional deaths from meningitis or stroke may have occurred through October 14, 2012 (Table 2).

Additional Use and Attack Rate Extrapolation

Table 2. Meningitis and stroke cases and deaths averted by lot

Lot no.	Cases averted, no. (95% CI)	Deaths averted, no. (range)*
05	0	NA
06	53 (32–88)	NA
08	100 (29–379)	NA
Total	153 (61–467)	124 (98–211)†

*NA, not applicable.
†Total deaths averted (124) derived from total cases averted (153) multiplied by the case fatality rate observed early in the outbreak (28%) plus case-patients who had a diagnosis of meningitis after October 4 and who were alive at 60 days (290) multiplied by the early case fatality rate: (153 × 0.28) + (290 × 0.28). Range derived from the upper and lower confidence intervals of cases averted (e.g., 98 = [61× 0.28] + [290 × 0.28]).

Table 3. Clinical characteristics and 60-day case fatality rates for meningitis and stroke patients given a diagnosis on or before October 4, 2012, versus after October 4, 2012

Characteristic	Diagnosis date on or before October 4, n = 82	Diagnosis date after October 4, n = 307	p value
CSF parameters*			
Median leukocyte count, cells/μL	1,064	29	<0.0001
Median total protein, g/dL	117	71	<0.0001
Median glucose, mg/dL	38	55	<0.0001
Median no. of symptoms	5	4	<0.0001
Antifungal treatment within 48 h of diagnosis	20 (59%)†	173 (84%)†	0.0006
Death within 60 d of diagnosis	23 (28%)‡	17 (6%)‡	<0.0001
Laboratory-confirmed cases only, n = 110	33	77	
Death within 60 d of diagnosis	13 (39%)§	6 (8%)§	<0.0001

*CSF, cerebrospinal fluid.
†The denominators used for percentages represent the number of case-patients for whom data on antifungal treatment was available (34 and 206, respectively) for each diagnosis period.
‡The denominators used for percentages are the total number of case-patients for each diagnosis period.
§The denominators used were the total laboratory-confirmed cases for each diagnosis period.

Extrapolation of the amount used from lot 08 suggests that, in the absence of a recall, its use would have continued until the week of December 2, 2012 (Figure 1). Estimates of attack rates and cases averted beyond October 14, 2012, are unreliable because of the short amount of time that lot 08 was in use before the recall. However, if lot 08 injections had continued through December 2 and if we assume an exponential rate of increase in attack rates (as was applied to lot 06), during the final week of injections, the attack rate for lot 08 might have been as high as 768.8 cases/1,000 persons exposed. Under these same assumptions, an additional 169 cases (total estimated cases = 322) and an additional 47 deaths (total estimated deaths = 171) would have occurred if lot 08 had been used through December 2.

Discussion

This outbreak of fungal meningitis and other infections triggered a public health response that involved thousands of public health officials, clinicians, and medical staff throughout the United States. This massive effort was commensurate with the scale of the public health crisis: a highly pathogenic fungus causing a clinical illness that had not been previously described and that initially resulted in a large number of deaths, with 13,534 persons potentially at risk. Despite the magnitude of this outbreak, we show that the effects of this outbreak, as measured by exposures, cases, and deaths, could have been far worse.

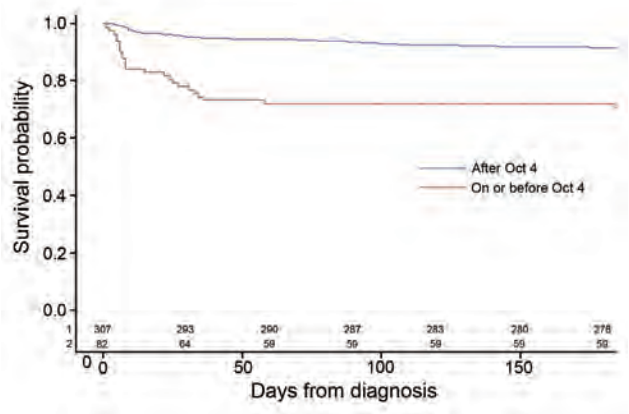

Figure 2. Kaplan-Meier (product limit) survival curves and persons at risk by date of diagnosis of meningitis and stroke case-patients among persons injected from 3 lots of methylprednisolone acetate contaminated with the fungus *Exserohilum rostratum*, United States, 2012. No patients were reported as lost to follow-up (e.g., censored) during the 6 months after their diagnosis.

The 22% risk reduction in the CFR described here among case-patients given a diagnosis after October 4 was likely due to the direct effects of patient notification and clinician outreach, highlighted by intense activity of public health authorities during a 2-week period: multistate patient notification efforts were initiated (beginning September 28), a Health Advisory with diagnostic guidance was issued (October 4) (*7*), CDC's first interim treatment guidance was developed and disseminated (October 4) (*6*), and the outbreak was publicized through a CDC–Food and Drug Administration joint telebriefing (October 4) (*8*) that publicly confirmed fungal contamination of MPA (*8*). Our analysis shows that these public health actions probably resulted in earlier diagnosis of infections at a less severe stage of disease and faster initiation of antifungal therapy; both actions may have contributed to a decreased number of deaths. The sharp decline in the 60-day CFR was probably not caused by inclusion of cases without fungal infection because the decline was also shown in laboratory-confirmed cases that had a CFR of ≈40% before October 4.

The projected case estimates through October 14, 2012, are likely underestimates because we could not accurately extrapolate attack rates and cases averted because lot 08 was not used beyond this date. With no recall, injections with lot 08 would likely have continued for many weeks beyond October 14 (Figure 1), resulting in additional cases. We also assumed that no further contaminated lots were produced after production of lot 08. However, because 3 sequential lots of MPA were contaminated and serious sterility breaches were found during the

onsite investigation at NECC (*11*), without a public health investigation and response, subsequent lots of contaminated MPA likely would have been produced, distributed, and administered. Ongoing contamination, leading to additional cases, would have occurred without rapid diagnosis and reporting of the index case (*12*), identification of the contaminated product, and subsequent public health actions. If we assume that subsequent MPA lots would be similar to the 06 lot in size, rate and amount of contamination, and attack rate, each additional contaminated MPA lot may have resulted in 275 additional cases of meningitis or stroke and 77 more deaths.

We found that the risk of meningitis and stroke was strongly influenced by the lot number and age of the MPA vial, with the latest produced lots (lots 06 and 08) and oldest vials conveying higher risk for disease than the earliest produced lot (lot 05) and younger vials of MPA (Figure 1). Because this MPA formulation lacked preservative, which inhibits fungal growth, fungus may have been better able to proliferate during extended storage times, possibly leading to high fungal contamination and attack rates. A study of compounded ophthalmic preparations reported increased fungal growth in preparations without common preservatives compared with preservative-containing solutions (*13*). Further studies are needed to confirm this observation for MPA.

Several areas of uncertainty surround our estimates. First, data on lot exposure were not always recorded in patient charts. When these data were missing, available data, including clinic shipping invoices and manufacture and injection dates, were used to extrapolate the most likely lot exposure. Second, uncertainty surrounds our model for and estimates of recalled and unused MPA (*2*), and we did not carry the uncertainty surrounding the volume estimates forward in our subsequent modeling. We also assumed that all MPA use ceased the day after the recall notice and that clinics using NECC MPA did not use MPA from other manufacturers. We know that some clinics used non-NECC MPA and that a few clinics ceased use before the recall date, while others extended use beyond that date; these areas of uncertainty could have resulted in higher or lower estimates for MPA injections averted than are presented here. Other areas of uncertainty include the choice of the log link function used in the Poisson regression model, which has a progressively stronger effect on model predicted values outside the time range of the observed data. Finally, uncertainty exists in assigning responsible injections for patients with multiple procedures and in the parameter estimates in our Poisson modeling. A multiple simulation approach was used to capture uncertainty in these 2 areas and is expressed in the resulting ranges for our estimates of attack rates and cases averted. These multiple areas of uncertainty mean that the true number of injections, cases, and deaths averted might be higher or lower than our estimates.

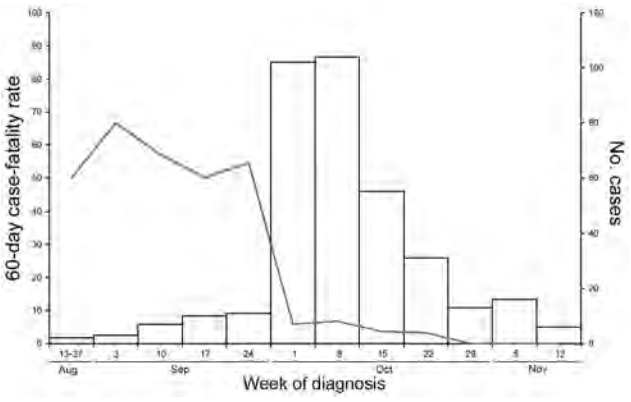

Figure 3. Sixty-day case-fatality rates (line) and case counts (bars) by week of diagnosis for meningitis case-patients among persons injected from 3 lots of methylprednisolone acetate contaminated with the fungus *Exserohilum rostratum*, United States, 2012.

This outbreak showed the devastating effect of contamination in a widely used product designated for sterile use. Public health actions, made possible by a strong existing public health infrastructure and rapid coordination among federal, state, and local partners, likely averted additional exposures, cases, and deaths. Maintaining this infrastructure and these partnerships is essential to preserve public health agencies' abilities to respond quickly and meaningfully to future public health emergencies.

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Global Burden of Invasive Nontyphoidal *Salmonella* Disease, 2010[1]

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Nontyphoidal *Salmonella* is a major cause of bloodstream infections worldwide, and HIV-infected persons and malaria-infected and malnourished children are at increased risk for the disease. We conducted a systematic literature review to obtain age group–specific, population-based invasive nontyphoidal *Salmonella* (iNTS) incidence data. Data were categorized by HIV and malaria prevalence and then extrapolated by using 2010 population data. The case-fatality ratio (CFR) was determined by expert opinion consensus. We estimated that 3.4 (range 2.1–6.5) million cases of iNTS disease occur annually (overall incidence 49 cases [range 30–94] per 100,000 population). Africa, where infants, young children, and young adults are most affected, had the highest incidence (227 cases [range 152–341] per 100,000 population) and number of cases (1.9 [range 1.3–2.9] million cases). An iNTS CFR of 20% yielded 681,316 (range 415,164–1,301,520) deaths annually. iNTS disease is a major cause of illness and death globally, particularly in Africa. Improved understanding of the epidemiology of iNTS is needed.

Nontyphoidal *Salmonella* (NTS) disease, a major cause of diarrheal disease globally, is estimated to cause 93 million enteric infections and 155,000 diarrheal deaths each year (*1*). The Institute for Health Metrics and Evaluation estimated that enteric NTS disease was associated with 4,847,000 disability-adjusted life years lost (70 disability-adjusted life years/100,000 population) and 81,300 diarrheal deaths (1.2 deaths/100,000 population) in 2010 (*2*,*3*). However, these estimates do not include invasive NTS (iNTS) disease, which is often not associated with diarrhea. A systematic review of community-acquired bloodstream infections in Africa showed that 29% were caused by *Salmonella enterica*, and a high proportion of these infections in some parts of Africa were caused by NTS: 88% in eastern Africa, 97% in southern Africa, and 87% in western and central Africa, compared with only 1% in northern Africa (*4*). Moreover, this review identified that the 2 most common serovars causing iNTS infections were *S. enterica* serovars Typhimurium and Enteritidis, which accounted for 65.2% and 33.1%% of all NTS serotyped isolates, respectively (*4*). iNTS disease appears to be more common in some parts of Africa than in other regions of the world (*5*). Host risk factors appear to play a major role in the epidemiology of iNTS disease in Africa (*6*), where the disease is closely associated with malaria and malnutrition among infants and children (*7*–*9*) and with HIV infection among adults (*4*,*10*,*11*).

An estimate of the global and regional burden of iNTS disease is needed to inform and stimulate efforts to prevent and manage the disease. Estimation of illness and death due to iNTS disease has been limited by a scarcity of population-based surveillance data on bloodstream infections, particularly in Africa. However, the availability of high-quality country-level data on 2 major host risk factors for iNTS (i.e., HIV and malaria [*6*]) provides a basis for extrapolation of the few population-based iNTS surveillance data that are available for other areas. We sought to estimate the global incidence of iNTS disease by age and region and to calculate the number of illnesses and deaths by extrapolating credible population-based incidence data and incorporating the effects of HIV and malaria to account for differences in the population at risk among countries.

Materials and Methods

Systematic Literature Review for iNTS Incidence Data

We conducted a systematic review for iNTS incidence data by following guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (*12*). We used standard primary search terms (*Salmonella*,

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Typhi/typhoid, Paratyphi/paratyphoid, non-typhoidal, foodborne, diarrhea, Typhimurium, and Enteritidis) and standard secondary search terms (morbidity, incidence, prevalence, sequelae, mortality, mode of transmission, outbreak, invasive, bacteremia, septicemia/septicemia, bloodstream infection, invasive, CSF/cerebral spinal fluid, bone marrow, and blood culture) in the following databases: PubMed, System for Information on Grey Literature in Europe, World Health Organization library, Food and Agriculture Organization of the United Nations, and Bath Information and Data Services. Each primary search term was combined with all secondary search terms by using the AND operator. The time period for the search was January 1990–December 2012.

The types of studies included population-based incidence studies, population-based surveillance systems, and national surveillance data. We consulted with a US Centers for Disease Control and Prevention (Atlanta, GA, USA) librarian during the development of the searches. Two teams of reviewers, each led by a co-author (T.T.A. or J.A.C.) and assisted by S.H. or D.B. (see Acknowledgments), processed the citations for relevant publications in 3 stages: title, abstract, and full-text review. If there was a disagreement during any stage, a tie-breaker from the other team (T.T.A. or J.A.C.) decided if the publication was appropriate. We included foreign language articles that had at least an English translation of the title for the first stage of review. If a citation met the requirement at this stage, we looked for the English translation of the abstract for the second and subsequent stages.

Extrapolation of Incidence to All Age Groups

We sought to obtain incidence data for all age groups. For studies identified through the systematic review that did not have incidence data for all age groups, we created incidence profile curves to extrapolate the available incidence data to other age groups in that particular population by using proportion curves that were calculated from 2 sources that had complete case counts for each age group (Figure 1): US FoodNet (Foodborne Diseases Active Surveillance Network) data (low iNTS incidence profile) (*13*) and Malawi and South Africa surveillance data (high iNTS incidence profile) (*14*). We divided the number of cases in the available age groups by the total number of cases and used these age-specific proportions and either the low or high incidence profile, according to that country's iNTS epidemiologic pattern, to extrapolate the incidence to the other age groups in that population. This yielded all age group iNTS incidence data for all studies identified in the systematic review (Figure 2).

Assignment of iNTS Incidence Data to 3-Level Matrix of HIV and Malaria Prevalence

We then assigned the iNTS incidence data identified from the systematic review to a 3 × 3 matrix based on the HIV seroprevalence and malaria population at risk category of the country of origin of the iNTS incidence data (Figure 3). When necessary, age incidence curves from the same matrix were also used to extrapolate data to all age groups. For HIV categorization in the matrix, we used the 2009 UNAIDS (Joint United Nations Program on HIV/AIDS) country-specific seroprevalence data (*15*) and classified countries into low (0 to <5.0%), moderate (5.0% to 10.0%), and high (>10.0%) HIV seroprevalence. For malaria categorization in the matrix, we used the Malaria Atlas Project country-specific population at risk for malaria (*16*,*17*). The population at risk is defined as the proportion of the total population living in an area of known *Plasmodium falciparum* transmission.

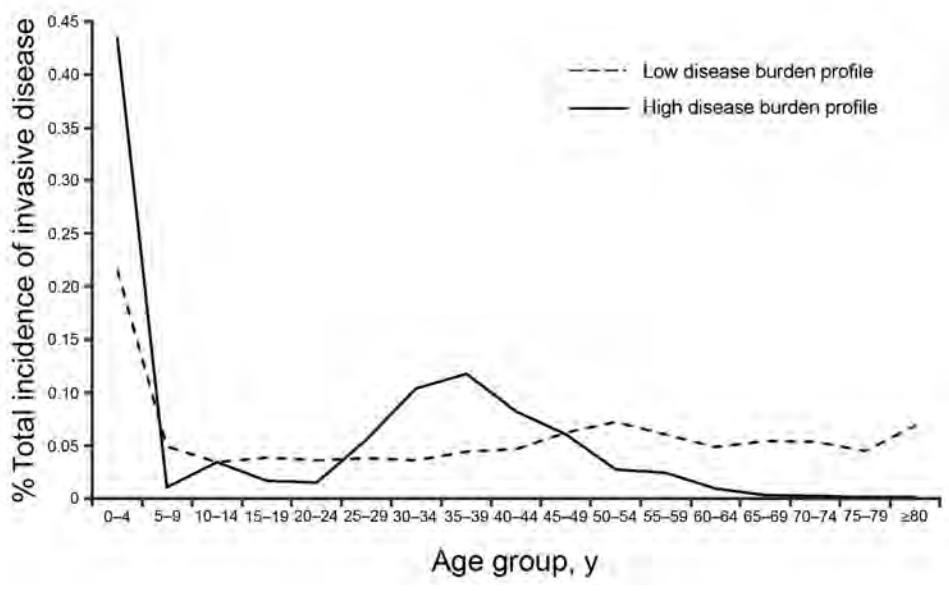

Figure 1. Proportion of invasive nontyphoidal *Salmonella* disease, by age group, from low-incidence settings in the United States and high-incidence settings in Malawi and South Africa.

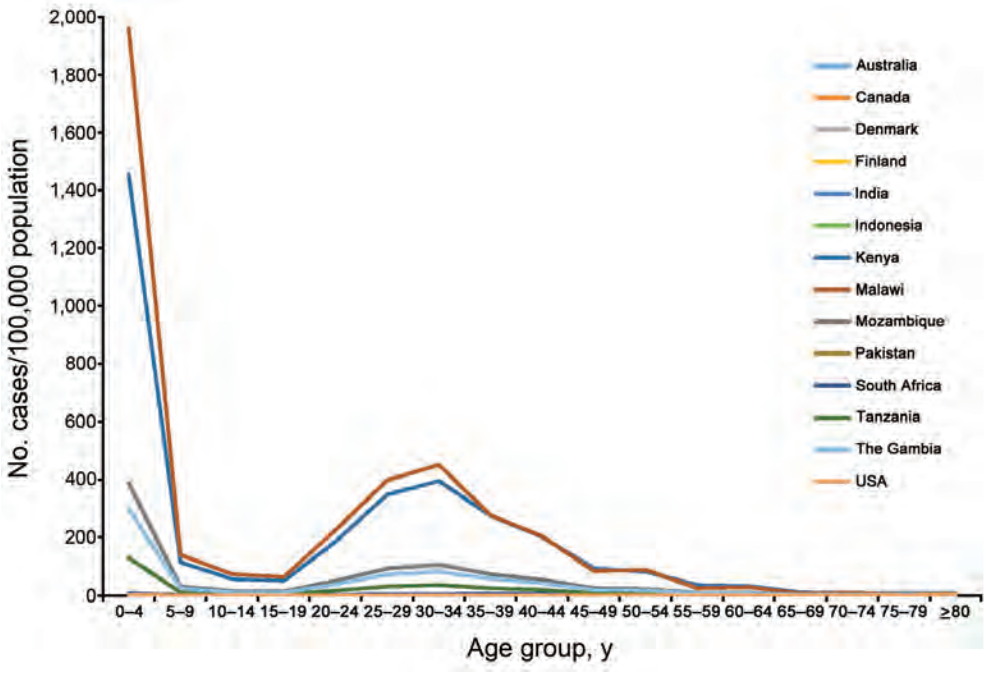

Figure 2. Incidence of invasive nontyphoidal *Salmonella* disease, by age group, in countries with data identified through a global systematic review of the literature.

Countries were classified as having low (0%), moderate (0.1%–10.0%), or high (>10.0%) proportions of their populations at risk for malaria. Each of the iNTS incidence data points from the systematic review (extrapolated to all age groups as described above) were assigned within the matrix, depending on the HIV and malaria epidemiology in the country source of the data. If a matrix cell had >1 data source after the iNTS incidence data were assigned, the mean incidence from all sources was calculated as the reference incidence for that matrix cell. A minimum to maximum range was also identified by using the respective values within a specific age group. If a matrix cell had only 1 incidence reference, the minimum and maximum incidences were calculated by using the median to mean ratio of cell A (Figure 3). If a cell did not have a source, we extrapolated incidence rates by using existing data, with the assumption that the middle cell was the mean of the 2 cells on either side. As shown in Figure 3, we first extrapolated cell C by assuming that cell B was the mean of cells A and C. Then we proceeded to calculate cells D, G, and H by using the same assumption. This yielded a reference all age group incidence rates for each cell in the 3 × 3 matrix (Figure 4).

Extrapolation to All Countries Worldwide

For the last round of extrapolation, we assigned all countries worldwide to a cell in the 3 × 3 matrix according to each country's HIV seroprevalence and malaria population at risk. We then calculated the number of iNTS cases for each country by using the reference incidence rates from the cell, according to the assignment of each country in the matrix and each country's population stratified by age groups. We used the United Nation's population data for 2010, medium variant, grouped by World Health Organization regions (*18*). Country classification by region, HIV, and malaria profile is available in online Technical Appendix 1 (http://wwwnc.cdc.gov/EID/article/21/6/14-0999-Techapp1.xlsx).

Case-Fatality Ratio and Number of Deaths

We conducted an additional literature review to identify reports of case-fatality ratios (CFRs) due to iNTS.

HIV seroprevalence* \ Malaria population at risk†	Low (0)	Moderate (0.1% to 10.0%)	High (>10.0%)
Low (0 to <5.0%)	Average of cases for Australia, Finland, Denmark, Pakistan, Canada, and US FoodNet‡ (A)	Average of cases for India, Indonesia, and The Gambia (B)	**Step 1:** Extrapolated from A and B (C)
Moderate (5.0% to 10.0%)	**Step 2:** Extrapolated from E and F (D)	Kenya (E)	Tanzania Mozambique (F)
High (>10.0%)	**Step 3:** Extrapolated from A and D (G)	**Step 4:** Extrapolated from B and E (H)	No countries in category (I)

Figure 3. HIV and malaria burden matrix and extrapolation strategy used to create reference age-specific incidence curves for invasive nontyphoidal *Salmonella* disease. *2010 Joint United Nations Program on HIV/AIDS (UNAIDS) HIV seroprevalence (*15*); †Malaria Atlas Project population at risk estimate, defined as the proportion of the population living in an area of known *Plasmodium falciparum* transmission (*16,17*); ‡US FoodNet (*13*).

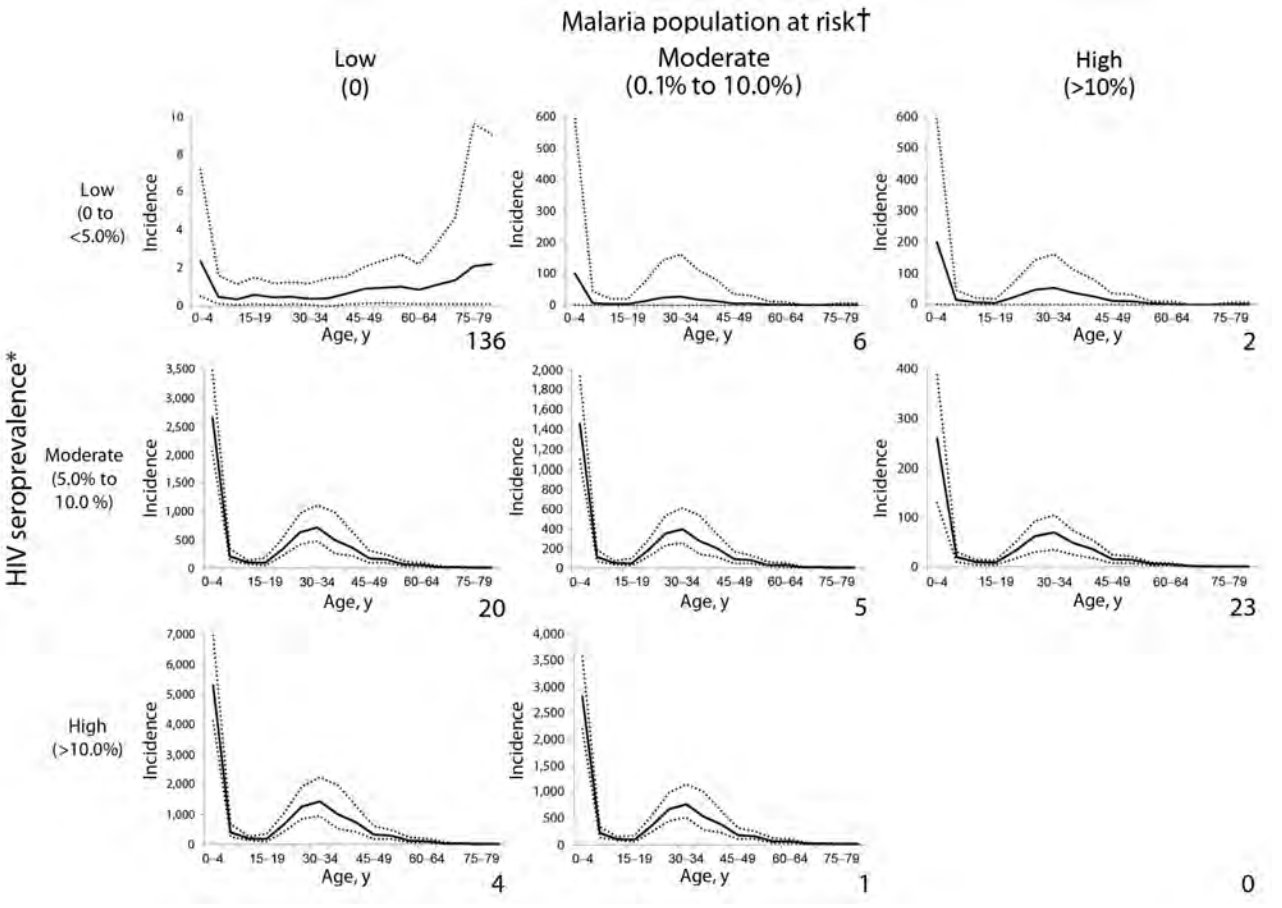

Figure 4. Age-specific invasive nontyphoidal *Salmonella* disease incidence (cases/100,000 population) for various HIV and malaria settings, 2010. Number at lower right corner of each chart represents the number of countries in the category. Incidence is cases/100,000 population. Solid lines on each graph represent the estimated age-specific invasive nonthyphoidal *Salmonella* (iNTS) disease incidence; dotted lines represent ranges. A country is classified into 1 of the 8 categories on the basis of national HIV seroprevalence and malaria population at risk. The age-specific iNTS incidence in that category (solid line in graph) is then applied to the country's population data to determine the number of cases and overall incidence of iNTS for the country. *2010 Joint United Nations Program on HIV/AIDS HIV seroprevalence (*15*); †Malaria Atlas Project population at risk estimate, defined as the proportion of the population living in an area of known *Plasmodium falciparum* transmission (*16,17*).

In addition, we solicited expert opinions in person at the 8th International Conference on Typhoid Fever and Other Invasive Salmonelloses in Dhaka, Bangladesh, 2013, and by email to develop a consensus CFR. We asked experts to provide a single value, taking into account variations by co-infections, geography, age, infection rate, strain type, and study setting (i.e., hospital based vs. community based). The consensus CFR was then applied to the estimated number of iNTS cases and the minimum and maximum range to estimate iNTS deaths and minimum and maximum range. We also varied the CFR to understand the effect of the consensus CFR on global iNTS deaths. To understand the effect of the consensus CFR, we performed a sensitivity analysis by applying the range of CFRs found in the systematic review.

Results

We identified 9,739 unique citations, 2,029 (20.8%) of which were from non–English language journals. We excluded 9,011 by title screening alone. Of the remaining 728 citations, 700 were excluded by abstract screening. Of the remaining 28 potential eligible citations with relevant abstracts, 10 were eligible for full text review (Figure 5; Table 1).

We found substantial geographic variation in age-specific incidence (Figure 2). Overall, Kenya and Malawi had higher incidence rates across all age groups compared with other countries. There was a bimodal distribution of incidence for these 2 locations, with peaks among for children <5 years of age and adults 30–35 years of age. In addition, for children <5 years of age, the incidence of disease for Kenya and Malawi was at least 3 times as high as that for other locations.

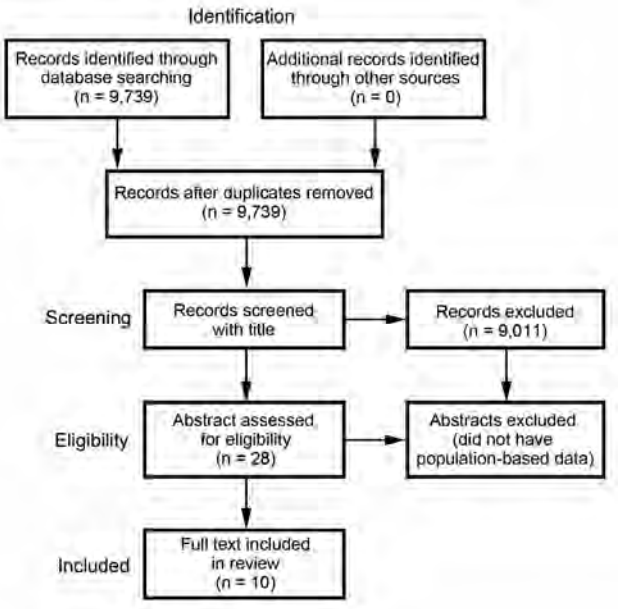

Figure 5. Results of a systematic review of the literature for the global burden of invasive nontyphoidal *Salmonella* disease. Reports published during January 1990–December 2012 were searched.

We estimate 3.4 (range 2.1–6.5) million cases of iNTS disease each year and an overall incidence of 49 (range 30–94) cases/100,000 population (Table 2). Africa has the highest incidence of iNTS disease (227 [range 152–341] cases/100,000 population) and the largest number of cases (1.9 [range 1.3–2.9]) million cases). The age group–specific incidence rate proportion profile graphs for 8 HIV and malaria scenarios suggest substantial changes in iNTS incidence rates as the HIV prevalence and proportion of population at risk for malaria increase (Figure 4). Most countries in the world (69%) belong to the low malaria and low HIV category. Among iNTS cases, 63.7% occurred in children <5 years of age globally, and 68.3% occurred in children <5 years of age in Africa. The global incidence ratio of enteric disease to invasive disease was 28:1 (range 14–45:1) (Table 2), indicating that for every iNTS case there were 28 enteric NTS cases. This ratio is highest in Asia and Oceania (3,851:1, range 1,317–23,806:1) and lowest in Africa (1:1, range 1–2:1).

CFR

A range of potential CFRs, from 3% (*27*) to 47% (*28*), was identified by the systematic review. Expert opinion identified a most likely CFR of 20%. Using a CFR of 20%, we estimated that 681,316 (range 415,165–1,301,520) deaths would result from the annual number of cases. Varying the CFR demonstrated a possible range of numbers of deaths due to iNTS from 102,197 at 3% CFR (range 62,275–195,228) to 1,703,290 at 50% CFR (range 1,037,912–3,253,800) (Table 3).

Discussion

We estimate that 3.4 (range 2.1–6.5) million cases of iNTS disease occurred in 2010, with a CFR of 20%, for an overall incidence of 49 (range 30–94) cases/100,000 population and 681,316 (range 415,164–1,301,520) deaths. For comparison, globally there were ≈1.2 million malaria-associated deaths and 1.5 million HIV-associated deaths in 2010 (*29*). Compared with the estimated number of 93.7 million enteric NTS illnesses in 2010, the number of iNTS cases is considerably lower (*1*). However, because of the high estimated CFR for iNTS, the number of deaths resulting from this disease (681,316 deaths) is considerably higher than that estimated for enteric NTS (155,000 deaths). Furthermore, the estimated number of deaths due to typhoid fever in 2000 was 216,510 (*30*), and the estimated number for typhoid and paratyphoid fever in 2010 was 190,200 (*29*).

The highest number of iNTS cases occurred in Africa, where in 2010, almost 2 million illnesses (227 cases/100,000 population) occurred, accounting for more than half of global cases. The region with the second highest number of cases in 2010 was Europe (763,191 cases cases/100,000 population), but the number was substantially lower than that for Africa. The high number of cases in Europe is mainly driven by cases in Russia, Ukraine, and

Table 1. Eligible studies of iNTS from systematic literature review, 1990–2012*

Reference	Time of study	Country (city)	Median iNTS cases/100,000 population (range)
Berkley et al. (*19*)	1998–2002	Kenya (Kilifi)	8 (4–1,457)
Tabu et al. (*8*)	2006–2009	Kenya	Lwak, 232 (24–2,085); Kibera, 0 (0–260)
Nadjm et al. (*20*)	2006–2007	Tanzania	7 (0–130)
Mtove et al. (*21*)	2006–2010	Tanzania	5 (0–82)
Sigaúque et al. (*22*)	2001–2006	Mozambique	22 (1–388)
Feasey et al. (*14*)	1998–2004	Malawi, South Africa	South Africa, 1.6 (0.3–7.2); Malawi, 84 (2–1,963)
Enwere et al. (*23*)	2000–2004	The Gambia	17 (1–300)
Khan et al. (*24*)	2001–2003	China, India, Indonesia, Pakistan, Viet Nam	Pakistan, 1.6 (1.2–7.2); Indonesia, 0.2 (0.2–1.0); India, 0.05 (0.03–1.8)
Gradel et al. (*25*)	1994–2003	Denmark	1.9 (0–9.6)
Laupland et al. (*26*)	2000–2007	Finland, Australia, Denmark, Canada	Finland, 0.2 (0.1–7.6); Calgary, Canada, 0.2 (0.1–6.5); Denmark, 0.4 (0.3–1.9); Sherbrooke, Canada, 0.5 (0.4–2.2); Victoria, Canada, 0.07 (0.05–0.3); Australia, 0.1 (0.09–0.5)

*iNTS, invasive nontyphoidal *Salmonella* disease.

Table 2. Global burden of invasive nontyphoidal *Salmonella* disease, 2010, compared with enteric nontyphoidal *Salmonella* disease, 2009*

	Enteric nontyphoidal *Salmonella*, 2009†			Invasive nontyphoidal *Salmonella*, 2010			
Region	Population, thousands	No. cases	Cases/ 100,000 population	Population, thousands	No. cases (range)	Cases/100,000 population (range)	Ratio of enteric to invasive disease (range)
N. Africa, MidEast	410,800	563,000	140	446,721	3,617 (660–10,483)	0.8 (0.1–2.3)	156:1 (54–853:1)
Africa	767,239	2,458,000	320	854,091	1,942,776 (1,301,399–2,910,768)	227 (152–341)	1:1 (1–2:1)
Asia, Oceania	1,628,815	53,610,000	3,280	1,693,046	13,920 (2,252–40,711)	0.8 (0.13–2.4)	3,851:1 (1,317–23,806:1)
SE Asia	2,072,274	29,839,000	1,440	2,220,248	472,263 (110,992–2,045,128)	21 (5–92)	63:1 (15–269:1)
Europe	738,071	5,065,000	690	746,372	763,191 (515375–1,179,778)	102 (69–158)	7:1 (4–10:1)
Americas	888,437	2,222,000	250	934,132	210,811 (145,145–320,732)	23 (16–34)	11:1 (7–15:1)
Global	6,511,638	93,757,000	1,140	6,894,610	3,406,579 (2,075,823–6,507,600)	49 (30–94)	28:1 (14–45:1)

*MidEast, Middle East; N. Africa, North Africa; SE Asia, Southeast Asia.
†Majowicz et al. (*1*).

Estonia, 3 eastern European countries with large populations and relatively higher iNTS incidence profiles compared with those for countries in western Europe. Our findings, together with contemporary longitudinal descriptive literature, provide additional evidence that a largely underappreciated epidemic of iNTS has been occurring in Africa, driven in part by co-infection with HIV or malaria. Antimicrobial drug resistance emerging across the continent is likely to influence the incidence of iNTS and associated deaths (*31*,*32*).

The incidence of iNTS is highest among children and young adults in sub-Saharan Africa. These groups should be a high priority for prevention efforts, including a potential NTS vaccine (*33*). Efforts to advance vaccines against the most prevalent serotypes of NTS, *Salmonella* serovars Typhimurium and Enteritidis, should be intensified.

Estimates of illness and deaths due to iNTS both in sub-Saharan Africa and globally are likely to be inaccurate (*6*,*34*). Invasive bacterial disease surveillance is essential for identifying cases and monitoring iNTS trends, yet it is not widely available in disease-endemic areas (*35*). As demonstrated by this review, few invasive bacterial disease surveillance studies have been conducted that can provide population-based incidence data. Most published articles identified by the literature search were clinical reports or case series. Population-based incidence data are vital for future refinements of our estimates.

Sources and modes of transmission of NTS in Africa are also poorly understood. The development of nonvaccine prevention efforts will require a more in-depth understanding of the basic epidemiology of NTS on the continent (*34*,*36*). It is possible that the relative importance of transmission through food, water, and contact with animals and their environments differs from patterns observed for enteric NTS infection in industrialized nations. Furthermore, genomic studies (*37*) and integrated human and animal studies (*38*) raise the hypothesis that infected humans may be an important source of infection. There is also evidence that in South Africa iNTS is often associated with health care facilities (*39*,*40*).

Invasive NTS is a leading cause of invasive bacterial disease in Africa. This finding has a range of implications for patient management. It is vital that recommendations for empiric management of sepsis incorporate antimicrobial agents suitable for the management of iNTS. Not only are aminoglycosides inappropriate for intracellular infections, such as iNTS, but resistance to traditional first-line drugs (ampicillin, trimethoprim sulfamethoxazole, and chloramphenicol) is now common among iNTS strains in Africa (*4*). Of further concern, resistance to traditional first-line drugs among iNTS strains in Asia occurs alongside resistance to fluoroquinolones and extended-spectrum cephalosporins in some areas (*41* in online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/21/6/14-0999-Techapp2.pdf). Antimicrobial resistance appears to have played a role in the emergence and proliferation of individual NTS serovars and strains in populations at risk for infection (*31*,*32*). In sub-Saharan Africa, evidence suggests that *Salmonella* Typhimurium ST313 has developed multiple drug resistance and has adapted itself to immunosuppressed persons, particularly those living with HIV. Prevention and management of host conditions predisposing to iNTS are also likely to be key to the control of iNTS. Reductions in malaria transmission have been ecologically associated with declines in iNTS disease in some areas (*21*) (*42*,*43* in online Technical Appendix 2). Declines in HIV seroprevalence and reductions in the proportion of HIV-infected persons with low CD4-positive T-lymphocyte

Table 3. Sensitivity analysis of invasive nontyphoidal *Salmonella* disease case-fatality ratio, 2010

Case-fatality ratio, %	Estimated no. annual deaths (range)
3	102,197 (62,275–195,228)
5	170,329 (103,791–325,380)
10	340,658 (207,582–650,760)
20	681,316 (415,165–1,301,520)
30	1,021,974 (622,747–1,952,280)
40	1,362,632 (830,329–2,603,040)
50	1,703,290 (1,037,912–3,253,800)

counts by successful antiretroviral drug therapy would be anticipated to have similar effects on iNTS disease (*44*,*45* in online Technical Appendix 2).

Our results demonstrate substantial differences in the ratio of enteric to invasive disease by geographic area. Whereas the incidence of enteric disease is highest in Asia and Oceania, the incidence of invasive disease is highest in Africa, where the incidence ratio of invasive to enteric disease was 1:1 (Table 2), which suggests that the magnitude of iNTS in Africa is comparable to that of enteric NTS.

Our study has several limitations. First, our rigorous systematic search for population-based incidence data in the literature resulted in a limited number of eligible sources. Despite this scarcity of eligible studies, the existing data are of high quality and, we believe, representative of the settings from where they were collected. Second, we did not consider other host risk factors, such as malnutrition and sickle cell disease. Excluding the effect of these conditions might result in underestimation of iNTS incidence rates and the number of cases and deaths. Third, we assumed that the country-level prevalence for the HIV- and malaria-infected population at risk is uniform, even though there is likely considerable subnational variation for these measures. Fourth, we did not take into account the declines in malaria worldwide in the past decade, nor did we account for changes in HIV seroprevalence and progress with provision of care and treatment globally. We used the most contemporary HIV and malaria data available. However, iNTS incidence data used in these estimations may have been collected under different HIV seroprevalence and malaria population at-risk conditions than those observed in 2010. In addition, the incidence curve for the low HIV and high malaria grouping had an artificially high peak in the 35- to 39-year-old age group, which was due to the assumption we made that, across the rows in our incidence reference grid, the middle cell is the average of the 2 cells on each side. Because we know that malaria is predominantly associated with iNTS disease among young children, we believe this artifact is a result of our assumption. However, because there were only 2 countries in this group (Comoros and Madagascar), and we believe that any potential overestimation of incidence in the 35- to 39-year-old age group in these countries will be negligible. In addition, HIV and malaria also contribute differently as risk factors in different ages in a given population. In populations with high HIV seroprevalence, the risk factors are highest among young adults and persons in younger age groups; in high malaria areas, the persons at highest risk for iNTS are those <5 years of age.

In the absence of a standard CFR for iNTS, we relied on expert opinion to estimate the most likely value. Our estimate of deaths from iNTS disease was high compared with the estimated number of deaths associated with HIV, malaria, and protein energy malnutrition. It is likely that many iNTS-associated deaths are currently counted as deaths resulting from these underlying conditions. This high estimate might have been biased by expert opinions from hospital-based studies and experiences, especially in established research programs that have better diagnostics and appropriate antimicrobial drug treatments and a higher level of care than in other settings.

Despite these limitations, we provide a baseline estimate of invasive nontyphoidal *Salmonella* disease burden globally, which is urgently needed to set the scientific and policy agenda . We hope that our estimate will be refined in the future by incorporating new population-based surveillance data, improved estimates of the CFR, and more sophisticated approaches to extrapolation and modeling. We have demonstrated that iNTS disease is a major cause of illness and death globally, particularly in Africa. Improved understanding of the epidemiology of iNTS is needed to underpin effective efforts for prevention, control, and improved patient management.

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Dose-Response Relationship between Antimicrobial Drugs and Livestock-Associated MRSA in Pig Farming[1]

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The farming community can be a vehicle for introduction of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in hospitals. During 2011–2013, an 18-month longitudinal study aimed at reducing the prevalence of LA-MRSA was conducted on 36 pig farms in the Netherlands. Evaluations every 6 months showed a slight decrease in MRSA prevalence in animals and a stable prevalence in farmers and family members. Antimicrobial use, expressed as defined daily dosages per animal per year, decreased 44% during the study period and was associated with declining MRSA prevalence in pigs. MRSA carriage in animals was substantially higher at farms using cephalosporins. Antimicrobial use remained strongly associated with LA-MRSA in humans regardless of the level of animal contact. A risk factor analysis outlined potential future interventions for LA-MRSA control. These results should encourage animal and public health authorities to maintain their efforts in reducing antimicrobial use in livestock and ask for future controlled intervention studies.

In 2005, sequence type (ST) 398 of methicillin-resistant Staphylococcus aureus (MRSA) emerged in Europe with proven transmission between pigs and humans (*1*,*2*). Since then, pigs, veal calves, and (to a lesser extent) poultry were increasingly found to harbor livestock-associated MRSA (LA-MRSA) (*3*).

ST398 is widely spread across Europe, and ≈70% of pig farms in the Netherlands test positive (*4*). After transfer to humans, it can be introduced into hospitals and the community (*5*–*8*). In 2011, ST398 accounted for 39% of all new MRSA detected through screening of patients in the Netherlands (*9*).

To our knowledge, no intervention studies have been undertaken to assess the efficacy of MRSA-reducing measures on farms. Trade of animals is a major risk factor for introducing MRSA into a negative herd (*10*–*12*). Larger herds have been associated with higher antimicrobial use (*4*). Antimicrobial use could not be identified as a clear determinant for MRSA (*4*). Transmission dynamics within herds vary by animals' ages and phase of production, potentially leading to endemicity (*13*).

In 2006, the European Union banned the use of antimicrobial drugs as growth promoters. In the Netherlands the most noticeable change started in 2010, when the government set objectives for a 50% reduction in antimicrobial use by 2013 and 70% by 2015, compared with 2009. This policy was combined with benchmarking of farms, and later veterinarians, to identify persistently high users of antimicrobial drugs (*14*). As part of this national program, farm treatment and health plans have to be drafted and reviewed annually (*15*), which has resulted in an almost 60% reduction for the major livestock industry sectors (*16*,*17*). Against the background of nationwide reduction of antimicrobial use, during 2011–2013, we evaluated MRSA carriage changes in pigs and humans and study the effect of introduction of an additional range of preventive measures on MRSA carriage in animals, and humans living and/or working on the farms.

Materials and Methods

Study Design, Sample Collection, and Laboratory Analysis

Thirty-six pig farms were enrolled in and completed the study; 15 were recruited from farmer cooperatives in the Netherlands, 20 were recruited by veterinarians in the cooperatives, and 1 was recruited by a farm health consultant. Farms were visited at the start of the study during

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March–September 2011. A questionnaire was completed during a walk-through survey with the farm veterinarian. The questionnaire contained items on farm characteristics, biosecurity, animal management and hygiene practices (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/6/14-0706-Techapp1.pdf). Then, tailor-made interventions were developed with the farmer for each farm to be implemented from the beginning of the study. Interventions focused on 1) further reducing antimicrobial use, 2) improving personnel and farm hygiene, and 3) changing animal contact structures.

Each farm was assessed 4 times during the 18-month period (6-month intervals). At each sampling time, the farm questionnaire was filled out again to monitor changes. Human participants completed another questionnaire (online Technical Appendix Table 2) focused on tasks performed, animal contact, and individual health status. Dry cotton nasal swabs (Copan, Brescia, Italy) were used to obtain samples from humans and animals. Persons self-sampled their nostrils, and veterinarians swabbed both anterior nares of 60 pigs per farm. Animal swab samples were analyzed in 10 pools of 6 animals. Each pool comprised pigs of the same age group in the same pen (suckling piglets, weaned piglets, gilts, sows, and finishing pigs). All animal and human samples were sent by courier to the Infectious Diseases and Immunology Department (Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands). The Medical Ethical Committee of the University Medical Centre Utrecht approved the study protocol, and all participants gave written informed consent.

Swab samples were pre-enriched in Mueller Hinton broth, followed by selective enrichment with ceftizoxime and aztreonam and culture on Brilliance MRSA agar (Oxoid, Badhoevedorp, the Netherlands) (*18*). Suspected colonies were subcultured on Columbia agar with sheep blood (Oxoid) and confirmed by using real-time PCR targeting *mecA*, *femA*, *nuc*, and C01 genes (*19*,*20*).

Farm Types

We classified production types as farrowing and farrow-to-finish. Farrowing farms did not produce fatteners and delivered growers (25 kg) to finishing farms (with the exception of 1 farm that delivered gilts for farrowing). Farrow-to-finish farms integrated farrowing and finishing production and delivered fattening pigs to the abattoir. A farm was defined as open when it received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when gilts were not supplied externally.

Data on Antimicrobial Use

In the Netherlands, all antimicrobial drug deliveries to each farm are compiled in national databases. Owners of the study farms gave written consent for retrieval of these antimicrobial use data over a 2-year period. Antimicrobial use was expressed as defined daily dosages per animal per year (DDDA/Y) per farm for the 4 periods preceding each sampling time. The DDDA/Y is a standard weighted measure indicating the number of days of antimicrobial drug use per year for an average animal on the farm. A more detailed description of the calculation of DDDA/Y has been described (*14,16*).

Data Analysis

We conducted all statistical analyses in SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). We explored changes in MRSA carriage in animals and humans and antimicrobial use over time using simple descriptive statistics. DDDA/Y was $\log_2$ transformed because of its right-skewed distribution. A total of 134 variables in the farm questionnaire and 59 in the human questionnaire were selected for longitudinal analysis together with antimicrobial use (criteria of <10% missing values and ≤10% of farms in each category). Odds ratios (ORs) for MRSA positivity in a pig or a human sample in the presence or absence of a determinant were obtained by using random intercept generalized linear mixed models (PROC GLIMMIX; SAS Institute, Inc.). Only associations from the selected variables with $p \leq 0.10$ in pigs (adjusting for age group of the pool) and $p \leq 0.20$ in humans (adjusting for hours worked on the farm) were presented. Goodness-of-fit of the models was described by using -2 log residual pseudo-likelihood estimation, and model assumptions were checked with diagnostic plots. Generalized additive mixed modeling (gamm4 package in R 3.0.2; R Foundation for Statistical Computing, Vienna, Austria) was used to assess the shape of the relationship between antimicrobial use and MRSA in human and animals.

Results

The number of farms was unequally distributed by type of farm (Table 1). Characteristics among persons from different farm types did not differ significantly (Table 2). All MRSA isolated from animals and humans was ST398.

Antimicrobial Use Reduction and Assessment of Particular Interventions

During the 4 periods, tetracyclines were the most used antimicrobial drugs (37.6% of total DDDA/Y), followed by penicillins (30.2%), trimethoprim/sulfonamides (12.3%), macrolides/lincosamides (12.0%), and polymyxins (4.6%). The remaining 3.3% corresponded mainly with cephalosporins, amphenicols, pleuromutilines, and fluoroquinolones. Most antimicrobial classes decreased in parallel during the study; only macrolides slightly increased in DDDA/Y (9.9% to 16.5% from the first to the fourth period), and tetracyclines and trimethoprim/sulfonamides decreased slightly (from 37.0% to 32.7% and from 14.9% to 11.2%,

Table 1. Characteristics of farms in a study of the dose–response relationship between antimicrobial drug use and livestock-associated methicillin-resistant *Staphylococcus aureus* in pig farming, the Netherlands, 2011–2013

Type of farm*	No. farms	Median no. (interquartile range)	
		Sows	Fatteners
All	36	350 (270–550)	773 (0–1,950)
Open	22	337 (300–500)	500 (0–1,300)
Farrowing†	9	533 (350–800)	0
Farrow-to finish	13	314 (242–380)	1,100 (600–2,010)
Closed	14	407 (232–698)	1,400 (450–2,725)
Farrowing†	3	439 (239–905)	0
Farrow-to finish	11	367 (200–673)	1,892 (1,025–2,950)

*Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts.
†No fattening pigs present.

respectively). Overall, 86% of the DDDA/Y were administered as batch or group treatment (i.e., animals were treated in groups mainly orally for prophylactic or metaphylactic reasons) and 14% as individual treatment (mainly by injection). These percentages did not significantly differ by type of farm. During the study, overall DDDA/Y decreased 44%, comparable with the national trend, across all farm types except open farrowing farms (Figure 1). Open and/or farrowing farms used at least twice as many antimicrobial drugs as closed and farrow-to-finish farms (Figure 1).

Farm management changes over time captured from the questionnaires were modest; just 10% of the intervention variables (median 9.7%, interquartile range [IQR] 6.0%–12.3%) per farm changed during the study. Thus, 27 farms had <12 of the 134 variables that changed. The median number of farms within a single change was 3 (IQR 1–4). Thus, 75% of the changes occurred in ≤4 farms. Changes over time did not differ by different farm type. Because of these limited and heterogeneous changes, an intervention effect could not be evaluated and we performed only a risk factor analysis.

MRSA in Pigs

The number of MRSA-positive farms decreased slightly during the study (from 31 to 29 positive farms). Twenty-eight farms were MRSA-positive at all sampling times. Most were open (21 farms; 13 farrow-to-finish and 8 farrowing farms), and 7 were closed (5 farrow-to-finish and 2 farrowing). Four closed farrow-to-finish farms remained MRSA-negative during the entire study. From the remaining 4 farms, 3 became negative and 1 became positive during the study.

Overall pool-prevalence per sampling time decreased slightly on all farms. Open and farrowing farms remained at higher prevalences than closed and farrow-to-finish farms (Figure 2).

MRSA carriage differed notably between different age groups. The average pool-prevalence was 45.6% for finishing pigs; it was highest for suckling and weaned piglets (52.2% and 66.2%, respectively) and lowest for gilts and sows (30.2% and 30.8%, respectively). These prevalences did not significantly differ by farm type.

MRSA in Humans

MRSA prevalence in humans did not change significantly over time (Figure 3, panels A, B). Prevalence and carriage dynamics differed by number of hours worked on the farm. Prevalence for persons working ≥20 hours per week was 5 times higher than for persons working <20 hours (Figure 3, panel B). Persons working ≥20 hours more frequently

Table 2. Characteristics of persons followed during the entire period of a study of the dose–response relationship between antimicrobial drug use and livestock-associated methicillin-resistant *Staphylococcus aureus* in pig farming, the Netherlands, 2011–2013*

Characteristic	Total study population	Farmers, employees	Partners	Children
Age, y (SD)	33.0 (17.8)	44.0 (13.6)	45.2 (8.9)	14.4 (5.6)
Mean time worked, h (SD)	21.8 (25.2)	46.0 (19.9)	10.1 (14.0)	2.2 (6.6)
Total no.	158	66	32	60
Sex				
M	91	58	0	33
F	67	8	32	27
Open farm	91	34	17	40
Farrowing†	26	11	5	10
Farrow-to finish	65	23	12	30
Closed farm	67	32	15	20
Farrowing†	14	8	3	3
Farrow-to finish	53	24	12	17

*Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts.
†No fattening pigs present.

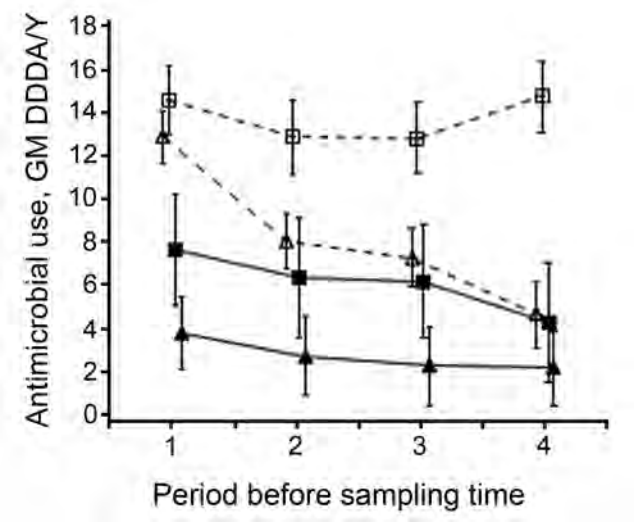

Figure 1. Antimicrobial use by type of farm during the 4 periods (≈6 months) before each sampling time in a study of the dose-response relationship between antimicrobial drug use and livestock-associated methicillin-resistant *Staphylococcus aureus* on pig farms, the Netherlands, 2011–2013. GM and 95% CI from $\log_2$ DDDA/Y. Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts. Closed triangles indicate closed farrow-to-finish farms; closed squares indicate closed farrowing farms; open triangles indicate open farrow-to-finish farms; open squares indicate open farrowing farms. DDDA/Y, defined daily dosages animal per year; GM, geometric mean. Error bars indicate 95% CIs.

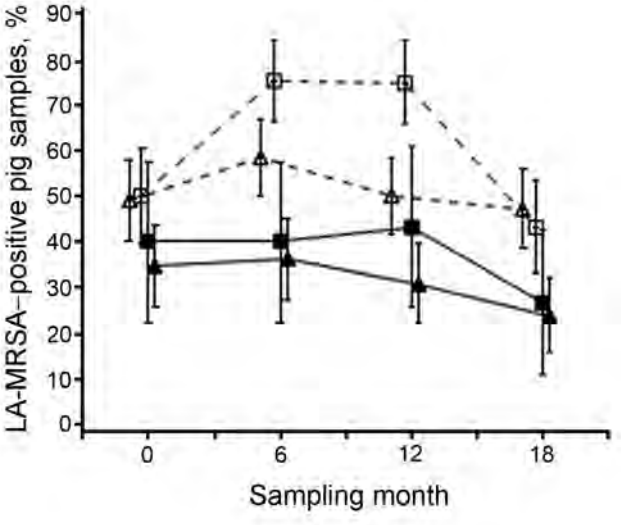

Figure 2. Prevalence of LA-MRSA–positive pooled samples from pigs during a study of the dose-response relationship between antimicrobial drug use and LA-MRSA on pig farms, the Netherlands, 2011–2013. Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts. Closed triangles indicate closed farrow-to-finish farms; closed squares indicate closed farrowing farms; open triangles indicate open farrow-to-finish farms; open squares indicate open farrowing farms. LA-MRSA, livestock-associated methicillin-resistant *Staphylococcus aureus*. Error bars indicate 95% CIs.

tested positive for MRSA at all sampling times (25%) or at least at 1 sampling time (48%), compared with those working <20 hours (2% and 24%, respectively). MRSA carriage dynamics did not significantly differ by level of antimicrobial use (data not shown) or by farm type (see overlap of 95% CIs in Figure 3, panel A).

Antimicrobial Use and MRSA Carriage in Pigs and Humans

Farms with higher antimicrobial use were more likely to have MRSA-positive pigs (Figure 4). The odds that a pool would be MRSA positive was 16% higher for a 2-fold increase in DDDA/Y (Table 3). MRSA in pigs from open and from farrowing farms (high users of antimicrobial drugs) showed a positive trend and a significant association, respectively, with antimicrobial use (Table 3). The odds for testing LA-MRSA positive was higher when the proportion of group treatments with antimicrobial drugs was >0.5 (odds ratio [OR] 1.79, 95% CI 1.12–2.88; p = 0.02). This association was also found on open and on farrow-to-finish farms but was stronger in farrowing farms (OR 2.9, 95% CI 0.98–8.60; p = 0.05). Changes in MRSA carriage in pigs over time were significantly associated with changes in antimicrobial use; the odds for a 2-fold increase in antimicrobial use per sampling time (antimicrobial use–time interaction) decreased from the second to the last sampling (ORs 0.94, 1.27, 1.26, and 1.14 in the 4 consecutive samplings; p = 0.01). The same was found in an analysis restricted to open farms (ORs 0.86, 1.33, 1.18, and 1.06; p = 0.01). In farrowing farms (with little reduction in antimicrobial use), the antimicrobial use–time interaction was also significant, but ORs increased over time (ORs 1.04, 1.38, 1.62, 1.62; p = 0.03).

We also observed a positive trend between antimicrobial use in animals and human MRSA carriage (Figure 4); the unadjusted OR for a 2-fold increase in DDDA/Y was 1.17 (95% CI 0.98–1.39; p = 0.09). The antimicrobial use–MRSA association did not significantly change after adjustment for hours worked (OR_{adj}) (Table 3). When stratified by working hours, antimicrobial use remained especially associated with MRSA for persons working ≥20 hours per week (OR_{adj} 1.25, 95% CI 1.01–1.54; p = 0.04), compared with those working <20 hours (OR_{adj} 1.21, 95% CI 0.92–1.59; p = 0.18). A similar trend was observed across farrow-to-finish, farrowing, and closed farms (Table 3). The probability of LA-MRSA carriage was higher when the proportion of antimicrobial group treatments was ≥0.5 (OR_{adj} 1.76, 95% CI 0.79–3.90; p = 0.17). Reduction in

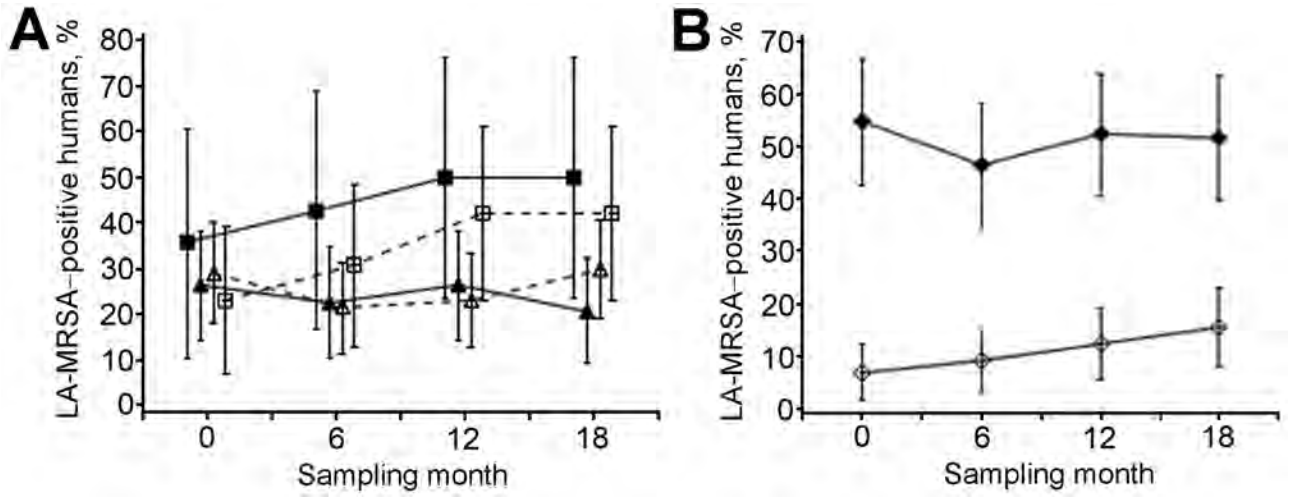

Figure 3. Prevalence of LA-MRSA in humans (n = 158) during a study of the dose-response relationship between antimicrobial drug use and LA-MRSA on pig farms, the Netherlands, 2011–2013. Results are stratified by type of farm (A) and number of hours worked on the farm (B). Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts. Closed triangles indicate closed farrow-to-finish farms; closed squares indicate closed farrowing farms; open triangles indicate open farrow-to-finish farms; open squares indicate open farrowing farms; open diamonds indicate persons working <20 hours per week; closed diamonds indicate persons working ≥20 hours per week. LA-MRSA, livestock-associated methicillin-resistant *Staphylococcus aureus*. Error bars indicate 95% CIs.

antimicrobial use over time was not associated with any change in MRSA carriage in humans.

Specific levels of DDDA/Y for tetracyclines and penicillins were positively associated (p values from 0.06 to 0.23) with MRSA in pigs and humans (data not shown). The use of cephalosporins (on 7 farms, 6 of them open) during the first sampling time, was strongly associated with MRSA carriage in pigs (OR 2.94, 95% CI 1.45–5.87; p = 0.002). This association was not found for humans. Associations with other antimicrobial classes were weaker and often not statistically significant.

Other Factors Determining MRSA in Humans and Pigs

Number of hours worked on the farm per week was strongly associated with MRSA in the human study population (univariate OR 1.82/10 hours worked increase, 95% CI 1.58–2.06; p<0.0001). Except for antimicrobial use, tasks related to animal contact and touching pigs from other farms were identified as risk factors for MRSA carriage in humans (Table 4). All variables in Table 4 were moderately or highly correlated (Spearman/Pearson r>0.5), and no multivariable model was built. We found no correlation between farm size, antimicrobial use, and hours worked.

More biosecurity items reducing MRSA carriage in pigs were found on closed farms (e.g., different compartments per production phase, boarding platform for sows, washing overalls) (Table 5, http://wwwnc.cdc.gov/EID/article/21/6/14-0706-T5.htm). Some variables had a similar effect on open and closed farms, increasing risk for MRSA (e.g., injection of antimicrobial drugs, clipping of teeth, and vaccination of piglets) or decreasing MRSA carriage (e.g., presence of a medication pipe separated from the water pipe, delivery room for materials, and keeping the sows in stable groups [i.e., not mixing]) (Table 5). However, other effects showed conflicting directions between strata (e.g., farm treatment plan, cleaning and disinfecting the carcass barrels, source of water supply) (Table 5). Low-level correlation existed between some variables (pairwise Spearman r<0.5) and with antimicrobial use or cephalosporin use (Table 5). A full multivariable model (online Technical Appendix Table 3) was fitted by using the significant determinants from Table 5 together with the use of antimicrobials and cephalosporins; results from the backward elimination of non-significant terms are presented in Table 6. The presence of external supply of animals, overall antimicrobial use, and use of cephalosporins were significant risk factors retained through all elimination steps.

Discussion

We found a quantitative association between antimicrobial use and MRSA in pigs and humans living and/or working on pig farms. Our findings indicate that a reduction in antimicrobial use is likely to be effective in reducing MRSA carriage in pigs. Risk for MRSA is higher for increased use of tetracyclines and penicillins but more so for use of cephalosporins. Except for the change in antimicrobial use over time, overall changes in farm management were modest and not sufficient to contribute to decreasing MRSA levels. Nevertheless, several factors were identified as possible candidates for future intervention studies.

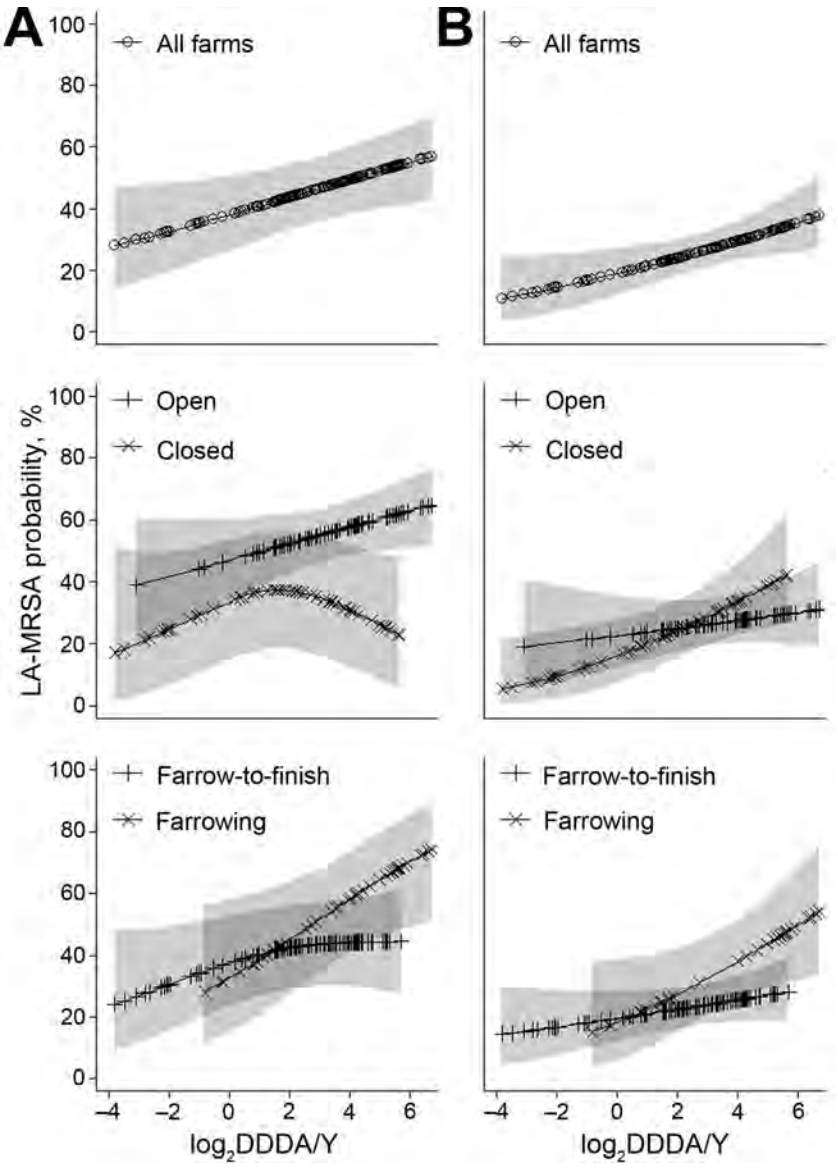

Figure 4. Dose–response relationships between antimicrobial use (log_2 DDDA/Y) and livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) predicted probabilities in pigs (A) and humans (B), the Netherlands, 2011–2013. Splines were obtained from generalized additive mixed models with random intercepts for farms in the analysis for pigs and humans. Models accounted for the repeated measurements design and were adjusted for age group of pigs and for animal contact (i.e., hours worked) for humans. DDDA/Y was determined by dividing the total number of kilograms treatable with a single mass unit of the antimicrobial drug concerned, in accordance with the package insert information, by the average number of animal kilograms on the farm. Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts. p values and maximum-likelihood (ML) scores for the splines in the models for pigs: all farms (p = 0.03; ML 1433.5); open farms (p = 0.09; ML 991.3); closed farms (p = 0.09; ML 407.9); farrowing farms (p = 0.02; ML 438.5); farrow-to-finish farms (p = 0.39; ML 936.5). p values and ML scores for the splines in the models for humans: all farms (p = 0.01; ML 573.9); open farms (p = 0.41; ML 337.8); closed farms (p = 0.01; ML 229.9); farrowing farms (p = 0.03; ML 170.3); farrow-to-finish farms (p = 0.17; ML 398.2). DDDA/Y, defined daily dosages per animal per year; ML, maximum likelihood. Shaded areas indicate 95% CIs.

The extent of representativeness of a convenient sample is difficult to evaluate. Nonetheless, descriptive results show the heterogeneity of farms included; the decreasing trend in use of antimicrobial drugs and the proportions by antimicrobial classes and by group and individual treatments mirror national data (*16*,*17*).

Levels of antimicrobial use differed considerably by farm type. Open and/or farrowing farms were high users of antimicrobial drugs and showed a strong positive dose–response relationship between antimicrobial use and MRSA in pigs. In particular, the use of cephalosporins was related to higher carriage rates of MRSA. The literature shows that selective pressure favors transmission and spread of MRSA in pigs (*13*,*21*). MRSA ST398 isolates have shown high diversity of resistance genes, and all of them are resistant to penicillin and tetracycline (*22*); the DDDA/Y of these antimicrobial classes was related to MRSA in our results. Although the use of cephalosporins represented a small proportion of total antimicrobial use, it was strongly associated with MRSA in pigs. These antimicrobial drugs are known to be important for generation and propagation of resistance in *S. aureus* and other microorganisms (*23*). The fact that they were administered before the first sampling time might be related to the initial increase in MRSA prevalence in pigs. We refrained from presenting detailed associations by antimicrobial classes because mostly all classes were used on all the farms and were correlated; thus, effects of individual classes of antimicrobial drugs were difficult to disentangle and require cautious interpretation. The higher risk posed by administering group treatments confirms previous findings in the literature (*4*,*12*). Interaction between antimicrobial use and time was significant, suggesting a decrease of

Table 3. ORs for livestock-associated MRSA in pigs and in humans with increasing use of antimicrobial drugs, the Netherlands, 2011–2013*

	ORs for a 2-fold increase in DDDA/Y							
	Pooled pig samples				Farmers and family members			
Characteristic	No.†	OR‡ (95% CI)	p value	–2 log RSPL§	No.¶	OR# (95%CI)	p value	–2 log RSPL§
All farms	1,421	1.16 (1.02–1.33)	**0.03****	6,937.5	626	1.22 (1.01–1.48)	**0.04**	3,196.9
Supply of gilts††								
Open	867	1.11 (0.97–1.27)	0.12**	3,828.9	365	1.08 (0.85–1.38)	0.53	1,806.9
Closed	554	0.86 (0.69–1.33)	0.79	3,132.2	261	1.31 (0.94–1.81)	0.11	1,424.3
Production type								
Farrowing	476	1.38 (1.03–1.86)	**0.03****	2,399.2	158	1.28 (0.85–1.94)	0.24	784.3
Farrow-to-finish	954	1.11 (0.95–1.30)	0.18	4,621.4	468	1.19 (0.95–1.50)	0.13	2,439.8

*Farm antimicrobial use was defined as 1 unit increase in the $\log_2$ DDDA/Y. Results from the random intercept generalized linear mixed models accounting for the repeated measurements design and adjusting for confounders. DDDA/Y indicates the number of days of antimicrobial use per year for an average animal on the farm. It was determined by dividing the total number of kilograms treatable with a single mass unit of the antimicrobial drug concerned, according to the package insert information, by the average number of animal kilograms on the farm. The denominator comprised sows and fatteners. DDDA/Y, defined daily dosages animal per year; MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odd ratio; RSPL, residual pseudo-likelihood. Bold type indicates significance (p<0.05).
†Number of observations at all sampling times together (10 pooled pig samples per farm on 36 farms in 4 sampling times). Values are missing for 19 observations.
‡For analysis in pigs, a farm random intercept was included in the mixed models and adjustment of ORs was made for sampling time and age group of pigs in the pool.
§RSPL from the generalized linear mixed models. Models per stratum of external supply or type of production are not nested and -2 log RSPL cannot be used for comparison.
¶Number of observations in all sampling times together (158 persons, 4 sampling times). Values are missing for 6 observations.
#For analysis in humans, a farm and a person random intercept were included in the mixed models, and number of hours worked on the farm and sampling time were used for adjustment of ORs.
**These models additionally showed significant antimicrobial use–time interaction indicating parallel change in antimicrobial use and livestock-associated MRSA prevalence over the study period (see extended explanation in text).
††Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts.

MRSA prevalence in pigs over time with decreasing antimicrobial use. These associations were not found on closed and farrow-to-finishing farms, indicating that below a certain level, antimicrobial use contributes less to MRSA prevalence. Nevertheless, it is important to consider that other studies have reported high MRSA transmission in the absence of antimicrobial agents (*24*,*25*). Thus, antimicrobial use should not be the only target for intervention.

Direct contact with positive animals has been widely reported as the major force driving MRSA carriage in persons living and/or working on farms (*26*–*28*). In our study, higher risk for MRSA in the human study population was strongly associated with the number of hours worked on the farm and to the variables related to tasks performed on the farm. However, antimicrobial use also showed a significant positive dose–response relationship to MRSA human carriage during the study, even after adjustment for hours worked. When antimicrobial drugs are administered to animals, substantial quantities of these drugs remain in manure, on surfaces of barns, and in dust as a potential risk source (*29*). The selective pressure exerted by exposure to dust containing antimicrobial drugs or directly to antimicrobial powder formulations would explain the higher risk for MRSA carriage in persons living or working on pig farms. However, this independent effect of antimicrobial use on susceptible bacteria in humans is difficult to disentangle from direct MRSA transmission from animals to humans.

The role of animal trade in introducing and spreading MRSA has been reported (*4*,*10*–*13*), but information about carriage status of animals entering the farm was not available in this study. Nevertheless, our results corroborate that external supply of animals is significantly associated with higher MRSA levels. A higher selective pressure for MRSA might also occur on open farms because they had higher overall antimicrobial use and 6 of them used cephalosporins. However, external supply of animals appeared to be a risk factor, even when evaluated together with antimicrobial use and cephalosporin use in the multivariate model.

A previous study in the Netherlands found that the prevalence of MRSA-positive pig farms steeply increased from 40% in 2007 to 70% in 2008 (*4*). Our results show that this prevalence remains high (>80%) but the slight increase since 2008 indicates that MRSA carriage in pigs might have reached a steady state. Herd size was identified as a risk factor when MRSA was emerging in livestock (*12*); however, we found no such association.

Several determinants could be targeted for specific interventions in the near future. Factors regarding biosecurity considerably reduced the risk for MRSA, especially on closed farms. It is remarkable that mostly variables related to management of piglets were associated with MRSA. Piglets are more susceptible to infection, and they receive larger amounts of antimicrobial drugs. Tooth clipping in piglets increased the probability for MRSA carriage; MRSA transmission from piglet to piglet might be higher when the same plier is used or through the worker. Unexpected risk factors could be the product of reverse

Table 4. ORs for determinants of livestock-associated MRSA in humans, adjusted for number of hours worked per week on the farm, the Netherlands, 2011–2013*

Variable	No.†	OR‡ (95% CI)	p value§	−2 log RSPL¶
Age, per 10 y increase	632	1.14 (0.93–1.41)	0.2	3,204.1
MRSA prevalence in pigs, %, per 10% increase	632	1.08 (0.97–1.21)	0.16	3,190.9
MRSA-negative farm				
Yes	114	0.06 (0.01–0.27)	**<0.01**	3,288.1
No	518	Ref		
Touching dogs in past 6–12 mo				
Yes	446	0.51 (0.27–0.96)	**0.04**	3,173.7
No	180	Ref		
Touching pigs from other farms in past 6–12 mo				
Yes	86	2.82 (1.35–5.91)	**0.01**	3,205.3
No	546	Ref		
Sorting of sows in past 7 d				
Yes	221	1.91 (0.97–3.77)	0.06	3,144.5
No	392	Ref		
Sorting of suckling piglets in past 7 d				
Yes	159	2.21 (1.16–4.22)	**0.02**	3,169.5
No	455	Ref		
Sorting of weaned piglets in past 7 d				
Yes	174	1.63 (0.83–3.20)	0.16	3,162.9
No	439	Ref		
Feeding sows in past 7 d				
Yes	220	2.03 (0.99–4.17)	0.05	3,126.0
No	390	Ref		
Cleaning and disinfecting weaned piglets section in past 7 d				
Yes	81	1.70 (0.76–3.80)	0.2	3,157.8
No	538	Ref		

*Results from the random intercept generalized linear mixed models accounting for the repeated measurements design and adjusted for number of hours worked. MRSA, methicillin-associated *Staphylococcus aureus*; OR, odds ratio; Ref, reference category; RSPL, residual pseudo-likelihood. Bold type indicates p values <0.05.
†Number of observations in all sampling times together (158 persons, 4 sampling times). Some variables have missing observations.
‡For analysis in humans, a farm and a person random intercept were included in the mixed models, and number of hours worked on the farm and sampling time were used for adjustment of ORs.
§Only variables with p≤0.2 in the mixed models are presented in the human analysis.
¶RSPL from the generalized linear mixed models.

causality such as vaccination of piglets, fatteners, or both and frequent change of needles. These possibilities need to be explored in other, independent studies. Observations for cleaning and disinfection were not consistent. It has been previously reported that disinfection has a short-lasting positive effect for MRSA reduction (*30*). Keeping the groups of sows stable was an interesting protective factor that might reduce MRSA spread within the farm. Animals that drank water from the public supply instead of from a private source had increased probability for MRSA. Zinc oxide specifically co-selects for MRSA ST398 (*31*,*32*), and concentrations can be higher in tap water as a result of leaching from pipes. A higher zinc intake in animals might have led to higher selection for MRSA, but this association needs further research.

Pooling of animal samples leads to less precise prevalence estimates (*33*,*34*) but is a low-cost alternative for individual sampling that enabled enlargement of the number of farms tested. Individual testing, however, would not be expected to lead to different outcomes.

This study shows the inherent difficulty in evaluating pragmatic interventions for MRSA control in pig farms under field conditions over a relatively short period. More farms and controlled interventions, together with longer follow-up periods to capture prevalence changes, are needed to assess intervention effects over time. Despite the limitations, we identified factors that can define attainable future interventions (e.g., avoiding tooth clipping, keeping sows in stable groups). Finally, we demonstrated that antimicrobial use has a strong and positive dose–response relationship with MRSA in pigs and humans living and/or working on pig farms. In particular, use of cephalosporins resulted in increased MRSA carriage rates in pigs. Animal and public health authorities should continue to promote the reduction of antimicrobial use. Different approaches for MRSA control might be needed in light of the differences by type of production and external supply of animals.

Acknowledgments

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Table 6. ORs for the most important determinants of livestock-associated MRSA positivity in 1,054 pooled pig samples from 32 farms (multivariable final model), the Netherlands, 2011–2013*

Characteristic	No.†	OR (95% CI)	p value
Sampling time			
0 mo	262	0.83 (0.48–1.43)	<0.001
6 mo	290	2.05 (1.25–3.37)	
12 mo	259	1.96 (1.20–3.20)	
18 mo	243	Ref	
Age group			
Gilts	212	1.08 (0.65–1.80)	<0.001
Finishers	140	4.09 (2.30–7.25)	
Suckling piglets	212	3.87 (2.34–6.39)	
Weaned piglets	280	9.89 (5.96–16.39)	
Sows	210	Ref	
External supply of gilts‡			
Open	630	5.54 (1.56–19.27)	0.008
Closed	424	Ref	
Delivery room for materials			
Yes	804	0.29 (0.13–0.62)	0.001
No	250	Ref	
Sows housed in stable groups			
Yes	594	0.53 (0.29–0.96)	0.038
No	460	Ref	
Antimicrobial drug use, per 2-fold increase, $\log_2$ DDDA/Y	1,054	1.22 (1.03–1.44)	0.024
Use of cephalosporins			
Yes	84	3.15 (1.47–6.74)	0.003
No	970	Ref	

*Model fit: −2 log RSPL estimation = 5331.7. Multivariable final model after backward elimination of non-significant variables from a full model (online Technical Appendix Table 3, http://wwwnc.cdc.gov/EID/article/21/6/14-0706-Techapp1.pdf) containing the significant associations (p<0.05) presented in Table 5 (http://wwwnc.cdc.gov/EID/article/21/6/14-0706-T5.htm) for all farms, together with antimicrobial drug use, use of cephalosporins, sampling time, and age group of the pool. MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odds ratio; DDDA/Y, defined daily dosages animal per year; Ref, reference category; RSPL, residual pseudo-likelihood.
†Multiple variables had missing values in the full model reducing the number of observations in the final model.
‡Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts.

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Cost-effectiveness of Chlamydia Vaccination Programs for Young Women

Kwame Owusu-Edusei Jr., Harrell W. Chesson, Thomas L. Gift, Robert C. Brunham, Gail Bolan

We explored potential cost-effectiveness of a chlamydia vaccine for young women in the United States by using a compartmental heterosexual transmission model. We tracked health outcomes (acute infections and sequelae measured in quality-adjusted life-years [QALYs]) and determined incremental cost-effectiveness ratios (ICERs) over a 50-year analytic horizon. We assessed vaccination of 14-year-old girls and catch-up vaccination for 15–24-year-old women in the context of an existing chlamydia screening program and assumed 2 prevaccination prevalences of 3.2% by main analysis and 3.7% by additional analysis. Estimated ICERs of vaccinating 14-year-old girls were $35,300/QALY by main analysis and $16,200/QALY by additional analysis compared with only screening. Catch-up vaccination for 15–24-year-old women resulted in estimated ICERs of $53,200/QALY by main analysis and $26,300/QALY by additional analysis. The ICER was most sensitive to prevaccination prevalence for women, followed by cost of vaccination, duration of vaccine-conferred immunity, and vaccine efficacy. Our results suggest that a successful chlamydia vaccine could be cost-effective.

Chlamydia remains a major public health problem; there were ≈105.7 million new cases of this disease among adults 15–49 years of age worldwide in 2008 (*1*). In the United States, >1.4 million cases of chlamydial infections were reported to the Centers for Disease Control and Prevention in 2012 (*2*). A recent study estimated that there were ≈2.8 million cases of chlamydia among all persons of all ages in 2008 (*3*) and that the estimated direct lifetime cost was >$500 million 2013 US dollars (*4*). Most infections in women are asymptomatic, and untreated infections can progress to serious sequelae, such as pelvic inflammatory disease (PID), ectopic pregnancy, tubal infertility, and chronic pelvic pain (*5*,*6*). In addition, untreated chlamydia may cause serious and costly sequelae, such as urethritis, epididymitis, proctitis, and Reiter syndrome in men (*5*).

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In this study, we explored the health and economic outcomes of a hypothetical chlamydia vaccine in the United States from a societal perspective. Although there currently is no chlamydia vaccine, the future development of an effective chlamydia vaccine is possible, and support for use of a vaccine for future chlamydia prevention efforts continues to increase (*7–10*). Models of the effect and cost-effectiveness of human papillomavirus (HPV) vaccine were developed before HPV vaccines were approved for use in the United States. These models, as well as subsequent models they helped to inform, proved valuable to public health officials and policy makers (*11–14*). Our exploratory model is intended to help advance the discussion surrounding development of a successful chlamydia vaccine, to inform the business case for investing in research and development of chlamydia vaccines, and to promote development of more detailed models so that the necessary tools are in place for chlamydia vaccine recommendations.

Methods

Model Summary

Institutional review board approval was not required for this study because we used only secondary data. To assess the health and economic outcomes of a hypothetical chlamydia vaccine for young persons (15–24 years of age), we accounted for herd effects by using a heterosexual transmission model. We constructed a relatively simple deterministic population-based compartmental model of chlamydia transmission (Figure 1) on the basis of previously published models (*15–17*). We assumed a population of 100,000 (50% men and 50% women) (*13*,*16*). To simplify our model, our population was made up of 1 age group (men and women 15–24 years of age) that has the highest risk for chlamydia infection in the United States (*3*). Thus, our model was not age-structured.

Given that our model population consisted of 10 birth cohorts (ages 15 to 24 years), we assumed that annual entry and exit into the population of 15–24-year-old persons was ≈10% of the population. In addition, we assumed that the age at sexual debut (first sexual intercourse) for girls and boys was 15 years. Thus, 14-year-old persons who

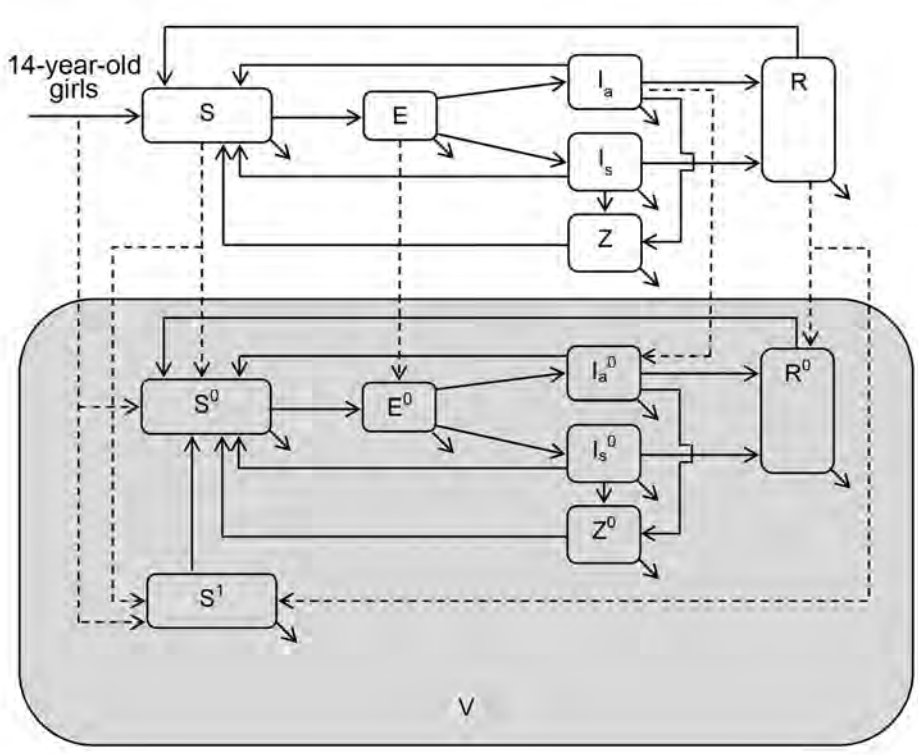

Figure 1. Schematic for exploring the cost-effectiveness of the hypothetical chlamydia vaccine. S, susceptible; E, exposed; I_a, infectious asymptomatic; I_s, infectious symptomatic; R, infection-conferred immunity; Z, sequelae; V (shaded area), vaccinated; superscripts, none, not vaccinated; 0, vaccinated but not effective; 1, vaccinated and effective. Infected persons move into the exposed (E, incubation compartment). From E, they move to either the infectious asymptomatic (Ia) or infectious symptomatic (Is) compartment on the basis of the probability of being symptomatic and the duration of incubation. Further details are provided in the online legend (http://wwwnc.cdc.gov/EID/article/21/6/14-1270-F1.htm).

turned 15 entered the model in susceptible compartments, and 24-year-old persons who turned 25 exited the model at the end of each year, such that the total population was constant at any given time over the analytic horizon (Figure 1). We accounted for heterogeneity in sexual behavior by assuming 2 classes of sexual activity (high and low) on the basis of the annual number of new sex partners. Other details of the model and associated equations are provided in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/21/6/14-1270-Techapp1.pdf). We assembled data for the model from published reports (Table 1).

Preliminary analyses, as well as results from other cost-effectiveness studies, indicated that the burden of chlamydia was an influential variable. Thus, we conducted 2 analyses: main analysis and additional analysis. In the main analysis, parameter values were selected from within published ranges such that the resulting chlamydia prevalence for women in the model was near the US national average for women 15–24 years of age (i.e., 3.2%) (*3*) after accounting for the current screening rate of 30%. In the additional analysis, we modified the model by using parameter values from within published ranges of key parameters such that the resulting chlamydia prevalence for women was 0.5% higher than was used in main analysis (i.e., 3.7% and a screening rate of 30%). Specifically, this was achieved by changing the proportion of women and men in the low sexual activity group from 97.9% to 97.6% and from 95.0% to 95.5%, respectively. Essentially, we increased the proportion of women in the high sexual activity group by 0.3% and decreased the proportion of men in the high sexual activity group by 0.5%. These changes were made to provide more information on the resulting health and economic outcomes in a population with a higher chlamydia prevalence.

Vaccine Characteristics

We assumed that vaccine efficacy was 75% at a cost of $547 (2013 US dollars, cost of complete vaccine series per person) and provided immunity for an average of 10 years. As has been performed in most published studies on vaccine cost-effectiveness (*8,13,14,28*), we repeated the analysis using 100% efficacy and lifelong duration of vaccine immunity. We assumed that the chlamydia vaccine was prophylactic; thus, there were no therapeutic benefits to recipients who were already exposed/infected. We also assumed that persons with symptomatic infections or sequelae were not vaccinated. On the basis of current coverage of HPV vaccine (*27*), we assumed that chlamydia vaccine coverage for girls 14 years of age and women 15–24 years of age would be 30% achieved by a linear increment during the first 5 years of the onset of the vaccination program and would remain at that rate over the analytic horizon.

Evaluation of Strategies and Health Outcomes

The 4 strategies assessed were A) no screening, no vaccination; B) screening women 15–24 years of age; C) screening

Table 1. Model parameters, base-case values, and ranges used in a model to assess health and economic outcomes of a hypothetical chlamydia vaccine*

Parameter	Value (range)		Reference
	Men	Women	
Duration of symptomatic infection, d	14 (10–21)	28 (10–35)	(*15*,*16*)
Duration of asymptomatic infection, d	182.5 (120–240)	365 (240–480)	(*15*,*16*)
Incubation period, d	14 (7–21)	14 (7–21)	(*15*,*16*)
Duration of sequelae, d	21 (10–30)	60 (45–75)	(*16*)
Probability of sequelae, %	2 (0–5)	15 (10–20)	(*16*,*18*)
Per-partnership transmission probability, %	70 (25–80)	68 (25–80)	(*19*)
Probability of symptomatic infection, %	50 (20–80)	20 (10–50)	(*15*,*16*)
Average no. partners in past year, high sexual activity	13.30 (10.00–16.00)	33.26 (30.00–40.00)	(*15*,*16*,*20*)
Average no. partners in past year, low sexual activity	0.90 (0.60–1.20)	0.88 (0.60–1.50)	(*15*,*16*,*20*)
Proportion in low sexual activity class, %	95.0 (90.0–99.0)	97.9 (95.0–99.0)	(*15*,*16*,*20*)
Annual screening rate, %	0	30 (10–50)	(*15*)
Probability of postscreening treatment, %	80 (50–99)	80 (50–99)	(*15*)
Probability of treatment, symptomatic, %	89 (80–100)	89 (80–100)	(*4*)
Test sensitivity, %	95 (90–100)	95 (90–100)	(*21*)
Test specificity, %	99 (95–100)	99 (95–100)	(*21*)
Treatment efficacy (doxycycline, azithromycin), %	92 (80–100)	92 (80–100)	(*15*,*22*)
QALYs lost/case			
Symptomatic infection	0.005646 ± 50%	0.009913 (± 50%)	(*16*)
Sequelae†	0.009530 ± 50%	0.497580 (± 50%)	(*16*)
Costs (2013 US dollars)			
Treatment of acute chlamydia‡	185.2 ± 50%	183.0 (± 50%)	(*4*,*23*–*25*)
Sequelae†	1,337 ± 50%	4,516 (± 50%)	(*4*,*16*,*26*)
Screening	55 ± 50%	55 (± 50%)	(*4*,*23*)
Vaccination	547 ± 50%	547 (± 50%)	Model assumption
Vaccine coverage, 14-y-old persons, %	0	30 (10–50)	Model assumption (*27*)
Vaccine coverage, 15–24-y-old persons, %	0	30	Model assumption (*27*)
Vaccine efficacy, %	75 (50–100)	75 (50–100)	Model assumption (*27*)
Duration of vaccine-conferred immunity, y	10 (1–100)	10 (1–100)	Model assumption
Duration of infection-conferred immunity, y	1 (0.5–5.0)	1 (0.5–5.0)	(*17*)
Relative size of the 14-y-old population entering model compared with overall population model, %	10 (5–15)		Model assumption
Sexual mixing parameter§	0.50 (0.10–0.90)		Model assumption
Discount rate, %	3 (0–10)		Model assumption

*QALYs, quality-adjusted life years.
†Includes productivity costs or QALYs (where applicable) for epididymitis for men and complications associated with pelvic inflammatory diseases (i.e., chronic pelvic pain, ectopic pregnancy, and infertility) for women.
‡Includes productivity costs associated with acute chlamydia and seeking treatment (*24*) and the reported youth (16–24-y-old persons) employment rate in 2010 (48.9%) (*25*).
§Used to determine the degree of mixing between the 2 (high and low) sexual activity groups (0, random mixing; 1, fully assortative).

women 15–24 years of age and vaccinating girls 14 years of age; and D) screening women 15–24 years of age, vaccinating girls (14-year-old), and catch-up vaccination for women 15–24 years of age. Thus, all persons vaccinated were also subject to annual screening at the same rate as persons who were not vaccinated. For cost purposes, it was assumed that screening would be conducted opportunistically when patients sought other care. Therefore, no productivity costs were assessed for screening.

Health outcomes were measured in quality-adjusted life-years (QALYs) estimated by using health state utility weights for acute infections and sequelae for men (epididymitis) and women (PID), including chronic pelvic pain, ectopic pregnancy, and infertility (*16*). Cumulative cost and effects (QALYs) were estimated over a 50-year time frame and analytic horizon for all strategies. All outcomes (cost and effects) were discounted at an annual rate of 3%. All costs were adjusted to 2013 US dollars by using the Medical Care component of the Consumer Price Index (*29*). To provide summaries of cost-effectiveness results from a societal perspective, we included productivity costs in the cost of diseases.

Sensitivity Analyses

We assessed the sensitivity of our results to numerous parameter values (n = 44) that we used in our model. Specifically, we first used the Latin hypercube sampling (*15*,*30*) method to create 120 random combinations of parameter values by randomly choosing (without replacement) from 120 equiprobable parameter value intervals from ranges provided in Table 1. To explore all values in specified ranges equally, we assumed uniform distribution for all variables. Next, we ran each simulation and checked to ensure that a steady-state was reached before and after introducing the strategy. We recorded the resulting prevalence (for men and women), costs, and QALYs before and after the vaccination program. We then ranked all values (i.e., parameter values, prevalence and incremental cost-effectiveness

ratios [ICERs]) and determined the partial rank correlation coefficients (PRCCs). The PRCCs provided the magnitude of the effect of the referent parameter on the ICER after partially eliminating effects of the other parameters.

In preliminary analyses, we found that prevaccination steady-state prevalence could vary substantially in the sensitivity analyses and that prevaccination prevalence for women was an influential determinant of the effect and cost-effectiveness of the vaccine program. Thus, we divided the PRCC analyses into 2 parts. In the first part, we determined the causal parameters for the prevaccination prevalence and then excluded these parameters from the second and final PRCC analysis, in which we determined the influential variables/parameters of the ICER. Thus, we determined the influential parameters of the prevaccination prevalence for women and included the prevaccination prevalence for women in the second and final PRCC analysis to determine the influential variables/parameters of the ICER. For the sensitivity analyses, we focused on the ICER for strategy C (screen women 15–24 years of age and vaccinate girls 14 years of age) when compared with strategy B (screen women 15–24 years of age).

Results

Main Analysis

In the base-case scenario, chlamydia prevalence in the strategy A scenario (no screening, no vaccination) was 3.73% in women and 2.90% in men. With annual chlamydia screening coverage of 30% (the approximate status quo in the United States), chlamydia prevalence decreased from 3.73% to 3.24% for women and from 2.90% to 2.79% for men (Figure 2). The estimated ICER of strategy B (screen women 15–24 years of age) when compared with strategy A (no screening, no vaccination) was $38,700/QALY gained (Table 2). When vaccinating 14-year-old girls only in addition to screening (i.e., strategy C: screen women 15–24 years of age and vaccinate girls 14 years of age), the chlamydia prevalence was reduced to 2.76% for women and to 2.55% for men, and the estimated ICER of vaccination when compared with the status quo strategy B (i.e., screening 15–24-year-old women) was $35,300/QALY gained (Table 2).

Including catch-up vaccination for 15–24-year-old women (i.e., strategy D, screen women 15–24 years of age, vaccinate girls 14 years of age, and catch-up vaccination for women 15–24 years of age) did not change the long-term reduction in chlamydia prevalence relative to strategy C (Figure 2). However, reductions in chlamydia prevalence were achieved more rapidly than without catch-up vaccination (Figure 2). The estimated ICER of adding catch-up vaccination when compared with strategy C (screen women 15–24 years of age and vaccinate girls 14 years of age) was $53,200/QALY gained. Throughout the analyses, although strategy B was weakly dominated, we did not eliminate it because we wanted to show how vaccine strategies compared with the status quo or existing strategy B (screen females 15–24 years of age).

When we applied values for perfect vaccine performance (i.e., 100% efficacy and lifelong duration of immunity), the chlamydia prevalence in strategy C (screen women 15–24 years of age and vaccinate girls 14 years of age) was reduced further, to 2.01% for women and to 2.14% for men (Figure 2), and the ICER when compared with strategy B (screen women 15–24 years of age) was reduced to $9,700/QALY gained. Adding catch-up vaccination for 15–24 year-old women (i.e., strategy D: screen women 15–24 years of age, vaccinate girls 14 years of age, and catch-up vaccination for women 15–24 years of age) compared with strategy C (screen women 15–24 years of age and vaccinate girls 14 years of age) had an ICER of $16,100/QALY gained (Table 2).

Table 2. Summary health and cost outcomes for a hypothetical population of 100,000 persons for the examined strategies for the main analysis (3.2% chlamydia prevalence for women 15–24 years of age)*

Strategy	Cumulative sequelae		Total cost†	QALYs lost	Incremental		
	Men	Women			Cost†	QALYs	$/QALY
A) No screening, no vaccination	1,654	7,458	54,159,500	4,268	Referent	Referent	Referent
B) Screening 15–24-year-old persons	1,593	6,515	72,823,100	3,786	18,663,600	482	38,700
75% efficacy lasting an average of 10 years							
C) Screening 15–24-year-old persons and vaccinating 14-year-old persons	1,487	5,767	87,480,600	3,371	14,657,600 ‡	415‡	35,300
D) Screening 15–24-year-old persons, vaccinating 14-year-old persons, and catch-up vaccination of 15–24-year-old persons	1,466	5,558	93,540,000	3,257	6,059,300	114	53,200
100% efficacy lasting for life							
Repeat C	1,352	4,903	81,495,900	2,889	8,672,800‡	897‡	9,700
Repeat D	1,297	4,423	85,773,100	2,624	4,277,200	265	16,100

*All outcomes (cumulative sequelae, quality-adjusted life-years [QALYs], and costs) have been discounted at an annual rate of 3%.
†Costs are in 2013 US dollars and rounded to the nearest hundred.
‡Incremental cost and QALYs when compared with strategy B (screening 15–24-year-old persons). Although this strategy was weakly dominated, we did not eliminate it because we wanted to show how the vaccine strategies compared with the status quo or existing strategy (B).

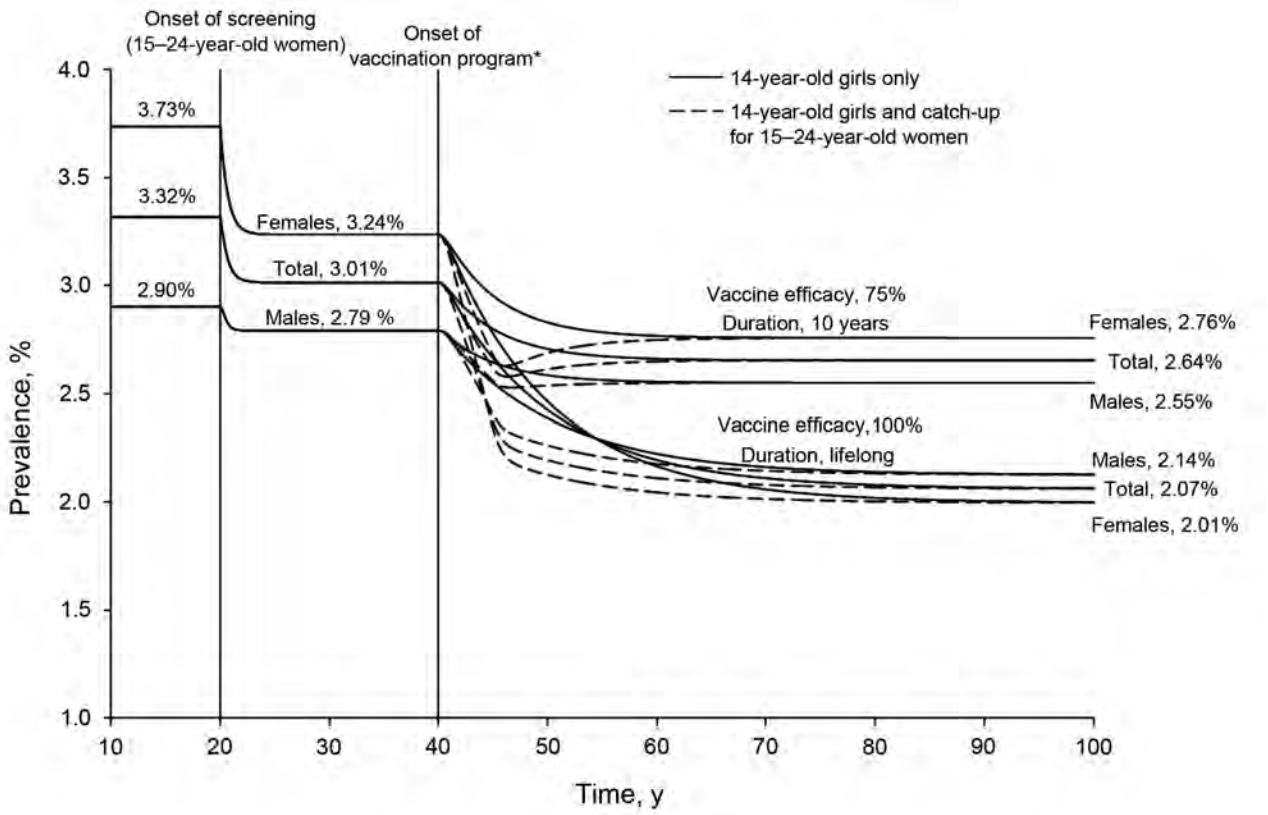

Figure 2. Time-prevalence chart for annual screening for 15–24-year-old women and a hypothetical chlamydia vaccine program for preadolescent girls (14 years of age) and women 15–24 years of age in the United States from the main analyses. We separated the start of the different programs (i.e., screening and vaccination) for illustrative purposes and to avoid clutter. When estimating the health and economic outcomes, we assumed that the strategy being analyzed started at the 20-year mark and the outcomes were tracked over a 50-year period (analytic horizon) ending at the 70-year mark. *Includes the existing annual screening (15–24-year-old women) strategy. Screening and vaccination coverage were 30% for all applicable age groups.

When we assumed perfect chlamydia vaccine performance (i.e., 100% efficacy and lifelong duration of immunity) and increased coverage for 14-year-old persons to ≥75%, our results indicated that overall illness from chlamydia decreased by ≈ 90% in 20 years. In addition, illness from chlamydia was eliminated in ≈30 years after onset of the vaccination program.

Additional Analysis

Results for additional analysis were similar in relative terms to what we found for main analysis. However, because of higher chlamydia prevalence in additional analysis, the estimated ICERs were substantially lower (<50%) than we found for main analysis (Table 3). When we applied values for perfect vaccine performance (i.e., 100% efficacy and lifelong duration of immunity), the estimated ICER for strategy C (screen women 15–24 years of age and vaccinate girls 14 years of age) was cost-saving (Table 3). Adding a catch-up vaccination program for 15–24-year-old women (i.e., strategy D: screen women 15–24 years of age, vaccinate girls 14 years of age, and catch-up vaccination for women 15–24 years of age) was also highly cost-effective ($1,500/QALY gained over strategy C [screen women 15–24 years of age and vaccinate girls 14-years of age]).

Sensitivity Analyses

A summary of results from the first part of the PRCC analyses used to determine the hierarchy of influential parameters for preintervention prevalence in women is shown in Table 4. Our results indicated that the preintervention prevalence for women was highly sensitive to the proportion of women in the low (or high) sexual activity category, followed by the duration of infection-conferred immunity, per-partner transmission probability (man to woman), duration of asymptomatic infections (woman followed by man), mixing parameter, probability of symptomatic infection (woman followed by man), annual screening coverage (women), number of partners in the past year for women with low sexual activity, number of partners in the past year for women with high sexual activity, number of partners in the past year for men with low sexual activity, duration of symptomatic infections in

Table 3. Summary health and cost outcomes for a hypothetical population of 100,000 persons for the examined strategies for the additional analysis (3.7% chlamydia prevalence for women 15–24 years of age)*

Strategy	Cumulative sequelae		Total cost†	QALYs lost	Incremental		
	Men	Women			Cost†	QALYs	$/QALY
A) No screening, no vaccination	1,720	8,610	63,744,600	5,161	Referent	Referent	Referent
B) Screening 15–24-year-old persons	1,635	7,465	82,743,300	4,282	18,998,700	879	21,600
75% efficacy lasting an average of 10 years							
C) Screening 15–24-year-old persons and vaccinating 14-year-old persons	1,568	6,931	87,498,800	3,989	4,755,500‡	293‡	16,200
D) Screening 15–24-year-old persons, vaccinating 14-year-old persons, and catch-up vaccination of 15–24-year-old persons	1,540	6,629	91,820,000	3,825	4,321,200	164	26,300
100% efficacy lasting for life							
Repeat C	1,457	6,122	82,059,500	3,541	–683,800‡	741‡	Cost-saving
Repeat D	1,368	5,252	82,750,200	3,067	690,700	474	1,500

*All outcomes (cumulative sequelae, quality-adjusted life-years [QALYs], and costs) have been discounted at an annual rate of 3%.
†Costs are in 2013 US dollars and rounded to the nearest hundred.
‡Incremental cost and QALYs when compared with strategy B (screening 15–24-year-old persons). Although this strategy was weakly dominated, we did not eliminate it because we wanted to show how the vaccine strategies compared with the status quo or existing strategy (B).

women, probability of postscreening treatment, and relative size of the population of persons 14 years of age entering the model each year.

The second and final part of the PRCC analyses used to determine the hierarchy of influential parameters/variables of the ICER is shown in Table 4. Our results showed that the most influential variable on the estimated ICER was the prevaccination prevalence in women, followed by 3 vaccine-related variables (vaccine cost, duration of vaccine-conferred immunity, and vaccine efficacy), probability of sequelae in women, and the discount rate.

The estimated prevaccination prevalence for women ranged from 0.06% to 8.51% (mean 2.06%, 95% CI 1.81%–2.31%). The overall average ICER was $86,349/QALY gained (95% CI $66,910–$105,789), but this value was largely attributable to scenarios with low prevalence of chlamydia. When looking at the ICERs for female prevaccination prevalence cutoffs (0.00–1.99, 2.00–3.99, and ≥4.00), the average ICERs were $125,087/QALY gained (95% CI $94,422–$155,752), $43,037/QALY gained (95% CI $32,824–$53,248), and $4,849/QALY gained (95% CI cost-saving–$28,344], respectively (Figure 3). When prevaccination prevalence for women was 2%–3%, the estimated average ICER was $44,486/QALY gained (95% CI $31,772–$57,202). Finally, when we limited the analysis to include only parameter sets that resulted in chlamydia prevalence within the CIs reported for chlamydia prevalence in the United States (i.e., 2.26%–4.52%) (*3*), the estimated average ICER was $42,378/QALY gained (95% CI $29,619–$55,136).

Discussion

We used a deterministic heterosexual transmission model that was relatively simple compared with previously published models (*11–14*,*20*,*31*) to explore the potential health and economic outcomes of a hypothetical chlamydia vaccine focusing on vaccination programs for 14-year-old girls and 15–24-year-old women in the United States. We repeated our analyses by using a higher disease burden. Overall, results from our exploratory analyses showed that a chlamydia vaccine could be cost-effective under many plausible scenarios. Interventions that reduce QALYs lost for <1–3 times per capita gross domestic product (≈$50,000 in the United States) are typically considered to be cost-effective (*32*). Our sensitivity analyses suggest that a highly efficacious chlamydia vaccine with long duration of immunity might be cost-saving in countries with high prevalence of chlamydia, as demonstrated by results of our additional analysis. Our results are consistent with preliminary, spreadsheet-based calculations, which suggested that a chlamydia vaccine would cost <$10,000/QALY saved (*28*).

Our analyses showed that a high-performance vaccine could potentially eliminate chlamydia infection when coverage was high (>75%) among susceptible persons before their sexual debut. These results were consistent with findings from previous studies (*17*,*33*), and our estimates of cost-effectiveness of chlamydia screening (versus no screening) were consistent with those of previous studies (*16*,*34*). In addition, the relative cost-effectiveness of targeting different age groups was consistent with results of previous studies on HPV vaccine (*11–14*). In particular, our study showed that catch-up vaccination of 15–24-year-old women, in addition to 14-year-old girls, resulted in an increase in the ICER, implying that additional QALYs are gained at higher costs. Consistent with results of Elbasha et al. (*13*) the addition of catch-up vaccination of 15–24-year-old women did not change the long-term prevalence of infection, but did shorten the time needed to realize the effects of vaccination.

An additional aspect of vaccination is that it is easier to implement than an intervention of routine screening

Table 4. Summary partial rank correlation coefficients for select parameters used in the model to determine the health and economic outcomes of a hypothetical chlamydia vaccine

Variable/parameter*	Rank coefficient†	p value
Dependent variable: prevaccination prevalence in women		
Proportion of women in low activity class	−0.85	0.0001
Duration of infection-conferred immunity	−0.77	0.0001
Per-partner probability of transmission, man to women	0.73	0.0001
Duration of asymptomatic infection in women	0.50	0.0001
Duration of asymptomatic infection in men	0.49	0.0001
Mixing parameter	−0.45	0.0001
Proportion of symptomatic infections for women	−0.40	0.0001
Proportion of symptomatic infections for men	−0.38	0.0001
Annual screening coverage for women	−0.36	0.0001
No. partners in past year, low sexual activity women	0.30	0.0001
No. partners in past year, high sexual activity women	0.30	0.012
No. partners in past year, low sexual activity men	0.27	0.013
Duration of symptomatic infection for women	0.21	0.047
Probability of postscreening treatment	−0.18	0.069
Relative size of the 14-y-old population	0.12	0.091
Dependent variable: incremental cost-effectiveness ratio		
Prevaccination prevalence for women	−0.77	0.0001
Vaccine cost	0.71	0.0001
Duration of vaccine-conferred immunity	−0.50	0.0001
Vaccine efficacy	−0.45	0.0001
Probability of sequelae for women	−0.32	0.0001
Discount rate	0.29	0.0001

*Only variables/parameters for which p<0.10 are shown.
†Presented in decreasing order of absolute magnitude.

because it does not need to be repeated annually. Although health services data have shown chlamydia screening rates ≥30% in young women (*35*), time-series insurance data have shown that <1% of women ≤25 years of age are consistently screened at least once per year (*36*).

Our exploratory study has several limitations. Notable among them is the inherent limitations associated with models in general because models are simplifications of real-world events. Thus, all limitations associated with models are applicable. Another major limitation is the high uncertainty surrounding the parameter values we used (including illness estimates). Because we focused on heterosexual transmission, our model was largely driven by parameters associated with women; prevaccination prevalence was calibrated to approximate reported illnesses for women, and prevaccination prevalence for men was determined by the model. Because a substantially high proportion of high-impact health outcomes of chlamydia infection are in women (i.e., PID and associated complications), it is reasonable to focus on illness in women in such analyses. Nonetheless, as was conducted for HPV (*11*,*13*), future studies should also assess cost-effectiveness of chlamydia vaccination for men.

The prevaccination prevalence rates for men determined by our model were substantially different from reported prevalence rates for men in the United States. For instance, the reported prevalence for men of a similar age group (15–24 years) in the United States was approximately half that of women (men 1.66%; women 3.21%) (*3*), and prevalence in men from our main analysis was substantially higher (men 2.79%; women 3.24%).

We excluded numerous possible outcomes of chlamydia vaccination, such as changes in the number of partners or screening practices, which might arise as a result of vaccination, health benefits for persons vaccinated while infected, and costs and loss in quality of life to persons who experience potential adverse vaccination outcomes, such as side effects (e.g., temporary pain at injection site) (*11*).

We did not explore potential broader properties of an effective chlamydia vaccine, such as degree (i.e., reducing susceptibility but not completely eliminating it) or infectiousness (i.e., breakthrough infections being less infectious than primary infections and shorter in duration). Future studies should consider assessing these 2 characteristics (degree and infectiousness). We assumed that all members of the hypothetical population (with substantially different sexual activity levels) have equal access to screening, treatment, and vaccination. Thus, treatment rate, screening rate, and vaccination coverage were applied equally across all eligible model compartments (subpopulations). However, this simplifying assumption is probably not realistic. Consequently, benefits of screening and vaccination might have been overestimated if women who are highly sexually active were less likely to be screened, treated, or vaccinated. In addition, it is also conceivable that persons vaccinated might be less likely to be screened for chlamydia annually. Further studies are needed to explore the potential health and economic benefits of a chlamydia vaccine that targets specific subpopulations, such as persons infected, those

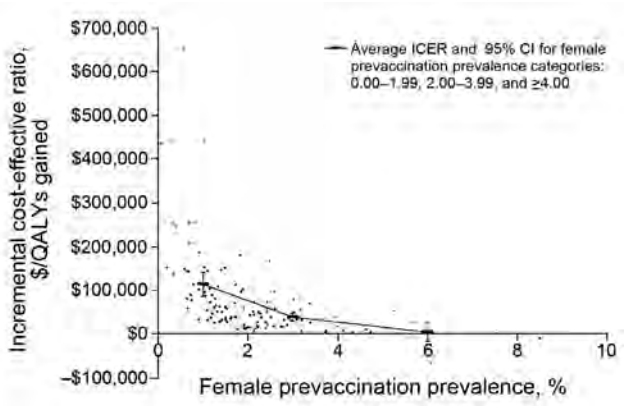

Figure 3. Sensitivity analyses (scatter diagram) showing incremental cost-effectiveness ratios (ICERs) versus female prevaccination prevalence for a hypothetical chlamydia vaccine program. QALYs, quality-adjusted life-years.

with limited access to health care, and those who have multiple sexual partners.

Because our model does not account for major factors, such as age-based mixing of sexual partners and ongoing sexual partnerships, our model is not of sufficient complexity to inform chlamydia vaccine recommendations. For example, our model assumed sexual debut at 15 years of age and that sex partners were chosen from a pool of 15–24 year-old persons, thereby ignoring heterogeneity in age at sexual debut, which is a simplification (*37*). Similarly, models such as ours that do not specifically keep track of ongoing sexual partnerships can overestimate the effect of chlamydia screening because reinfection of treated women by their untreated sex partner is not specifically taken into account (*38*,*39*). If the effect of chlamydia screening is overestimated, then the marginal effect of adding chlamydia vaccination to an existing chlamydia screening program might be underestimated. Development of more complex models will be needed over time, and these models would be better suited to examine the effect of vaccination over a wide range of assumptions regarding vaccine coverage, efficacy, and duration of protection.

Notwithstanding these limitations, our model provides useful information on the potential cost-effectiveness of a chlamydia vaccine, as well as a useful basis for future chlamydia vaccine cost-effectiveness analyses and other modeling studies. In particular, determination of the hierarchy of influential parameters in our model would be useful for future analyses, and assist in understanding the relative roles played by numerous variables that are used in models to facilitate discussions around simple and complex model inputs. Finally, our study suggests that a successful chlamydia vaccine could have a substantial effect on chlamydia prevalence, thereby reducing the health and economic burden associated with chlamydia.

Dr. Owusu-Edusei is an economist at the Centers for Disease Control and Prevention, Atlanta, GA. His primary interests include cost and cost-effectiveness analysis of sexually transmitted infection interventions and policies.

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Hospitalization Frequency and Charges for Neurocysticercosis, United States, 2003–2012

Seth E. O'Neal, Robert H. Flecker

Neurocysticercosis, brain infection with *Taenia solium* larval cysts, causes substantial neurologic illness around the world. To assess the effect of neurocysticercosis in the United States, we reviewed hospitalization discharge data in the Nationwide Inpatient Sample for 2003–2012 and found an estimated 18,584 hospitalizations for neurocysticercosis and associated hospital charges totaling >US $908 million. The risk for hospitalization was highest among Hispanics (2.5/100,000 population), a rate 35 times higher than that for the non-Hispanic white population. Nearly three-quarters of all hospitalized patients with neurocysticercosis were Hispanic. Male sex and age 20–44 years also incurred increased risk. In addition, hospitalizations and associated charges related to cysticercosis far exceeded those for malaria and were greater than for those for all other neglected tropical diseases combined. Neurocysticercosis is an increasing public health concern in the United States, especially among Hispanics, and costs the US health care system a substantial amount of money.

Neurocysticercosis is a leading cause of acquired epilepsy in the developing world (*1*,*2*). The disease occurs when larvae of the pork tapeworm, *Taenia solium*, encyst in the human brain; this process causes a broad range of neurologic signs and symptoms, including seizures, headache, obstructive hydrocephalus, encephalitis, stroke, and cognitive and other mental health disorders (*3*,*4*). Neurocysticercosis is endemic in poor rural communities in Latin America, sub-Saharan Africa, and Asia, where pigs can access and ingest human feces (Figure 1). However, the disease is also of increasing public health concern in the United States, especially in the immigrant population and among persons who have traveled to regions where cysticercosis is endemic (*5*).

The World Health Organization designates cysticercosis as a neglected tropical disease (NTD) and has called for international efforts to strengthen surveillance (*6*–*8*). The disease remains neglected partly because the scale of the problem has not been well defined (*2*). In most disease-endemic regions, population-level data are sparse because surveillance for neurocysticercosis is nonexistent and diagnostic neuroimaging is typically unavailable. In the United States, there is an opportunity to collect population-based data on neurocysticercosis because of the large immigrant population at risk for infection, the widespread availability of neuroimaging, and the well-established disease surveillance infrastructure. However, only Alaska, Arizona, California, New Mexico, Oregon, and Texas require reporting of neurocysticercosis.

Death rates due to neurocysticercosis in the United States have been reported previously (*9*), but national-level assessments of neurocysticercosis that use population–based data are lacking. The objective of our study was to evaluate the frequency and total associated charges for hospitalizations due to neurocysticercosis in the United States and to compare these against other tropical diseases of potential importance in the United States.

Methods

Data Source

We analyzed hospital discharge data contained in the Nationwide Inpatient Sample (NIS) for 2003–2012 (*10*,*11*). The NIS, a stratified weighted sample of short-term and nonfederal hospitals, is designed to approximate a 20% sample of all community hospitals in the United States. As of 2012, 47 states participated in reporting discharge data to the NIS (only Alabama, Delaware, Idaho, and the District of Columbia had not participated), creating a sample representing 95% of the national population. The NIS is the largest collection of longitudinal inpatient care data in the United States and holds information on ≈8 million hospitalizations from >1,000 hospitals each year (*10*). NIS data are de-identified and include information on demographics, diagnostic and procedural codes, length of stay, discharge status, total charges, and expected payees associated with each hospitalization.

Case Definitions

We based our case definitions for hospitalization on diagnostic and procedural codes from the International Classification

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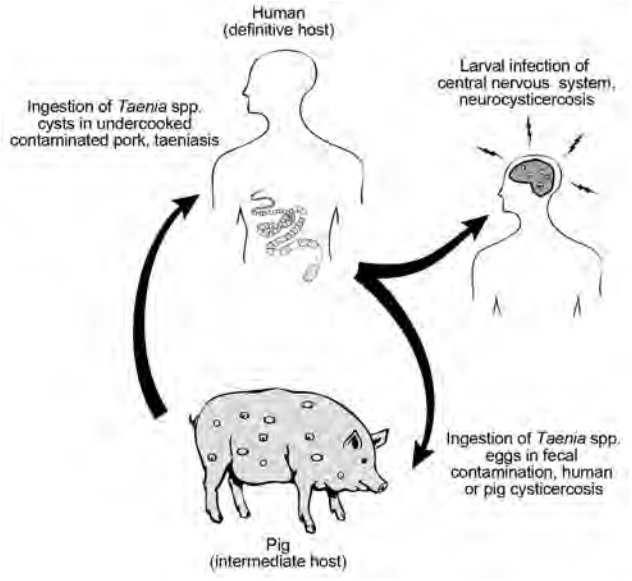

Figure 1. The lifecycle of the *Taenia solium* cestode parasite.

of Diseases, 9th Revision, Clinical Modification (ICD-9-CM). The ICD-9-CM code listed in the first diagnostic field is intended to capture the primary reason for hospitalization. However, there is no specific ICD-9-CM code for neurocysticercosis, so coding patterns may vary. For example, a hospitalization for neurocysticercosis might be coded with a first diagnostic field of 123.1 (cysticercosis) or with a neurologic code, such as 345.9 (epilepsy unspecified), in combination with 123.1 in a different diagnostic field.

We used 2 case definitions in this analysis. The first was a conservative case definition for reporting hospitalizations associated with neurocysticercosis. This definition required the ICD-9-CM code for cysticercosis (123.1) in any of the 15 available diagnostic fields and a supporting diagnostic or procedural code associated with a clinical manifestation of neurologic disease in any of the first 5 diagnostic or procedural fields (Table 1). We used individual ICD-9-CM codes and coding groups defined by Clinical Classification Software to define these additional diagnostic or procedural codes (*13*). This conservative case definition was designed to reduce the likelihood of including hospitalizations for persons carrying an existing diagnosis of neurocysticercosis who were hospitalized for an unrelated condition.

The second case definition was designed to facilitate comparison of hospitalizations for cysticercosis with those for the 16 other NTDs and malaria. The case definition for cysticercosis included all hospitalizations with an ICD-9-CM diagnostic code for cysticercosis (123.1) in any of the first 15 diagnosis fields, but it did not require an additional supportive diagnostic or procedural code. Similarly, the case definitions for the other tropical diseases in the comparative analysis relied on ICD-9-CM codes specific to the disease without a requirement for an additional supportive diagnostic or procedural code. This approach ensured consistency of case definitions across the various diseases at the expense of greater specificity. We assumed that the likelihood of capturing unrelated hospitalizations was similar for the diseases we compared. We excluded Buruli ulcer from our comparison because there is no ICD-9-CM code specific for this disease. However, to our knowledge, Buruli ulcer has not been reported in the United States (*14*). We did not report hospitalizations for rabies, African trypanomiasis, or dracunculiasis because the numbers of hospitalizations were too low (<10/year) to provide accurate estimates. A list of ICD-9-CM codes used in all case definitions is provided in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/21/6/14-1324-Techapp1.pdf).

Table 1. Supporting diagnostic or procedural codes from ICD-9-CM used for conservative case definition for reporting hospitalizations associated with neurocysticercosis*

CCS code	Diagnosis or procedure
Diagnostic code	
76	Meningitis
77	Encephalitis
78	Other CNS infection and poliomyelitis
83	Epilepsy; convulsions
84	Headache; including migraine
85	Coma; stupor; and brain damage
90	Inflammation; infection of eye
95	Other nervous system disorders
109	Acute cerebrovascular disease
111	Other and ill-defined cerebrovascular disease
112	Transient cerebral ischemia
245	Syncope
650	Adjustment disorders
651	Anxiety disorders
652	Attention-deficit, conduct, and disruptive behavior disorders
653	Delirium, dementia, and amnestic and other cognitive disorders
656	Impulse control disorders, NEC
657	Mood disorders
658	Personality disorders
659	Schizophrenia and other psychotic disorders
662	Suicide and intentional self-inflicted injury
670	Miscellaneous mental disorders
Procedural codes	
1	Incision and excision of CNS
2	Insertion; replacement; or removal of extracranial ventricular shunt
177	Computerized axial tomography (CT) scan head
198	Magnetic resonance imaging
199	Electroencephalogram (EEG)

*A complete list of ICD-9-CM codes used in this study is provided in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/21/6/14-1324-Techapp1.pdf). ICD-9-CM, International Classification of Diseases, 9th Revision, Clinical Modification; CCS, Clinical Classification Software groupings of ICD-9-CM codes (*12*); CNS, central nervous system; NEC, not elsewhere classified.

Statistical Methods

To account for the sampling design of the NIS, we analyzed all data by using the survey family of commands in

Stata 13 (StataCorp LP, College Station, TX, USA). We applied hospital discharge weights provided in the NIS to estimate total national hospitalizations on the basis of the stratified sample. All sampled hospitals, regardless of whether they had a patient who was hospitalized with neurocysticercosis, were included for calculation of SEs and CIs. We examined neurocysticercosis hospitalizations by patient age, sex, race, place of service, discharge status, and length of stay; US region; associated diagnostic and procedural codes; and hospitalization charges. State-level assessment was not possible because of the sampling and stratification strategy used in the NIS. Mean annual hospitalization rates were calculated as the weighted number of hospitalizations per 100,000 population on the basis of the US Census Bureau data for each year during the study period (*15*). Age- and sex-adjusted rates were calculated by using the direct standardization method and the 2005 US Census population as the reference population.

We used Gaussian family generalized linear models with logarithmic function link within the Stata survey framework to estimate the crude and adjusted mean length of stay and mean hospitalization charges. We first constructed univariate generalized linear models to evaluate demographic variables of interest, retaining those that were significant at the $p<0.2$ level (Wald test) in the final multivariate models. The independent categorical variables we evaluated were sex, age, race, hospital region, and year of hospitalization. Once we built the final models, we estimated the mean length of stay and mean hospitalization charges for diagnoses and procedures commonly seen with neurocysticercosis (i.e., seizures, obstructive hydrocephalus, headache, stroke, mental health disorder, encephalitis/meningitis, cerebral edema, syncope, neuroimaging, ventricular shunt management, and central nervous system surgery) by individually introducing dummy variables encoding these clinical variables into the models. Inflation-adjusted charges were used in all models.

Hospital Charges

We analyzed hospital charges that were billed to private insurance, Medicaid, Medicare, and other sources from the payer's perspective. Charges represent the amount that hospitals billed for services, not the actual cost of providing these services. Generally, total charges did not include professional fees, noncovered charges, or charges incurred in the emergency department unless the patient was admitted directly from the emergency department into the hospital. We adjusted all charges for inflation by using the Consumer Price Index, setting the base year to 2012.

Results

During 2003–2012, an estimated 23,266 hospitalizations (95% CI 21,741–24,792) in the United States were assigned an ICD-9-CM code of 123.1 in any of the first 15 diagnostic fields. Of these hospitalizations, 18,584 (95% CI 17,322–19,846), approximately 80% of the total, met our case definition of hospitalization due to neurocysticercosis. The number of hospitalizations due to neurocysticercosis per year ranged from a high of 2,247 in 2006 to a low of 1,495 in 2012. The largest proportion of hospitalizations due to neurocysticercosis occurred in the western region (n = 8,026, 42.9% [95% CI 39.2%–46.7%)], followed by the southern region (n = 5,860, 31.8% [95% CI 28.6%–35.1%]), the northeastern region (n = 2,902, 15.5% 95% CI [13.5%–17.6%]) and the midwestern region (n = 1,796, 9.8% [95% CI 8.2%–11.7%]).

We found distinct differences in the mean annual incidence rates of hospitalization stratified by age, sex, and race (Table 2). The mean annual incidence of hospitalization was highest in 20- to 44-year-old age group (1.04 hospitalizations/100,000 population). Hospitalization rates were 33% higher among male patients than female patients. The age- and sex-adjusted mean annual incidence of hospitalizations was highest among Hispanics (2.50 hospitalizations/100,000

Table 2. Number and rate of hospitalizations for neurocysticercosis in the United States, by demographic group, 2003–2012*

Characteristic†	No. hospitalizations (SE)	% All hospitalizations (95% CI)	Rate (95% CI)‡
Age, y			
<20	1,493 (103)	8.0 (7.1–9.1)	0.18 (0.16–0.21)
20–44	10,827 (394)	58.3 (56.5–60.1)	1.04 (0.97–1.12)
45–64	4,357 (232)	23.5 (22.0–25.0)	0.56 (0.51–0.62)
≥65	1,889 (136)	10.2 (9.0–11.5)	0.49 (0.42–0.56)
Sex			
M	10,377 (373)	56.3 (54.5–58.2)	0.70 (0.65–0.75)
F	8,043 (349)	43.7 (41.8–45.5)	0.52 (0.48–0.57)
Race/ethnicity			
Hispanic	12,030 (551)	74.0 (71.5–76.3)	2.50 (2.27–2.73)
White	1,530 (104)	9.4 (8.2–10.7)	0.07 (0.06–0.08)
Black	900 (95)	5.5 (4.5–6.8)	0.25 (0.21–0.30)
Asian/Pacific Islander	377 (61)	2.3 (1.7–3.2)	0.31 (0.23–0.39)
Overall	18,584 (644)		0.61 (0.57–0.66)

*National estimates were based on the Nationwide Inpatient Sample, by using diagnosis code 123.1 from the International Classification of Diseases, 9th Revision, Clinical Modification.
†Missing data not presented
‡Rate for age and sex are unadjusted. Rate for race/ethnicity is adjusted for age and sex by direct standardization method by using 2005 US Census data. Rates expressed as mean annual incidence per 100,000 population.

Table 3. Source of admission, disposition, and expected payer for hospitalizations due to neurocysticercosis, United States, 2003–2012*

Characteristic	No. hospitalizations (SE)	% All hospitalizations (95% CI)
Source of admission		
Emergency department	9,436 (484)	74.7 (72.3–76.9)
Routine	2,210 (145)	17.5 (15.6–19.6)
Transfer	947 (80)	7.5 (6.4–8.7)
Disposition		
Routine	15,693 (562)	84.5 (83.1–85.8)
Transfer	1,617 (107)	8.7 (7.7–9.8)
Home health	834 (81)	4.5 (3.8–5.4)
Against medical advice	205 (33)	1.1 (0.8–1.5)
Died	218 (32)	1.2 (0.9–1.6)
Expected primary payer		
Medicare	2,025 (139)	10.9 (9.7–12.3)
Medicaid	5,543 (316)	29.9 (27.8–32.1)
Private insurance	4,335 (206)	23.4 (21.4–25.4)
Self-pay	4,753 (224)	25.6 (23.8–27.5)
Other payer	1,883 (160)	10.2 (8.9–11.6)

*National estimates were determined on the basis of the Nationwide Inpatient Sample, by using diagnosis code 123.1 from the International Classification of Diseases, 9th Revision, Clinical Modification.

population); the rate was 35 times higher than that for non-Hispanic whites, 10 times higher than that for blacks, and 8 times higher than that for Asian/Pacific Islanders. Unadjusted rates by race were similar: Hispanic, 2.57/100,000; white, 0.06/100,000; black, 0.23/100,000; and Asian/Pacific Islander, 0.26/ 100,000.

Length of Stay, Total Charges, and Payees

The mean length of hospitalization was 6.0 (95% CI 5.7–6.4) days and did not show a significant trend over the study period (p = 1.0). Total inflation-adjusted hospitalization charges over the 10-year study period were US $908,238,000 (95% CI US $814,483,000–$1,001,992,000), increasing 27% from US $72,560,000 in 2003 to US $91,959,000 in 2012. The mean charge per hospitalization was US $50,976 (95% CI US $47,492–$54,716), increasing 50% over the 10-year study period from US $41,874 in 2003 to US $62,986 in 2012. After we adjusted for demographic variables, mean length of stay and mean hospitalization charges were substantially higher for male patients, middle-aged adult patients, and patients from the western region (online Technical Appendix). Publically funded insurance (Medicaid or Medicare) was the primary payer in 40% of the hospitalizations (Table 3).

Associated Diagnoses and Procedures

The most common diagnosis group associated with hospitalization for neurocysticercosis was epilepsy/convulsions, which occurred in 57.3% of hospitalizations, followed by obstructive hydrocephalus (17.7%) and headache (12.4%) (Table 4). After we controlled for year and patient demographics, the diagnoses associated with the longest mean length of stay and the highest mean charges were encephalitis/meningitis (12.2 days and US $78,984) and hydrocephalus (11.4 days and US $79,084). Diagnostic codes for syncope and headache were associated with the shortest

Table 4. Diagnostic and procedure codes for hospitalizations due to neurocysticercosis, United States, 2003–2012*

Associated diagnoses and procedures	No. hospitalizations (SE)	% All hospitalizations (95% CI)	Mean length of stay, d†	Mean charges, US$†
Diagnoses				
Epilepsy; convulsions	10,652 (360)	57.3 (55.3–59.3)	5.4 (4.2–6.9)	33,058 (21,846–50,023)
Obstructive hydrocephalus	3,292 (208)	17.7 (16.2–19.3)	11.4 (8.1–16.2)	79,084 (46,139–135,552)
Headache, including migraine	2,308 (126)	12.4 (11.3–13.6)	3.8 (2..9–4.9)	19,893 (12,422–31,857)
Cerebrovascular disease	1,650 (121)	8.9 (7.9–10.0)	7.5 (5.3–10.6)	45,183 (26,027–78,436)
Mental health disorder	1,843 (132)	9.9 (8.8–11.2)	6.4 (4.7–8.8)	24,436 (13,578–43,979)
Encephalitis/meningitis	1,033 (81)	5.6 (4.8–6.4)	12.2 (8.1–18.6)	78,983 (46,851–133,151)
Cerebral edema	931 (79)	5.0 (4.3–5.9)	7.5 (5.6–10.0)	40,639 (23,449–70,429)
Syncope	573 (56)	3.1 (2.6–3.7)	3.4 (2.6–4.6)	20,017 (11,934–33,577)
Procedures				
Neuroimaging, CT of head or MRI	3,087 (330)	16.6 (13.9–19.8)	6.1 (4.8–7.7)	34,905 (22,177–54,937)
Ventricular shunt, insert, remove, or repair	1,661 (137)	8.9 (7.9–10.2)	16.3 (10.6–25.1)	86,272 (48,313–154,054)
CNS incision or excision	1,499 (111)	8.1 (7.1–9.2)	10.3 (7.9–13.5)	89,893 (56,625–142,709)

*National estimates were based on the Nationwide Inpatient Sample, by using diagnosis code 123.1 from the International Classification of Diseases, 9th Revision, Clinical Modification. CNS, central nervous system; CT, computed tomography; MRI, magnetic resonance imaging.
†Mean length of stay and mean inflation-adjusted hospitalization charges for diagnostic and procedure codes after adjusting for year, patient age, sex, race, and hospital region. Diagnostic and procedure codes were evaluated individually as independent variables in the final generalized linear models built for length of stay and charges.

stays and lowest charges (3.4 days and US $20,017 and 3.8 days and US $19,893, respectively). Procedure codes for shunt management (insertion, removal, or repair) were associated with a mean length of stay of 16.3 days and mean hospitalization charges of US $86,272; codes for brain surgery (central nervous system incision or excision) were associated with a mean length of stay of 10.3 days and mean hospitalization charges of US $89,893. Only 17% of hospitalizations included a procedural code for either computed tomography scans or magnetic resonance imaging of the head.

Comparison of NIS Data for Cysticercosis with that for NTDs and Malaria

The frequency of and total charges for hospitalizations due to cysticercosis exceeded those for all other NTDs combined (Figure 2). During 2003–2012, an estimated 23,266 (95% CI 21,741–24,792) hospitalizations were associated with a diagnosis code for cysticercosis, resulting in US $1,149,044,000 in total hospital charges (95% CI US $1,038,730,000–$1,259,357,000). In contrast, there were 20,029 hospitalizations and US $1,043,109,000 in total charges for all of the other NTDs combined (Table 5).

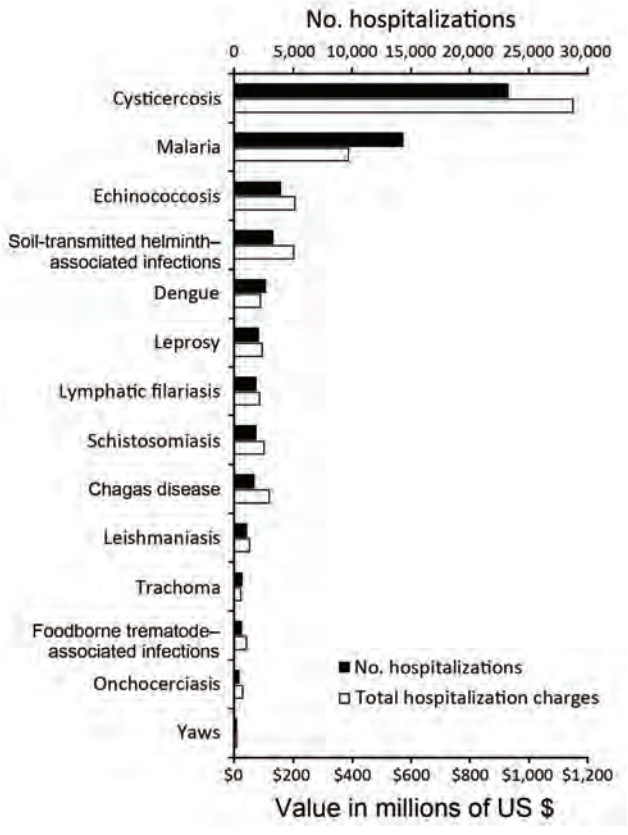

Figure 2. Frequency and total charges of hospitalizations in the United States during 2003–2012 for 13 of the World Health Organization (WHO)–designated neglected tropical diseases (NTDs) and malaria. Estimates were determined by using the Nationwide Inpatient Sample, which codes diagnoses according to the International Classification of Diseases, 9th Revision, Clinical Modification. Frequency of and total charges for hospitalizations for the other NTDs (i.e., Buruli ulcer, rabies, African trypanomiasis, and dracunculiasis) are not shown because there were too few hospitalizations for these diseases for accurate estimation. Frequency and total charges of hospitalizations for malaria, although it is not one of the WHO–designated NTDs, are shown for comparison.

Discussion

The study findings demonstrate that neurocysticercosis poses considerable health and economic problems in the United States, especially among the Hispanic population. Over the 10-year study period, >18,500 hospitalizations for neurocysticercosis occurred, totaling hospital charges of >US $908 million, of which 40% was billed to publicly funded insurance programs. Hospitalization stays were prolonged and expensive, reflecting the complicated nature of acute disease management. Hospitalizations and associated charges for cysticercosis exceeded the totals for malaria and for all of the other NTDs combined.

The hospitalization rates we report in this nationwide study are comparable to those reported in previous state- or county-level studies, providing support for the case definition we used (*12*, *15*–*20*). Because there is no ICD-9-CM diagnostic code specific for neurocysticercosis, the case definitions varied slightly among these studies. The estimated overall hospitalization rate of 0.65/100,000 population that we report falls between the rates previously observed in California (0.8–1.1 hospitalization/100,000 population) and Oregon (0.2–0.5 hospitalizations/100,000 population) (*12*, *16*–*18*). Risk for hospitalization was highest among Hispanic, male, and young to middle-aged adult patients in all studies.

Nearly three quarters of all patients hospitalized for neurocysticercosis in the United States were Hispanic. The Hispanic population is the largest minority group in the United States and among the fastest growing US population groups. Because the hospitalization rate for the Hispanic population is 36 times greater than that of the non-Hispanic white population, the effect of neurocysticercosis on the US economy is likely to increase substantially in the coming years. The US Census Bureau projects that the Hispanic population will grow from 53 million in 2012 to >78 million by 2030 (*21*). Without changes in the rate of hospitalization or the increase in mean hospitalization charges, there could be >1,900 hospitalizations and US $250 million total charges related to neurocysticercosis among Hispanics alone in the year 2030. Changing immigration patterns may also bring an influx of cases in persons from other regions of the world where neurocysticercosis is endemic, particularly Asia and sub-Saharan Africa.

Table 5. Hospitalizations and total charges for neglected tropical diseases and malaria, United States, 2003–2012*

Disease	Hospitalizations		Total charges	
	No. (SE)	95% CI	US$, millions (SE)	95% CI
Cysticercosis	23,266 (778)	21,741–24,792	1,149 (56)	1,039–1,259
Malaria	14,319 (434)	13,469–15,169	387 (18)	351–423
Echinococcosis	3,919 (170)	3,586–4,252	206 (16)	174–237
Soil-transmitted helminth–associated infections	3,256 (151)	2,959–3,552	201 (19)	162–239
Dengue	2,644 (135)	2,379–2,909	89 (9)	70–107
Leprosy	2,055 (135)	1,791–2,319	94 (9)	76–111
Lymphatic filariasis	1,836 (106)	1,629–2,044	86 (9)	68–103
Schistosomiasis	1,811 (120)	1,576–2,046	101 (12)	78–125
Chagas disease	1,686 (151)	1,389–1,982	118 (17)	84–152
Leishmaniasis	1,022 (92)	841–1,203	52 (7)	38–66
Trachoma	649 (69)	514–784	20 (4)	13–28
Foodborne trematode–associated infections	610 (60)	492–729	41 (7)	28–54
Onchocerciasis	380 (47)	287–473	29 (12)	5–53
Yaws	161 (28)	106–216	7 (2)	3–11

*National estimates were determined on the Nationwide Inpatient Sample by using diagnostic codes from the International Classification of Diseases, 9th Revision, Clinical Modification. A complete list of ICD-9-CM codes used in this study is provided in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/21/6/14-1324-Techapp1.pdf).

Several hospital-based studies have shown that seizures are the most frequent reason for hospitalization for neurocysticercosis (*3*,*4*,*22*). In this study, epilepsy was the most frequent diagnosis associated with hospitalization for neurocysticercosis; it was coded in more than half of all hospitalizations for the disease. Seizures in neurocysticercosis are typically amenable to therapy with antiepileptic and anti-inflammatory drugs, resulting in relatively uncomplicated and short hospital stays. In contrast, more severe disease may require intensive interventions and longer hospitalizations, resulting in higher charges (*23*–*25*). While diagnoses of obstructive hydrocephalus or encephalitis/meningitis occurred in ≈20% of persons hospitalized for neurocysticercosis, these more severe presentations accounted for 40% of the total charges incurred.

We report hospitalization diagnostic codes that may not represent the distribution of disease manifestations experienced by individual patients. For example, although a diagnostic code for headache was listed for 11% of hospitalized patients, only patients with headaches associated with underlying pathology requiring acute intervention, such as obstructive hydrocephalus, are likely to be admitted and therefore represented in this study. Even then, the diagnosis of headache may be underrepresented. There were twice as many hospitalizations with diagnostic codes for hydrocephalus and encephalitis than for headache, although both of these manifestations would be expected to be associated with headache (*22*). Similar caution is suggested in interpreting the frequency of other diagnoses presented here. It may seem contradictory that only 17% of hospitalizations had a procedural code for neuroimaging. However, because most imaging for neurocysticercosis would be expected to occur in the emergency department before admission, the infrequent coding for neuroimaging may reflect exclusion of these procedural codes from the hospital discharge summary.

This study documents the substantial costs of hospitalizations due to neurocysticercosis in the United States, but the true effect of neurocysticercosis on the US health care system is likely much greater. Only those emergency department visits that result directly in inpatient admission are captured in the hospital discharge databases in the NIS. In Oregon (*15*), over 40% (31/72) of all patients with neurocysticercosis were seen only in the emergency department and were not admitted to the hospital. While nonadmissions likely represent cases of less clinical severity, substantial charges are still incurred in the emergency department and in outpatient follow-up. Neurocysticercosis is also likely to be substantially underdiagnosed and misdiagnosed because of the lack of a definitive diagnostic test and limited provider awareness of the disease.

Neurocysticercosis also often results in chronic disease that requires outpatient follow-up with infectious disease or neurology specialists, none of which is captured in this study. Management of neurocysticercosis may involve long-term antiepileptic therapy, prolonged regimens of antiparasitic drugs and high-dose corticosteroids, monitoring and repair of ventriculoperitoneal shunts, and treatment of frequent complications resulting from these interventions (*26*,*27*). A chart review at the outpatient neurology clinic in a Houston hospital showed that 2% of all patients were seen for management of neurocysticercosis (*28*). A few states are now collecting comprehensive claims data covering health care provided in inpatient, outpatient, and long-term care settings. Data from these programs could provide more complete information about health care and associated costs related to management of neurocysticercosis in all settings. The high neurocysticercosis hospitalization rate we noted in young adults and men suggests substantial indirect costs to the US domestic workforce. Loss of worker productivity should also be considered in the overall costs of neurocysticercosis.

The use of administrative databases, such as the NIS, to obtain data for this study does have drawbacks, including several limitations we already described. An additional drawback to using the NIS was the inability to identify multiple hospitalizations for a single person, which precludes the ability to estimate the prevalence or incidence of disease. Although the number of states participating in NIS has grown over the years, several states still do not participate in reporting hospital discharge data. In addition, the regional sampling structure of the NIS does not allow for accurate state-level estimates, limiting the ability to identify specific states whose populations are at increased risk for neurocysticercosis. Furthermore, the lack of in-depth demographic and clinical information in the NIS limits the type of questions that can be addressed. For example, knowing the country of birth or travel history of patients with neurocysticercosis could help understand their source of exposure.

Although the primary purpose of this study was to evaluate hospitalizations for neurocysticercosis, we also compared hospitalizations for cysticercosis with those for other NTDs and malaria. Our findings showed that the number of hospitalizations for cysticercosis was nearly 2 times the number for malaria, and the associated hospital charges were nearly 3 times higher. In addition, hospitalizations and charges for cysticercosis were higher than those for all other NTDs we evaluated combined. This comparative analysis was not meant to be exhaustive; we recognize that many factors other than hospitalization contribute to the public health effect of any particular disease. However, the markedly higher number of hospitalizations and associated charges related to cysticercosis, compared with those for other NTDs and malaria in the United States, merits attention and further exploration.

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Additional Drug Resistance of Multidrug-Resistant Tuberculosis in Patients in 9 Countries

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Data from a large multicenter observational study of patients with multidrug-resistant tuberculosis (MDR TB) were analyzed to simulate the possible use of 2 new approaches to treatment of MDR TB: a short (9-month) regimen and a bedaquiline-containing regimen. Of 1,254 patients, 952 (75.9%) had no resistance to fluoroquinolones and second-line injectable drugs and thus would qualify as candidates for the 9-month regimen; 302 (24.1%) patients with resistance to a fluoroquinolone or second-line injectable drug would qualify as candidates for a bedaquiline-containing regimen in accordance with published guidelines. Among candidates for the 9-month regimen, standardized drug-susceptibility tests demonstrated susceptibility to a median of 5 (interquartile range 5–6) drugs. Among candidates for bedaquiline, drug-susceptibility tests demonstrated susceptibility to a median of 3 (interquartile range 2–4) drugs; 26% retained susceptibility to ≤2 drugs. These data may assist national TB programs in planning to implement new drugs and drug regimens.

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In 2009, the World Health Organization (WHO) estimated that ≤5% of patients with multidrug-resistant tuberculosis (MDR TB) were receiving appropriate diagnostic and therapeutic services. This proportion increased to ≈20% by 2012 (*1*,*2*). After decades of relative neglect, health care services for persons with drug-resistant TB are scaling up worldwide at an unprecedented pace (*2*). Part of the delay had been that treatment of MDR TB required a combination of 4–6 expensive, relatively toxic drugs administered for ≈2 years (*3*,*4*). However, treatment guidelines are based on expert opinion, observational studies, in vitro drug-susceptibility testing (DST), and analogies with other mycobacteria because few clinical trials have been conducted of treatment for MDR TB. Treatment success rates average only 55%–65% (*5*–*7*).

Two recent advances have the potential to revolutionize treatment of MDR TB. First, during 2012–2013, two new drugs, bedaquiline and delamanid, were approved provisionally (by the US Food and Drug Administration and the European Medicines Agency, respectively) to treat MDR TB; these drugs are the first truly new anti-TB drugs since rifampin was developed during the 1960s. Because of the urgency of the global MDR TB situation, these drugs were approved on the basis of phase II controlled clinical trials data that used a short-term proxy for long-term treatment outcomes: sputum culture conversion at 2 months (delamanid) or 6 months (bedaquiline). Phase III trials, currently underway, will take years to complete (*8*). The Food and Drug Administration and WHO guidelines recommend adding bedaquiline when an effective regimen (containing at least 4 effective second-line drugs plus pyrazinamide) cannot be designed because of drug toxicity or resistance to fluoroquinolones or second-line injectable drugs (*9*,*10*).

Second, in 2010, Van Deun et al. published their experience with programmatic management of MDR TB in Bangladesh (*11*) and suggested that an intensive 9-month regimen was more effective than treatment success rates reported worldwide and was also considerably less

expensive. The regimen consisted of 7 drugs for the first 4 months, followed by 4 drugs for the remaining 5 months of treatment. The results were compelling enough that the regimen is being evaluated in a randomized controlled clinical trial, STREAM (the Evaluation of a Standardised Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR TB); results are expected in 2017–2018 (*12*). Because the original study focused on patients without previous use of second-line drugs, both the STREAM trial and countries that are adopting the 9-month regimen exclude patients who have baseline resistance to fluoroquinolones or a second-line injectable drug.

Thus, the exclusion criteria for the 9-month regimen—resistance to a fluoroquinolone or a second-line injectable drug—are virtually the same as the inclusion criteria for the use of bedaquiline. To simulate the extent to which MDR TB patients might qualify either for treatment with a 9-month regimen or for treatment with bedaquiline, we analyzed data from a large observational cohort of patients with MDR TB from 9 countries.

Methods

Patient Population

Dalton et al. described the Preserving Effective TB Treatment Study (PETTS) (*13*). In brief, PETTS was a prospective cohort study of consecutive consenting adults who had locally confirmed pulmonary MDR TB and who started treatment with second-line drugs during January 1, 2005–December 31, 2008, in 9 countries (the Philippines, South Africa, Peru, Russia, South Korea, Latvia, Thailand, Taiwan, and Estonia). Patients were treated in accordance with WHO and local treatment guidelines at that time, using regimens of at least 18 months duration, and were followed with monthly sputum cultures throughout treatment (*3*). Eight countries used individualized MDR TB regimens. The study was approved by the US Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) Institutional Review Board and by institutional review boards at all participating sites. Informed consent for participation in the study was obtained from all patients.

Laboratory Methods

Baseline isolates of *Mycobacterium tuberculosis* were shipped in batches to CDC for DST using the indirect agar proportion method on Middlebrook 7H10 agar according to the Clinical Laboratory Standards Institute standard as previously described (*13*). DST was conducted for 10 drugs: rifampin (1.0 μg/mL); isoniazid (0.2, 1.0, and 5.0 μg/mL); ethambutol (5.0 μg/mL); ofloxacin (2.0 μg/mL); ciprofloxacin (2.0 μg/mL); kanamycin (5.0 μg/mL); capreomycin (10.0 μg/mL); amikacin (4.0 μg/mL); para-aminosalicylic acid (2.0 μg/mL); and ethionamide (10.0 μg/mL). Cycloserine was not tested at CDC because it requires Lowenstein-Jensen medium, and CDC uses Middlebrook agar. Pyraznamide susceptibility testing and *pncA* sequencing are under way.

CDC's DST results were used in all analyses. For analysis purposes, ciprofloxacin and ofloxacin were counted as the same drug ("a fluoroquinolone") and kanamycin and amikacin counted as the same drug ("an aminoglycoside"). High-dose isoniazid resistance was defined as resistance at a concentration of 1.0 μg/mL.

Definitions

Second-line injectable drugs were kanamycin, amikacin, and capreomycin. Extensively drug-resistant (XDR) TB was defined as MDR TB plus resistance to any fluoroquinolone and at least 1 of 3 second-line injectable drugs. Pre-XDR TB was defined as MDR TB plus resistance to either any fluoroquinolone or at least 1 of 3 second-line injectable drugs. Effective drugs were defined according to susceptibility demonstrated by CDC's phenotypic DST results. Standard WHO treatment outcomes were used in all sites (*3*). Treatment success was defined as cure or completion of treatment (*3*). Poor outcome was defined as treatment failure, patient death, or loss to follow-up.

The short regimen was defined as treatment of MDR TB with duration of ≤12 months (*14*). Candidates for the short regimen were defined as patients whose *M. tuberculosis* isolates had no baseline resistance to fluoroquinolones and second-line injectable drugs. Candidates for bedaquiline-containing regimen were defined as patients whose baseline *M. tuberculosis* isolates had resistance to a fluoroquinolone or second-line injectable drug (i.e., pre-XDR or XDR) (*10*).

Data Analysis

We conducted statistical analyses using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). General descriptive data, including frequencies of basic demographic and clinical variables, were calculated in aggregate and stratified by drug-resistance patterns. Continuous variables were summarized with standard descriptive statistics. We compared proportions using the χ^2 test or Fisher exact test, as appropriate. Confidence intervals for binomial proportions were calculated by using the 1-sample Wald method. We considered $p \leq 0.05$ statistically significant.

Results

A total of 1,659 patients were enrolled in PETTS strictly according to protocol. For 1,254 (75.6%) of these, a baseline *M. tuberculosis* isolate was shipped to, and DST results were available from, CDC. A total of 64.1% of patients were male, and the median age of all patients was 37 (interquartile range [IQR] 28-47) years. HIV co-infection was confirmed in 159 (12.7%) patients, of whom 135 (85%) were in 1

country. A total of 168 (13.4%) patients had new MDR TB; 879 (70.1%) previously received first-line anti-TB drugs; 185 (14.8%) received second-line drugs in the past; and 22 (1.8%) had unknown treatment history. DST was performed in local laboratories in countries to a median of 6 (IQR 4–8) drugs. Overall treatment outcomes were as follows: cure, 659 (52.5%); completion of treatment, 66 (5.2%); treatment failure, 80 (6.4%); death, 170 (13.6%); loss to follow-up, 235 (18.7%); and unknown, 44 (3.5%).

Of the 1,254 patients, 952 (75.9%) had no resistance to fluoroquinolones and second-line injectable drugs and would qualify as candidates for the short regimen on the basis of the drug resistance indication. On the other hand, 302 (24.1%) patients had resistance to a fluoroquinolone or second-line injectable drug and would qualify as candidates for a bedaquiline-containing regimen. The proportion of patients with no baseline resistance to fluoroquinolones and second-line injectable drugs varied among countries from 47.2% to 92.5%; the proportion of patients with pre-XDR or XDR varied from 7.5% to 52.8% (Table 1).

We assessed baseline resistance to individual drugs among potential candidates for the 9-month short regimen and for a bedaquiline-containing regimen and stratified the results by resistance to fluoroquinolones and second-line injectable drugs (Table 2). Susceptibility to high-dose isoniazid was observed in 8.6% of candidates for the short regimen and 3.0% of candidates for a bedaquiline-containing regimen ($p<0.001$), susceptibility to ethambutol in 41.9% and 27.8% ($p<0.001$), susceptibility to ethionamide in 81.9% and 80.1% ($p = 0.48$), and susceptibility to para-aminosalicylic acid in 95.0% and 76.2% ($p<0.001$), respectively.

Overall, a median of 5 (IQR 5–6) drugs remained effective in candidates for the short regimen and 3 (IQR 2–4) drugs in candidates for a bedaquiline-containing regimen (Table 3). However, in XDR TB cases, a median of only 2 (IQR 1–2) drugs were effective, whereas in pre-XDR cases, a median of 4 (IQR 2–4) drugs were effective ($p<0.001$). The initial isolate was susceptible to ≥3 drugs in all of the candidates for the short regimen and in 73.8% (223/302) of candidates for a bedaquiline-containing regimen. Among candidates for a bedaquiline-containing regimen, 90.4% (206/228) of pre-XDR patients had susceptibility to ≥3 drugs, compared with 23.0% (17/74) of XDR patients ($p<0.001$).

Several patient characteristics were significantly associated with higher probability of resistance to fluoroquinolones or second-line injectable drugs (and thus candidacy for bedaquiline-containing regimen). These characteristics were unemployment, history of imprisonment, alcohol abuse, history of treatment with second-line drugs, and pulmonary cavities (Table 4).

Overall treatment success among candidates for a short regimen was 66.1% (95% CI 63.0%–69.1%), varying from 90% to 50% among countries. Among candidates for a bedaquiline-containing regimen, treatment success was 39.9% (95% CI 34.3%–45.6%), varying from 90% to 10% among countries.

Discussion

Data from our large prospective observational cohort study of 1,254 MDR TB patients showed that about three fourths would qualify as candidates for the short regimen and about one fourth would qualify as candidates for a bedaquiline-containing regimen on the basis of the drug resistance indication. DST demonstrated susceptibility to a median of 5 (IQR 5–6) drugs among candidates for the 9-month regimen, whereas among candidates for bedaquiline, a median of 3 (IQR 2–4) drugs remained effective. Overall treatment success was 66% for candidates for the short regimen and 40% for candidates for a bedaquiline-containing regimen. This analysis has several practical implications for national TB control programs that plan to start using short regimens or bedaquiline for programmatic management of drug-resistant tuberculosis.

First, data from this report may assist national TB control programs in planning the number of patients to be enrolled for short or bedaquiline-containing regimen. Not all countries have representative data from routine drug resistance surveillance or surveys; thus, data from this report can be used to estimate numbers of candidate patients for certain regimens. The 2013 WHO report included combined representative data from 75 countries and 4 territories showing that the 32% of persons had MDR TB resistant to a fluoroquinolone, a second-line injectable drug, or both, and thus these persons might be eligible to receive bedaquiline (*1*). Our data showed a relatively similar proportion (26%). The remaining 74% of patients could be considered as candidates for the short regimen. However,

Table 1. Proportion of patients with multidrug-resistant tuberculosis who would have qualified for treatment with the short (9-month) regimen or with bedaquiline-containing regimen, 2005–2008*

	Country, no. patients (%)									
Candidate regimen	Estonia, n = 24	Latvia, n = 89	Peru, n = 194	Philippines, n = 386	Russia, n = 96	South Africa, n = 281	South Korea, n = 96	Taiwan, n = 40	Thailand, n = 48	Total, N = 1,254
Short†	13 (54.2)	42 (47.2)	154 (79.4)	357 (92.5)	59 (61.5)	195 (69.4)	58 (60.4)	31 (77.5)	43 (89.6)	952 (75.9)
BDQ‡	11 (45.8)	47 (52.8)	40 (20.6)	29 (7.5)	37 (38.5)	86 (30.6)	38 (39.6)	9 (22.5)	5 (10.4)	302 (24.1)

*Percentages show proportion of patients in cohort form each country who would be candidates for a particular regimen. BDQ, bedaquiline.
†Candidates for short regimen were patients without baseline resistance to fluoroquinolones and second-line injectable drugs.
‡Candidates for BDQ-containing regimen were patients with baseline resistance to fluoroquinolones or second-line injectable drugs.

Table 2. Individual drug resistance in relation to the profile of fluoroquinolone and SLI resistance among patients with multidrug-resistant tuberculosis who could have been candidates for a short (9-month) treatment regimen or a bedaquiline-containing regimen*

		Candidate patients, no. (%)				
		Short regimen,	BDQ-containing regimen			
Drug, DST result	Total, N = 1,254	FQ-S and SLI-S, n = 952	FQ-R or SLI-R,† n = 302	FQ-R only, n = 73	SLI-R only, n = 155	XDR, n = 74
INH, high-dose						
NA	11 (0.9)	11 (1.2)	0	0	0	0
R	1,152 (91.9)	859 (90.2)	293 (97.0)	69 (94.5)	154 (99.4)	70 (94.6)
S	91 (7.3)	82 (8.6)	9 (3.0)	4 (5.5)	1 (0.6)	4 (5.4)
EMB						
R	771 (61.5)	553 (58.1)	218 (72.2)	51 (69.9)	113 (72.9)	54 (73)
S	483 (38.5)	399 (41.9)	84 (27.8)	22 (30.1)	42 (27.1)	20 (27)
FQ						
R	147 (11.7)	0	147 (48.7)	73 (100)	0	74 (100)
S	1,107 (88.3)	952 (100)	155 (51.3)	0	155 (100)	0
KAN/AMK						
R	219 (17.5)	0	219 (72.5)	0	150 (96.8)	69 (93.2)
S	1,035 (82.5)	952 (100)	83 (27.5)	73 (100)	5 (3.2)	5 (6.8)
CAP						
NA	9 (0.7)	9 (0.9)	0	0	0	0
R	140 (11.2)	0	140 (46.4)	73 (100)	88 (56.8)	140 (46.4)
S	1,105 (88.1)	943 (99.1)	162 (53.6)	0	67 (43.2)	162 (53.6)
THA						
R	232 (18.5)	172 (18.1)	60 (19.9)	8 (11.0)	30 (19.4)	22 (29.7)
S	1,022 (81.5)	780 (81.9)	242 (80.1)	65 (89.0)	125 (80.6)	52 (70.3)
PAS						
R	120 (9.6)	48 (5.0)	72 (23.8)	13 (17.8)	28 (18.1)	31 (41.9)
S	1,134 (90.4)	904 (95.0)	230 (76.2)	60 (82.2)	127 (81.9)	43 (58.1)

*AMK, amikacin; BDQ, bedaquiline; CAP, capreomycin; DST, drug-susceptibility testing; EMB, ethambutol; FQ, fluoroquinolone; INH, isoniazid; KAN, kanamycin; NA, not available; PAS, para-aminosalicylic acid; R, resistant; S, susceptible; SLI, second-line injectable drug; THA, ethionamide; XDR, extensively drug-resistant.
†Comprises FQ-R only, SLI-R only, and XDR cases combined.

the proportions of patients in our study who might qualify for the 9-month or bedaquiline-containing regimen varied widely among countries: 47%–93% and 8%–53%, respectively. This variation in baseline drug resistance probably reflected previous use of anti-TB second-line drugs in these countries and the consequent epidemiology of MDR TB (*13*).

Second, we report on the prevalence of resistance to first- and second-line drugs by the status of resistance to fluoroquinolones or second-line injectable drugs. Candidates for the short regimen had, on average, susceptibility to 5 anti-TB drugs. We did not have DST for pyrazinamide and clofazimine (which are part of the 9-month regimen). Susceptibility to high-dose isoniazid, also included in the short regimen, was found in only 8.6% of candidates for that regimen. To our knowledge, only 1 randomized clinical trial has documented improved interim treatment outcomes (reduced time to sputum culture conversion and higher proportion with sputum culture negative 6 months after treatment started) among patients receiving high-dose isoniazid (16–18 mg/kg) as an adjuvant to second-line drugs in documented MDR TB (*15*). More clinical research is needed about use of high-dose isoniazid to treat MDR TB (*16*). Susceptibility to ethambutol was found in 42% and to thioamides in 82% of candidates for the short regimen. On the other hand, candidates for a bedaquiline-containing regimen frequently had resistance to other first- and second-line drugs with a median of 3 drugs that remained effective; the number of effective drugs progressively decreased from 3 or 4 in patients with pre-XDR to 2 drugs in XDR TB. Therefore, countries planning to implement bedaquiline for programmatic use should include plans to procure third-line drugs, such as linezolid and clofazimine. These additional drugs are needed to build regimens with the minimum 4

Table 3. Resistance patterns of drugs effective in vitro for multidrug-resistant tuberculosis*

		Candidate effective drugs				
		Short regimen,	BDQ-containing regimen			
	Total, N = 1,254	FQ-S and SLI-S, n = 952	Combined FQ-R or SLI-R,† n = 302	FQ-R only, n = 73	SLI-R only, n = 155	XDR, n = 74
Median	5	5	3	4	3	2
Interquartile range	4–6	5–6	2–4	4–5	3–4	1–2
Range	0–7	3–7	0–6	2–6	1–5	0–4

*Drug susceptibility test results were analyzed for 7 drugs: high-dose isoniazid, ethambutol, FQ, aminoglycoside, capreomycin, ethionamide, and para-aminosalicylic acid; BDQ, bedaquiline; FQ, fluoroquinolone; R, resistant; S, susceptible; SLI, second-line injectable drug; XDR, extensively drug-resistant.
†Includes FQ-R only, SLI-R only, and XDR cases combined.

Table 4. Association of patient characteristics with candidacy for a short (9-month) regimen or a BDQ-containing regimen among patients with MDR TB*

Characteristic	Patient candidate, no. (%)			p value
	Total	Short regimen	BDQ-containing regimen	
Sex				
M	804	613 (76.2)	191 (23.8)	0.72
F	450	339 (75.3)	111 (24.7)	
Employment status				
Unemployed	475	328 (69.1)	147 (30.9)	**<0.001**
Disabled, retired, student, housewife	192	150 (78.1)	42 (21.9)	0.37
Employed	581	471 (81.1)	110 (18.9)	
History of imprisonment				
Yes	81	50 (61.7)	31 (38.3)	**0.001**
Unknown	181	130 (71.8)	51 (28.2)	0.08
No	992	772 (77.8)	220 (22.2)	
Homeless				
Yes	29	21 (72.4)	8 (27.6)	0.54
Unknown	137	91 (66.4)	46 (33.6)	**0.005**
No	1,088	840 (77.2)	248 (22.8)	
Alcohol abuse				
Yes	186	108 (58.1)	78 (41.9)	**<0.001**
Unknown	52	31 (59.6)	21 (40.4)	**<0.001**
No	1,016	813 (80)	203 (20)	
HIV status				
Positive	159	108 (67.9)	51 (32.1)	0.88
Unknown	468	422 (90.2)	46 (9.8)	**<0.001**
Negative	627	422 (67.3)	205 (32.7)	
Any co-morbidity other than HIV infection				
Yes	335	257 (76.7)	78 (23.3)	0.69
No	919	695 (75.6)	224 (24.4)	
Classification by prior treatment history				
Received first-line drugs	879	719 (81.8)	160 (18.2)	**<0.001**
Received second-line injectable drugs	185	103 (55.7)	82 (44.3)	**0.04**
New case	168	112 (66.7)	56 (33.3)	
Classification by prior TB treatment outcome				
Relapse	181	124 (68.5)	57 (31.5)	0.66
Failure, loss to follow up, change to second-line regimen	598	444 (74.2)	154 (25.8)	**0.04**
Chronic	190	172 (90.5)	18 (9.5)	**<0.001**
Unknown	119	102 (85.7)	17 (14.3)	**<0.001**
New case	166	110 (66.3)	56 (33.7)	
Body mass index				
<18.5	471	369 (78.3)	102 (21.7)	0.12
≥18.5	783	583 (74.5)	200 (25.5)	
Site of TB disease				
Extrapulmonary and pulmonary	57	45 (78.9)	12 (21.1)	0.59
Pulmonary only	1,195	906 (75.8)	289 (24.2)	
Bilateral TB disease				
Yes	993	748 (75.3)	245 (24.7)	0.48
No	236	183 (77.5)	53 (22.5)	
Cavities on chest radiograph				
Unilateral	477	339 (71.1)	138 (28.9)	**<0.001**
Bilateral	293	210 (71.7)	83 (28.3)	**<0.001**
No	484	403 (83.3)	81 (16.7)	
AFB smear at the start of MDR treatment				
Positive	1,063	810 (76.2)	253 (23.8)	0.37
Negative	155	113 (72.9)	42 (27.1)	

*Row percentages show proportion of patients with each characteristic that would be candidates for a particular regimen. AFB, acid-fast bacilli; BDQ, bedaquiline; MDR, multidrug-resistant; TB, tuberculosis.Bold type indicates statistical significance.

effective drugs because of the extent of resistance to other second-line drugs among patients who might qualify for bedaquiline. Alternatively, this study's results can be used to infer what might happen if bedaquiline were introduced solely on the basis of the result of fluoroquinolone or second-line injectable drug resistance. Without the capacity for full-spectrum DST, widespread acquired bedaquiline resistance would be expected quickly.

Finally, when countries implement the short regimen or bedaquiline under programmatic conditions, a comparator arm is unlikely to be evaluated to determine the effectiveness of the regimens. Patients placed on a standard

WHO MDR TB regimen will not be an appropriate control group to make valid comparisons. In this study, overall treatment success was 66% among candidates for the short regimen and 40% among candidates for a bedaquiline-containing regimen. A meta-analysis of the 18- to 24-month regimen outcomes reported by Falzon et al. (*17*) included data from 26 treatment centers and reported treatment success of 64% among 4,763 patients with MDR TB and no additional resistance to fluoroquinolones or second-line injectable drugs (this group of patients would be most comparable to candidates for the short regimen) and 40%–56% among patients with additional resistance to second-line injectable drugs or fluoroquinolones and with XDR TB (similar to candidates for bedaquiline). Thus, the proportions of patients with treatment success in the respective groups defined by drug resistance status can be used as historical comparators to assess the effectiveness of treatment in cohorts on the short regimen or the bedaquiline-containing regimen. Also, these studies clearly demonstrate the tremendous capacity for improvement of MDR TB treatment outcomes; new drugs and regimens are desperately needed.

Our report emphasizes the crucial need for strong laboratory capacity to manage drug-resistant TB. A few years ago, the Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) for detecting *M. tuberculosis* and rifampin resistance became widely available. Many countries are adopting this diagnostic tool and thus increasingly diagnosing MDR TB. However, for implementation and programmatic use of short and bedaquiline-containing regimens, national TB control programs would need capacity for rapid, accurate DST for both first- and second-line drugs to appropriately select candidates for the respective regimens and ensure the availability of at least 3 other effective drugs. This need should be considered during planning of the implementation of these new regimens.

Our study findings are subject to several major limitations in interpretation and applicability to programs. This study did not evaluate the frequency of drug-related toxicities leading to permanent discontinuation of fluoroquinolones or second-line injectable drugs. These patients would be candidates for bedaquiline-containing regimens even if their isolates remained susceptible to these drugs. Thus, we might have underestimated the proportion of patients eligible for bedaquiline. DST results were not available for pyrazinamide by the time of this analysis. On the basis of published data, pyrazinamide resistance in patients with MDR TB in countries included in PETTS varied from 49% in Thailand, 52% in South Africa, and 55% in Taiwan to 85% in South Korea (*18*–*21*). Thus, our study might overestimate the proportion of patients with MDR TB who would respond to the 9-month regimen because >50% of isolates could be resistant to pyrazinamide. CDC did not test for moxifloxacin when this work was conducted because standardized procedures had not yet been established. CDC does not test for clofazimine because standardized procedures using currently available technology have not been established. CDC does not test for cycloserine, which requires Lowenstein-Jensen medium. The intrinsic reproducibility of phenotypic DST for several drugs tested routinely including ethambutol, thioamides, cycloserine, and para-aminosalicylic acid is poor. All patients in the PETTS study were treated under WHO-recommended regimens during 2005–2010, when new treatment options were not available; thus, treatment outcomes might not be able to be extrapolated to new regimens.

Current standards for treating patients with MDR TB pose at least 2 major problems that reduce the effectiveness of treatment and treatment success rates. First, the long duration of treatment—a total of at least 20 months—places a major burden on patients and health care systems. Second, common serious adverse drug reactions contribute to reduced adherence or suspension of treatment. Given the global prevalence of MDR TB and low treatment success rates, better TB drugs and shorter regimens are urgently needed to enable more effective, less toxic, and less expensive treatment for persons with MDR TB.

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Oral Cholera Vaccine Coverage, Barriers to Vaccination, and Adverse Events following Vaccination, Haiti, 2013[1]

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In 2013, the first government-led oral cholera vaccination (OCV) campaign in Haiti was implemented in Petite Anse and Cerca Carvajal. To evaluate vaccination coverage, barriers to vaccination, and adverse events following vaccination, we conducted a cluster survey. We enrolled 1,121 persons from Petite Anse and 809 persons from Cerca Carvajal, categorized by 3 age groups (1–4, 5–14, ≥15 years). Two-dose OCV coverage was 62.5% in Petite Anse and 76.8% in Cerca Carvajal. Two-dose coverage was lowest among persons ≥15 years of age. In Cerca Carvajal, coverage was significantly lower for male than female respondents (69% vs. 85%; p<0.001). No major adverse events were reported. The main reason for nonvaccination was absence during the campaign. Vaccination coverage after this campaign was acceptable and comparable to that resulting from campaigns implemented by nongovernmental organizations. Future campaigns should be tailored to reach adults who are not available during daytime hours.

Since October 2010, Haiti has endured one of the largest cholera epidemics ever recorded in a single country, accounting for 54% of all cholera cases and 41% of all cholera deaths reported to the World Health Organization (WHO) during 2010–2013 (*1*–*4*). Contributing to this sustained, ongoing epidemic were inadequate drinking water and sanitation infrastructure, worsened by the 2010 earthquake, and an immunologically naive population. In February 2013, the Haiti Ministry of Health and Population launched the 2013–2022 national plan of action for elimination of cholera (*5*). The plan outlined long-term interventions such as improving water quality, sanitation, and waste management. However, because these interventions will require years to implement, the Haitian government proposed vaccinating 600,000 persons during 2013–2015 as a short-term approach to help control the cholera epidemic (*6*). This decision was consistent with World Health Assembly Resolution 64.15, which calls for implementation of an integrated and comprehensive approach to cholera control that includes the use of oral cholera vaccine (OCV) (*7*).

OCVs are increasingly being used as part of preemptive and reactive vaccination strategies (*8*–*18*). Before 2011, Dukoral vaccine (Crucell, Stockholm, Sweden), licensed for use in persons ≥2 years of age (2 doses given 7 days to 6 weeks apart), was the only available WHO-prequalified vaccine approved for purchase by United Nations agencies on the basis of safety and efficacy. However, its use in vaccination campaigns was limited by the need to mix the vaccine in a buffer solution diluted in clean water and by its relatively high cost (US$3–6/dose). In September 2011, Shanchol vaccine (Shantha Biotechnics, Hyderabad, India) was prequalified by WHO (2 doses given 14 days apart). Shanchol offered several advantages over Dukoral, including approval for use in persons ≥1 year of age, administration without buffer or water, and lower price (US$1.85/dose). Recent data from Kolkata, India, indicated that the 5-year protective efficacy of 2 doses of Shanchol was 65% (95% CI 52%–74%) (*19*), and effectiveness 6 months after a vaccination campaign for outbreak control in Guinea was 86% (95% CI 56.7%–95.8%) (*20*). These findings further support the use of OCV in response to epidemic and endemic cholera.

In 2012, the first pilot OCV campaign was conducted in Haiti by 2 Haitian nongovernmental organizations (NGOs) in a rural area in Artibonite Department (target population for vaccination 50,000) and in an urban area in

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Port-au-Prince (target population 69,185) (*12*,*13*). In 2013, the Haiti Ministry of Health and Population conducted the first government-run OCV campaign as part of the national plan for the elimination of cholera. Shanchol was used, and the target population included (per manufacturer recommendations) persons ≥1 year of age, with the exception of pregnant women. Because only 200,000 doses of the vaccine were available, the Ministry of Health and Population chose to target Petite Anse, an urban area in the commune of Cap Haitian in the North Department (estimated target population 86,989), and Cerca Carvajal, a rural area in the Centre Department (estimated target population 20,917). These areas were chosen because they had the required target population for the available OCV doses, poor water and sanitation infrastructure, difficult access to health care services, and historically high cholera attack rates (10.1%–37%) (*21*) (Figure). The first vaccination round was conducted August 5–9, 2013. The second round was conducted August 26–30 in Cerca Carvajal and was split between August 26–28 and September 9–10 in Petite Anse because of depleted vaccine supplies and the time needed to receive additional doses. The campaign was conducted at fixed and mobile sites and through house-to-house visits. Vaccination cards specific for the campaign were used to document vaccination. Printed pamphlets including information about water, sanitation, and hygiene (WASH) and the need to receive 2 doses of the vaccine were distributed during the campaign. However, messages delivered orally varied between areas and included no details about the vaccine. Administrative coverage (coverage reported by the country) with 2 OCV doses was 92% in Petite Anse and 104% in Cerca Carvajal. Previously, administrative vaccination coverage estimates have been shown to be unreliable in Haiti because the number of persons in the target populations was not always known (*22*,*23*).

To inform planning for future OCV campaigns in Haiti and other countries, we conducted a vaccination coverage survey. Compared with use of administrative coverage results, this method enables better assessment of the success of vaccination campaigns (evaluation of vaccine coverage, barriers to vaccination, and adverse events reported following vaccination).

Methods

Sampling and Study Population

We conducted a multistage cluster survey by using the 2011 household and population estimates provided by the Haitian Institute of Statistics and Information. The sampling frame consisted of 116 enumeration areas in Petite Anse and 25 in Cerca Carvajal. Enumeration areas are the primary sampling units, clearly delineated and mapped by the census bureau in Haiti, and are used as a sampling frame for major surveys in Haiti, including the Demographic and Health Survey. Sample size was calculated to estimate coverage by age group (1–4, 5–14 and ≥15 years) by using the following assumptions: 1) a desired precision of ±0.05, 2) an expected 2-dose OCV coverage of 85%, 3) a child 1–4 years of age in 65% of households, 4) a nonparticipation rate of 5%, and 5) a design effect of 1.7 in Petite Anse and 1.5 in Cerca Carvajal. These age groups were chosen for the purpose of comparison with OCV surveys conducted in

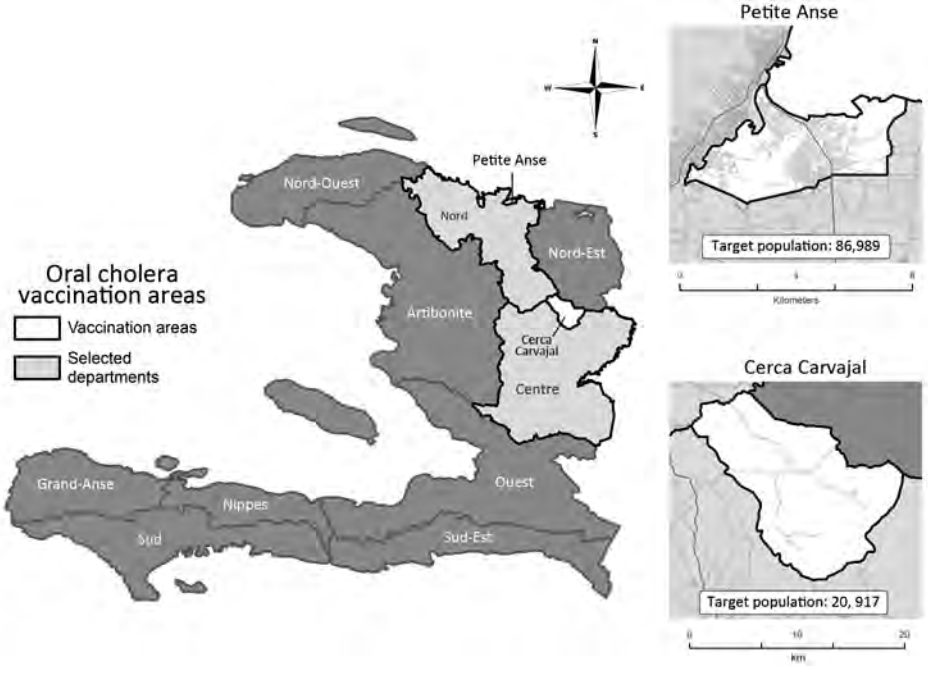

Figure. Areas selected for the first government-implemented oral cholera vaccination campaign in Haiti, 2013. Data source: Haiti Ministry of Health and Population, Centre National de l'Information Géo-Spatiale, and Institut Haïtien de Statistique et d'Informatique, OpenStreetMap.

other countries (*8*,*9*,*18*,*24*). A total of 564 households were needed in Petite Anse, and 353 households were needed in Cerca Carvajal.

We selected 30 (26%) enumeration areas from Petite Anse and 18 (72%) from Cerca Carvajal by systematic random sampling without replacement. In each enumeration area, 20 households were selected by systematic sampling. Finally, in each selected household, 1 person in each age group, if available, was randomly selected (by use of a random number table) for interview.

A household was defined as a group of persons who ate together and lived under the same roof. Persons in households were eligible to participate if they 1) were ≥1 year of age and not pregnant during the OCV campaign, 2) resided in a selected household during the OCV campaign, and 3) gave oral consent (for participants <18 years of age, consent was provided by a responsible adult member of the household). Responses for children were provided by the mother and the child if child was >5 years of age.

The protocol was approved by the national ethics committee in Haiti. It was classified as a program evaluation activity by the Centers for Disease Control and Prevention.

Data Collection

Team members (2 interviewers and 1 supervisor) who had experience with Demographic and Health Surveys were trained on survey and household selection methods, interviewing, use of smartphones for data collection, and use of global positioning system units. The survey was conducted during September 13–27, 2013, <1 month after campaign completion.

Interviews were initiated with the first household in 1 of the corners of the enumeration area noted on the global positioning system device. Subsequent households were selected by using the systematic method of traversing the enumeration area by moving in a clockwise manner and skipping households according to a precalculated sampling interval (estimated total number of households in cluster divided by 20). Contact was initiated with an adult, usually the female head of household, who was interviewed by use of a standardized questionnaire. Information collected included general household information: access to treated water and health care facilities, previous history of cholera infection in the household, awareness of the OCV campaign, and the number of eligible persons in the household who were vaccinated with OCV during the campaign and the number of doses received. Next, for each household, 1 person was randomly chosen from each of the 3 age groups for an in-depth interview (for younger children, the mother provided the information). Each interview collected information about the interviewee's age, sex, previous history of cholera, number of OCV doses received during the campaign (documented by card or by recall if the card was not available), vaccine administration (route, location, and whether person spat out the vaccine), adverse events within 14 days of receipt of the first and second OCV dose, and general knowledge about OCV (duration of protection, need for other measures for protection against cholera). Those who had not received the first or second dose were asked their reasons for not being vaccinated. Interviewers asked all questions without prompting for answers. Interviews were conducted in Haitian Creole, and answers were recorded on smartphones.

Households were visited at least 2 times if no one was at home or if a randomly selected person was unavailable during the first visit. Selected households were not replaced if they were not eligible or if no one was at home (no response) after at least 2 attempted visits.

Statistical Analyses

Estimated percentages and 95% CIs were calculated by using SAS-callable SUDAAN version 10.01 (RTI International, Research Triangle Park, NC, USA) to account for the finite population at the first stage cluster sampling. Statistical weights for each household were based on the sampling probabilities of the first 2 stages, and statistical weights for each person were based on the sampling probabilities of all 3 stages. For each area, we estimated 1- and 2-dose OCV campaign coverage (including 95% CI) by age group and sex. Satterthwaite-adjusted χ^2 tests were used to compare coverage between subpopulations. For each area we also calculated rates of dropout between receipt of first and second vaccine doses, reports of any adverse events, and reasons for not receiving vaccine.

Results

Household Characteristics

Of 960 visited households, 925 (96%) consented to participate (568 in Petite Anse, 357 in Cerca Carvajal). Of the participating households, 79% in Petite Anse and 46% in Cerca Carvajal were within a 15-minute walking distance of a drinking water source. In Petite Anse, the most common source of drinking water was bottled water or water purchased from a company (84%); in Cerca Carvajal, it was unprotected spring water (42%) and public piped water (34%).

For 56% and 21% of households in Petite Anse and Cerca Carvajal, respectively, the closest heath facility was located within 30 minutes of travel by the mode of transportation available in the household (walking, driving, motorcycle, or other). Overall, for 11% and 59% of the households in Petite Anse and Cerca Carvajal, respectively, the nearest health facility was >1 hour away. For ≈16% and 27% of households in Petite Anse and Cerca Carvajal, respectively, at least 1 household member had been infected

with cholera during the past 2 years. At least 1 person had died of cholera in ≈3% of households (3.4% in Petite Anse and 3.2% in Cerca Carvajal).

Campaign Awareness and Vaccination of Household Members

Of the 568 households in Petite Anse and 357 in Cerca Carvajal, 511 (91%, 95% CI 87%–93%) and 335 (93%, 95% CI 89%–96%), respectively, were aware of the 2013 OCV campaign. Of those who were aware, the principal sources of information were social mobilizers who used megaphones, followed by health care workers and friends/family. In Petite Anse, 79.9% (95% CI 75.5%–83.7%) of households had at least 1 eligible person who had received 2 OCV doses; in Cerca Carvajal, 89% (95% CI 83%–93%) of households had at least 1 person who had received 2 doses. All eligible household members had received 2 OCV doses in 23% (95% CI 17.4%–28.7%) of households in Petite Anse and 37% (95% CI 31%–44%) of households in Cerca Carvajal.

Vaccination among Enrolled Household Members

A total of 1,121 and 809 persons in Petite Anse and Cerca Carvajal, respectively, who were eligible for vaccination were enrolled and categorized into 1 of the 3 age groups (Table 1). Overall, 62.5% (95% CI 57.9%–66.9%) of eligible persons from selected households in Petite Anse and 76.8% (95% CI 71.1%–81.8%) from Cerca Carvajal received both doses of OCV (Table 2). Of those who received 2 doses, 51% from Petite Anse and 70% from Cerca Carvajal had card documentation of both doses. In Petite Anse, the dropout rate between the first and second OCV dose was 9.6% (95% CI 7.1%–12.9%) and was significantly higher among persons ≥15 years of age (12.0%) than among children 1–4 years of age (3.4%; p = 0.008). In Cerca Carvajal, the dropout rate between the first and second OCV dose was 8.4% (95% CI 5.5%–12.6%) and was significantly higher among male than female respondents (12.6% vs. 4.5%; p = 0.002). For both regions, 2-dose coverage was significantly lower among persons ≥15 years of age than among younger persons (p<0.01). In Cerca Carvajal, coverage was significantly lower among male than among female respondents overall (69.0% vs. 84.9%; p<0.001), among those 5–14 years of age compared with those in other age groups (76.5% vs. 92.9%; p = 0.005), and among those ≥15 years of age compared with those in other age groups (57.9% vs. 82.7%; p<0.001).

In Petite Anse, two thirds of respondents reported having received OCV at home (66.4%, 95% CI 58.5%–73.5%) and nearly a quarter at mobile posts (23.9%, 95% CI 18.1%–30.8%). In Cerca Carvajal, almost half of respondents reported having received OCV at mobile posts (49.6%, 95% CI 41.5%–57.8%) and 19.4% (95% CI 15.3%–24.3%) at health centers. About 7% and 5% of respondents in Petite Anse and Cerca Carvajal, respectively, reported spitting out part of the first dose because of its bad taste; <5% in both areas reported spitting out part of the second OCV dose.

Knowledge about OCV

Of 1,459 respondents who had received at least 1 OCV dose, almost one third (34% in Petite Anse, 33% in Cerca Carvajal) reported that they thought OCV alone was enough to protect them from cholera. Most (73%) respondents did not know the duration of protection provided by OCV; <2% thought protection lasted 3–5 years, and 16% in Petite Anse and 10% in Cerca Carvajal thought protection lasted a lifetime.

Adverse Events following Vaccination and Reasons for Nonvaccination

Among respondents who reported having received at least 1 dose of OCV, minor adverse events following the first dose were reported by 8% and following the second dose by almost 5%. The most commonly reported adverse events were nausea, vertigo, and abdominal pain (Table 3). No major adverse events were reported. The most common reason for not receiving the first or the second dose in both regions was absence during the campaign (Table 4).

Discussion

We report OCV coverage, barriers to vaccination, and adverse events after the first government-implemented OCV campaign in Haiti. The overall rates of 2-dose OCV coverage in rural Cerca Carvajal (77%) and urban Petite Anse (63%) were lower than the reported administrative

Table 1. General characteristics of participants in oral cholera vaccine coverage survey, Haiti, 2013

Characteristic	Area	
	Petite Anse, n = 1,121	Cerca Carvajal, n = 809
Sex, no. (%)		
M	499 (43.1)	407 (50.6)
F	622 (56.9)	402 (49.4)
Age, y, no. (%)		
1–4	206 (10.3)	192 (13.8)
5–14	353 (24.8)	263 (35.0)
≥15	562 (64.9)	354 (51.1)
History of cholera, % (95% CI)	38 3.5 (2.4–5.2)	48 6.6 (4.5–9.6)

Table 2. Estimated oral cholera vaccination coverage, Haiti, 2013*

No. doses Received	Area, % (95% CI)					
	Petite Anse			Cerca Carvajal		
	Total, n = 1,118	Male, n = 497	Female, n = 621	Total, n = 808	Male, n = 407	Female, n = 401
Total						
2	62.5 (57.9–66.9)	59.8 (53.9–65.5)	64.5 (58.8–69.7)	76.8 (71.1–81.8)	69.0 (60.4–76.4)	84.9 (80.0–88.8)
1	6.6 (4.9–8.9)	7.1 (4.7–10.4)	6.3 (3.8–10.2)	7.0 (4.7–10.5)	10.0 (6.3–15.4)	4.0 (2.4–6.8)
0	30.9 (26.8–35.3)	33.1 (27.6–39.1)	29.2 (24.8–34.1)	16.1 (12.4–20.6)	21.1 (15.3–28.3)	11.0 (7.9–15.2)
Age group, y						
1–4	n = 206	n = 102	n = 104	n = 191	n = 91	n = 100
2	67.9 (60.2–74.8)†	63.9 (52.8–73.6)	71.8 (62.5–79.5)	81.6 (72.9–88.0)†	87.5 (75.5–94.1)	76.1 (63.1–85.6)
1	2.4 (0.8–6.9)	4.5 (1.4–13.4)	0.3 (0.0–2.4)	5.8 (2.7–12.0)	3.5 (0.8–13.9)	7.9 (3.2–18.5)
0	29.7 (23.3–37.0)	31.6 (22.3–42.5)	27.9 (20.3–37.1)	12.6 (7.9–19.6)	9.1 (4.1–18.8)	16.0 (9.1–26.5)
5–14	n = 351	n = 163	n = 188	n = 263	n = 148	n = 115
2	77.9 (71.7–83.0)†	75.5 (65.1–83.6)	79.8 (72.4–85.6)	83.8 (75.0–89.9)†	76.5 (63.6–85.9)	92.9 (85.1–96.7)
1	5.8 (3.7–9.0)	6.1 (3.1–11.3)	5.6 (3.0–10.3)	5.5 (2.8–10.8)	8.5 (4.0–17.2)	1.8 (0.6–5.5)
0	16.3 (12.1–21.7)	18.4 (11.5–28.2)	14.6 (9.8–21.2)	10.7 (6.2–17.7)	15.0 (8.3–25.5)	5.4 (2.0–13.7)
≥15 y	n = 561	n = 232	n = 329	n = 354	n = 168	n = 186
2	55.7 (50.0–61.3)†	52.5 (44.8–60.0)	58.0 (50.7–65.0)	70.8 (63.9–76.9)†	57.9 (47.4–67.8)	82.7 (75.7–88.0)
1	7.6 (5.3–10.9)	7.9 (4.5–13.6)	7.4 (4.0–13.2)	8.4 (5.3–13.1)	12.9 (7.6–21.1)	4.3 (2.1–8.6)
0	36.7 (31.5–42.1)	39.6 (32.4–47.2)	34.6 (28.7–41.0)	20.8 (15.7–27.0)	29.2 (20.5–39.7)	13.0 (8.6–19.2)

*Vaccination status was assessed from special cards distributed to document doses administered during the campaign, if available, or by recall; in every selected household, 1 person was randomly selected from each age group. Statistical analyses accounted for the weights and the study design.
†Design effect (DE) and estimated intraclass correlations (ICC) = (DE–1)/(b–1), where b is the average number of responses per cluster. DE is based on accounting for clustering only (The finite population correction and weighting are ignored). Petite Anse: age group: 1–4 y, DE = 1.3 and ICC = 0.05; 5–14 y, DE = 1.4 and ICC = 0.04; ≥15 y, DE = 2.1 and ICC = 0.06. Cerca Carvajal: age group 1–4 y, DE = 2.4 and ICC = 0.15; 5–14 y, DE = 3.9 and ICC = 0.22; ≥15 y; DE = 2.3 and ICC = 0.07.

coverage. Potential explanations could be the inaccurate population denominators used to calculate administrative coverage because the most recent census data were for 2003. In addition, several persons came from other areas to receive the vaccine, especially in Petite Anse, a crowded urban area, leading to overestimation of administrative coverage. Furthermore, the splitting and delaying of the second round of vaccination in Petite Anse created some confusion among the population regarding the vaccination dates and could have contributed to the high dropout rates. Nevertheless, the 2-dose OCV coverage achieved in Haiti is considered acceptable because herd immunity after 2-dose coverage with Shanchol as low as 28% has been reported (*25*), and mathematical models have shown that cholera might be controlled in disease-endemic settings starting with 2-dose OCV coverage of 50% (*26*).

Our results are comparable to those reported after a pilot OCV vaccination campaign conducted in 2012 by 2 NGOs in Haiti, for which coverage was 77% in rural Bocozel (*12*) and 69% in urban slums in Port-au-Prince (*13*). Two-dose OCV coverage rates in rural Haiti are similar to those reported in Bangladesh (72%) during the cholera off season (*11*) and rural Guinea (76%) during a cholera outbreak (*18*). Furthermore, OCV coverage rates in Haiti are among the highest observed thus far, compared with those reported after NGO-implemented campaigns in South Sudan, India, Mozambique, and Zanzibar (*9*,*10*,*14*,*15*). This campaign is one of the few OCV campaigns implemented by a government in a cholera-endemic setting; when the governments of Vietnam and Micronesia conducted OCV vaccinations in disease-endemic or outbreak settings, coverage rates were <80% and 50%, respectively (*16*,*17*).

OCV coverage was much lower among persons ≥15 years of age in both regions and lower among male than female respondents in Cerca Carvajal. Similar findings have been reported for Mozambique, Bangladesh, India, South Sudan, Guinea, and Vietnam (*9*,*11*,*14*,*15*,*18*,*27*). However, as in other countries (*8*,*9*,*12*,*18*,*28*), awareness and acceptance of OCV was relatively high. The major reason for not receiving the vaccine was absence during the campaign. Unlike previous vaccination campaigns in Haiti, which primarily targeted children or women of reproductive age, OCV campaigns targeted all nonpregnant persons ≥1 year of age. Vaccination campaigns focused on adults need to include vaccination sessions either very early in the morning or in the evenings, when working men and women are more likely to be at home. More than two thirds of persons in Petite Anse were vaccinated at home. Vaccinators visited homes during the day, when several respondents might have been at work or at the market. In addition, adults in general and men in particular may believe that vaccines are intended for children and might not seek vaccination. Hence, additional efforts are needed to explain the need for adults to receive OCV. If vaccines are available, additional activities to reach those who were not vaccinated because of absence during the campaign might help increase coverage.

Most respondents did not know the duration of protection provided by the vaccine, and almost one third thought that vaccine alone would be enough to protect them from cholera. This poor knowledge about the vaccine may result from limited messaging about the vaccine

Table 3. Adverse events reported within 14 days of receipt of oral cholera vaccine, by area, Haiti, 2013*

Adverse event	Petite Anse, no. (%)	Cerca Carvajal, no. (%)
First dose		
No. who received dose	768	691
Total events reported	68 (7.9; 95% CI 6.0–10.3)	56 (8.0; 95% CI 5.4–11.7)
Common events reported†		
Nausea	20 (2.6)	17 (2.5)
Vertigo	15 (2.0)	11 (1.6)
Abdominal pain	13 (1.7)	17 (2.5)
Weakness/fatigue	11 (1.4)	4 (0.6)
Diarrhea	9 (1.2)	9 (1.3)
Vomiting	5 (0.7)	5 (0.7)
Bloating	3 (0.4)	7 (1.0)
Fever	8 (1.0)	5 (0.7)
Headache	2 (0.3)	4 (0.6)
Rash	4 (0.5)	Not reported
Second dose		
No. who received dose	697	637
Total events reported	35 (4.7; 95% CI 3.0–7.3)	29 (4.1; 95% CI 2.4–6.8)
Common events reported‡		
Vertigo	6 (0.9)	7 (1.1)
Nausea	7 (1.0)	5 (0.8)
Abdominal pain	6 (0.9)	9 (1.4)
Vomiting	1 (0.1)	2 (0.3)
Diarrhea	2 (0.3)	4 (0.6)
Fever	4 (0.6)	3 (0.5)
Weakness/fatigue	4 (0.6)	1 (0.2)
Headache	3 (0.4)	3 (0.5)
Rash	6 (0.9)	Not reported
Bloating	2 (0.3)	Not reported

*The categories for adverse events are not mutually exclusive as participants had the option to report multiple adverse events.
†Denominator incudes persons who received the first dose. Percentages are unweighted for the purpose of description only.
‡Denominator includes persons who received the second dose. Percentages are unweighted for the purpose of description only.

during the campaign; most of the information was spread through pamphlets, which probably were of limited usefulness because of low literacy rates in the target communities. These findings are concerning because persons who believe they are completely protected from cholera after vaccination might abandon protective behavior such as treating drinking water and practicing good hygiene. OCV campaigns should offer an opportunity to promote hygiene and safe water and food practices; an OCV campaign conducted in 2012 by an NGO in rural Haiti included a strong cholera and WASH education component and was associated with improved cholera knowledge and hygiene practices (*29*). Future campaigns in Haiti should focus on word-of-mouth messaging to spread cholera prevention educational information. Health care workers and trained social mobilizers with megaphones could transmit these messages before, during, and after the vaccination campaign.

Although the 200,000 OCV doses for the 2013 campaign were purchased directly from the manufacturer by the United Nations Children's Fund, future OCV for use in cholera-endemic and -epidemic settings will be mainly obtained through the OCV stockpile managed by the International Coordinating Group and the Global Task Force on Cholera Control (*30*–*32*). Given the limited amount of vaccine available, epidemiologic, technical, and operational evidence, as well as local capacity to conduct OCV campaigns, will be assessed for optimal stockpile vaccine use. Moreover, the International Coordinating Group highlights the need to integrate OCV use with early case detection, appropriate case management, provision of adequate WASH infrastructure, and raising of awareness in the affected communities. Therefore, the cornerstones for cholera prevention and control remain safe water, improved sanitation, and adequate hygiene; WHO recommends that OCV use should complement traditional cholera control measures, including WASH interventions (7).

As has been noted for other Shanchol campaigns (*12*,*13*,*15*,*18*,*33*), no major adverse events were reported. Although the rates of minor adverse events were not higher than those reported by the manufacturer and in Bangladesh (*33*), they were higher than adverse events reported within 48 hours of vaccination during 2 pilot NGO-run OCV campaigns in Haiti (0.5%–1.3%) (*12*,*13*) and a campaign in Guinea (1%) (*18*).

This survey has 2 main limitations. Only half of respondents who received both OCV doses in Petite Anse and 70% of those in Cerca Carvajal could document OCV vaccination by card. Therefore, in some instances we based vaccine status on a patient's verbal report, which could have led to overestimation of vaccine coverage. However, the extent of this bias was probably limited because the

Table 4. Principal reasons for not receiving oral cholera vaccine, by area, Haiti, 2013*

Reason	Petite Anse, no. (%)	Cerca Carvajal, no. (%)
First dose		
No. who did not receive dose	348	117
Absent during the campaign	141 (40.5)	63 (53.8)
Did not hear about the vaccination activities	41 (11.8)	15 (12.8)
Busy/no time	34 (9.8)	7 (6.0)
Sick during the campaign	14 (4.0)	4 (3.4)
Didn't think vaccination was important/necessary	20 (5.7)	2 (1.7)
Don't think vaccines are safe/vaccines can harm	10 (2.9)	2 (1.7)
Vaccines not available	5 (1.4)	4 (3.4)
Didn't know when or where to go	6 (1.7)	2 (1.7)
Clinic closed/vaccinator not there/vaccinator refused to vaccinate	6 (1.7)	2 (1.7)
Other	71 (20.4)	16 (13.7)
Second dose†		
No. who did not receive dose	73	54
Absent during second campaign	21 (28.8)	13 (24.1)
Clinic closed/vaccinator refused to vaccinate	5 (6.8)	1 (1.9)
Busy/no time	4 (5.5)	11 (20.4)
Bad experience/ adverse event after first dose	6 (8.2)	2 (3.7)
Did not know needed a second dose	3 (4.1)	1 (1.9)
Sick during the campaign	2 (2.7)	Not reported
Didn't know when or where to go	Not reported	2 (3.7)
Forgot to go	2 (2.7)	1 (1.9)
Vaccines not available	1 (1.4)	7 (13.0)
Other	29 (39.7)	9 (16.7)

*Categories are mutually exclusive because respondents were allowed to give only 1 primary reason. Percentages are unweighted for the purpose of description only.
†Of those who received the first dose.

survey was conducted shortly after the campaign, and respondents correctly identified campaign dates and route of OCV administration. Second, overestimating coverage in the sample size calculation and underestimating the design effect in Cerca Carvajal contributed to the wide confidence intervals. However, we have reported the estimated design effect caused by clustering and the estimated intraclass correlation (Table 2, footnote). Future OCV surveys in Haiti can use these intraclass correlations, along with the expected number of responses in each cluster, to estimate the design effect caused by clustering.

In conclusion, coverage rates after the first government-implemented OCV campaign in Haiti were acceptable. As part of the national plan for the elimination of cholera, results from this survey would be essential for planning future OCV campaigns in Haiti to reach those who remain nonvaccinated. Given the lack of accurate data about target population estimates and high vaccine demand from nearby areas, it may be useful to overestimate required vaccine doses to improve vaccination coverage and avoid running out of vaccine during future campaigns. Printing enough vaccination cards and emphasizing the value of keeping the card and bringing it back when receiving the second vaccine dose are needed. Vaccination sessions should be tailored to reach persons who work during the day and men in general. Furthermore, OCV campaigns should be coordinated with WASH activities to ensure a comprehensive approach to cholera control and prevention and to promote the elimination of cholera from Haiti.

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Acquired Drug Resistance in *Mycobacterium tuberculosis* and Poor Outcomes among Patients with Multidrug-Resistant Tuberculosis

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Learning Objectives

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

- Assess the epidemiology of complicated tuberculosis
- Determine the epidemiology and risk factors for acquired resistance in tuberculosis in the current study
- Distinguish variables associated with a higher risk for acquired resistance in tuberculosis
- Evaluate outcomes of complicated tuberculosis.

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Rates and risk factors for acquired drug resistance and association with outcomes among patients with multidrug-resistant tuberculosis (MDR TB) are not well defined. In an MDR TB cohort from the country of Georgia, drug susceptibility testing for second-line drugs (SLDs) was performed at baseline and every third month. Acquired resistance was defined as any SLD whose status changed from susceptible at baseline to resistant at follow-up. Among 141 patients,

acquired resistance in *Mycobacterium tuberculosis* was observed in 19 (14%); prevalence was 9.1% for ofloxacin and 9.8% for capreomycin or kanamycin. Baseline cavitary disease and resistance to ≥6 drugs were associated with acquired resistance. Patients with *M. tuberculosis* that had acquired resistance were at significantly increased risk for poor treatment outcome compared with patients without these isolates (89% vs. 36%; p<0.01). Acquired resistance occurs commonly among patients with MDR TB and impedes successful treatment outcomes.

The World Health Organization (WHO) reported that control efforts are off-track in managing multidrug-resistant tuberculosis (MDR TB) and that addressing this problem is a priority (*1*). In 2013, WHO estimated that there were 480,000 new cases of MDR TB and 210,000 MDR TB–related deaths (*2*). MDR TB, defined as resistance to first-line drugs isoniazid and rifampin, has been associated with worse treatment outcomes than for drug-susceptible TB (*3*). A primary reason for worse treatment outcomes for MDR TB is use of second-line drugs (SLDs), which are costly, poorly tolerated, and suboptimally effective and require a prolonged treatment duration.

SLD treatment for MDR TB might also increase risk for further acquired drug resistance. Acquired resistance among *Mycobacterium tuberculosis* isolates from MDR TB patients is a concern because this resistance would leave clinicians with few effective drugs and might lead to development of extensively drug-resistant (XDR) TB, which is defined as resistance to a fluoroquinolone and ≥1 injectable drug (amikacin, kanamycin, or capreomycin) (*4*), in addition to isoniazid and rifampin. XDR TB has been associated with treatment outcomes much worse than outcomes for MDR TB (*5*).

A case of drug-resistant TB occurs by primary transmission of drug-resistant *M. tuberculosis* strains or acquired resistance during TB treatment. For acquired resistance, *M. tuberculosis* is believed to develop resistance by spontaneous chromosomal mutations (*6*). Given that frequencies of *M. tuberculosis* mutations that correlate with drug resistance occur infrequently and resistance mutations for different drugs are believed to be unlinked, additional drug resistance is unlikely when ≥3 effective drugs are used in combination (*6*). For inadequate drug treatment caused by poor regimen selection, inadequate drug supply, nonadherence, or subtherapeutic drug concentrations, subpopulations of drug-resistant *M. tuberculosis* might be selected for, amplified, and become the predominant strain. Limited data suggest that the risk for acquired resistance is higher among persons with MDR TB than drug-susceptible TB (*7–10*). Data for risk factors for acquired resistance among MDR TB patients during treatment and their effect on outcomes are limited to a few studies (*8*,*11*,*12*).

On the basis of prior results from our group, we hypothesized that cavitary disease would increase the risk for acquired resistance (*13*). Because infection with *M. tuberculosis* strains with increasing drug resistance is associated with worse patient outcomes, we also hypothesized that acquired resistance would be associated with a poor outcome (*5*).

To address research questions generated by these hypotheses, we studied a cohort of MDR TB patients in Georgia, 1 of 27 countries with a high incidence of MDR TB, as designated by WHO (*1*). In 2012, 9% of newly diagnosed cases and 31% of re-treatment TB cases in Georgia were MDR TB (*1*). In 2008, with support from the Global Fund (http://www.theglobalfund.org/en/?gclid=CO2244zBqsQCFdgNgQodQAkANA) and the Green Light Committee (GLC) (http://www.who.int/tb/challenges/mdr/greenlightcommittee/en/), Georgia was the first low-to-middle income country to achieve universal access to diagnosis and treatment of MDR TB. However, despite availability of culture and molecular diagnostic methods and use of recommended SLD regimens, MDR TB treatment outcomes have remained suboptimal compared with other similar settings (*14*). By assessing prevalence of acquired resistance and its effect on treatment outcomes, we aimed to obtain data that might lead to improved management of MDR TB patients in Georgia and other countries that have drug-resistant TB.

Methods

Study Population

We conducted a retrospective study of patients with pulmonary MDR TB treated through the National Center for Tuberculosis and Lung Diseases (NCTLD) in Tbilisi, Georgia. All patients were sputum smear–positive for acid-fast bacilli (AFB) and culture-positive for *M. tuberculosis* at baseline and had MDR TB confirmed by conventional drug susceptibility testing (DST). Patients with MDR TB were given a diagnosis during March 2009–October 2012, as described in a study that evaluated the clinical effect of a rapid diagnostic test for detection of MDR TB (*15*). Approval for this study was obtained from the Georgia NCTLD and Emory University (Atlanta, GA, USA) Institutional Review Boards.

Cultures and Drug Susceptibility Testing

Direct sputum smears with Ziehl-Neelsen staining were examined by light microscopy at a sputum microscopy center, and 1 sputum smear sample positive for acid-fast bacilli (AFB) was sent to the National Reference Laboratory (NRL) at NCTLD, where it was processed as reported (*16*). Cultures were prepared by using Löwenstein-Jensen–based solid medium or the MGIT 960 broth culture system (Becton Dickinson, Franklin Lakes, NJ, USA). Cultures with positive results by either method were confirmed to be

M. tuberculosis complex by using the MTBDR*plus* assay (Hain Lifescience, Nehren, Germany) and the Capilia TB assay (Tauns Laboratories, Inc., Shizuoka, Japan) (*16*).

DST for first-line drugs was performed as described (*16*). DST for SLDs was performed by using the proportion method and Löwenstein-Jensen medium with the following drug concentrations: ethionamide, 40.0 μg/mL; ofloxacin, 2.0 μg/mL; p-aminosalicylic acid, 0.5 μg/mL; capreomycin, 40.0 μg/mL; and kanamycin, 30.0 μg/mL (*17*). The NRL has undergone external quality assessment by the WHO Supranational TB Reference Laboratory (Antwerp, Belgium) annually since 2005. In 2012, a certificate from the WHO Supranational TB Reference Laboratory was received by the NRL for successfully passing DST quality assurance testing for isoniazid, rifampin, ethambutol, kanamycin, capreomycin, and ofloxacin.

As per standard of care, follow-up sputum smears and cultures were obtained monthly during the intensive phase of MDR TB treatment (minimum 6 months). Second-line DST was performed at baseline and was recommended at 3 and 6 months if culture results remained positive. During the continuation phase, sputum smears and cultures were obtained every 3 months, and second-line DST was recommended for all positive cultures.

Data Collection

A standardized data form was used to abstract data from medical charts, patient treatment cards, the NCTLD surveillance database, and the NRL database. Information was collected about sociodemographic characteristics, TB history, signs and symptoms, treatment regimens, outcomes; and all sputum smear, culture, and DST results. Data were entered into a REDCap database (*18*).

Definitions

Acquired resistance was defined as any SLD that was susceptible on baseline DST and resistant on any subsequent DST result. Time to MDR TB treatment initiation was defined as time from initial diagnostic sputum collection to start of SLD therapy. Initial MDR TB treatment was defined as any drug received ≤30 days of starting an SLD regimen. Treatment interruption was defined as a continuous interruption for ≥1 SLDs for ≥1 week. Final treatment outcomes were determined using WHO criteria (*19*). A favorable outcome was defined as cure or treatment completion; a poor outcome was defined as treatment failure, death during treatment, or loss to follow up (LFU) (formerly known as default). Two alternative classifications were used in secondary analyses: 1) patients with a negative culture result at time of LFU were included as a favorable outcome, and 2) patients with a poor outcome were defined as treatment failure or death. LFU patients were excluded from analysis.

Treatment

The NCTLD Drug Resistance TB Treatment Committee provided initial guidance on choosing an empiric SLD regimen for patients initiating MDR TB treatment. After second-line DST results were available, treatment regimens were individualized if needed and guided by WHO recommendations. When possible, regimens were designed to include at least 4 drugs to which an *M. tuberculosis* isolate was susceptible. All treatment regimens included a fluoroquinolone, pyrazinamide, and capreomycin or kanamycin for ≥6 months. All patients received directly observed therapy. Patients initiated therapy as inpatients before transitioning to outpatient treatment. Patients were recommended to remain hospitalized until showing sputum smear or culture conversion and clinical improvement.

Data Analysis

Data were analyzed by using SAS software version 9.3 (SAS Institute, Cary, NC, USA). For descriptive statistics, differences in categorical variables were tested by using the χ^2 or Fisher exact tests. The Wilcoxon-Mann-Whitney test was used for comparing non–normally distributed continuous variables. A 2-sided p value <0.05 was considered significant. A logistic regression model was used to estimate the independent association of potential risk factors with acquired resistance and the adjusted association of acquired resistance with a poor outcome. Logistic model building and covariate selection was based on purposeful selection of patient-level factors as described (*20*). Additional logistic regression models using our alternative definitions of poor outcome as defined above were used.

Results

Study Cohort

A total of 158 patients with pulmonary MDR TB were included in the study. For analysis of acquired resistance, 17 patients were excluded because of XDR TB at baseline or absence of a 2-month or later follow-up sputum examination (Figure 1). The remaining 141 patients had a mean age of 37.9 years; most (73%) were men. Less than half (44%) had a history of TB treatment. *M. tuberculosis* isolates with baseline resistance to capreomycin or kanamycin were found in 33% of patients, and isolates with baseline resistance to ofloxacin were found in 6%. Other patient characteristics are shown in Table 1.

Acquired Resistance

Of the 141 patients evaluated for acquired resistance, 32 patients had ≥1 follow-up DST performed, including 40% of patients with a positive 4-month culture and 52% with a positive 6-month culture. A total of 22 patients had different follow-up DST results that showed a change in resistance

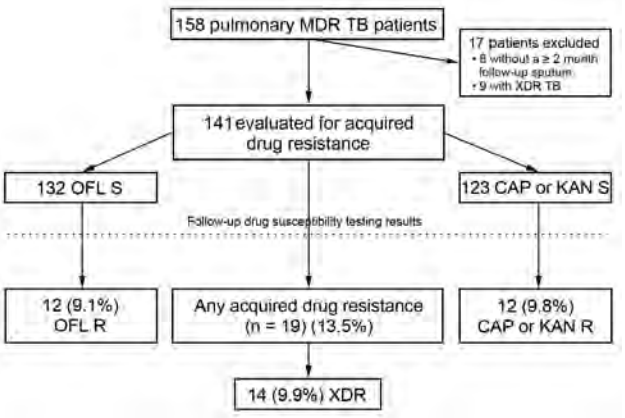

Figure 1. Cohort diagram of patients with multidrug-resistant tuberculosis (MDR TB) depicting rates of acquired drug resistance, Georgia, March 2009–October 2012. XDR TB, extensively drug-resistant tuberculosis; OFL, ofloxacin; S, susceptible; CAP, capreomycin; KAN, kanamycin; R, resistant.

pattern, including 19 (13.5%) with acquired resistance and 3 (2.1%) with follow-up DST showing a reversion to susceptibility for 1 SLD. Median time to initial development of acquired resistance was 142 days (range 85–480 days), including 200 days for capreomycin or kanamycin and 149 days for ofloxacin. Of 132 patients infected with *M. tuberculosis* that had baseline ofloxacin susceptibility, ofloxacin resistance developed in isolates from 12 (9.1%) patients. Among 123 patients infected with isolates that had baseline susceptibility to capreomycin, kanamycin, or both, resistance to capreomycin or kanamycin developed in 12 (9.8%) (Figure 1). Of 12 patients infected with isolates that had acquired resistance to an injectable drug, resistance to capreomycin and kanamycin developed in isolates from 5 patients, resistance to capreomycin developed in isolates with baseline kanamycin resistance from 3 patients, and susceptibility to capreomycin or kanamycin remained in isolates from 4 patients.

Among 19 patients infected with isolates that had acquired resistance, increasing resistance to 1 drug developed in isolates from 9 patients, to 2 drugs in isolates from 8 patients, and to 3 and 4 additional drugs in isolates from 1 patient each. Almost all acquired resistance was to ofloxacin, capreomycin, or kanamycin; 2 patients each had isolates with acquired resistance to ethionamide and p-aminosalicylic acid. Acquired resistance led to emergence of XDR TB in 14 (74%) of 19 patients.

Patients with and without isolates that had acquired resistance were similar in regards to age, sex, prior TB, and other characteristics (Table 1). In contrast, patients who had isolates with acquired resistance had a lower mean baseline body mass index (19.1 vs. 20.7 kg/m^2; p = 0.02) and a higher prevalence of baseline cavitary disease (58% vs. 16%; p<0.01) and were more likely to have isolates resistant to ≥6 drugs at baseline DST (74% vs. 38%; p = 0.01) than patients who had isolates without acquired resistance. Patients with isolates that had acquired resistance were less likely to receive kanamycin (32% vs. 62%; p = 0.01) as part of their initial MDR TB treatment regimen. Regarding sputum examinations, patients with isolates that had acquired resistance were more likely to have baseline AFB sputum smear values ≥3+ (63% vs. 28%; p<0.01) and to be sputum smear and culture positive at 4 and 6 months (Table 1) than patients without isolates that had acquired resistance.

Factors associated with acquired resistance by univariate analysis were baseline cavitary disease, high baseline drug resistance, baseline ofloxacin resistance, number of known active drugs in the initial MDR TB treatment regimen, initial AFB sputum smear result ≥3+, and sputum smear or culture positivity at 4 and 6 months (Table 2). By multivariate analysis, factors associated with acquired resistance were baseline cavitary disease (adjusted odds ratio [aOR] 5.21, 95% CI 1.56–17.38); resistance to ≥6 drugs at baseline DST (aOR 5.31, 95% CI 1.50–18.77); and sputum smear positivity at 4 months (aOR 6.54, 95% CI 1.23–34.88).

Risk Factors for Poor Outcomes

A total of 140 patients were evaluated for final treatment outcomes; 1 patient was excluded because he was still receiving treatment (Figure 2). Of the remaining 139 patients, 61 (44%) had a poor outcome. Poor outcomes were more frequent among patients with isolates that had acquired resistance than patients without these isolates (89% vs. 36%; p<0.01). Of 2 patients who had favorable outcomes and isolates that had acquired resistance, 1 underwent adjunctive surgery and 1 had an isolate with acquired resistance to ethionamide; these isolates remained susceptible to ofloxacin, kanamycin, and capreomycin. Most (44 of 61) poor outcomes were caused by LFU, and the remaining poor outcomes were caused by deaths (11) and treatment failure (6). Of LFU patients, 15 (34%) of 44 had positive sputum cultures at the time of LFU, including 6 with isolates that had acquired resistance. The 29 patients with negative cultures at the time of LFU received a median of 111 days of treatment for MDR TB (range 42–325 days), and the 15 patients with positive cultures received a median of 91 days of treatment (range 80–360 days).

Patients with a poor outcome were significantly more likely to have isolates with acquired resistance (28% vs. 3%; p<0.01) and be sputum smear positive at 4 and 6 months and sputum culture positive at 2, 4, and 6 months (Table 3) than patients with a favorable outcome. There were no other differences between groups (Table 3).

Risk factors associated with a poor outcome by univariate analysis were acquired resistance, high baseline

Table 1. Characteristics of patients with multidrug-resistant tuberculosis stratified by acquired resistance to second-line drugs, Georgia, March 2009–October 2012*

Characteristic	Overall, n = 141	Acquired resistance to second-line drugs		p value†
		Yes, n = 19	No, n = 122	
Median age, y (IQR)	34.9 (27–46)	41.4 (30–53)	37.3 (27–45)	0.28
Male sex	103 (73)	16 (84)	87 (71)	0.24
Married	73 (52)	10 (53)	63 (52)	0.94
Employed	19 (14)	1 (5)	18 (15)	0.26
History of imprisonment	40 (28)	6 (32)	34 (28)	0.74
Diabetes mellitus	16 (11)	1 (5)	15 (12)	0.37
Hepatitis C	16 (11)	3 (16)	13 (11)	0.51
HIV positive	6 (4)	1 (5)	5 (4)	0.82
Median BMI, kg/m^2 (IQR)	20.5 (2.7)	20.0 (17.5–21.1)	20.2 (18.9–22.5)	0.049
BMI ≤18.5 kg/m^2	35 (25)	7 (37)	28 (23)	0.19
History of TB	62 (44)	10 (53)	52 (43)	0.41
Prior TB treatment				0.27
None	79 (56)	9 (47)	70 (57)	NA
First-line	52 (37)	7 (37)	45 (37)	NA
Second-line	10 (7)	3 (16)	7 (6)	NA
Baseline cavitary disease	30 (21)	11 (58)	19 (16)	<0.01
Median no. drugs to which baseline isolate was resistant (IQR)	5 (5–6)	6 (5–7)	5 (5–6)]	0.02
Resistant to ≥6 drugs on baseline DST	60 (43)	14 (74)	46 (38)	<0.01
Baseline drug resistance category				
MDR only	85 (60)	8 (42)	77 (63)	0.01
MDR + ofloxacin resistant	9 (6)	4 (21)	5 (4)	NA
MDR + capreomycin or kananmycin resistant	47 (33)	7 (37)	40 (33)	NA
Initial treatment inpatient	45 (32)	9 (47)	36 (30)	0.12
Starting SLDs >30 days after TB diagnosis	66 (47)	6 (32)	60 (49)	0.15
Median known active drugs in initial regimen (IQR)‡	3 (2–3)	2 (1–3)	3 (2–4)	0.05
Initial MDR TB treatment				
Pyrazinamide	139 (99)	19 (100)	120 (98)	0.57
Prothionamide	141 (100)	19 (100)	122 (100)	1.00
Capreomycin	65 (46)	13 (68)	52 (43)	0.04
Kanamycin	82 (58)	6 (32)	76 (62)	0.01
Levofloxacin	134 (95)	18 (94)	116 (95)	0.95
Cycloserine	135 (96)	19 (100)	116 (95)	0.32
p-aminosalicyclic acid	140 (99)	19 (100)	121 (99)	0.69
Treatment interruption	57 (40)	12 (63)	45 (37)	0.03
Baseline AFB sputum smear value ≥3+	46 (33)	12 (63)	34 (28)	<0.01
Sputum culture positive, mo§				
2	121 (86)	19 (100)	102 (84)	0.06
4	80 (57)	17 (90)	63 (52)	<0.01
6	48 (34)	16 (84)	32 (26)	<0.01
Sputum smear positive, mo§				
2	115 (82)	19 (100)	96 (79)	0.03
4	77 (55)	17 (90)	60 (49)	<0.01
6	39 (28)	15 (79)	24 (20)	<0.01

*Values are no. (%) unless otherwise indicated. IQR, interquartile range; BMI, body mass index; TB, tuberculosis; NA, not applicable; DST, drug susceptibility testing; MDR, multidrug resistant; SLDs, second-line drugs; AFB, acid-fast bacilli.
†Comparing persons with and without acquired resistance by using χ^2 or Fischer exact tests for categorical variables and Wilcoxon-Mann-Whitney test for continuous variables.
‡DST was performed for streptomycin, isoniazid, rifampin, ethambutol, ofloxacin, ethionamide, kanamycin, capreomycin, and p-aminosalicyclic acid.
§Time from initiation of SLD treatment for MDR TB.

drug resistance, and sputum smear or culture positivity at 4 and 6 months (Table 4). Multivariate analysis showed that acquired resistance (aOR 8.05, 95% CI 1.56–41.66) and sputum smear positivity at 6 months (aOR 3.43, 95% CI 1.36–8.63) remained associated with a poor outcome. In our first alternative multivariate model, when we classified patients with a negative culture at time of LFU as a favorable outcome, acquired resistance was associated with a poor outcome (aOR 24.91, 95% CI 4.21–147.48). In a second alternative multivariate model, in which poor outcome was defined as treatment failure or death and LFU patients were excluded, acquired resistance remained associated with a poor outcome (aOR 38.44, 95% CI 5.96–247.73).

Discussion

In a country with high rates of MDR TB, we found an alarmingly high rate of acquired drug resistance during SLD treatment (13.5%), including development of XDR TB (9.9%) and a strong association between acquired resistance and poor treatment outcomes (aOR 8.05, 95% CI 1.56–41.66). These high rates of acquired resistance were observed even though Georgia is a GLC-approved country, thus receiving

Table 2. Risk factors for acquired resistance to second-line drugs among patients treated for multidrug-resistant tuberculosis, Georgia, March 2009–October 2012*

Risk factor	Univariate analysis, OR (95% CI)	p value	Multivariate analysis, aOR (95% CI)	p value
Baseline characteristic				
Median age >35 y	1.96 (0.72–5.30)	0.19	–	–
Male sex	2.15 (0.59–7.83)	0.25	–	–
BMI ≤18.5 kg/m^2	1.96 (0.70–5.45)	0.20	3.73 (0.98–14.14)	0.053
History of TB	1.50 (0.57–3.94)	0.42	–	–
Prior receipt of second-line TB drugs	3.08 (0.72–13.13)	0.13	–	–
Diabetes	0.40 (0.05–3.19)	0.38	–	–
Hepatitis C	1.57 (0.40–6.13)	0.52	–	–
HIV	1.30 (0.14–11.78)	0.82	–	–
Cavitary disease	7.45 (2.65–20.96)	<0.01	5.21 (1.56–17.38)	<0.01
No. of drugs to which baseline isolate was resistant/drug (IQR)	1.63 (1.05–2.51)	0.03	–	–
Resistant to ≥6 drugs by baseline DST	4.63 (1.56–13.68)	<0.01	5.31 (1.50–18.77)	0.01
Baseline ofloxacin resistant	6.24 (1.51–25.83)	0.01	–	–
Baseline capreomycin or kanamycin resistant	1.20 (0.44–3.27)	0.73	–	–
Known active drugs in initial regimen per drug	0.58 (0.35–0.99)	0.045	–	–
Follow-up characteristic				
Initial MDR TB treatment				
Capreomycin	2.92 (1.04–8.18)	0.04	–	–
Treatment interruption	2.93 (1.08–7.99)	0.04	–	–
>30 d to start SLDs	0.48 (0.17–1.34)	0.16	–	–
Baseline AFB sputum smear value ≥3+	4.44 (1.61–12.22)	<0.01	2.21 (0.66–7.48)	0.20
Sputum smear positive, mo†				
4	8.78 (1.95–39.66)	<0.01	6.54 (1.23–34.88)	0.03
6‡	15.31 (4.66–50.32)	<0.01	–	–

*OR, odds ratio; aOR, adjusted OR; –, not included in multivariate analysis; BMI, body mass index; TB, tuberculosis; IQR, interquartile range; DST, drug susceptibility testing; MDR, multidrug resistant; SLDs, second-line drugs; AFB, acid-fast bacilli.
†Time from initiation of second-line drug treatment for MDR TB.
‡Significant by an alternative multivariate analysis model when replacing the variable sputum smear positive at 4 mo.

quality-ensured SLDs and providing all treatment by directly observed therapy. In our study, baseline cavitary disease, high-grade smear positivity, increased drug resistance, and persistent smear positivity at follow-up sputum examinations were associated with acquired resistance. Although these risk factors might assist physicians in identifying those patients at increased risk for acquired resistance (and consequently poor outcomes), further evaluation is needed in evaluating optimal methods to treat patients who have isolates with acquired resistance.

Information on rates of acquired resistance among patients receiving second-line treatment is limited. A retrospective study of 536 MDR TB patients in western Siberia, Russia, found that XDR TB developed in 34 (6.4%); no information was provided on acquired resistance to quinolones or injectable drugs (*12*). Another study from the autonomous region of Abkhazia found that in a subpopulation of 47 MDR TB patients, XDR TB developed in 5 (11%) (*21*).

In the recently published Preserving Effective TB Treatment Study (PETTS), 832 MDR TB patients from 9 countries were prospectively followed up for acquired resistance (*11*). In that study, in comparison with our results, the rate for acquired XDR TB was similar (8.9%), that for acquired resistance to fluoroquinolones was slightly higher (11.4% vs. 9.1%), and that for an injectable drug was lower (7.8% vs. 10.6%). In PETTS, rates for acquired XDR TB in GLC-approved countries ranged from 0.6% to 9.8% compared with 6.3% to 18.0% for non–GLC-approved countries (*11*). On the basis of these findings, Georgia is on the higher end of acquired resistance rates for GLC-approved countries. Another recent study of patients in the United States found that among MDR TB patients, the rate of acquired resistance was 6.4% for fluoroquinolones and 6.6% for injectable drugs (*7*). These findings are probably overestimates because only patients with an initial and follow-up DST were included (<30% of all MDR TB patients during the study). Our results, along with the above

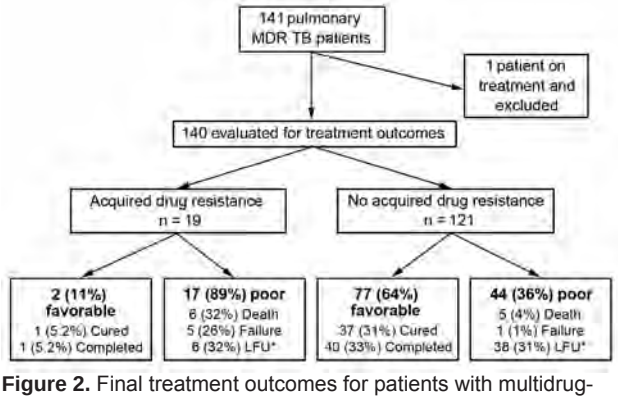

Figure 2. Final treatment outcomes for patients with multidrug-resistant tuberculosis (MDR TB), by acquired drug resistance status, Georgia, March 2009–October 2012. LFU, loss to follow up. *15 of 44 patients were culture positive at time of LFU, including all 6 patients with acquired resistance.

Table 3. Characteristics of patients with multidrug-resistant tuberculosis, by treatment outcome, Georgia, March 2009–October 2012*

Characteristic	Poor outcome, n = 61	Favorable outcome, n = 79	p value†
Acquired resistance to any second-line drug	17 (28)	2 (3)	<0.01
Median age, y	39.7	33.7	0.21
Male sex	49 (80)	53 (67)	0.08
History of imprisonment	21 (34)	19 (24)	0.18
Diabetes mellitus	6 (10)	10 (13)	0.60
Hepatitis C	9 (15)	7 (9)	0.28
HIV	3 (5)	3 (4)	0.75
BMI ≤18.5 kg/m^2	14 (23)	21 (27)	0.63
History of TB	29 (48)	33 (42)	0.50
Prior TB treatment			0.77
None	32 (53)	46 (58)	NA
First-line	24 (39)	28 (35)	NA
Second-line	5 (8)	5 (6)	NA
Baseline cavitary disease	17 (28)	13 (17)	0.11
Median no. drugs to which baseline isolate was resistant	6	5	0.18
Resistant to ≥6 drugs on baseline DST	32 (53)	28 (35)	0.04
Baseline ofloxacin resistant	6 (10)	3 (4)	0.15
Baseline capreomycin kanamycin resistant	24 (39)	23 (29)	0.20
Starting SLDs >30 days	28 (46)	38 (48)	0.80
Initial MDR TB treatment regimen included			
Capreomycin	25 (41)	40 (51)	0.26
Kanamycin	35 (57)	46 (58)	0.92
Ever received			
Moxifloxacin	11 (18)	9 (12)	0.27
Clarithromycin	3 (5)	2 (3)	0.45
Augmentin	3 (5)	2 (3)	0.45
Clofazimine	3 (5)	3 (4)	0.75
Treatment interruption	29 (48)	27 (34)	0.11
Adjunctive surgery performed	3 (5)	4 (5)	0.97
Baseline sputum AFB smear value ≥3+	23 (38)	23 (29)	0.28
Sputum culture positive, mo‡			
2	58 (95)	62 (79)	<0.01
4	44 (72)	36 (46)	<0.01
6	34 (56)	14 (18)	<0.01
Sputum smear positive, mo‡			
2	53 (87)	61 (77)	0.14
4	42 (69)	35 (44)	<0.01
6	28 (46)	11 (14)	<0.01

*BMI, body mass index; TB, tuberculosis; NA, not applicable; DST, drug susceptibility testing; SLDs, second-line drugs; MDR, multidrug resistant; AFB, acid-fast bacilli.
†Comparing persons with and without acquired resistance by using χ^2 or Fischer exact tests for categorical variables and *t*-test or median test for continuous variables.
‡Time from initiation of second-line drug treatment for MDR TB.

findings, indicate that acquired resistance occurs at fairly high rates across diverse settings and stress the need for repeating DST among patients with persistent positive sputum cultures. As DST and molecular drug-resistance testing become more available, we will probably see additional reports of acquired resistance and its effects.

Our results provide novel data on risk factors for acquired resistance among MDR TB patients and indicate that severity of disease at baseline and persistent AFB smear positivity were predictors of acquired resistance. Patients with a higher AFB smear microscopy grade (indicating higher bacillary load), baseline cavitary disease, and increasing baseline drug resistance had higher rates of acquired resistance. Persons with a lower baseline body mass index tended to have higher acquired resistance, but this result was not significant (p = 0.053). Shin et al. also found that baseline cavitary disease was associated with acquired XDR TB (aOR, 3.47, 95% CI 1.32–9.14), and although baseline drug resistance was not modeled, they found that a history of treatment with an injectable drug was a risk factor for acquired XDR TB (*12*). PETTS results corroborate our findings of increasing baseline drug resistance leading to higher rates of acquired resistance. Although that study reported that cavitary disease was associated with acquired XDR TB by univariate analysis (relative risk 1.84, 95% CI 1.04–3.26), it was included only as part of a propensity score for multivariate analysis and not modeled separately (*11*).

A novel finding of our study was the association of persistent smear positivity at 4 and 6 months with acquired resistance. Because AFB smear testing is more widely available than culture, this is a practical test that can help clinicians target high-risk patients who might need a regimen change, improved adherence, or other intervention.

Table 4. Risk factors for poor treatment outcomes among patients treated for multidrug-resistant tuberculosis, Georgia, March 2009–October 2012*

Risk factor	Univariate analysis, OR (95% CI)	p value	Multivariate analysis, aOR (95% CI)	p value
Acquired resistance to any second-line drug	14.88 (3.28–67.42)	<0.01	8.05 (1.56–41.66)	0.01
Baseline characteristics				
Increasing age per year	1.02 (0.99–1.05)	0.14	1.02 (0.99–1.05)	0.26
Male sex	2.00 (0.91–4.40)	0.08	–	
BMI ≤18.5 kg/m^2	0.82 (0.38–1.79)	0.62	–	–
History of TB	1.26 (0.65–2.48)	0.50	–	–
Prior receipt of second-line TB drugs	1.32 (0.37–4.79)	0.67	–	-
Diabetes mellitus	0.75 (0.26–2.20)	0.60	–	–
Hepatitis C	1.78 (0.62–509)	0.28	–	–
HIV	1.31 (0.26–6.73)	0.75	–	–
Baseline cavitary disease	1.96 (0.87–4.44)	0.11	0.72 (0.25–2.05)	0.54
Resistant to ≥6 drugs by baseline DST	2.01 (1.02–3.98)	0.04	1.45 (0.68–3.11)	0.34
No. drugs to which baseline isolate was resistant (IQR)	1.20 (0.90–1.59)	0.21	–	–
Drug resistance category				
Baseline ofloxacin resistant	2.76 (0.66–11.53)	0.16	–	–
Baseline capreomycin or kanamycin resistant	1.58 (0.78–3.20)	0.21	–	–
Follow-up characteristics				
Treatment interruption	1.75 (0.88–3.46)	0.11	–	–
>30 d to start SLDs	0.92 (0.47–1.79)	0.80	–	–
Initial capreomycin treatment	0.68 (0.35–1.33)	0.26	–	–
Baseline sputum smear AFB value ≥3+	1.47 (0.73–3.00)	0.28	–	–
Sputum smear positive, mo†				
4‡	2.78 (1.38–5.60)	<0.01	–	–
6	5.25 (2.33–11.81)	<0.01	3.43 (1.36–8.63)	0.01
Sputum culture positivity,mo				
4‡	3.09 (1.51–6.31)	<0.01	–	–
6‡	5.85 (2.71–12.59)	<0.01	–	–

*OR, odds ratio; aOR, adjusted OR; –, not included in multivariate analysis; BMI, body mass index; TB, tuberculosis; IQR, interquartile range; DST, drug susceptibility testing; SLDs, second-line drugs; AFB, acid-fast bacilli.
†Time from initiation of second-line drug treatment for MDR TB.
‡Significant by alternative multivariate analysis models when replacing the variable sputum smear positive at 6 mo.

We previously demonstrated acquired resistance among *M. tuberculosis* isolates from resected cavitary tissue compared with sputum samples (*13*). The cavitary lesion is an ideal setting for acquired resistance, given high bacterial loads, active mycobacterial replication, reduced exposure to host defenses, and potentially low penetration by drugs. The fibrotic wall of the cavity and variable vascularization might decrease SLD drug penetration, result in drug-selection pressure, and lead to emergence of acquired resistance (*22*). We are currently conducting a pharmacologic study to measure cavitary penetration of SLDs to assess the association between drug penetration and acquired resistance. It has been shown in an in vitro system that pharmacokinetic variability can lead to emergence of MDR TB (*23*). Consistent with this finding is a study that showed that among drug-susceptible TB patients, low isoniazid and rifampin concentrations preceded all cases of drug resistance (*24*). However, no clinical studies of SLD pharmacokinetics have examined their relationship with acquired resistance and treatment outcomes.

High rates of poor outcomes among MDR TB patients with isolates that have acquired resistance in our cohort are a concern and stress the need for prevention of acquired resistance. Only 2 patients with isolates that had acquired resistance had favorable outcomes, 1 who had adjunctive surgery and 1 whose isolate remained susceptible to ofloxacin, capreomycin, and kanamycin. An increasing number of reports have found that adjunctive surgery might be beneficial for MDR TB patients with cavitary disease (*25*,*26*). However, these studies were observational, and a randomized controlled clinical trial is needed to demonstrate if adjunctive surgery improves MDR TB treatment outcomes, including among patients with isolates that had acquired resistance.

The few other studies reporting some association of acquired resistance and outcomes found results mirroring our findings; however, our study found that acquired resistance associated with a negative outcome in adjusted analysis when controlling for other potential confounders. In a study from Abkhazia, all patients with isolates that had acquired resistance to ofloxacin had a poor outcome (*21*). In a report of 87 MDR TB patients from Uzbekistan, only 5 (28%) of 18 patients with isolates that had acquired resistance to ofloxacin to ofloxacin were successfully treated (*27*). In the study by Shin et al., only 14.7% of MDR TB patients in whom XDR TB developed were cured or completed treatment compared with 68.5% among those in whom XDR TB did not develop (*12*).

A limitation of our study was lack of genotyping, which prevented distinguishing isolates that had acquired

resistance from reinfection with another strain. Other reports found that potential reinfection with an exogenous strain accounted for 0%–34% of acquired drug resistance (*8*,*11*,*21*,*27*). It has also been estimated that certain strains of *M. tuberculosis* have higher mutation rates and are more likely to acquire drug resistance (*28*). In addition, we did not have detailed information on treatment adherence, which prevented us from measuring the association of different levels of adherence with isolates that had acquired resistance. Shin et al. found that cumulative months with <80% treatment adherence were associated with acquired resistance (*12*). The high rate of LFU also prevented determining the association of isolates that had acquired resistance with failure and death in these patients. In addition, lack of DST for many patients who were culture positive at 4 and 6 months might have led to an underestimation of isolates that had acquired resistance and biased the association of isolates that had acquired resistance to ofloxacin and poor outcomes if DST was selectively performed for sicker patients.

In summary, our results provide novel findings on risk factors for *M. tuberculosis* isolates developing acquired resistance and complete analysis of isolates that had acquired resistance and treatment outcomes among MDR TB patients. The need is urgent to further elucidate mechanisms of acquired resistance among *M. tuberculosis* isolates to improve treatment outcomes among MDR TB patients and to ensure that we preserve the effectiveness of newly introduced TB drugs.

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Risk Factors for Acquisition of Drug Resistance during Multidrug-Resistant Tuberculosis Treatment, Arkhangelsk Oblast, Russia, 2005–2010

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Acquired resistance to antituberculosis drugs decreases effective treatment options and the likelihood of treatment success. We identified risk factors for acquisition of drug resistance during treatment for multidrug-resistant tuberculosis (MDR TB) and evaluated the effect on treatment outcomes. Data were collected prospectively from adults from Arkhangelsk Oblast, Russia, who had pulmonary MDR TB during 2005–2008. Acquisition of resistance to capreomycin and of extensively drug-resistant TB were more likely among patients who received <3 effective drugs than among patients who received ≥3 effective drugs (9.4% vs. 0% and 8.6% vs. 0.8%, respectively). Poor outcomes were more likely among patients with acquired capreomycin resistance (100% vs. 25.9%), acquired ofloxacin resistance (83.6% vs. 22.7%), or acquired extensive drug resistance (100% vs. 24.4%). To prevent acquired drug resistance and poor outcomes, baseline susceptibility to first- and second-line drugs should be determined quickly, and treatment should be adjusted to contain ≥3 effective drugs.

Treatment of multidrug-resistant tuberculosis (MDR TB) is complicated by the length of treatment, toxicity, and expense involved in use of second-line drugs. The latest World Health Organization (WHO) recommendations for treatment of MDR TB include use of a second-line injectable agent for 8 months, a fluoroquinolone, pyrazinamide, and ≥2 additional effective second-line drugs for almost 2 years (*1*). Fluoroquinolones and second-line injectable agents are essential for treatment of MDR TB because of their bactericidal activity relative to other second-line drugs (*2*,*3*). The second-line companion drugs are bacteriostatic and are used mainly to prevent amplification of resistance to the 2 key bactericidal drugs (*4*–*6*).

Mycobacterium tuberculosis drug resistance occurs by 2 mechanisms: initial infection with a resistant strain (primary resistance) or emergence of a resistant population of bacilli in a patient who initially had drug-susceptible TB (acquired resistance). Acquired drug resistance develops when inadequate treatment kills drug-susceptible *M. tuberculosis* bacilli while allowing bacilli with spontaneously occurring mutations that confer drug resistance to flourish until they predominate (*7*). Inadequate treatment can be a consequence of insufficient dosing, poor gastrointestinal absorption of oral medications, substandard quality of drugs, poor adherence to treatment, unsatisfactory duration of treatment, or treatment with a regimen containing ≥1 drugs to which the organism is already resistant (*7*,*8*). Acquisition of additional drug resistance, especially to fluoroquinolones or second-line injectable agents, leaves few treatment options, complicating an already difficult treatment (*9*).

WHO estimates that almost half of all TB cases in countries of the former Soviet Union involve resistance to ≥1 drug and that 1 in 5 TB patients has MDR TB (*10*). Furthermore, in this region, prevalence of extensively drug-resistant (XDR) TB, defined as MDR TB with additional resistance to any fluoroquinolone and ≥1 of the 3 second-line injectable agents, is among the highest in the world (*10*,*11*). In Russia, the proportion of new cases that are MDR TB varies from 8.8% to 15% across regions (*10*). Reported proportions of MDR TB in new (13.5%–19%) and previously treated (45%–60%) case-patients have been among the highest in Arkhangelsk Oblast, which is in northwestern Russia (*12*,*13*). Although the overall rate of TB notification in this oblast is declining, especially among new cases, the relative proportion of MDR TB is increasing (*14*).

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Before 2000, the Arkhangelsk TB Control Program had limited access to second-line drugs because of their high cost (*15*). In early 2002, this program applied to the Green Light Committee (GLC), which was created to evaluate, lend guidance, and approve TB control programs for access to reduced-price, quality-assured, second-line drugs (*16*). The GLC led the development of WHO guidelines for programmatic management of drug-resistant TB, at the time called DOTS–Plus. The GLC required programs to follow these guidelines, which were designed to minimize the risk for acquired drug resistance for MDR TB patients and to improve treatment outcomes (*17*). In May 2003, the GLC approved Arkhangelsk TB Control Program procurement of quality-assured second-line drugs.

Even programs that follow WHO guidelines for programmatic management of drug-resistant TB have reported detection of XDR TB in patients undergoing treatment for MDR TB, suggesting that *M. tuberculosis* is acquiring additional drug resistance over the course of treatment (*18*). Our goals with this study were to determine the frequency of acquired drug resistance, the risk factors for acquisition of additional drug resistance over the course of MDR TB treatment, and which treatment regimens for MDR TB will decrease the risk for acquired resistance and lead to better treatment outcomes.

Methods

Study Population and Data Collection

The study prospectively enrolled 2 cohorts of consecutively seen, consenting, nonimprisoned adult patients in Arkhangelsk Oblast, Russia, who had confirmed pulmonary MDR TB and were starting treatment with second-line drugs. MDR TB was confirmed by sputum culture and drug-susceptibility testing (DST) at the regional TB laboratory. Patients in cohort 1 were enrolled from January 1, 2005, through December 31, 2006; patients in cohort 2 were enrolled from January 1, 2007, through December 31, 2008. The 2 cohorts were approved in 2 separate applications to the GLC. Most patients in cohort 1 had been on a waiting list for MDR TB treatment for an extended amount of time at the time of treatment initiation, whereas most patients in cohort 2 had a recent diagnosis of MDR TB at the time of treatment initiation. Study inclusion criteria required having ≥1 *M. tuberculosis*–positive culture result within 1 month (before or after) of starting second-line drugs for the treatment for MDR TB (baseline isolate) and ≥1 month of treatment with second-line drugs.

Standardized forms were used to prospectively collect sociodemographic, clinical, and laboratory data from patients' medical charts. Chest radiographs were read by experienced chest physicians and radiologists, and results were recorded in a standardized manner. Sputum specimens were collected from each patient at the start of second-line drug treatment (baseline isolate) and then monthly until treatment outcome was known.

The study protocol was approved by ethics committees at the US Centers for Disease Control and Prevention (CDC), Northern State Medical University in Arkhangelsk, and the State Research Center for Applied Microbiology and Biotechnology (SRCAMB) in Obolensk, Russia. All patients provided written informed consent.

Laboratory Methods

Baseline and follow-up sputum specimens were cultured on Lowenstein-Jensen solid media in the Arkhangelsk Regional TB Dispensary Laboratory. Frozen *M. tuberculosis* isolates were shipped to SRCAMB in Obolensk, Russia, for first- and second-line DST, genotyping, and DNA sequencing. The analysis reported in this article is based on the SRCAMB results. Testing at SRCAMB was conducted months to years after patients were enrolled, and results were not available in real time.

At SRCAMB, each isolate was cultured in 6 mL of Middlebrook 7H9 broth to an optical density of ≥1.0 McFarland standard and on Lowenstein-Jensen medium. Susceptibility testing for the baseline and the last follow-up (final) isolates from each patient were determined for isoniazid, rifampin, ethambutol, streptomycin, kanamycin, amikacin, capreomycin, ofloxacin, ethionamide, and para-aminosalicylic acid. Drug susceptibility was determined by the proportion method according to CDC protocol (*19*). When drug susceptibility of a patient's baseline and final isolates differed, isolates were genotyped by mycobacterial interspersed repetitive units–variable number of tandem repeats analysis and by restriction fragment-length polymorphism–IS6110 analysis to determine whether the isolates were the same strain.

Definitions

Definitions of pulmonary TB, MDR TB, and treatment outcomes were based on WHO guidelines (*20*). Third-line drugs refer to drugs classified by WHO as group 5 drugs (*1*). "Effective treatment" was defined as treatment with a drug or combination of drugs to which baseline DST reported susceptibility. "Ineffective treatment" was considered use of said drug(s) despite reported resistance. Effectiveness of treatment was considered unknown and the patient was not included in the analysis when the patient never received said drugs or baseline DST results were not available. "Acquired resistance" was defined as occurring when baseline DST result showed susceptibility in vitro, the final DST result showed resistance in vitro, and genotypes matched for the initial and final isolates. Acquired resistance was considered absent in each of the following

3 scenarios: 1) baseline and final isolates were susceptible to a drug; 2) DST result changed from susceptible to resistant, but genotyping indicated different strains; or 3) no follow-up positive culture results were available because the patient's sputum culture results sustainably converted to negative after the baseline DST, the patient died, or the patient was lost to follow-up. In each instance, the denominator for each group refers to the number of isolates with baseline DST results indicating susceptibility to the given drug. Patients whose baseline isolate was resistant to a given drug were excluded from the acquired resistance analysis for that drug. Successful treatment outcome was defined as cure and treatment completion (*20*). Poor treatment outcome was defined as treatment failure or death. The second-line companion drugs were para-aminosalicylic acid or ethionamide. Being underweight was defined as having a body mass index <18.5 kg/m^2.

Data Management and Statistical Analyses

The data from standardized forms were double-entered into an Epi Info (CDC, Atlanta, GA, USA) database in Arkhangelsk and sent to CDC for checking and analysis. Laboratory data were sent directly from the SRCAMB laboratory to CDC.

Statistical analyses were performed by using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The main outcome of interest was acquired resistance to specific second-line drugs. The secondary outcome of interest was end-of-treatment outcome. Bivariate associations between the potential risk factors and the outcome variable for each respective analysis were examined by using the Fisher exact test with a significance level of 0.05. Multivariate logistic regression was used to assess associations of acquired resistance and treatment with treatment outcomes while controlling for the potential confounding effects of extent of drug resistance at baseline, disease severity, and previous treatment for MDR TB.

Results

Patient Population

A total of 202 MDR TB patients were enrolled in the study: 81 in cohort 1 and 121 in cohort 2. Median patient age was 42 years, 171 (84.7%) patients were male, and none were HIV infected (HIV test results were available for all patients). Most patients had previously received treatment for TB: 69 (34.7%) had received first-line drugs and 73 (36.7%) had received additional second-line drugs. Almost all patients (189 [93.6%]) had pulmonary cavities, 162 (80.2%) had bilateral lung involvement, 161 (80.9%) had sputum smears with acid-fast bacilli seen with microscopy, and 45 (22.3%) had a body mass index <18.5 kg/m^2 at the start of MDR TB treatment.

A total of 740 *M. tuberculosis* isolates from 202 patients were shipped to SRCAMB. Of these 202 patients, baseline DST results from SRCAMB were available for 171 (84.7%) and were included in analysis of baseline drug resistance (Figure). Of the 171 patients for whom baseline DST results were available, follow-up DST results at SRCAMB were available for 117. Among the other 54 patients (without a final DST result), cultures converted after the initial isolate for 45, follow-up cultures were contaminated or did not grow for 5, and a reason was not documented for 4.

Baseline Drug Resistance

Among 171 patients for whom baseline isolate DST results were available (Table 1), MDR TB was not confirmed by DST for 4 (2.3%) isolates, and 130 (76.0%) baseline isolates were resistant to 4 first-line drugs tested (rifampin, isoniazid, ethambutol, and streptomycin). In addition, 74 isolates were resistant to ≥1 of the 3 second-line injectable agents: 72 (42.1%) to kanamycin, 30 (17.5%) to amikacin, 13 (7.6%) to capreomycin, and 10 (5.8%) to all 3. A total of 10 (5.8%) isolates were resistant to ofloxacin, and 7 (4.1%) were XDR. Of the second-line companion drugs tested, resistance to ethionamide was found for 46 (26.9%) isolates and to para-aminosalicylic acid for 54 (31.6%) isolates.

Acquired Drug Resistance

Among 117 patients for whom final DST results from SRCAMB were available, results for the baseline and final isolates differed for 32 (27.3%) and the isolates were successfully genotyped. Of these, genotype results for isolate

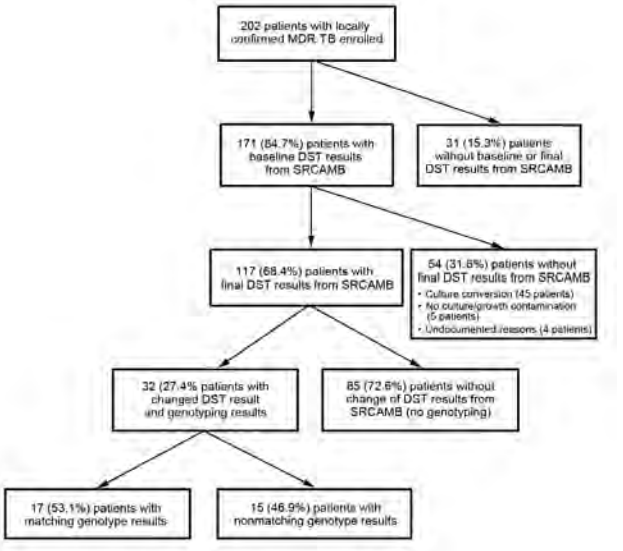

Figure. Patient enrollment and reference laboratory drug susceptibility and genotype testing results. DST, drug-susceptibility testing; MDR TB, multidrug-resistant tuberculosis; SRCAMB, State Research Center for Applied Microbiology and Biotechnology.

Table 1. Drug susceptibility and acquired resistance of *Mycobacterium tuberculosis* from 171 patients with MDR TB, Arkhangelsk Oblast, Russia, 2005–2010*

Drug(s) tested	Baseline, no. (%)		Acquired resistance no. (% of susceptible)
	Resistant	Susceptible	
RIF	167 (97.7)	4 (2.3)	1 (25.0)
INH	170 (99.4)	1 (0.6)	0
MDR TB drugs†	167 (97.7)	4 (2.3)	1 (25.0)
EMB	135 (79.0)	36 (21.0)	2 (5.6)
STR	163 (95.3)	8 (4.7)	1 (12.5)
All 4 first-line drugs‡	130 (76.0)	41 (24.0)	3 (7.3)
KAN	72 (42.1)	99 (57.9)	4 (4.0)
AMK	30 (17.5)	141 (82.5)	1 (0.7)
CAP	13 (7.6)	158 (92.4)	3 (1.9)
Any second-line injectable§	74 (43.3)	97 (56.7)	Not applicable
All 3 second-line injectables	10 (5.8)	161 (94.2)	7 (4.4)
OFX	10 (5.8)	161 (94.2)	6 (3.7)
XDR TB drugs¶	7 (4.1)	164 (95.9)	4 (2.4)
ETA	46 (26.9)	125 (73.1)	6 (4.8)
PAS	54 (31.6)	117 (68.4)	2 (1.7)
Both second-line companion drugs	18 (10.5)	153 (89.5)	6 (3.9)
All second-line drugs	1 (0.6)	170 (99.4)	14 (8.2)
All drugs	1 (0.6)	170 (99.4)	16 (9.4)

*AMK, amikacin; CAP, capreomycin; EMB, ethambutol; ETA, ethionamide; FQ, fluoroquinolone; INH, isoniazid; KAN, kanamycin; OFX, ofloxacin; MDR TB, multidrug-resistant tuberculosis; RIF, rifampin; STR, streptomycin; PAS, para-aminosalicylic acid; XDR, extensively drug-resistant tuberculosis.
†RIF and INH.
‡RIF, INH, EMB, and STR.
§KAN,AMK, and CAP.
¶RIF, INH, a second-line injectable drug, and an FQ.

pairs matched for 17 (53.1%) patients and were eligible for inclusion in numerators for respective analyses of acquired drug resistance (Figure). Of 41 paired isolates with baseline susceptibility to ≥1 first-line drug, resistance to a first-line drug was acquired in 3 (7.3%) (Table 1). Of 161 paired isolates with baseline susceptibility to ≥1 second-line injectable agents, resistance to ≥1 second-line injectable agents was acquired in 7 (4.4%): resistance to kanamycin by 4 (4.0%) of 99, to amikacin by 1 (0.7%) of 141, and to capreomycin by 3 (1.9%) of 158. Resistance to ofloxacin was acquired by 6 (3.7%) of 161, and XDR TB was acquired by 4 (2.4%) of 164 over the course of treatment for MDR TB.

Risk Factors for Acquisition of Drug Resistance

Acquired resistance to capreomycin was significantly associated with receiving the following drugs or drug groups: <3 effective drugs (p = 0.008), an ineffective fluoroquinolone (p = 0.009), or ineffective para-aminosalicylic acid (p = 0.02). Furthermore, acquired resistance to capreomycin was associated with not having received ofloxacin (regardless of baseline DST results) (p = 0.003), baseline resistance to ofloxacin (p = 0.008), and baseline resistance to para-aminosalicylic acid (p = 0.03) (Table 2). In addition, patients whose isolates acquired resistance to capreomycin were more likely to have received moxifloxacin (instead of ofloxacin) in the treatment regimen.

Acquired resistance to ofloxacin was significantly more common among patients who were underweight (p = 0.02) (Table 3). Patients with acquired ofloxacin resistance were more likely to have received moxifloxacin (p = 0.006), to have had fluoroquinolones switched during treatment (p = 0.05), and to be receiving a third-line drug during the current episode (p = 0.01).

Acquired XDR TB was more frequent among those receiving <3 effective drugs than among those receiving ≥3 effective drugs (p = 0.03) and among those who were underweight (p = 0.03) (Table 4). Those who acquired XDR TB were more likely to be receiving moxifloxacin during the current episode (p = 0.02). Patients in whom isolates acquired resistance to any second-line companion drug (ethionamide or para-aminosalicylic acid) were less likely to have received ofloxacin (p = 0.003) and more likely to be receiving moxifloxacin (p<0.001) during the current episode (Table 5).

Treatment Outcomes

Of 171 patients for whom baseline DST results were available, treatment was successfully completed for 94 (55.0%), treatment failed for 18 (10.5%), 20 (11.7%) died, and 39 (22.8%) defaulted from treatment. Poor treatment outcomes (treatment failure or death) were more likely among patients whose MDR TB acquired resistance to capreomycin (100% vs. 25.9%; p = 0.02) or ofloxacin (83.3% vs. 22.7%; p = 0.004) or became XDR TB (100% vs. 24.4%; p = 0.004) than among those in whom the respective resistance was not acquired (Table 6). Patients who received an effective fluoroquinolone were statistically less likely to have poor treatment outcomes than were those who received an ineffective fluoroquinolone (25.6% vs. 85.7%; p = 0.002). Patients who received any third-line drug were more likely to have previously

Table 2. Risk factors for acquired resistance to CAP while receiving treatment for MDR TB, 158 patients, Arkhangelsk Oblast, Russia, 2005–2010*

Variable†	Total	Acquired capreomycin resistance, no. (%)		p value‡
		Yes	No	
Received ≥3 effective drugs				
Yes	126	0	126 (100)	0.008
No	32	3 (9.4)	29 (90.6)	
Ever received effective FQ treatment§				
Yes	148	1 (0.7)	147 (99.3)	0.009
No	9	2 (22.2)	7 (77.8)	
Ever received effective PAS treatment§				
Yes	110	0 (0)	110 (100)	0.02
No	46	3 (6.5)	43 (93.5)	
Previous treatment with FQ				
Yes	28	2 (7.1)	26 (92.9)	0.08
No	130	1 (0.8)	129 (99.2)	
Previous PAS treatment¶				
Yes	23	2 (8.7)	21 (91.3)	0.08
No	112	1 (0.9)	111 (99.1)	
First time patient treated for MDR TB				
Yes	134	1 (0.7)	133 (99.3)	0.06
No	24	2 (8.3)	22 (91.7)	
Baseline OFX DST result				
Resistant	9	2 (22.2)	7 (77.8)	0.008
Susceptible	149	1 (0.7)	148 (99.3)	
Baseline PAS DST result				
Resistant	47	3 (6.4)	44 (93.6)	0.03
Susceptible	111	0 (0)	111 (100)	
Received OFX during episode				
Yes	135	0 (0)	135 (100)	0.003
No	23	3 (13)	20 (87)	
Received MOX during episode				
Yes	31	3 (9.7)	28 (90.3)	0.007
No	127	0 (0)	127 (100)	

*CAP, capreomycin; DST, drug-susceptibility test; FQ, fluoroquinolone; MDR TB, multidrug-resistant tuberculosis; MOX, moxifloxacin; OFX, ofloxacin; PAS, para-aminosalicylic acid.
†Certain variables were tested for association but omitted from table because results were not statistically significant at α = 0.1.
‡Fisher exact test.
§Patient(s) who did not receive treatment with the respective drug during the current episode of MDR TB were not included in the analysis.
¶For 23 patients, history of treatment with PAS was unknown.

received treatment for MDR TB (21.6% vs. 8.4%; p = 0.02), have resistance to >4 drugs at baseline (72.7% vs. 47.0%; p<0.001), and experience treatment failure or die (42.0% vs. 20.6%; p = 0.01) than those who did not receive any third-line drug. According to multivariable analysis, compared with no acquired resistance, acquired resistance to ofloxacin was associated with 10.2-fold (95% CI 1.1–95.1) increased odds of poor outcome when confounding was controlled for (Table 6). Compared with not receiving a third-line drug, treatment with a third-line drug was associated with 2.7-fold (95% CI 1.2–5.7) increased odds of poor treatment outcome when confounding was controlled for. Compared with not receiving effective fluoroquinolone treatment, effective treatment with a fluoroquinolone was associated with 16.7-fold (95% CI 1.9–100.0) increased the odds of successful treatment outcome when confounding was controlled for.

Discussion

This study measured the frequency with which drug resistance was acquired during MDR TB treatment and identified statistically significant associations for acquiring resistance to a specific drug or group of drugs in a population of MDR TB patients being managed in a high-quality TB program. The rates of acquired resistance to the 2 essential groups of drugs for MDR TB treatment were 4.3% for second-line injectable agents and 3.7% for fluoroquinolones, the middle of the range recently reported for GLC-approved programs (*21*). In this study, odds of treatment failure or death were 10.2-fold higher among those with acquired resistance to ofloxacin than among those without, further supporting the value of this class of drugs in successful MDR TB treatment. Although this study focused on 1 region of Russia, it reflects the broader global context of increasing use of second-line drugs and rapidly emerging resistance as exemplified by the global phenomenon of XDR TB reported in 2006 (*22*,*23*).

We found that the highest proportion of acquired second-line drug resistance was to any second-line injectable agent (4.3%), most frequently kanamycin (4.0%). Given the high baseline level of kanamycin resistance, the cross-resistance between second-line injectable agents, and the

Table 3. Risk factors for acquiring resistance to OFX during MDR TB treatment, 161 patients, in Arkhangelsk, Russia, 2005–2010*

Variable†	Total	Acquired ofloxacin resistance, no. (%)		p value‡
		Yes	No	
Enrollment cohort				
2005–2006	64	0 (0)	64 (100)	0.08
2007–2008	97	6 (6.2)	91 (93.8)	
Body mass index <18.5 at MDR TB diagnosis				
Yes	35	4 (11.4)	31 (88.6)	0.02
No	126	2 (1.6)	124 (98.4)	
Hospitalized at time of enrollment				
Yes	159	5 (3.1)	154 (96.9)	0.07
No	2	1 (50)	1 (50)	
Ever received MOX during current episode				
Yes	26	4 (15.4)	22 (84.6)	0.007
No	135	2 (1.5)	133 (98.5)	
Changed FQ during current episode				
Yes	10	2 (20)	8 (80)	0.05
No	151	4 (2.6)	147 (97.4)	
Ever received a third-line drug during episode				
Yes	79	6 (7.6)	73 (92.4)	0.01
No	82	0 (0)	82 (100)	

*FQ, fluoroquinolone; OFX, ofloxacin; MDR TB, multidrug-resistant tuberculosis; MOX, moxifloxicin.
†Certain variables were tested for association but omitted from table because results were not statistically significant at α = 0.1.
‡Fisher exact test.

rate of acquired resistance to second-line injectable agents illustrated in this study, treating MDR TB with second-line injectable agents is becoming less of an effective option (*24*). Furthermore, because of the common baseline resistance to kanamycin, most of the acquired XDR TB was the result of acquired ofloxacin resistance. Historically in Arkhangelsk Oblast, kanamycin was widely used for TB treatment along with 2–3 other drugs, including first- and second-line drugs, whereas fluoroquinolones were rarely used for TB treatment before GLC approval in 2003 (*12*,*25*). Acquired resistance to fluoroquinolones during MDR TB treatment was reported for 11.2% of cases in 9 countries, including Russia, possibly because of the high mutation frequency of the *gyrA* and *gyrB* genes (*21*,*26*–*28*). Of any single second-line drug tested in this study, acquired resistance to ofloxacin occurred second most often. Most patients whose isolates acquired resistance to either of the second-line companion drugs tested also experienced acquired resistance to a second-line injectable agent, ofloxacin, or both (i.e., acquired extensive drug resistance), making TB in these patients virtually untreatable with available drugs (*28*).

As seen elsewhere and in this population of MDR TB patients for whom prevalence of baseline resistance

Table 4. Risk factors for acquiring extensive drug resistance during MDR TB treatment, 164 patients, Arkhangelsk, Russia, 2005–2010*

Variable†	Total	Acquired extensive drug resistance, no. (%)		p value‡
		Yes	No	
Treated with ≥3 effective drugs				
Yes	129	1 (0.8)	128 (99.2)	0.03
No	35	3 (8.6)	32 (91.4)	
Ever received effective FQ§				
Yes	160	3 (1.9)	157 (98.1)	0.07
No	3	1 (33.3)	2 (66.7)	
Body mass index <18.5 at MDR TB diagnosis				
Yes	36	3 (8.3)	33 (91.7)	0.03
No	128	1 (0.8)	127 (99.2)	
Baseline OFX susceptibility result				
Resistant	3	1 (33.3)	2 (66.7)	0.07
Susceptible	161	3 (1.9)	158 (98.1)	
Ever received OFX during current episode				
Yes	144	2 (1.4)	142 (98.6)	0.07
No	20	2 (10)	18 (90)	
Ever received MOX during current episode				
Yes	29	3 (10.3)	26 (89.7)	0.02
No	135	1 (0.7)	134 (99.3)	

*FQ, fluoroquinolone; OFX, ofloxacin; MDR TB, multidrug-resistant tuberculosis; MOX, moxifloxicin
†Certain variables were tested for association but omitted from table because results were not statistically significant at α = 0.1.
‡Fisher exact test.
§Patients who did not receive treatment with the respective drug during the current episode of MDR TB were not included in the analysis.

Table 5. Risk factors for acquiring resistance to second-line companion drugs during MDR TB treatment, 153 patients, Arkhangelsk Oblast, Russia, 2005–2010*

Variable†	Total	Acquired resistance to ETA or PAS, no. (%)		p value‡
		Yes	No	
Enrollment cohort				
2005–2006	58	0 (0)	58 (100)	0.08
2007–2008	95	6 (6.3)	89 (93.7)	
Thoracic surgery during current episode				
Yes	2	1 (50)	1 (50)	0.08
No	151	5 (3.3)	146 (96.7)	
Ever received OFX during current episode				
Yes	133	2 (1.5)	131 (98.5)	0.003
No	20	4 (20)	16 (80)	
Ever received MOX during current episode				
Yes	28	5 (17.9)	23 (82.1)	<0.001
No	125	1 (0.8)	124 (99.2)	
Ever received a third-line drug during current episode				
Yes	77	6 (7.8)	71 (92.2)	0.03
No	76	0 (0)	76 (100)	

*OFX, ofloxacin; MDR TB, multidrug-resistant tuberculosis; MOX, moxifloxacin.
†Certain variables were tested for association but omitted from table because results were not statistically significant at α = 0.1.
‡Fisher exact test.

to kanamycin, ethionamide, and para-aminosalicylic acid was high, baseline susceptibility to and use of fluoroquinolones were essential for preventing further resistance to second-line injectable agents, preventing acquired XDR TB, and increasing treatment success (*29*,*30*). With fewer effective treatment options, the risk for acquired resistance to additional drugs increases (*28*). This study illustrates the value of effective use of bactericidal drugs such as fluoroquinolones and companion drugs (especially para-aminosalicylic acid) in preventing acquired resistance to second-line injectable agents during treatment for MDR TB. Other studies reported a significant association between use of thioamides and treatment success but not with para-aminosalicylic acid (*31*).

WHO recommends that MDR TB be treated with ≥4 second-line drugs to which *M. tuberculosis* is likely to be susceptible plus pyrazinamide, creating a regimen of ≥5 drugs during the intensive phase of treatment (*1*). Many factors make creating such a regimen challenging, including availability of timely DST results for second-line drugs and availability of multiple drugs within a class of second-line drugs. In this setting, in which baseline DST results for multiple second-line drugs were available, *M. tuberculosis* treated with ≥3 effective drugs were less likely to acquire resistance to each of the drugs or drug groups tested than were *M. tuberculosis* treated with <3 effective drugs. However, this association was only statistically significant for acquired resistance to capreomycin and for acquired extensive drug resistance. Treatment with ≥4 effective drugs had a similar, but not statistically significant, inverse association with acquired drug resistance.

Acquired resistance to ofloxacin was not associated with any of the effective treatment variables. The treatment and patient management characteristics that were associated with acquired ofloxacin resistance may be an artifact of clinical management practices when treatment regimens fail and probably reflect confounding. The treatment for severe disease or a failing regimen will often be switched to a newer generation fluoroquinolone because these are thought to be more effective and because cross-resistance within the class is not complete (*32*,*33*). Therefore, the only significant risk factor for acquired ofloxacin resistance in this population was being underweight, which is a risk factor for incident TB and an indicator of disease severity, regardless of drug susceptibility (*34*).

Other studies have found that the main risk factors for acquired drug resistance included empiric re-treatment (i.e., without reference to DST) and unsupervised treatment (*35*,*36*). In this study population, drug resistance was acquired among patients with MDR TB even though the patients had received individualized treatment, and directly observed therapy was mandatory for all patients in the program.

The MDR TB treatment outcomes for this population are consistent with previously reported outcomes. Treatment success for this population (55%) was greater than the WHO-reported worldwide average (48%) but less than published results of individualized MDR TB treatment programs (*31*,*37*). This study indicates that treatment failure and death are significantly more common among patients who experienced acquired resistance to capreomycin or ofloxacin or who acquired XDR TB than among patients who did not, providing even more evidence that these drugs are essential for successful treatment of MDR TB (*38*).

This study had several limitations. First, the relatively small sample size limited statistical power of our analyses. Second, testing of *M. tuberculosis* for susceptibility to second-line drugs is difficult and not well standardized (*39*), which could have caused patient misclassification for both

Table 6. Effect of acquired resistance and treatment on MDR TB treatment outcomes, 132 patients, Arkhangelsk Oblast, Russia, 2005–2010*

Variable†	Total	Successful treatment outcome, no. (%)‡	Poor treatment outcome, no. (%)§	Fisher exact p value	aOR (95% CI)¶
Overall	132	94 (71.2)	38 (28.8)		
Acquired resistance to any second-line drug#					
Yes	13	6 (46.2)	7 (53.8)	0.05	1.93 (0.54–6.88)
No	118	88 (74.6)	30 (25.4)		
Acquired resistance to CAP#					
Yes	3	0	3 (100)	0.02	NR
No	116	86 (74.1)	30 (25.9)		
Acquired resistance to OFX#					
Yes	6	1 (16.7)	5 (83.3)	0.004	10.18 (1.09–95.08)
No	119	92 (77.3)	27 (22.7)		
Acquired XDR#					
Yes	4	0 (0)	4 (100)	0.004	NR
No	123	93 (75.6)	30 (24.4)		
Ever received effective FQ**					
Yes	125	93 (74.4)	32 (25.6)	0.002	0.06 (0.01–0.53)††
No	7	1 (14.3)	6 (85.7)		
Ever received CAP during current episode					
Yes	104	67 (64.4)	37 (35.6)	0.08	1.42 (0.49–4.05)
No	33	27 (81.8)	6 (18.2)		
Ever received MOX during current episode					
Yes	27	14 (51.9)	13 (48.1)	0.06	1.48 (0.56–3.90)
No	110	80 (72.7)	30 (27.3)		
Ever received third-line drug during current episode					
Yes	69	40 (58)	29 (42)	0.01	2.68 (1.25–5.75)
No	68	54 (79.4)	14 (20.6)		

*aOR, adjusted odds ratio; CAP, capreomycin; FQ, fluoroquinolone; MDR TB, multidrug-resistant tuberculosis; MOX, moxifloxacin; NR, no result; OFX, ofloxacin; XDR, extensively drug-resistant tuberculosis.
†Certain variables were tested for association but omitted from table because results were not statistically significant at α = 0.1.
‡Cure or treatment completion.
§Death or treatment failure.
¶aOR for poor treatment outcome versus successful treatment outcome controlling for extent of drug resistance at baseline, severity of disease, and previous treatment for MDR TB.
#Patient(s) with baseline TB resistance to the respective drug (or drug groups) were not included in the analysis.
**Patient(s) who did not receive treatment with the respective drug during the current episode of MDR TB were not included in the analysis
††16.67 (95% CI 1.89–100.0) aOR of successful treatment outcome vs. poor treatment outcome.

the effective treatment and acquired resistance variables because both sets of variables involve DST results. Third, the effective treatment variables did not consider dosage or length of time the drug was given—all key components of effective treatment. Last, the prevalence of cavitary disease in this population was unusually high, and because cavitation is associated with acquired resistance, the results might not be directly applicable to patient populations with less chronic or destructive disease (*21*).

Knowing the drug resistance pattern in the community and risk factors for acquired resistance to second-line drugs can help TB programs initiate effective treatment regimens, prevent additional acquired resistance, and improve treatment outcomes for patients for whom MDR TB is suspected before DST results are available. The likelihood of treatment success can be further improved by adjusting treatment after receipt of DST results for second-line drugs. The need for rapid diagnosis of drug resistance and effective treatment is crucial.

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Ebola Risk Perception in Germany, 2014

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Ebola virus disease (EVD) outbreaks have occurred during the past 5 decades, but none has affected European countries like the 2014 epidemic in West Africa. We used an online questionnaire to investigate risk perceptions in Germany during this epidemic peak. Our questionnaire covered risk perceptions, knowledge about transmission routes, media use, reactions to the outbreak, attitudes toward measures to prevent the spread of EVD and vaccination against EVD, and willingness to volunteer for aid missions. Of 974 participants, 29% indicated that they worried about EVD, 4% correctly stated virus transmission routes, and 75% incorrectly rated airborne transmission and transmission by asymptomatic patients as possible. Many indicated that if a patient were flown to Germany for treatment in a nearby hospital, they would adapt preventive behavior. Although most participants were not worried about EVD at the current stage of the epidemic, misperceptions regarding transmission were common and could trigger inappropriate behavior changes.

Misperceptions of risk can lead to inappropriate reactions during epidemics (*1*,*2*), such as stigmatization of those who are perceived as possible sources of infection (*3*). With regard to Ebola virus disease (EVD) in the West African countries most affected by the outbreak in 2014, indications are strong that societal misperceptions contributed to the outbreak spread (*4*). Public perceptions even in countries not directly affected by the EVD outbreak might influence outbreak response (e.g., by the priorities governments will set or by the willingness of persons to volunteer for aid missions in the affected countries) (*5*,*6*).

National authorities in countries outside of Africa responded differently to the potential risks of importing EVD into their countries. In November 2014, Australia and Canada imposed entry restrictions for persons from Guinea, Sierra Leone, and Liberia (*7*,*8*). At the same time, the United Kingdom introduced entry screening for international flight and train passengers (*9*,*10*). Because evidence for these public health actions is difficult to evaluate (*11*,*12*), public opinions might have played a role in political decision making. As of November 2014, Germany had not implemented any travel or entry restriction. As of October 14, 2014, a total of 3 patients who had acquired EVD in West Africa have been evacuated to hospitals in Germany for treatment. These evacuations to Germany were intensively covered by the media in Germany.

Several previous EVD outbreaks have occurred, but none was comparable in size and spread to the 2014 epidemic in West Africa and none directly or indirectly affected European countries, until now. To understand public reactions during an emerging epidemic in a country not directly affected by EVD, but one that is exposed to media coverage of the epidemic and involved in actions to contain the epidemic, we conducted an online survey about EVD for residents of Lower Saxony, Germany. Our goal was to improve our understanding of risk perceptions and potential changes in behavior during epidemics.

Methods

Participants

We implemented this survey by using a longitudinal online panel, which was created in March 2014 to address human hygiene and preventive behavior regarding infectious diseases (*13*,*14*). The panel consists of 1,376 persons 15–69 years of age, who complete short, online questionnaires once a month. Panel members come from 4 districts in Lower Saxony, Germany (Braunschweig, Salzgitter, Vechta, and Wolfenbüttel). The districts were chosen by convenience: Braunschweig is the location of our research institute (the Helmholtz Centre for Infection Research), Vechta is its rural counterpart, and Salzgitter and Wolfenbüttel are 2 neighboring districts of Braunschweig. In each district, potential participants were invited to the panel by means of proportional stratified random sampling from the population registry. Of 26,895 invited, 9% were successfully recruited.

Questionnaire

We used an open-source online survey application (Limesurvey; *15*) to develop a knowledge-attitude-practice survey

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for online use (*16*). In November 2014, the questionnaire was about EVD (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/6/15-0013-Techapp1.pdf.). The EVD questionnaire consisted of 27 questions with 2–11 items each, totaling 123 items. The questions covered 7 topics: worries about EVD and perceived personal probability of infection, knowledge about transmission routes of Ebola virus, media use to obtain information about EVD, personal reactions to the EVD outbreak, attitudes toward specific measures to prevent the spread of EVD to Europe, willingness to volunteer to fight EVD in West Africa, and attitudes toward vaccination against EVD.

Risk perceptions were operationalized by asking participants if they worry about EVD ("yes" or "no") and how they perceive their personal probability of acquiring EVD in the following 9 scenarios: at work, in public transport, in public places, at an airport in Germany, as a patient in a hospital in Germany, at a doctor's office in Germany, during travel to affected countries, by food imported from West African countries, or by other products originating in West Africa. Responses were chosen from a Likert scale with 5 options: "yes," "rather yes," "rather no," "no," and "does not apply." "Worry about EVD" describes thinking about threatening scenarios in the absence of actual danger (*17*), and "perceived personal probability of infection" describes participants' estimation of the actual risk for infection.

Knowledge about the transmission of Ebola virus was assessed with regard to the following 11 potential transmission routes: by direct contact with bodily fluids of infected persons, dead or living; through material heavily contaminated with such fluids; by direct contact with infected but asymptomatic persons; through air; through material that has been heavily contaminated with bodily fluids of infected persons, dead or living; through drinking water; through food produced in Germany; by casual contact with someone already sick, such as sitting next to someone (and without any direct contact of bodily fluids); by wild animals in Africa; by insects in Africa; or by wild animals/insects in Germany. Response choices were "true," "false," and "don't know." We computed a cumulative knowledge score (1 point for each answer in agreement with current scientific knowledge, range 0–11). In addition, participants were invited to rate their personal knowledge of EVD as "very good," "good," "moderate," or "not good." They were also asked whether they increased their use of media to inform themselves about EVD.

To assess behavioral implications, participants were asked if they had changed their behavior as a result of the EVD outbreak, how they would change behavior if an EVD patient were flown from Africa to Germany for treatment in a nearby hospital, and whether they would cancel an already booked flight to Africa. Participants were also asked if they thought that specific prevention measures should be introduced to prevent the spread of EVD to Europe (Figure). The survey also included questions about aid missions in affected countries and about the potential vaccine against EVD.

This study was approved by the Ethics Committee of the Hannover Medical School and the Federal Commissioner for Data Protection and Freedom of Information. All participants gave written informed consent before entering the study.

Statistical Analyses

To test differences among groups, we used the χ^2 test for categorical variables and the Wilcoxon test for continuous variables. To assess associations of sociodemographic factors with worrying about EVD, we used the Spearman correlation coefficient. In addition, we performed explorative multivariable logistic regression analyses to assess the effect of knowledge about transmission routes and sociodemographic factors on worries about EVD and on willingness to volunteer for aid missions. Analyses were performed by using Stata 12 (StataCorp LP, College Station, TX, USA).

Results

Risk Perceptions

A total of 974 participants, 15–69 years of age, completed the questionnaire. Sociodemographic characteristics of participants who completed the questionnaire (Table 1) did not differ from those of other panel members who did not (data not shown). In response to the question about whether they worried about EVD, 29% of participants answered in the affirmative; of those, 79.0% rated the strength of their worries as average (score ≤3 on a scale of 1 [a little] to 5 [very strong]) (data not shown). In response to another question, 68% of the participants reported that they perceived acquiring EVD as possible in at least 1 of the 9 scenarios specified (data not shown). In response to a question asking whether in the next 6 months EVD could spread to the general population of Germany in a similar way as occurred in some West African countries, 8% of participants worried about EVD and 1.6% of those not worried about EVD answered in the affirmative (Figure).

Knowledge

Although 25% of participants rated their personal knowledge about EVD as good or very good, only 3.9% correctly answered all questions about transmission routes. The most common misperception (by 73.7% of participants) was that airborne transmission of Ebola virus is possible; moreover, 74.0% believed that human-to-human transmission by infected but asymptomatic persons is possible. Among those who specified airborne transmission as being possible, 18.5% reported that they perceived that acquiring EVD while using public transportation was possible compared

Figure. Personal behavior and attitudes toward measures against the spread of Ebola virus disease (EVD) and toward vaccination against EVD. Black, worried about EVD; gray, not worried about EVD; NS, not significant. *χ^2 test $p<0.05$. †"Yes" to at least 1 of 5 items (avoid contact with African acquaintances; avoid contact with African persons in public places; avoid going to public events; avoid using public transportation; engage in precautionary purchases). ‡"Yes" to at least 1 of 7 items (avoid public events/crowded places; avoid using public transportation; avoid physical contact with other persons; increase hygiene behavior; wear face mask outside of the home; avoid admission to the same hospital; avoid visiting friends admitted to the same hospital).

with 9.4% of those who did not consider airborne transmission as being possible (p = 0.001). Education was positively associated with knowledge scores about Ebola virus transmission routes (Spearman correlation coefficient 0.18, p<0.001) and rating of personal knowledge about EVD (Spearman correlation coefficient 0.39, p<0.001). After controlling for the rating of personal knowledge about EVD, education was no longer associated with the score for knowledge about Ebola virus transmission routes (partial correlation coefficient –0.003, p = 0.91).

Media Use

Increased use of media to learn about EVD was reported by 43% of participants. These participants most commonly used the Internet (45.5%), television (53.1%), and print media (45.7%). Increased use of television was more common among participants with a low level of vocational or secondary education than among participants with a higher level of education (data not shown). Increased media use was not associated with a higher knowledge score (median score for both groups = 7, p = 0.37). Personal knowledge about EVD was self-rated as good or very good by 28.7% of those who increased their media use and by 21.8% who did not (p = 0.01).

Multivariable logistic regression analyses that included age, sex, education, increased media use, and knowledge score showed that those who increased their media use were more likely to be worried about EVD than were those who

Table 1. Characteristics of 974 participants in survey about risk for EVD, Lower Saxony, Germany, 2014*

Characteristic	Total, no. (%)	Not worried about EVD, no. (%)†	Worried about EVD, no. (%)‡	p value§
Sex				0.06
F	534 (54.8)	365 (52.7)	169 (60.1)	
M	414 (42.5)	311 (44.9)	103 (36.7)	
Missing information	26 (2.7)	17 (2.4)	9 (3.2)	
Median age, y (IQR)	46 (34–56)	47 (34–58)	46 (35–54)	0.10¶
Education#				0.02
Low	410 (42.1)	275 (39.7)	135 (48.0)	
Intermediate	117 (12.0)	80 (11.5)	37 (13.2)	
High	411 (42.2)	314 (45.3)	97 (34.5)	
Missing information	36 (3.7)	24 (3.5)	12 (4.3)	
Country of birth				0.59
Germany	891 (91.5)	638 (92.1)	253 (90.1)	
Other	45 (4.6)	30 (4.3)	15 (5.3)	
Missing information	38 (3.9)	25 (3.6)	13 (4.6)	
Median knowledge score (IQR)	7 (6–9)	7 (6–9)	7 (5–8)	<0.001¶

*EVD, Ebola virus disease; IQR, interquartile range.
†n = 693 (71.1%).
‡n = 281 (28.9%).
§χ^2 test comparing those worried about EVD with those not worried (missing values were not considered).
¶Wilcoxon rank-sum test comparing those worried about EVD with those not worried.
#Low, <12 y of vocational or secondary education and/or completed apprenticeship; intermediate, at least 12 y of vocational or secondary education and/or degree of a specialized vocational school; high, university training (bachelor degree and higher academic level).

did not increase their media use and that knowledge about Ebola virus transmission routes was negatively associated with being worried about EVD (Table 2). Worrying about EVD was not affected by age, sex, or education (Table 2).

Personal Reactions

Among all participants, 7% changed behavior in response to the EVD outbreak (Figure). Among those, 68.8% avoided contact with African persons in public places and 26.6% avoided using public transportation.

If an EVD patient were to be flown from Africa to Germany and treated in a nearby hospital, 86.9% of all participants stated that they would change their behavior. Of these, 16.4% would avoid using public transportation, 74.9% would increase their hygiene behavior (e.g., washing hands more often), and 30.2% would not visit friends admitted to the same hospital.

Participants were also asked about travel to Africa. As many as 95% of all participants would cancel an already booked flight to affected countries in West Africa, and 35.6% would cancel a flight to nonaffected countries in Africa.

Attitudes toward Specific Measures to Prevent the Spread of EVD to Europe

Asked about specific measures to prevent the spread of EVD to Europe, 97.0% of participants replied that all travelers from affected areas should receive information about EVD and advice on what to do if signs and symptoms of EVD developed (Figure). Entry restrictions for persons from affected countries were supported by 17.0% of participants. Mandatory quarantine for volunteers returning from aid missions in West Africa was supported by 37.6% of participants; the difference between those worried about EVD (51.6%) and those not worried (31.9%) was significant ($p<0.001$). Prohibiting return to Germany of persons who acquired Ebola infection during aid missions was supported by 10%.

Willingness to Volunteer to Fight EVD in West Africa

Of all participants, 38.7% would volunteer to fight EVD in West Africa if their experience and their knowledge were needed and if their personal situation and their health allowed them to do so. Multivariable logistic regression analyses including age, sex, education, increased media use, and knowledge score showed that older persons were less likely than younger persons to volunteer for aid missions and that women were less likely than men to volunteer (Table 3). Willingness to volunteer was not associated with education level.

Vaccination against EVD

If a vaccine against EVD existed, 18.3% would opt for vaccination even if they did not plan to visit affected countries in West Africa and did not have contact with EVD patients. Of those who wanted to get vaccinated, 41.1% would still do so if the vaccine were associated with occasional mild side effects and 15.2% if it were associated with rare but severe side effects.

Of all participants, 85.9% stated that compulsory vaccination against EVD should be implemented in affected countries. A total of 36.4% would support compulsory vaccination against EVD for medical staff in Germany, and 51.5% would support compulsory vaccination against EVD for the general population of Germany if the number of EVD cases in Germany increased.

Discussion

We report public perceptions of EVD in Germany, a country not directly affected by the current epidemic. Among the participants of our study, a substantial proportion were worried about EVD; however, among those worried, most did not report strong worries. Only one quarter of participants rated their knowledge of Ebola as good or very good. In addition, a large majority had poor knowledge about the transmission routes of the virus. A particularly common misperception was that Ebola virus can be transmitted by the airborne route or that it can be transmitted from human to human by infected but asymptomatic persons. These misperceptions were strongly associated with perceived personal probability of becoming infected while using public transportation. At the peak of the epidemic (November 2014), we identified inappropriate, unjustified, and stigmatizing attitudes in only a small proportion of participants. In contrast, treatment of a patient flown from Africa to a nearby hospital would induce worrying and inappropriate behavior in most participants. This response might be attributable to the fact that persons intuitively overestimate the risk for rare events (*18*). Our findings indicate a potential for inappropriate reactions to the epidemic should cases of EVD occur in Germany or should evacuations of EVD patients to Germany increase (*19*). For either of these 2 scenarios, trusted institutions (e.g., government) should spread information on the cause and the risk for infection (*20*).

As expected, participants who were worried about EVD were more likely to support measures preventing its spread to Europe. The difference between those worried and those not worried was particularly large for measures that can be considered inappropriate or even counterproductive to fighting the epidemic. For example, the stigmatization of returning health care workers and other volunteers can lead to fewer persons being willing to volunteer for aid missions (*21*). It is crucial that those worried about EVD remain a minority so that society will not be paralyzed by worries. Thus, misperceptions regarding transmission routes of Ebola virus should be resolved, and the media should contribute to a balanced, rational response rather than fuel worries. The observation that increased media use was not associated with better knowledge of transmission routes indicates the need for qualitative improvement of media reporting of such situations. However, the direction of the association between increased media use and worries cannot be determined from our data, so conclusions on worries and increased media use should be made cautiously. Not only the media but also public health experts might have contributed to mixed messages regarding airborne transmission of Ebola virus (*22*).

Almost 39% of participants indicated that they would volunteer to fight EVD in West Africa, but some of those participants would at the same time support prevention measures that are likely to negatively affect willingness to participate in aid missions. The high percentage of volunteers might result from the specific question that the participants were asked. The question included 2 preconditions that would qualify persons to volunteer: having the required experience and having a personal situation that would enable going to Africa. Most participants probably did not fulfill these preconditions, so their willingness to volunteer was only hypothetical. Therefore, they might not have realized that the restriction regarding return of volunteers would hamper their own return.

The changes in personal daily behavior reported or forecasted by the study participants (change of contact structure and mode of transportation, support of rapidly introduced vaccines) have consequences for understanding future emerging epidemics. Mathematical models constructed on the basis of contact structures and health perceptions obtained outside an epidemic setting will not be able to provide helpful insights if they do not take these factors

Table 2. Association between knowledge, media use, sociodemographic factors, and worries about EVD, Lower Saxony, Germany, 2014*

Characteristic	Odds ratio (95% CI)	p value†
Age, per 10-y increase	0.97 (0.87–1.08)	0.52
Sex		0.11
F	1.28 (0.94–1.74)	
M	Reference	
Education‡		0.39
Low	1.28 (0.92–1.78)	
Intermediate	1.33 (0.83–2.14)	
High	Reference	
Increased media use		<0.001
Yes	2.14 (1.59–2.88)	
No	Reference	
Knowledge score (per 1-point increase)	0.87 (0.81–0.93)	<0.001

*Multivariable logistic regression. EVD, Ebola virus disease.
†Wald test.
‡Low, <12 y of vocational or secondary education and/or completed apprenticeship; intermediate, at least 12 y of vocational or secondary education and/or degree of a specialized vocational school; high, university training (bachelor degree and higher academic level).

Table 3. Association between knowledge, media use, and sociodemographic factors and willingness to volunteer in aid missions in Africa, Lower Saxony, Germany, 2014*

Characteristic	Odds ratio (95% CI)	p value†
Age (per 10-y increase)	0.86 (0.77–0.96)	0.07
Sex		0.03
F	0.72 (0.53–0.98)	
M	Reference	
Education‡		0.94
Low	1.09 (0.79–1.52)	
Intermediate	1.05 (0.64–1.70)	
High	Reference	
Increased media use		0.90
Yes	0.98 (0.73–1.32)	
No	Reference	
Knowledge score (per 1-point increase)	1.06 (0.99–1.14)	0.09

*Multivariable logistic regression. EVD, Ebola virus disease.
†Wald test.
‡Low, <12 y of vocational or secondary education and/or completed apprenticeship; intermediate, at least 12 y of vocational or secondary education and/or degree of a specialized vocational school; high, university training (bachelor degree and higher academic level).

into account. Problems in modeling the further course of the influenza A(H1N1)pdm09 outbreak might be attributable to these factors (*1*,*2*), and the experience with models made for the current EVD epidemic might be similar.

A large majority of participants supported compulsory vaccination against EVD for persons in affected countries. About half also stated that EVD vaccination should be compulsory for the general population should the number of cases in Germany increase. This finding is astonishing because no compulsory vaccination exists in Germany, and during the 2009 influenza A(H1N1)pdm09 pandemic, it was regarded as completely unacceptable (*23*). It is possible that the acceptability of drastic and compulsory measures is high only if the likelihood that such measures will be implemented is low, as is now the situation for EVD. On the contrary, the perception of associated risks might be scored much higher for EVD than for influenza, thereby increasing the acceptance of compulsory vaccination.

This study has some limitations. Regional data collected in an online survey might not represent perceptions of the general population in Germany. Furthermore, because the respondents in our survey were participating in a study on hygiene and behavior regarding infectious diseases, their level of motivation and knowledge about health-related topics might be higher than that of the general population. The education level of participants was also higher than that of the general population (42.2% of the study participants had university training compared with only 17.2% of the general population of Germany; *24*). The panel members were also older, and the percentage of female panel members was higher than that of the general population.

For some characteristics in our analyses we did not have baseline data. For example, we did not have baseline information about which types of media are generally used by participants, so we cannot tell whether participants increased their media use or whether they used additional media sources that they did not use before. Because we do not have information about participants' professions, we cannot assess whether risk groups for exposure to EVD (e.g., medical staff) are overrepresented in the study sample.

The reported risk perceptions and attitudes are conditional for the situation in Germany as of November 2014 and assume no transmission of Ebola virus in Germany. In the case of real exposure, persons might not act as they predicted they would. We also cannot assess how much the responses are influenced by the current status and how persons would react when media attention is less. However, having access to the study population of the larger infectious diseases study will enable us to ask the same persons again several months later and to examine temporal changes of risk perceptions.

In conclusion, a substantial proportion of the study population demonstrated poor knowledge about the transmission modes of Ebola virus and about the actual risks in a European country during the 2014 EVD epidemic in West Africa. Increased media use was not associated with better knowledge, underscoring the need to improve quality of content reported by the media. Although inappropriate or unjustified attitudes in the current situation were not demonstrated by most participants, the treatment of flown-in EVD patients in a nearby hospital would trigger inappropriate behavioral changes.

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Acute Middle East Respiratory Syndrome Coronavirus Infection in Livestock Dromedaries, Dubai, 2014

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Camels carry Middle East respiratory syndrome coronavirus, but little is known about infection age or prevalence. We studied >800 dromedaries of all ages and 15 mother–calf pairs. This syndrome constitutes an acute, epidemic, and time-limited infection in camels <4 years of age, particularly calves. Delayed social separation of calves might reduce human infection risk.

Middle East respiratory syndrome coronavirus (MERS-CoV) causes outbreaks and isolated cases of severe respiratory disease in humans. The virus is transmissible from human to human, but the focus of infection has remained in countries on the Arabian Peninsula. Recent reports have shown that dromedaries (*Camelus dromedarius*) across the Arabian Peninsula and parts of eastern and northern Africa have MERS-CoV antibodies (*1–4*). Virus detection by reverse transcription PCR (RT-PCR) and sequencing has confirmed that these antibodies are likely to be caused by infection with the same virus strains that infect humans (*5*). In singular cases, strong evidence for virus transmission between camels and humans was found (*6,7*). Infection of dromedaries in the laboratory has confirmed susceptibility and efficient shedding (*8*). MERS-CoV antibodies were not found in other species of livestock and leisure animals, including cattle, goats, sheep, and horses (*9*).

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In the absence of a MERS-CoV vaccine, the prevention of human infections relies on knowledge of acute infection in camels. Available serologic studies indicate a high prevalence of MERS-CoV in adult camels, suggesting that MERS-CoV infection in camels may target young animals (*1–4*). However, only limited data on the age of animals at infection and the degree of age-specificity are available (*5*).

To best approximate the actual infectivity of virus in camels, testing should include RT-PCR and systematic virus isolation in cell culture (*10*). We recently analyzed a small group of camels in Saudi Arabia and found signs of recent acute MERS-CoV infection by demonstrating seroconversion, indicating a method for the serologic diagnosis of acute infection (*7*). To increase knowledge of acute MERS-CoV in dromedaries, we analyzed acute- and convalescent-phase MERS-CoV infections in similarly sized groups of camels of the same age in Dubai, United Arab Emirates.

The Study

We investigated dairy, racing, and breeding dromedaries from 3 flocks on farms 20–40 km apart. When possible, blood and nasal swab specimens were obtained from all camels in the flocks during March–June 2014. Samples were grouped according to the camels' ages rather than sampling site because livestock ages differed between sites. Serologic testing by ELISA yielded evidence of MERS-CoV antibodies in >96% of all dromedaries >2 years of age (Table 1). Seroprevalence among dromedaries <1 year of age (calves) was significantly lower but still exceeded 80%. Using cross-sectional testing, we could not discriminate between maternal and autonomous antibodies in calves. RT-PCR testing of nasal swab specimens showed a considerable prevalence of MERS-CoV RNA among all dromedaries <4 years of age but particularly in calves. Similarly, virus isolation conducted on all samples, including those RT-PCR–negative for MERS-CoV (*14*), was successful only for animals <4 years of age but particularly for calves. The prevalence of virus RNA and the rate of virus isolation were significantly higher in calves than subadults (2–4 years of age) (χ^2, $p<0.05$). The higher rate of virus isolation among calves suggests increased infectivity of calves.

To understand MERS-CoV infection in dromedary calves, we investigated 24 mother–calf pairs from the

Table 1. Results of cross-sectional study of MERS-CoV antibodies and RNA and MERS-CoV isolation in dromedary camels at 3 sampling sites, Dubai, March–June, 2014*

	Mean no. positive/no. tested (% positive)		
Age group, y	Serum antibody detection by ELISA†	RNA detection by RT-PCR‡	Virus isolation§
Adults, >4	298/310 (96.1)	0/250 (0)	0/12 (0)
Subadults, 2–4	328/340 (96.5)	10/344 (2.9) ¶	1/14 (7.1)
Calves, <1	92/108 (85.2)¶	24/68 (35.3)¶	6/44 (13.6) ¶
Unknown	68/85 (80)	11/209 (5.3)	1/12 (8.3)
Total	786/843 (93.2)	45/871 (5.1)	8/82 (9.6)

*MERS-CoV, Middle East respiratory syndrome coronavirus; RT-PCR, reverse transcription PCR.
†ELISA used a recombinant MERS-CoV globular spike (S1) domain as described by Drosten et al. (*11*), modified by the reagent manufacturer (EUROIMMUN, Lübeck, Germany) for application with camel serum as validated by Corman et al. (*12*) and Memish et al (*7*). The anti-human conjugate was replaced by an anti-camel conjugate. The test was not selective for IgG.
‡RT-PCR targeting regions upstream of the envelope gene, as described by Corman et al. (*13*).
§Method as described by Drosten et al. (*14*).
¶Significantly different from grand mean (shown under Total), $p<0.05$ (χ^2 test)

breeding flock. The investigations were all conducted in May 2014. At the time of sampling, mother camels were >12–15 years of age, and calves were 4–6 months of age. As shown in Table 2, all cows were MERS-CoV antibody positive and had no signs of active MERS-CoV infection by RT-PCR and virus isolation. Of the 15 calves studied, 4 showed evidence of ongoing seroconversion during sampling days 0 and 8; on day 8, all calves were seropositive by ELISA. On sampling day 0, virus was detected in 11/15 (73.3%) calves, and on sampling day 8, it was detected in 4/15 (26.7%) calves. This overall pattern was suggestive of a recent infection peak in the flock that was

Table 2. Results of testing for the presence of MERS-CoV and MERS-CoV antibody in 15 mother–calf pairs in a dromedary breeding flock, Dubai, May 2014*

	Antibody ELISA		Virus isolation		PCR, threshold cycle	
Camel	Day 0	Day 8	Day 0	Day 8	Day 0	Day 8
Mother						
M1	+	ND	–	ND	–	ND
M2	+	ND	–	ND	–	ND
M3	+	ND	–	ND	–	ND
M4	+	ND	–	ND	–	ND
M5	+	ND	–	ND	–	ND
M6	+	ND	–	ND	–	ND
M7	+	ND	–	ND	–	ND
M8	+	ND	–	ND	–	ND
M9	+	ND	–	ND	–	ND
M10	+	ND	–	ND	–	ND
M11	+	ND	–	ND	–	ND
M12	+	ND	–	ND	–	ND
M13	+	ND	–	ND	–	ND
M14	+	ND	–	ND	–	ND
M15	+	ND	–	ND	–	ND
Total	15	NA	0	NA	0	NA
Calf						
C1	±	+	+	–	19.5	–
C2	+	+	–	–	–	–
C3	+	+	–	–	24.3	–
C4	–	+	–	–	26.8	32.3
C5	±	+	–	–	30.4	–
C6	–	+	–	–	26.5	–
C7	+	+	–	–	–	–
C8	+	+	–	–	–	–
C9	+	+	+	–	23.8	–
C10	+	+	+	–	24.1	–
C11	+	+	+	–	22.3	34.2
C12	+	+	–	–	–	–
C13	+	+	–	–	22.8	34.2
C14	+	+	+	–	20.7	–
C15	+	+	–	–	32.4	35.3
Total	11 (+2)	15	5	0	11	4

*MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not applicable ND, not done; +, positive; –, negative; ±, weak positive (borderline optical density range as identified by the reagent manufacturer, EUROIMMUN, Lübeck, Germany).

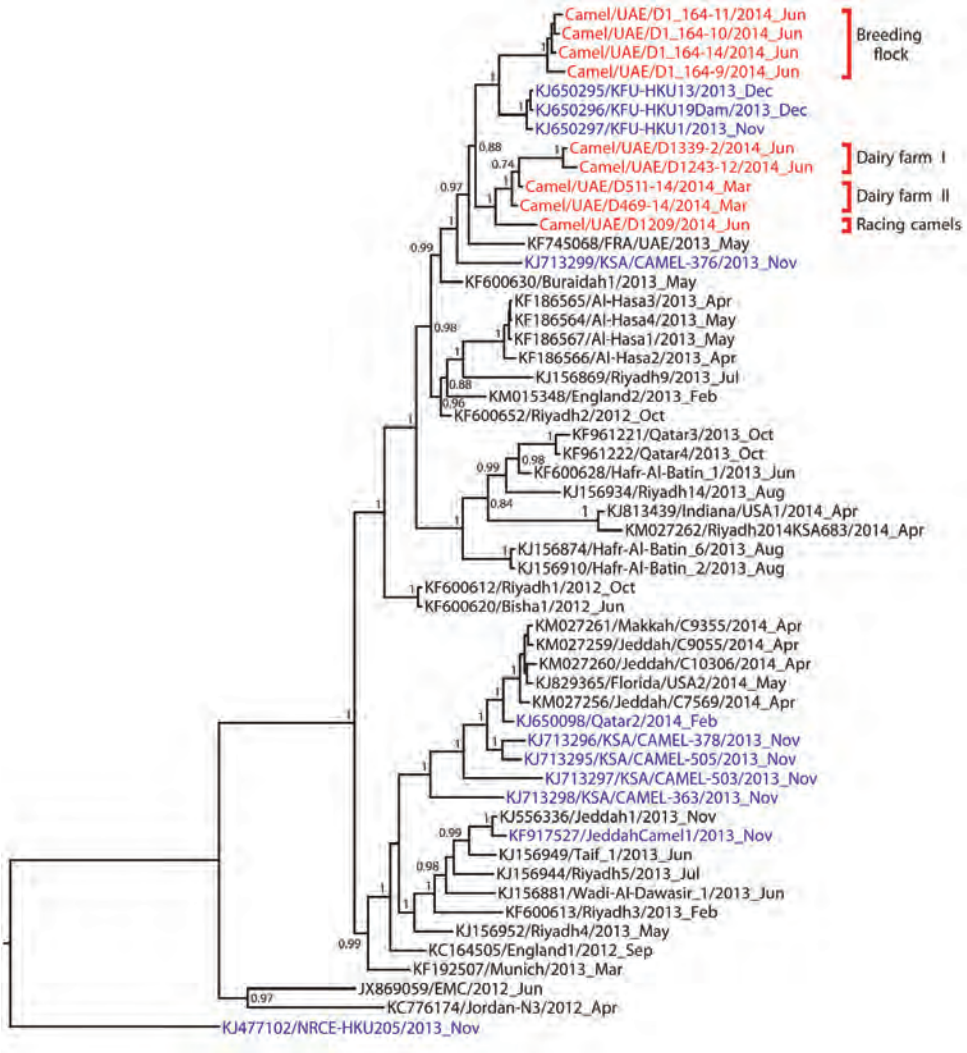

Figure. Phylogenetic analyses of the complete concatenated coding sequences of available Middle East respiratory syndrome coronavirus (MERS-CoV) genomes were done by using MrBayes v3.1 (http://mrbayes.sourceforge.net/) and a general time-reversible plus gamma distribution plus invariable site nucleotide substitution model with 2,000,000 generations sampled every 100 steps. Trees were annotated by using the last 75% of all generated trees in TreeAnnotator v.1.5 (http://beast.bio.ed.ac.uk/TreeAnnotator/) and visualized with FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/). Statistical support of grouping based on Bayesian posterior probabilities is shown at all nodes (95% highest posterior density; shown if value >0.7). Red indicates the 9 camel MERS-CoV strains characterized in this study; blue indicates MERS-CoV sequences obtained from other camels. EMC, Erasmus Medical Centre; FRA, France; HKU, Hong Kong University; KFU, King Faisal University; KSA, Kingdom of Saudi Arabia; UAE, United Arab Emirates; USA, United States of America.

already on the decline at the time of sampling. The ongoing infection in most calves suggests a general susceptibility to infection in 4- to 6-month-old dromedaries.

We sequenced genomes of 9 virus isolates, representing 3 different phylogenetic lineages, from dromedaries on the 3 farms. Phylogeny of full genomes showed that all viruses clustered according to their place of origin. The phylogenetic position of 1 of these clades suggested recent separation from viruses circulating in the eastern part of Saudi Arabia; some of the animals in the breeding flock from which the viruses were isolated had been moved temporarily to Saudi Arabia for grazing. The other clade separated from these viruses somewhat earlier, but it shared recent common ancestors with other viruses from the eastern part of the Arabian Peninsula. Samples collected in June from animals on the dairy farm yielded viruses from the same clade as that for viruses derived from different animals sampled on the same farm in March (dairy farm samples I and II) (Figure).

Conclusions

Our findings provide evidence of infection of camel flocks in Dubai with MERS-CoV of contiguous virus clade. Similar to findings from earlier studies, we found evidence of new introductions of virus in flocks, such as the flock that temporally grazed in Saudi Arabia and was infected with a virus strain typical for Saudi Arabia (*7*,*15*). Acute MERS-CoV infection, rather than the long-term presence of virus in the dromedaries, was supported by testing mother–calf pairs. Because cows were not acutely infected before their calves, perennial persistence of MERS-CoV in adult dromedaries is unlikely. Titration and longitudinal serologic studies might have shown increases antibody titers in adult dromedaries after calves were infected. However, such studies were not possible for technical and logistical reasons, which is a clear limitation of our study.

Although we did not designed our study to cover the duration of virus shedding in young dromedaries, our

results suggest excretion to be short lived in individual camels. Of the 11 virus-positive calves, 5 had high virus RNA concentrations in their first samples (cycle threshold values <25) but no RNA in samples tested 8 days later. An infection experiment in adult dromedaries showed shedding occurred for ≤35 days after virus inoculation (*8*), which seems longer than the length of virus shedding observed for young camels in our study. However, detection sensitivity in the defined conditions of a laboratory trial might have been higher than in our study. Both studies agreed in their finding of short-lived infectivity of excreted virus: in our study, we did not detect virus in any calf on day 8, and none were detected beyond day 7 in the study by Adney et al. (*8*). Nevertheless, virus can be maintained in flocks over several weeks or months, as exemplified by the detection of the same virus clade in March and June on 1 dairy farm.

The restricted and highly compartmentalized social structure of livestock camels would provide population niches in which viruses can differentiate in isolation after bottleneck-type transmission events. This situation holds promise for control of the spread of MERS-CoV through flock management practices, and it also suggests a rather simple way of avoiding camel-to-human transmission by avoiding camels <2 years of age. Camel calves are not easily accessible by humans and instinctually avoid humans. They are generally separated from their mothers after 12 months of age (i.e., at an age when they are still likely to be infected with MERS-CoV). Humans normally come into contact with calves only after the animals have been separated from their mothers. A change in this practice (i.e., postponing separation until the calves are older) might reduce the risk for camel-to-human MERS-CoV transmission.

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Invasion Dynamics of White-Nose Syndrome Fungus, Midwestern United States, 2012–2014

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White-nose syndrome has devastated bat populations in eastern North America. In Midwestern United States, prevalence increased quickly in the first year of invasion (2012–13) but with low population declines. In the second year (2013–14), environmental contamination led to earlier infection and high population declines. Interventions must be implemented before or soon after fungal invasion to prevent population collapse.

Invasion of novel wildlife diseases has caused widespread declines or species extinction among birds, amphibians, and mammals (*1*–*4*). White-nose syndrome (WNS), caused by the fungal pathogen *Pseudogymnoascus destructans*, is a recently emerged disease of hibernating bats (*5*) that has caused substantial declines in 6 species; bats of 2 species are predicted to become globally extinct (*3*). In little brown bats (*Myotis lucifugus*), tissue damage from fungal infection results in a cascade of physiologic disruptions resulting in death 70–100 days after infection (*6*).

Although the seasonal dynamics of *P. destructans* were recently characterized (*7*), the dynamics of *P. destructans* invasion of new sites has yet to be described. In the 2 years since the identification of *P. destructans*, the extent of the population decline differed each year and among species for unknown reasons (*3*). Furthermore, the role of *P. destructans* in the environment remains unclear (*8*) because no study has reported co-occurring patterns of *P. destructans* in bats and on substrates. We hypothesized that yearly differences in death rates result from changes in the timing of infection as *P. destructans* becomes established and that the environment serves as a source of infection for bats (bats that leave summer maternity sites are not infected; *7*).

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The Study

To test our hypothesis, we studied the invasion dynamics of the WNS fungus by sampling bats of 5 species at 2 hibernacula in central Illinois, USA. We collected samples twice each winter for 2 years (2012–13 and 2013–14). The hibernacula were moderately sized (5–10 hectares, 2–5 m high) abandoned limestone mines that bats use for fall mating and hibernation from September through April. During each visit, we counted all visible bats at each site, which produced complete census data for 4 of the 5 species. Accurately collecting census data for bats of the remaining species (*Eptesicus fuscus*) was difficult because these bats, unlike those of other species, roosted primarily behind crumbling slabs of rock around mine entrances, which were dangerous and difficult to survey.

During each site visit we sampled 15–20 bats of each species by epidermal swabbing (*7*). We also sampled the wall or ceiling of hibernacula under, near (10–20 cm), and far from (>2 m) roosting bats by using the same swabbing technique. Samples were tested for *P. destructans* by using real-time PCR (*9*); according to a serial dilution experiment, the limit of detection was ≈50 conidia.

We obtained 611 samples from bats and 444 from substrate. In early winter of 2012–13, only 1 individual (*Myotis septentrionalis*) of 129 bats of 5 species sampled was positive for *P. destructans*, and none of the 46 substrate samples were positive (Figure 1, panels A, C, E). Just 4 months later, in March 2013, prevalence was >85% for bats of 2 species (*M. septentrionalis*, *M. lucifugus*), 40%–75% for 2 species (*E. fuscus*, *Myotis sodalis*), and 15%–60% for 1 species (*Perimyotis subflavus*) at the 2 sites (Figure 1, panel A). The prevalence of *P. destructans* on the substrate under these bats varied from 0% to 67%, and substrate prevalence paralleled fungal prevalence for bats of each species (Figure 1, panel C). Despite widespread apparent infection of bats at this time, none of the 36 substrate samples taken just 10–20 cm from bats were positive for *P. destructans* (Figure 1, panel E).

In early winter of the next year (late November 2013), patterns differed markedly from those of the previous early winter. *P. destructans* was already widespread in the environment, found in 70% of samples from under bats, 22% of samples 10–20 cm from bats, and 14% of samples >2 m from bats (Figure 1, panels D, F). Prevalence among bats of 4 species was already ≥70%, and prevalence among bats

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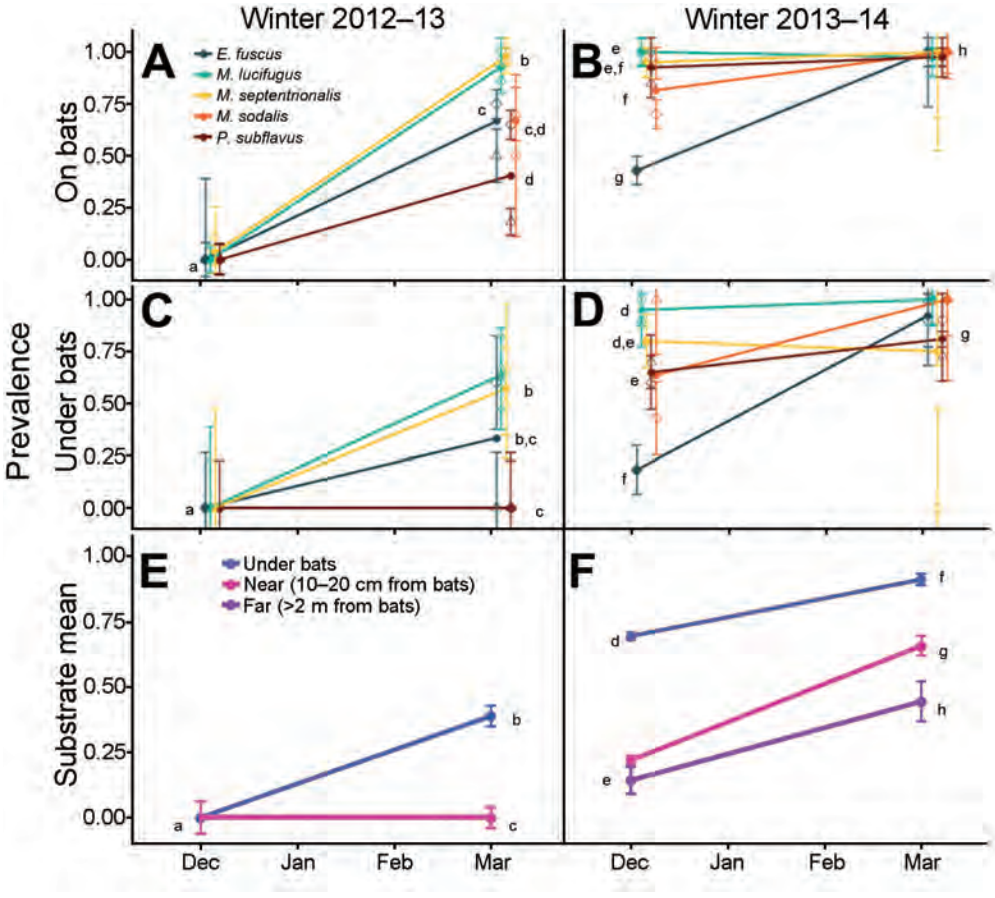

Figure 1. *Pseudogymnoascus destructans* prevalence (±1 SE, calculated from the variance of a binomial distribution sample) over 2 winters, 2012–13 and 2013–14, at 2 sites (diamonds and triangles) in Illinois, USA, on bats of 5 species (A, B); prevalence of *P. destructans* on substrate under bats of each species (C, D), and prevalence of *P. destructans* under, near (10–20 cm), and far from (>2 m) bats (E, F). No substrate samples far from bats were taken in the first winter. Lines join observed mean prevalence for each species (solid circles) to facilitate presentation but do not indicate trajectories between time points. Prevalence of species or substrate means indicated by the same letter did not differ significantly ($p>0.05$) in a logistic regression analysis with either species and site as fixed effects at each sampling point (A, B) or substrate sample type at each sampling point (C–F); effect of site was not significant in any of these comparisons. *E.*, *Eptesicus*; *M.*, *myotis*; *P.*, *perimyotis*.

of 1 of these species (*P. subflavus*), for which prevalence at the end of the previous winter had been lowest, was already 85%–100% (Figure 1, panel B). By the end of the second winter, 109 (98%) of 111 bats were positive for *P. destructans*, and *P. destructans* was present throughout the hibernacula (in 91% of samples from under bats, 66% of samples near bats, and 44% of samples far from bats) (Figure 1, panels B, D, F).

Over these 2 years, the effect of WNS on bat populations mirrored the patterns of *P. destructans* prevalence. During the first winter, declines were limited at the larger site and moderate (50%–75%) at the smaller site (Figure 2). In contrast, over the second winter, counts of *M. septentrionalis* bats declined by 95%–99% and *M. lucifugus* bats by 81%–88% (20,000 bats of this species disappeared) (Figure 2, panel A). Populations of bats of the 2 other species also experienced moderate to severe declines in the second year (*M. sodalis*, 16%–96%; *P. subflavus*, 47%–73%) (Figure 2, panel B). Declines probably resulted from disease-related deaths because high hibernacula site fidelity makes emigration unlikely (*10*) and substantial numbers of dead bats were observed at both sites.

Conclusions

Early in the first winter studied, prevalence of *P. destructans* was very low, and although transmission resulted in most bats harboring *P. destructans* by winters' end, declines in bat populations were limited. In contrast, early in the second winter, fungal prevalence among bats was already high and severe communitywide declines occurred over the next 4 months. The earlier timing of exposure in the second year would be expected to increase the effects of WNS because by winter's end most bats would have been infected and in hibernation for at least 70–100 days (the approximate time between infection and death; *5*). Few would be able to survive until spring, when bats cease hibernating and clear the fungus (*7*).

Patterns of *P. destructans* distribution in the environment mirrored prevalence among bats and population declines. Early in the first year, when *P. destructans* was rare on hibernacula substrates, most bats were not infected in

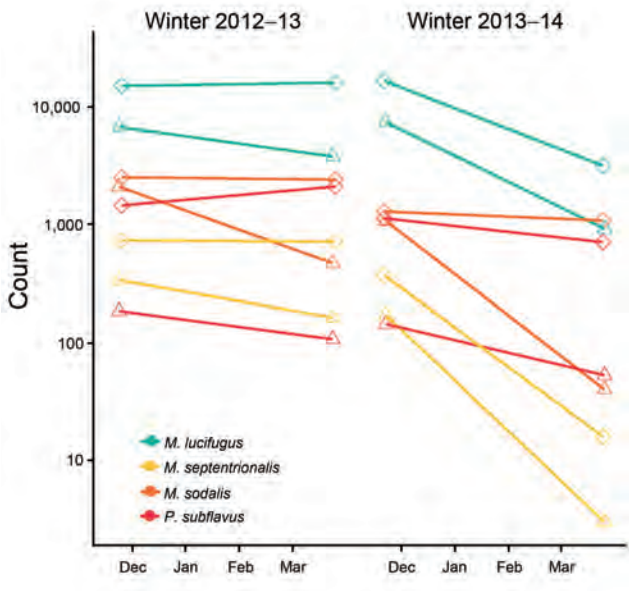

Figure 2. Complete population counts on a log scale of 4 species of bats at 2 sites in Illinois, USA, over 2 winters, 2012–13 and 2013–14. Diamonds and triangles indicate sites.

early winter, and 4 months later, *P. destructans* was not detectable in one third of bats of 3 species. However, by the end of the first winter, *P. destructans* was present on hibernacula substrate under bats, probably resulting from bats shedding *P. destructans* into the environment. At the beginning of the following winter, *P. destructans* was widespread in the environment, and nearly all bats had fungus on them. The widespread occurrence of *P. destructans* in the environment at this time may have contributed to higher prevalence among bats because most bats clear infections during the summer, when their body temperature is too high for *P. destructans* growth (*7*,*11*). Long-term persistence of *P. destructans* in the absence of bats (*8*,*12*) suggests that an environmental reservoir of *P. destructans* may contribute to WNS persistence, as occurs for other diseases, such as cholera (*13*).

WNS continues to spread south, west, and north from New York, where it was first detected in 2006, and continues to cause widespread bat population declines. Potential control strategies include development of probiotic treatments (*14*) and alteration of hibernacula microclimates to make them cooler and drier (*3*,15). Our results suggest that if *P. destructans* invasion in other sites is similar to what we documented in Illinois, interventions must be implemented proactively, or quickly after *P. destructans* invasion, to prevent collapse of bat communities. Reduced bat populations will probably have a negative effect on humans because bats play a useful role in ecosystems by consuming disease vectors and many forest and agricultural insect pests.

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Extensively Drug-Resistant New Delhi Metallo-β-Lactamase–Encoding Bacteria in the Environment, Dhaka, Bangladesh, 2012

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Carriage of the New Delhi metallo-β-lactamase variant 1 (NDM-1) enables drug resistance to move between communities and hospitals. In Bangladesh, we found the *bla*$_{NDM-1}$ gene in 62% of environmental waters and in fermentative and nonfermentative gram-negative bacteria. *Escherichia coli* sequence type (ST) 101 was most commonly found, reflecting a common global relationship between ST101 and NDM-1.

Carbapenemases, bacterial enzymes that typically inactivate most of the β-lactam class of antimicrobial drugs, have emerged rapidly over the past decade (*1*). These resistance mechanisms are often accompanied by other resistance alleles, and together they can confer extensive drug resistance, leaving minimal treatment options (*2*). The New Delhi metallo-β-lactamase variant 1 (NDM-1), a chimera formed by the fusion of 2 resistance genes, is unique among the carbapenemases (*3*). Since its description in 2009, NDM-1 has spread rapidly to many countries worldwide and appears to be endemic in South Asia (*1*,*4*,*5*). A study of the environment in New Delhi, India, showed that ≈30% of surface waters and sewage was contaminated with NDM-1; the enzyme was also detected in drinking water (*6*). In addition, high rates of NDM-1 gut carriage have been found in the community and in hospitals in Pakistan (*7*). High rates of gut carriage can lead to contamination of drinking water and food through inadequate sewage treatment. Furthermore, gut carriage of NDM-1–encoding *Escherichia coli* can lead to common community-acquired infections (e.g., urinary tract infections), which often require hospitalization (*8*) and enable resistance mechanisms to move between community and hospital sectors. Indirect studies in 2009 and 2010 showed that NDM-1 was not present in the Bangladesh environment (*9*,*10*). To determine whether NDM-1 is now present in Bangladesh, we surveyed the environmental waters of Dhaka.

The Study

During October 19–27, 2012, we collected environmental water/sewage samples from 7 regions (58 sites) in Dhaka, Bangladesh (Figure 1). Control samples were from the United Kingdom. Each sample was investigated for bacterial growth on UTI brilliance agar plates (Thermo Fischer Scientific, Basingstoke, UK) containing vancomycin (30 mg/L) plus meropenem (0.5 mg/L). The species of individual colonies of different colors and morphologies were determined by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Bacteria were genetically characterized by *bla*$_{NDM-}$specific PCR. Genetic location of the *bla*$_{NDM-1}$ gene was determined by probing S1 nuclease pulsed-field gels. A subset of isolates of each species was further investigated for MICs of relevant antimicrobial drugs. All *E. coli* isolates were genotyped to determine multilocus sequence typing group; examples of each group were characterized for additional relevant resistance mechanisms. Details are provided in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/21/6/14-1578-Techapp1.pdf).

The carbapenemase and extended-spectrum β-lactamase genes *bla*$_{NDM-1}$ and *bla*$_{CTX-M-15}$ were detected by PCR in 36 (62%) and 41 (71%), respectively, of the 58 water samples. Both genes were found at all 7 sample region sites in Dhaka. Gene *bla*$_{CTX-M-15}$, but not *bla*$_{NDM-1}$, was detected in sewage samples from the United Kingdom; neither was detected in UK water samples from the River Thames.

We identified 226 gram-negative NDM-1–producing isolates to the species level (Figure 1; online Technical Appendix Table 1); 15 isolates harboring *bla*$_{NDM-1}$ could not be identified and were not investigated further. The most widely disseminated bacteria in samples from Dhaka were pseudomonads (6/7 regions) and *Klebsiella pneumoniae* (4/7 regions). Nine different species of *Pseudomonas* spp. and 5 *Acinetobacter* spp., mostly belonging to nonpathogenic strains, were among the nonfermentative bacteria (online Technical Appendix Table 1). Carbapenem resistance in the *Pseudomonas* spp. isolates was unstable; all strains lost the *bla*$_{NDM-1}$ gene after 2 days' growth or when frozen for storage.

With the exception of 4 isolates, all bacterial isolates contained the original *bla*$_{NDM-1}$ allele; 3 *E. coli* sequence type (ST) 101 isolates carried the *bla*$_{NDM-3}$ variant, and 1 ST648 isolate carried the *bla*$_{NDM-4}$ variant (online Technical Appendix Table 2). S1 nuclease pulsed-field gel electrophoresis combined with *bla*$_{NDM-1}$ probes detected *bla*$_{NDM-1}$ on plasmids of limited size diversity in *E. coli* (ST101, 160 kb; ST405, 100 kb; ST648, 150 kb); however, other species included *bla*$_{NDM-1}$–positive plasmids in

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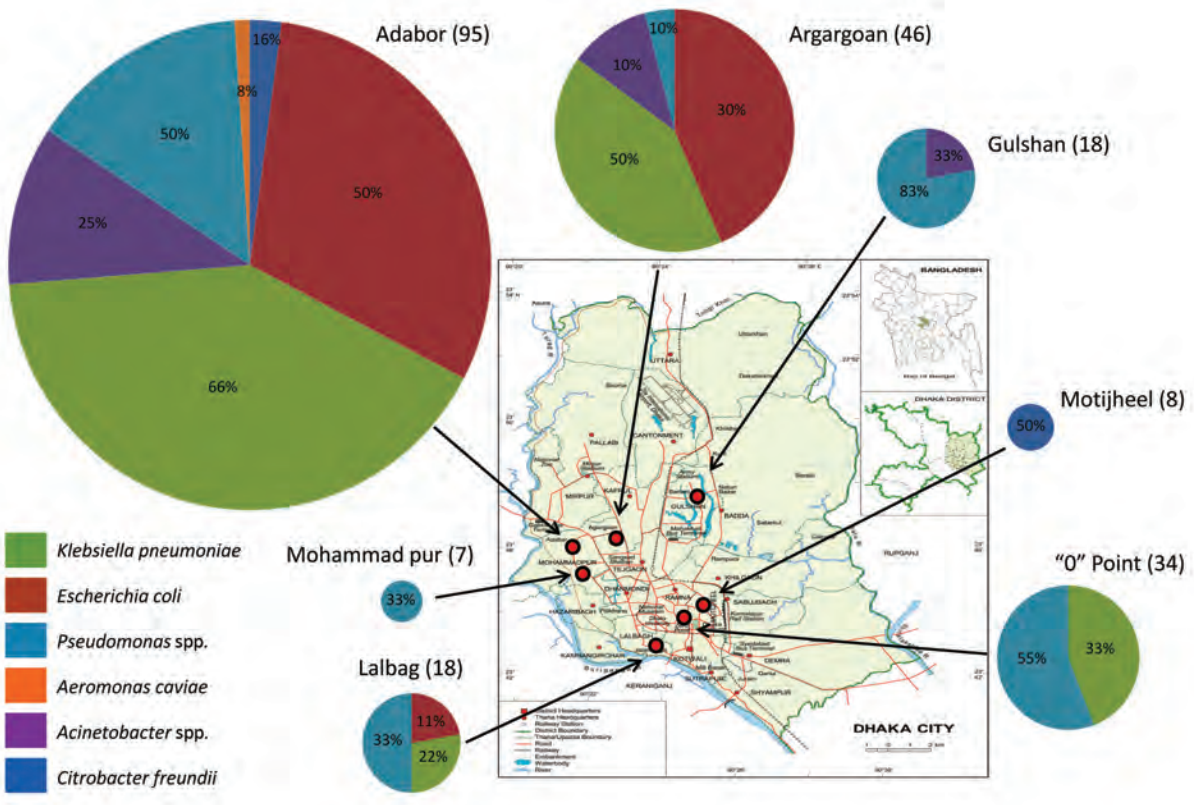

Figure 1. Diversity of New-Delhi metallo-β-lactamase variant 1–encoding species and the number found in 58 locations in 7 regions (red circles on map) of Dhaka, Bangladesh, October 2012. Individual sampling sites were within 2 km of each sampling region, and the number of sites varied from 6 to 12 per region. Pie charts indicate the proportions of different bla_{NDM-1}–positive bacteria isolated in each region; colors indicate specific species. The diameter of each pie chart is directly proportional to the number of bla_{NDM-1}–positive isolates collected in each region; actual numbers are shown in parentheses after the region name. Numbers within pie charts indicate the percentage of sites in each region in which the individual positive bla_{NDM-1}–positive species were found. bla_{NDM-1} was detected in samples from all 7 regions and from 36 (62%) of the 58 sampling sites.

a wide diversity of sizes (30 kb–450 kb); some of these species had multiple positive plasmids, and bla_{NDM-1} was also found on the chromosome (online Technical Appendix Table 1 and Figure 1).

The *E. coli* isolates were further analyzed by PCR to identify additional resistance mechanisms often associated with bla_{NDM-1} (Table). $bla_{CTX-M-15}$ and 16s ribosomal methylase genes (*armA* or *rmtB*) were associated with most *E. coli* strains, which explains the extensively drug-resistant phenotype of the *E. coli* isolates (online Technical Appendix Table 3). Plasmids of plasmid incompatibility groups *inc*FII (ST101, ST405, ST648) and *inc*X (ST405, ST648) were also closely associated with *E. coli* strains (Table). *E. coli* harboring bla_{NDM-1} were isolated from 10 sampling sites (Figure 1; online Technical Appendix Table 1). *The E. coli* isolates belonged to 3 different multilocus sequence typing groups: ST101 (phylogroup B1, 20/53 samples); ST405 (phylogroup D, 5/53 samples); and ST648 (phylogroup D, 28/53 samples) (online Technical Appendix Table 2). ST101, which was found in samples from 6 (10.3%) of the 58 sites, was the most prevalent NDM-1–encoding *E. coli* genotype. ST648 represented an intermediate prevalence (5/58 [8.6%] sites), and ST405 was the least prevalent (1/58 [1.7%] sites) (online Technical Appendix Tables 1, 2).

Conclusions

Our findings indicate that NDM-1 is widespread in the Dhaka environment. We detected 241 NDM-1–encoding bacterial isolates; they were found in all 7 sampled regions and at 36 (62%) of the 58 sampling sites. This high level of environmental bla_{NDM-1} contamination is of concern, especially because drinking water in Bangladesh usually carries high levels of sewage-derived bacteria (*11*). It is therefore likely that bla_{NDM-1} carriage rates will rise rapidly. Future environmental studies could provide

Table. Resistance genes and plasmid profiles for a subset of *Escherichia coli* strains in a study of extensively drug-resistant New Delhi metallo-β-lactamase–encoding bacteria in the environment, Dhaka, Bangladesh, October 2012*

	Resistance genes								
E. coli strain, ST	$bla_{CTX-M-15}$	bla_{NDM}	16S methylase	bla_{ampC}	*inc*X	*inc*FII	*inc*L/M	*inc*A/C	*inc*N2
18, ST101	+	NDM-3	*rmtB*	–	–	+	–	–	–
24, ST101	+	NDM-3	*rmtB*	–	–	+	–	–	–
25, ST101	+	+	*rmtB*	–	–	+	–	–	–
28, ST101	+	NDM-3	*rmtB*	–	–	+	–	–	–
221, ST101	+	NDM-1	*rmtB*	–	–	+	–	–	–
34, ST648	+	+	*armA*	*cmy*, *dha*	+	+	–	–	–
192, ST648	+	NDM-4	*armA*	*cmy*, *dha*	+	+	–	–	–
346, ST648	+	+	*armA*	*cmy*, *dha*	+	+	–	–	–
43, ST405	+	NDM-1	*armA*	*cmy*, *dha*	+	+	–	–	–
54, ST405	+	NDM-1	*armA*	*cmy*, *dha*	+	+	–	–	–

**armA* and *rmtB*, aminoglycoside methylase genes; CTX-M-15, *cmy*, and *dha,* β-lactamases; *inc*, plasmid incompatibility group; NDM, New-Delhi metallo-β-lactamase; ST, sequence type; –, negative; +, positive.

indicators of epidemics of emerging resistant bacteria before they are realized in hospitals.

Despite the widespread presence of NDM-1 in Dhaka, it appears that this carbapenemase has recently emerged in the Bangladesh environment. Studies in northern Bangladesh did not find NDM-1 in wild ducks and poultry in 2009 (*9*) or in crow and gull feces in 2010 (*10*). Similarly, NDM-1 was not detected in drinking water in Dhaka during 2008–2009 (*11*) even though all samples had high levels of fecal and $bla_{CTX-M-15}$ contamination. Furthermore, a study of 1,879 clinical *E. coli* and *Shigella* spp. isolates collected during 2009–2010 in Bangladesh did not detect bla_{NDM-1} (*12*). The first known clinical isolates date from 2008 (*12*), and the first evidence of human gut carriage of bla_{NDM-1} was found in samples collected in Dhaka (*13*) a month before our study.

Because *E. coli* is the leading cause of human urinary tract infections, bloodstream infections, and neonatal meningitis, the ability of NDM-1 to give this bacterium clinical resistance to carbapenems is of concern (*14*). *E. coli* is also universally carried in the human gut. Therefore, we focused on this species because it is likely to be the greatest threat to human health. *E. coli* encoding NDM-1 were found in 3 of the 7 sampled regions, and genotyping showed they belonged to only 3 STs: ST648, ST101, and ST405. These same 3 *E. coli* genotypes are

Figure 2. Sites where New-Delhi metallo-β-lactamase variant 1 (NDM-1)–encoding *Escherichia coli* sequence type (ST) 101 isolates have been detected worldwide. Stars indicate countries where NDM-encoding *E. coli* ST101 has been detected: Australia, Bangladesh, Belgium, Bulgaria, China, Canada, Denmark, France, Germany, India, Korea, New Zealand, Pakistan, the United Kingdom, and the United States.

responsible for 80% of clinical NDM-1–encoding *E. coli* isolates in the United Kingdom (*15*). Furthermore, ST101 is the most common *E. coli* genotype in the Bangladesh environment (10.3% prevalence) and in clinical isolates from the United Kingdom (50%). Results of a literature search for NDM-1–encoding *E. coli* belonging to ST101 showed that this genotype has been detected in 15 nations (Figure 2). Thus, *E. coli* ST101 appears to be a successful global genotype that is often associated with NDM-1. This association with a single global genotype is analogous to the association between *E. coli* ST131 and the cephalosporinase CTX-M-15. Because of the critical nature of extensively drug-resistant bacteria, we are investigating the underlying factors responsible for the success of these particular antimicrobial drug–resistant strains.

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Dr. Toleman is a senior lecturer at Cardiff University. His recent work includes the discovery of the *ISCR* (insertion sequence common region) elements, NDM-1, and the formation of NDM-1 by an unusual genetic fusion event.

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Coccidioides Exposure and Coccidioidomycosis among Prison Employees, California, United States

Marie A. de Perio, R. Todd Niemeier, Gregory A. Burr

Responding to a request by corrections agency management, we investigated coccidioidomycosis in prison employees in central California, a coccidioidomycosis-endemic area. We identified 103 cases of coccidioidomycosis that occurred over 4.5 years. As a result, we recommended training and other steps to reduce dust exposure among employees and thus potential exposure to *Coccidioides*.

Coccidioidomycosis, also known as Valley fever, is caused by inhalation of spores of the fungus *Coccidioides*, which grows in soil in semiarid areas. Coccidioidomycosis is endemic to the southwestern United States, the Central Valley of California, Mexico, and parts of Central and South America (*1*). An estimated 150,000 new infections occur annually in the United States (*2*). In disease-endemic areas, workers involved in soil disturbance, including agricultural, construction, and archeological workers, are at high risk for coccidioidomycosis (*1*).

As part of a health hazard evaluation requested by corrections agency management (*3*), we investigated the incidence of coccidioidomycosis among employees at 2 prisons in California's Central Valley. To reduce exposure to *Coccidioides*, we recommended ways to improve coccidioidomycosis-related occupational health practices at the prisons.

The Study

Prison A, a minimum–maximum security facility, and prison B, a minimum–medium security facility, employed ≈1,300 and 1,500 custody and support staff, respectively. The prisons are located on 640 acres in 2 (1 each) of 6 coccidioidomycosis-hyperendemic California counties. In those counties, annual case rates for coccidioidomycosis are consistently higher than rates for the state (*4*).

We identified confirmed cases of coccidioidomycosis among prison staff by using agency employee rosters and the Confidential Morbidity Reports from the California Department of Public Health for 2009–2013. We used the coccidioidomycosis case definition of the Council of State and Territorial Epidemiologists and defined a case as a clinically compatible illness that was laboratory confirmed (*5*) in a person employed at either prison at symptom onset. We calculated the crude (unadjusted) average annual incidence of coccidioidomycosis for prison A and B employees. Using information from the California Department of Public Health and from the corrections agency, we described personal and work characteristics of the employee case-patients.

During visits to the prisons in June 2013, we interviewed a convenience sample of 172 employees across all job categories about their work practices and exposures and met with staff to learn about dust mitigation efforts. As a public health response, according to Title 45 Code of Federal Regulations Part 46, this evaluation did not require review by an institutional review board.

We identified 65 confirmed cases of coccidioidomycosis among prison A employees and 38 confirmed cases of among prison B employees from 2009 to mid-2013. These cases were reported by 9 counties where the employees resided. All but 3 employees resided in 1 of the 6 coccidioidomycosis-hyperendemic counties. For 2009–2012, the crude average annual incidence for prison A employees was 1,039 cases/100,000 employees, and for prison B employees, 511 cases/100,000 employees. The 12-month combined incidence for 2009–2012 showed no clear seasonal patterns (Figure 1).

Of 103 employees with confirmed illness, 84 (82%) were male; median age was 43 (range 24–67) years. The median time worked at the prison before symptom onset was 5 years (range 2 months–23 years). The most common job categories were custody (72%), health care (11%), administration (10%), and plant operations (2%).

We interviewed 172 (99%) of 173 invited employees at both prisons. Median age was 47 (range 25–74) years; 67% were male. Most (59%) reported their race as white, 5% as Filipino, and 3% as black or African American; 42% reported their ethnicity as Hispanic or Latino. The median number of years living in any coccidioidomycosis-hyperendemic California county was 35 (range 0.5–67) years. Thirty-five (20%) interviewed employees reported having ≥1 underlying medical condition that placed them at high risk for severe or disseminated disease, including asthma, emphysema, diabetes mellitus, immunosuppression resulting from medication, heart disease, kidney disease, and cancer requiring chemotherapy or radiation therapy.

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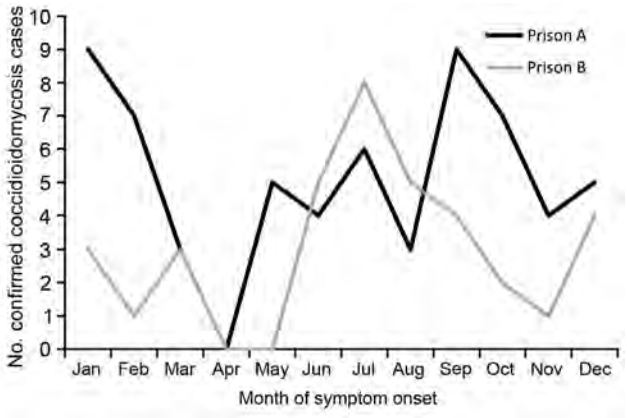

Figure 1. Number of coccidioidomycosis cases among prison A and B employees by month of symptom onset, California, USA, 2009–2012.

Job categories of interviewed employees were custody (45%), administration (17%), plant operations (13%), health care (13%), education or vocational training (7%), and other (5%). Of interviewed employees, 171 (99%) reported outdoor work activities, including walking around prison grounds, performing security checks, patrolling, performing maintenance, and landscaping. The median time spent outdoors during the work day was 1.5 hours (range 10 minutes–9 hours). Forty-two (24%) employees reported conducting soil disruption activities during their job, including grid searches, digging for contraband, and drilling or digging for maintenance and repairs. The median time spent performing outside work was 2 hours per day (range 0–9 hours).

During meetings with facilities and engineering staff at both prisons, we learned that efforts to reduce potential dust exposures on prison grounds already included wetting soil before soil disruption, reducing soil disking (shallow plowing), applying a soil stabilizer, and planting grass and other vegetation. Prison B's grounds and surrounding areas have little natural vegetation (Figure 2).

Conclusions

Our investigation revealed 103 cases of confirmed coccidioidomycosis in prison A and B employees from 2009 to mid-2013. The crude average annual incidence among prison employees seems to be higher than that reported among the general noninmate adult population in the surrounding counties (county A, 40 cases/100,000 persons; county B, 110 cases/100,000 persons) (J. Mohle-Boetani, pers. comm.; F. Tabnak, pers. comm.). However, comparisons of crude incidence rates between the prison employee and noninmate populations should be interpreted cautiously because of unmeasured confounding factors. We were unable to determine age-, sex-, and race-adjusted incidence rates for prison employees because these data were unavailable for this population; therefore, we could not make statistical comparisons. Also, reporting or testing bias is possible because of heightened awareness among employees and could have contributed to the difference in crude incidence rates.

Most employees with confirmed coccidioidomycosis were custody workers. However, because these workers represent most of the employee population, cases for this group did not seem to be disproportionate. We also could not determine if each confirmed coccidioidomycosis case in an employee was because of exposure at work or outside of work. Most prison employees with confirmed coccidioidomycosis resided in a coccidioidomycosis-hyperendemic area, and our interviews revealed that employees are probably exposed to *Coccidioides* at work and outside of work. Almost all interviewed employees at each prison reported spending time outdoors at work, and almost one third, specifically custody and plant operations employees, reported work involving soil disruption.

The prisons face ongoing challenges of maintaining or restoring vegetation and grass on the grounds because of water restrictions. Environmental mitigation efforts, such as reducing soil disking, paving roads, and wetting soil before disturbing it, can reduce dust levels and therefore may lower the risk for localized airborne dispersion of *Coccidioides* spores. However, little data exist to demonstrate the effectiveness of these measures in reducing airborne dispersal and occupational coccidioidomycosis (*6*). Airborne spores can travel for miles, and focal *Coccidioides* sites may be small and unevenly distributed within coccidioidomycosis-endemic areas (*6*–*8*). Environmental mitigation measures neither eradicate the organism from soil nor prevent exposure to dust from areas outside the prison grounds.

Figure 2. Prison B, located in an arid, coccidioidomycosis-hyperendemic area of the Central Valley of California, USA. Little natural vegetation grows on the grounds and in surrounding areas. Photograph courtesy of National Institute for Occupational Safety and Health.

Nevertheless, reducing dust is a reasonable risk-reduction strategy for addressing occupational coccidioidomycosis.

On the basis of our findings, we recommended that prison management weigh the advantages and disadvantages of various environmental mitigation efforts to reduce dust exposures. Our recommendations included providing employees with education and training about coccidioidomycosis symptoms and transmission, risk factors for disseminated disease, and ways to minimize exposures; closing the prison yards during dust storms; and using respirators approved by the National Institute for Occupational Safety and Health as part of a respiratory protection program for employees who must work outside during unusually dusty days or who may disturb soil. The recently available coccidioidal spherulin skin test may also be a potentially useful tool for identifying at-risk employees.

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Dr. de Perio is a medical officer and infectious diseases physician at the National Institute for Occupational Safety and Health at the Centers for Disease Control and Prevention in Cincinnati, Ohio, USA. Her primary research interest is infectious diseases in the workplace.

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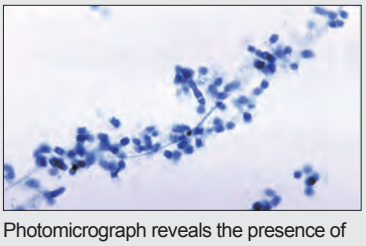
etymologia

Coccidioides [kok-sid"e-oi'dēs]

A soil fungus found in the western United States and parts of Mexico and Central and South America, *Coccidioides* was discovered in 1892 by Alejandro Posadas, a medical student, in an Argentinian soldier with widespread disease. Biopsy specimens revealed organisms that resembled the protozoan *Coccidia* (from the Greek *kokkis*, "little berry"). In 1896, Gilchrist and Rixford named the organism *Coccidioides* ("resembling *Coccidia*") *immitis* (Latin for "harsh," describing the clinical course). Ophüls and Moffitt proved that *C. immitis* was a fungus rather than a protozoan in 1900. In 2002, *C. immitis* was divided into a second species, *C. posadasii*, after Alejandro Posadas.



Photomicrograph reveals the presence of thick-walled arthroconidia and arthrospores of *Coccidioides immitis* fungal organisms.

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KPC and NDM-1 Genes in Related *Enterobacteriaceae* Strains and Plasmids from Pakistan and the United States

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To characterize the genomic context of New Delhi metallo-b-lactamase-1 (NDM-1) and *Klebsiella pneumoniae* carbapenemase (KPC), we sequenced 78 *Enterobacteriaceae* isolates from Pakistan and the United States encoding KPC, NDM-1, or no carbapenemase. High similarities of the results indicate rapid spread of carbapenem resistance between strains, including globally disseminated pathogens.

Pathogenic *Enterobacteriaceae*, including *Escherichia coli* and *Klebsiella pneumoniae*, are major causes of multidrug-resistant (MDR) infections in hospitals worldwide. These pathogens have recently been shown to have acquired resistance to carbapenems, and the US Centers for Disease Control and Prevention identified carbapenem-resistant *Enterobacteriaceae* as 1 of the 3 most urgent MDR threats (*1*). Among the *Enterobacteriaceae*, β-lactam resistance, including carbapenem resistance, is primarily caused by enzymatic degradation by β-lactamases. Two carbapenemase subclasses are especially problematic: *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase-1 (NDM-1). KPC, identified in 2001 (*2*), has become endemic to several noncontiguous areas of the world, including the United States, Israel, Greece, South America, and China (*3*). NDM-1 was first described in 2008, although retrospective studies identified NDM-1 from 2006 (*4*) and is abundant in New Delhi water samples (*5*). Most patients from whom NDM-1 is isolated have an epidemiologic link to the Indian subcontinent, but NDM-1 has also recently become endemic to the Balkans and Middle East (*6*).

The spread of antibiotic resistance genes such as NDM-1 and KPC is facilitated by horizontal gene transfer (HGT) between bacteria (*7*). Among globally disseminated pathogens, HGT facilitates combination of the most effective antibiotic resistance genes from diverse geographies into multidrug resistance plasmids that spread between strains. Recombination and transposition have created populations of these plasmids that have related architectures but vary in their composition of antibiotic drug resistance cassettes (*8*). This effect has enabled both KPC and NDM-1 to rapidly expand within the *Enterobacteriaceae* and other proteobacterial pathogens, such as *Acinetobacter baumanii* (*9,10*). Antibiotic resistance genes can also spread through clonal expansion in successful pathogenic strains, for example, KPC in *K. pneumoniae* sequence type (ST) 258 (*11*), and the extended-spectrum β-lactamase CTX-M-15 in E. coli ST131 (*12*). Both HGT and clonal expansion have enabled KPC and NDM-1 to rapidly spread to distant locations after their emergence (*6,8*).

The similarities in the spread and resistance spectra of KPC and NDM-1 (both provide resistance to nearly all β-lactam antimicrobial drugs) leads to the hypothesis that similar mobile elements will make both genes available to similar pathogen populations. We tested this hypothesis by examining clinical *Enterobacteriaceae* isolates from Pakistan and the United States encoding NDM-1, KPC, or no carbapenemase.

The Study

We collected 450 bacterial isolates (including 195 Enterobacteriaceae) in Pakistan during February 2012–March 2013 from Pakistan Railway General Hospital in Rawalpindi and the Pakistan Institute of Medical Sciences in Islamabad. From this collection, we randomly selected 55 *Enterobacteriaceae* isolates for whole-genome sequencing. We then selected 23 isolates from samples collected in the United States during January 2010–June 2013 from patients in Barnes Jewish Hospital in St. Louis, Missouri, that had similar proportions of β-lactam susceptibility and resistance to the isolates collected in Pakistan for sequencing. All isolates were de-identified and retrieved from existing strain banks. The combined set included 33 *E. coli*, 30 *K. pneumoniae*, 9 *Enterobacter cloacae*, and 6 *Enterobacter aerogenes* (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/6/14-1504-Techapp1.pdf). We extracted plasmid DNA from 9 isolates encoding NDM-1, 11 isolates encoding KPC, and 3 isolates encoding CTX-M-15 and performed shotgun sequencing on those plasmid preparations. Detailed methods are described in the online Technical Appendix.

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Using antibiotic resistance gene predictions from the Resfams database (*13*) and core genome alignment, we constructed a phylogenetic tree for each species in our set, overlaid by the β-lactamases encoded by each isolate (Figure 1). Isolates from both locations were found to be members of the same subspecies clades (online Technical Appendix Figure 1) and to contain similar repertoires of β-lactamases (Figure 1), indicating that geography is not a discriminating variable for these isolates. Many of these isolates were also MDR: resistance to ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, doxycycline, and chloramphenicol occurred in 63%, 65%, 45%, 54%, and 56% of isolates, respectively. As expected from results of previous work (*8*), *E. coli* ST131 isolates had high rates of CTX-M carriage (82%; Figure 1, panel A) and ciprofloxacin resistance (100%).

The variety of strains that we discovered encoding KPC and NDM-1 is consistent with existing evidence that HGT is a major factor in their spread. All KPC genes were proximal to Tn4401 and all NDM-1 genes were carried on ISAba125, mobile elements with which each gene has respectively been previously associated (*14*). We observed multiple examples of NDM-1 within the *K. pneumoniae* ST11 clade (*15*) (Figure 1, panel B; online Technical Appendix Figure 1, panel B), a close relative of ST258. This association could be caused by clonal expansion or multiple HGT events and emphasizes that lineages known to encode KPC are now also acquiring NDM-1. We also observed high rates of NDM-1 carriage in *Enterobacter* isolates (Figure 1, panels C and D), which in general showed a high number (maximum 8) and wide variety of β-lactamases. These isolates were also MDR: 57% of the *Enterobacter* isolates were resistant to all or all

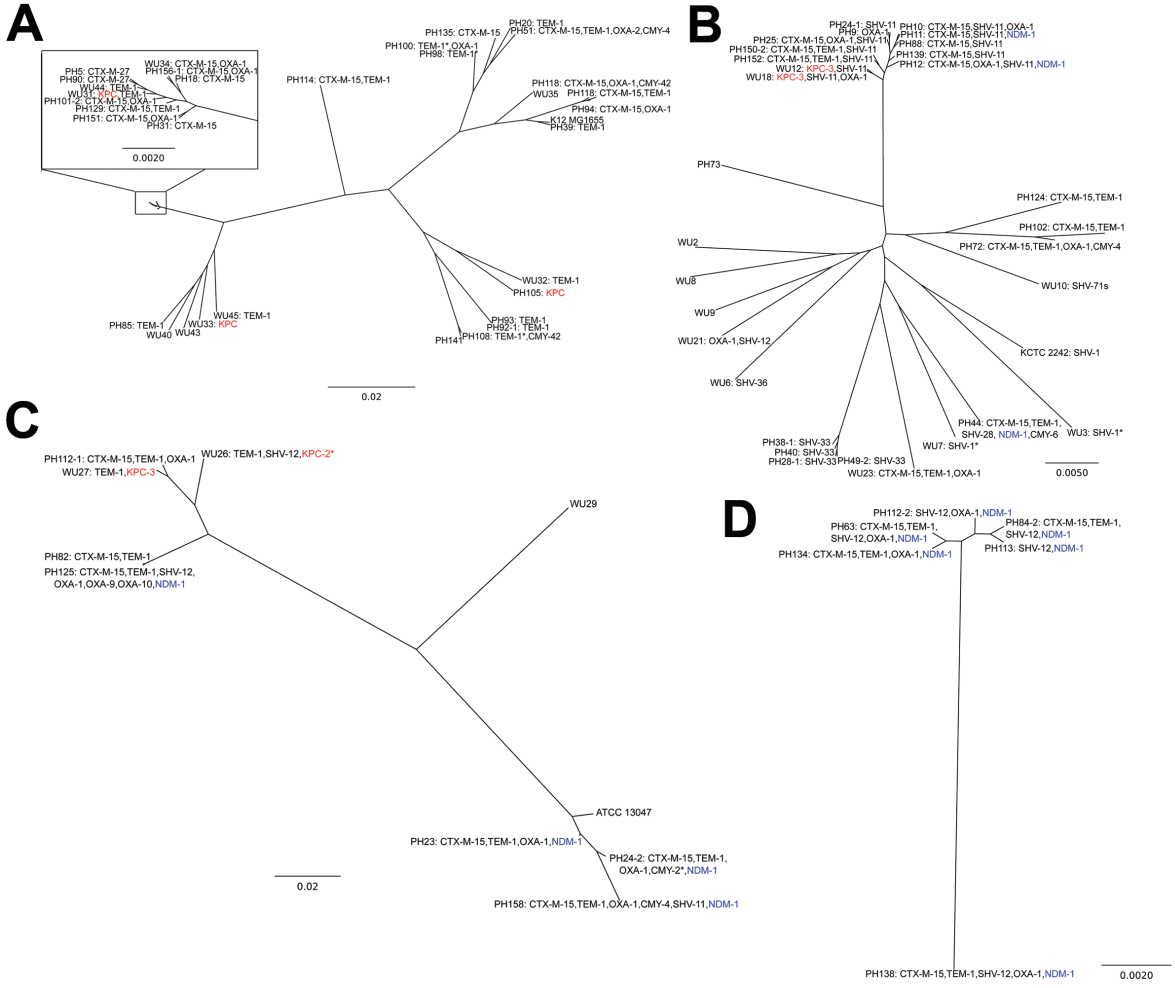

Figure 1. Distribution of antimicrobial drug resistance genotypes of Klebsiella pneumoniae carbapenemase (KPC) and New Delhi metallo-β-lactamase-1 (NDM-1) genes in related Enterobacteriaceae strains and plasmids in Pakistan and the United States. A) Escherichia coli; B) K. pneumoniae; C) Enterobacter cloacae; D) E. aerogenes. Phylogenetic trees have been annotated with the specific β-lactamases encoded by those isolates. *Denotes an unnamed single nucleotide variant of the named β-lactamase. Scale bars indicate nucleotide substitutions per site.

but 1 of the antimicrobial drugs tested. At best, these *Enterobacter* strains are a reservoir for resistance in Pakistan; at worst, they are the vanguard of an expansion of carbapenem-resistant *Enterobacter* infections.

Previous observations have predominantly found KPC and NDM-1 to be expressed from plasmids (*6*,*11*). To characterize the sequence similarity of plasmids within the NDM- and KPC-carrying plasmid populations, we purified and sequenced plasmid DNA from 9 isolates encoding NDM-1, 11 encoding KPC, and 3 encoding CTX-M-15. Sequencing showed that these plasmids include representatives from IncHI2, IncY, IncN, IncFIA, IncFIB, IncFIC, and IncI1 incompatibility groups. Using reciprocal BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) alignment between each pair of plasmid preparations, we calculated the percentage of each plasmid shared using a 99% identity threshold. We performed this same analysis for all sequenced plasmids containing NDM-1, KPC, or CTX-M available in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov) together with our set (Figure 2) and separately (online Technical Appendix Figure 2). Certain components, primarily mobile elements, were abundant within these plasmids: the average plasmid shared 500 contiguous bases with 58 of the other plasmids; however, median BLAST identity for this pairwise comparison was <12%, even when considering plasmids with the same β-lactamase, suggesting that both carbapenemases exist within a variety of plasmid configurations.

To visualize this comparison of carbapenemase plasmids, we generated a network diagram in which each node represented a plasmid and each line represented shared sequence between 2 plasmids (Figure 2, panel B). Node size and line width correlate to the number of nucleotides contained in the plasmid or sharing interaction. This visualization shows the abundant small, shared regions that exist between most plasmid pairs, represented as thin background lines. This visualization also highlights the larger shared regions that indicate highly similar plasmids, represented by the few wide lines. These outliers were often between pairs of plasmids encoding the same β-lactamase but were also observed between NDM-1 and KPC containing plasmids (maximum 79% of smaller plasmid length).

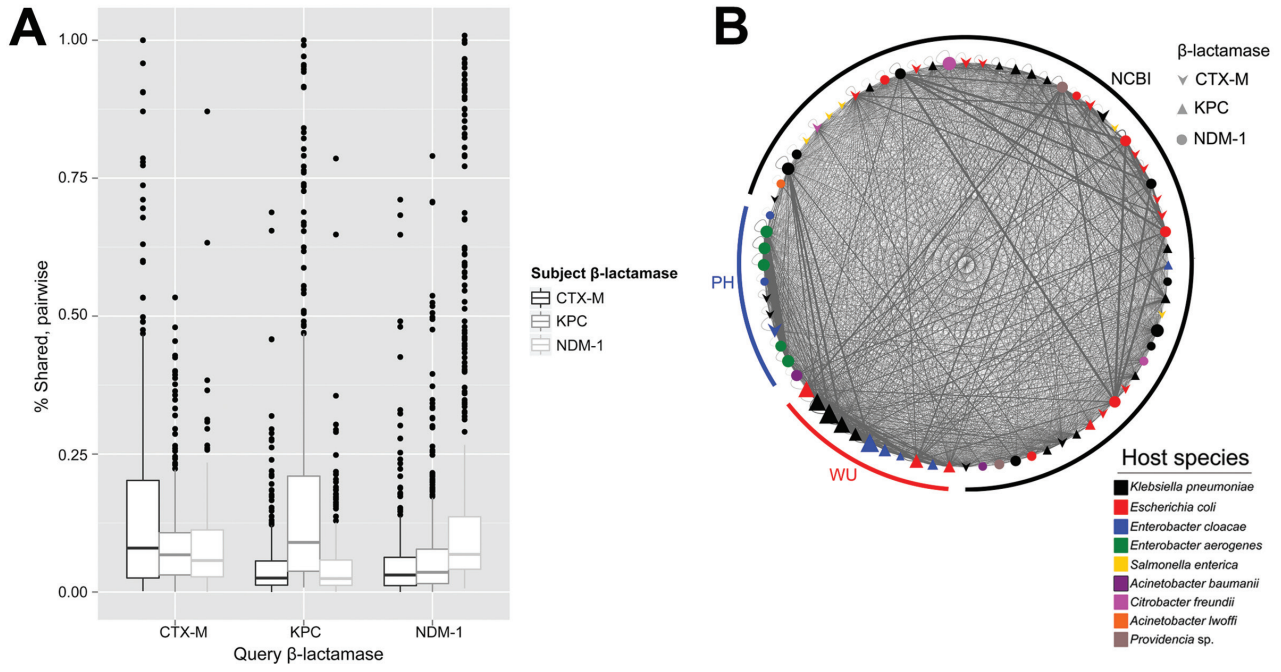

Figure 2. Pairwise BLAST identity (http://blast.ncbi.nlm.nih.gov/Blast.cgi) of all CTX-M genes, *Klebsiella pneumoniae* carbapenemase (KPC), and New Delhi metallo-β-Lactamase-1 (NDM-1) plasmids from isolates collected in Pakistan and the United States plasmid preparations, and the National Center for Biotechnology Information database complete plasmids. An all-against-all plasmid BLAST was performed and plasmid interactions were defined by the percentage of the query plasmid conserved (at ≥99% identity) in the subject plasmid. A) Plasmid interactions collected based on the defining β-lactamase of their query and subject plasmids. Box and whisker plots represent the range of pairwise sharing values within this population of plasmids. Upper and lower boundaries of the box correspond to the first and thirds quartiles; whiskers (error bars) represent 1.5 times the interquartile range; points beyond the whiskers represent outliers. B) Network map in which nodes represent individual plasmids and lines represent regions shared between plasmids. Line width is proportional to the number of nucleotides contained in fragments >500 bp in length at >99% sequence identity. Genetic elements repeated within the same plasmid DNA are represented by lines that leave and return to the same node. Plasmid sequence origin is indicated in arcs around the network.

Conclusions

Together, this evidence supports our hypothesis that strains and plasmids known to carry either carbapenemase also have access to the other. Given the similarity of carbapenemase-negative strains to those carrying KPC or NDM-1 and the high diversity of plasmids in which they can be found, we anticipate that global carbapenem usage will encourage HGT of both of these carbapenemases into additional strain and plasmid backgrounds. Because KPC and NDM-1 are poised to cross genetic and geographic boundaries, we recommend that hospitals routinely screen *Enterobacteriaceae* strains for both genes, even in regions where they are not yet endemic. We further advocate reduced carbapenem use to limit the selection for resistance against this vital antibiotic class.

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Endemic Melioidosis in Residents of Desert Region after Atypically Intense Rainfall in Central Australia, 2011

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After heavy rains and flooding during early 2011 in the normally arid interior of Australia, melioidosis was diagnosed in 6 persons over a 4-month period. Although the precise global distribution of the causal bacterium *Burkholderia pseudomallei* remains to be determined, this organism can clearly survive in harsh and even desert environments outside the wet tropics.

Melioidosis, a tropical disease caused by the bacterium *Burkholderia pseudomallei*, is endemic to Southeast Asia and northern Australia and is being increasingly recognized in other locations globally (*1*,*2*). During 1989–2009, a total of 540 cases of melioidosis were documented in a prospective melioidosis study based at Royal Darwin Hospital (latitude 12.4°S) in the tropical north of the Northern Territory of Australia (*3*). During that 20-year period, 4 of the study participants were considered to have been infected in the central Australia region of the Northern Territory; the other 536 were infected in the tropical north. During the same period, 2 persons who were not included in that study tested positive for *B. pseudomallei* in the central Australian region and were treated at Alice Springs Hospital (23.8°S), making a total of 6 cases of melioidosis during 20 years attributed to *B. pseudomallei* infection acquired in Central Australia. The ongoing Darwin prospective melioidosis study is approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (approval 02/38).

The Study

Central Australia (also known as the "Red Centre") is the most inland part of the arid interior of Australia and features low average annual rainfall, a desert environment, and rivers that are often dry. During March–July 2011, a total 6 cases of melioidosis were diagnosed in patients from Central Australia who resided south of latitude 20°S (Figure) and had not traveled to the tropical north of the country or to overseas regions to which melioidosis is endemic. These cases occurred after exceptionally heavy rainfall in February and March 2011, which resulted in widespread flooding in this normally parched region. The heavy rainfall in Central Australia was linked to a La Niña–associated, record-breaking wet season during 2010–2011 in the tropical north of the Northern Territory (Figure). Tropical Cyclone Carlos formed over the Beagle Gulf north of Darwin on February 15. At Darwin airport, the total rainfall during February 2011 of 1,110.2 mm and during the October–April wet season of 2,926.8 mm were the highest on record for that weather station. At Alice Springs airport, rainfall was 107.6 mm during February 2011, compared with a February mean of 40.3 mm during 1981–2010. The February 2011 rainfall at Tennant Creek airport (19.6°S) was 309.6 mm, compared with a February mean of 122.3 mm during 1981–2010. During the month of March 2011, when the first 3 patients became ill, rainfall amounts at Alice Springs and Tennant Creek airports were 120.6 mm and 222.6 mm, respectively; the annual mean March rainfalls in these locations during 1981–2010 were 35.4 mm and 53.8 mm, respectively.

The 6 persons whose illnesses were diagnosed as endemic melioidosis were treated at the central Australia hospitals in Alice Springs and Tennant Creek (Table). Of the 6 patients, 3 were male and 5 were indigenous Aboriginal Australians; 3 became bacteremic and required intubation and ventilation in intensive care for severe sepsis. The only patient <50 years of age and no identified risk factor had the mildest disease, characterized by a localized skin abscess. All patients were treated according to standard guidelines (*1*): with initial intravenous ceftazidime or meropenem, then with oral eradication therapy, which is usually with trimethoprim/sulfamethoxazole. All 6 patients survived.

Rainfall in the region returned to normal patterns and there have been no further locally acquired cases of melioidosis in Central Australia. Two persons with confirmed melioidosis were treated at Alice Springs Hospital in August 2011 and December 2014; both were attributed to infection acquired while traveling to the tropical north of the Northern Territory.

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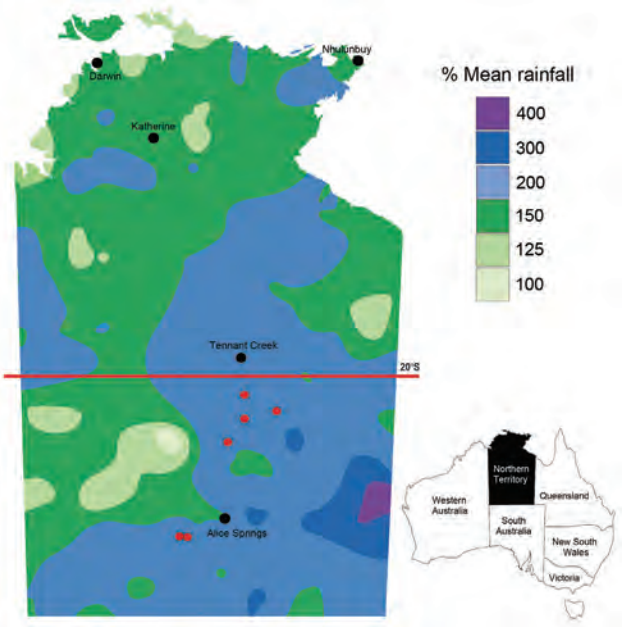

Figure. Rainfall in the Northern Territory of Australia during August 1, 2010–July 31, 2011. Rainfall is expressed as percentages of historical mean; the lowest rainfall total was 100% of mean. Cities and towns are indicated by black dots; locations of 6 persons with melioidosis in central Australia are indicated by red dots. Adapted from the National Climate Centre, Australian Bureau of Meteorology (http://www.bom.gov.au). Inset shows location of Northern Territory in Australia.

B. pseudomallei isolates from the 6 patients were subjected to multilocus sequence typing (MLST) as described by Godoy et al (*4*). On interrogation of the global *B. pseudomallei* MLST database (http://bpseudomallei.mlst.net/), each of the 6 isolates was unique and a novel sequence type (ST) (Table). ST907 has 2 novel alleles (gmhD, 54 and lipA, 48) and ST905 has 1 novel allele (lepA, 44); the remaining 4 STs contained unique configurations of existing alleles. None of the 6 STs was a single-locus variant of any ST in the MLST database (i.e., sharing 6/7 alleles), but all in the database except ST904 had double locus variants (i.e., sharing 5/7 alleles) that were *B. pseudomallei* isolates from northern Australia. Subsequent environmental soil sampling from Central Australia has confirmed the endemic presence of *B. pseudomallei* in several locations, and further studies are planned to characterize the environmental correlates of such *B. pseudomallei*–positive sites in Central Australia (M. Mayo, B.J. Currie, unpub. data).

Historically, melioidosis has been found to occur in the wet tropics between latitudes 20°S and 20°N (*5*,*6*). Nevertheless, the first recognition of melioidosis in Australia was in an outbreak among sheep in 1949 at Winton, Queensland (22.4°S) (*7*), an arid location with geographic similarities to Central Australia and where flooding also sporadically occurs. Case clusters of melioidosis have also occurred even further south in Australia: 1 cluster spanned 25 years in temperate southwestern Western Australia (31°S) (*8*). Severe weather events with heavy rainfall have dramatically increased the case numbers in regions in Australia and overseas to which the organism is endemic (*9*,*10*) and have also unmasked melioidosis in locations where it was uncommon or not previously recognized as being endemic (*11*–*13*).

Although genotype profiles showed the 6 isolates to be closer to STs of other Australian *B. pseudomallei* than to STs from Southeast Asia and the rest of the world, the extensive diversity among *B. pseudomallei* in Central Australia is evident in that each isolate is a novel ST and that none has any known single-locus variants. This diversity is similar to the situation seen in tropical northern Australia and in northeast Thailand (*14*) and contrasts with endemic melioidosis identified in Puerto Rico, which was recently shown to be mostly restricted to a single ST (*15*). The limited genetic diversity of *B. pseudomallei* seen in Puerto Rico to date is consistent with more recent introduction of *B. pseudomallei* to that location, potentially through importation of infected animals, as has occurred elsewhere (*2*). Importation of infected animals from the tropical north of Australia was also considered a possible source of the clonal cluster of melioidosis seen in various animals and in a person in southwestern Western Australia (*8*).

Although the presence of *B. pseudomallei* in central Australia is clearly not a recent phenomenon, the origins and longevity of *B. pseudomallei* in the region and the phylogenetic relationships to *B. pseudomallei* in tropical Australia and elsewhere globally require further studies using whole genome sequencing. Furthermore, the geographic boundaries of *B. pseudomallei* across the vast interior of the Australian continent and the extent of incursion into southern Australia remain entirely unclear.

Table. Details of 6 residents of desert region in whom melioidosis was diagnosed after heavy rainfall in Central Australia, 2011*

Age, y, sex, ethnicity	Month of illness onset	Risk factors	Clinical manifestation	*B. pseudomallei* culture source	MLST sequence type
22y, F, indigenous	March	Hazardous alcohol use	Brain abscess	Abscess pus	ST 897
32y, M, indigenous	March	Chronic renal disease	Axillary abscess	Abscess pus	ST 894
68y, F, indigenous	March	Elderly	Pneumonia, septic shock	Blood, sputum	ST 903
36 y, M, indigenous	April	Type 2 diabetes mellitus	Septic arthritis, septic shock	Blood, joint aspirate	ST 904
44 y, F, indigenous	May	Type 2 diabetes mellitus	Septic shock, no focus	Blood	ST 905
23y, M, caucasian	July	None	Skin abscess	Skin swab sample	ST 907

*Indigenous, Aboriginal people of Australia; MLST, multilocus sequence typing.

Conclusions

Heavy rains and flooding in the normally arid interior of Australia early in 2011 were followed by 6 cases of melioidosis over a 4-month period. Although the true extent of the environmental presence of *B. pseudomallei* remains to be determined, both regionally and globally (*1*,*2*), these bacteria can clearly survive in harsh and even desert environments outside the traditionally recognized melioidosis-endemic regions of the wet tropics. Melioidosis should therefore be considered in such regions, especially after heavy rainfall and flooding.

Acknowledgments

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Prospective Multicenter International Surveillance of Azole Resistance in *Aspergillus fumigatus*

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To investigate azole resistance in clinical *Aspergillus* isolates, we conducted prospective multicenter international surveillance. A total of 3,788 *Aspergillus* isolates were screened in 22 centers from 19 countries. Azole-resistant *A. fumigatus* was more frequently found (3.2% prevalence) than previously acknowledged, causing resistant invasive and noninvasive aspergillosis and severely compromising clinical use of azoles.

Azole resistance is increasingly recognized as a problem in aspergillus diseases (*1*). Within the *Aspergillus fumigatus* species complex, new sibling species have been reported to cause invasive aspergillosis; these species are generally intrinsically less susceptible than *A. fumigatus* sensu strictu to azole compounds (*2*). Acquired resistance to azoles in *A. fumigatus* has become a public health concern because of the presumed fungicide-driven route of resistance selection and the associated risk for geographic migration. Surveillance studies show that, in areas to which *Aspergillus* is endemic, the environmental route of resistance selection contributes to >90% of resistance mechanisms in azole-resistant aspergillus diseases (*1*,*3*). Azole resistance has been observed in patients with no recent history of azole therapy, and the mortality rate for patients with azole-resistant invasive aspergillosis was 88% (*3*).

The Study

Our objective was to investigate the prevalence of azole resistance in clinical *Aspergillus* isolates. A multicenter international surveillance network was established (Surveillance Collaboration on *Aspergillus* Resistance in Europe [SCARE-network]), comprising 22 centers from 19 countries (18 European and 4 non-European sites) (Figure 1). To detect azole-resistant *A. fumigatus*, we developed a phenotypic screening-method using a 4-well plate format with agar supplemented with itraconazole, voriconazole, and posaconazole (*4*). Each center was asked to screen for azole resistance for 12 consecutive months. For each screened isolate, patient characteristics were registered through an online questionnaire, and patients with invasive aspergillosis were classified according to the European Organization for the Research and Treatment of Cancer/Mycoses Study Group consensus definitions (*5*).

For every *A. fumigatus* isolate that grew on any of the azole-containing wells, the primary culture isolate was sent both to the Radboud University Medical Centre (Nijmegen, the Netherlands) and Statens Serum Institute (Copenhagen, Denmark) for molecular species identification, susceptibility testing according to the EUCAST (European Committee

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Figure 1. Study characteristics (number of isolates/patients screened) from 22 centers in 19 countries participating in a study of azole resistance in *Aspergillus fumigatus*. *Period screened was 8 months instead of 1 year in this center for unknown reason. †Total number of screened patients is unknown because this center is a reference laboratory that does not have access to patient characteristics.

on Antimicrobial Susceptibility Testing) broth microdilution reference method (*6*), and determination of the full coding sequence of both strands of the *cyp51A* gene and the promoter region by PCR amplification. For every resistant isolate, a susceptible control isolate was assigned; this control isolate was the first susceptible isolate screened on the 4-well plate format in the same center after the resistant isolate, and they received molecular species identification and susceptibility testing according to the EUCAST broth microdilution reference method (*6*).

During January 2009–January 2011, a total of 3,788 *Aspergillus* isolates were screened for azole resistance by using the 4-well plates (Figure 1). Clinical information was available from 1,911 patients from 21 centers in 18 countries. Most (2,941 [77.6%]) isolates were classified as *A. fumigatus* species complex and were recovered from 1,450 patients. The most common underlying disease was chronic lung disease (30.0%), followed by cystic fibrosis (22.1%) and hemato-oncologic diseases (12.9%). A total of 204 (14.1%) patients had undergone hematopoietic stem cell transplantation or solid organ transplantation, and 265 (18.3%) had been treated with corticosteroids within 3 months before culture of the isolate. A total of 223 (15.4%) of 1,450 patients had received antifungal drugs within 3 months before, or at the time of the positive culture. For 806 (55.6%) patients, the clinical relevance of the cultured *A. fumigatus* sc isolate was reported (Figure 2).

For 60 *A. fumigatus* species complex isolates, the resistant phenotype was confirmed in vitro (Table 1). Forty-seven (78.3%) azole-resistant isolates were identified as *A. fumigatus* sensu strictu. The other 13 azole-resistant isolates were identified as *A. lentulus* (7 isolates), *Neosartorya pseudofisheri* (4 isolates), and *N. udagawae* (2 isolates). Sequence analysis of the *cyp51A* gene of *A. fumigatus* showed TR_{34}/L98H in 23 (48.9%) azole-resistant *A. fumigatus* isolates (Table 2). All TR_{34}/L98H isolates were cultured from patients from European centers. In 3 isolates from the Netherlands, the TR_{46}/Y121F/T289A resistance mechanism was found (*7*).

A total of 60 azole-resistant isolates were recovered from 46 patients. The overall prevalence of azole resistance among patients with *A. fumigatus* species complex isolates was 3.2% (range 0.0%–26.1% among the centers). Azole resistance was detected in 11 (57.9%) of 19 participating countries. Acquired resistance in *A. fumigatus* was found at European sites: Austria, Belgium, Denmark, France, Italy, the Netherlands, Spain, Sweden, and the United Kingdom. In 5 countries (Australia, Germany, Spain, Sweden, and United Kingdom), azole-resistant *A. fumigatus* sibling species were recovered.

From the 46 patients with resistant isolates, 8 patients had azole-resistant *A. fumigatus* sibling isolates, and 38 had a resistant *A. fumigatus* isolate. Of these 38 patients, 19 had an isolate that harbored a fungicide-driven resistance mechanism (i.e., TR_{34}/L98H or TR_{46}/Y121F/T289A). When comparing patients with isolates harboring presumed

fungicide-driven resistance mechanisms with "non–fungicide driven" resistance mechanisms (i.e., point mutations or non–*Cyp51A*-mediated mechanisms), azole exposure differed significantly: 4 (21.1%) of 19 patients with an isolate with the fungicide-driven resistance mechanism had a history of azole therapy, compared with 16 (84.2%) of 19 patients with isolates with other or no *cyp51A* mutations (p = 0.001). Of the 195 cases with invasive aspergillosis, azole resistance was documented in 10 (5.1%) (3 proven, 1 probable, and 6 possible infections). Among the patients with resistant isolates, 28 patients had documented aspergillus disease (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/6/14-0717-Techapp1.pdf). The case-fatality rate for this cohort was 70%.

Conclusions

Acquired azole resistance in *A. fumigatus* was detected in 11 of 17 European centers in 9 countries. Overall prevalence of azole resistance was 3.2%; TR_{34}/L98H was the predominant mechanism of resistance (48.9%) in *A. fumigatus* sensu strictu isolates. This finding substantiates our concern that azole resistance is an emerging problem in *A. fumigatus* and that resistance selection in the environment contributes significantly to azole-resistant aspergillus diseases. A predilection of isolates harbored the TR_{34}/L98H mutation for patients with acute invasive diseases over patients with aspergilloma and chronic pulmonary aspergillosis. Azole-resistant invasive aspergillosis was documented in 5.1% of cases of invasive aspergillosis, which is not lower than the percentage of the prevalence of azole resistance among the *A. fumigatus* isolates (3.2%). This finding might indicate that resistance does not come with a significant fitness cost and that azole-resistant isolates that harbored TR_{34}/L98H or TR_{46}/Y121F/T289A are at least as capable of causing invasive aspergillosis as nonresistant wild-type isolates. Although the clinical implications of sibling species of *A. fumigatus* are less well understood, our study confirms that these species are generally less susceptible than *A. fumigatus* to azole antifungal drugs.

Our study shows that azole resistance is widespread in Europe. Azole-resistant *A. fumigatus* caused aspergillus diseases in the patients in our study, and azole resistance was associated with a worsened outcome (*3*). A rapid and convenient screening method for resistance is indispensable, and centers that care for patients with aspergillus diseases should perform surveillance to determine their local epidemiology. Furthermore, azole resistance has become a public health problem that needs continued international surveillance and research on the mechanisms that enable its selection in the environment. This report of the emergence of resistance has launched a new phase in the management of aspergillus diseases. Unless we can implement measures that prevent the fungicide-driven route of resistance development, the clinical use of azoles will be severely compromised.

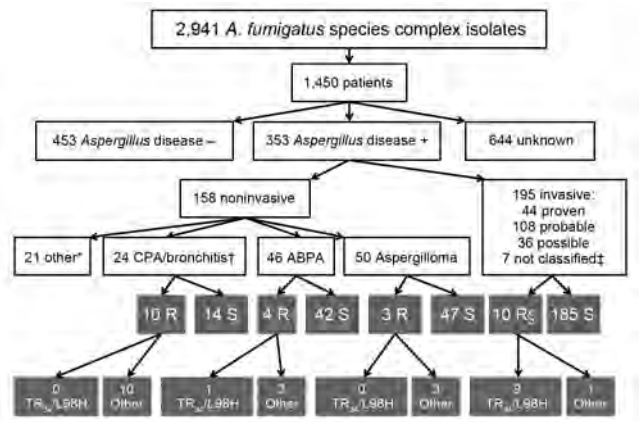

Figure 2. Patient characteristics and underlying resistance mechanisms of patients with invasive and noninvasive *Aspergillus* disease. *Otomycosis, dermatomycosis, or onychomycosis; 1 patient had a resistant isolate and otomycosis (patient 9 in the online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/6/14-0717-Techapp1.pdf). †One patient had chronic pulmonary aspergillosis and ABPA. ‡Not classified according to European Organization for the Research and Treatment of Cancer/Mycoses Study Group criteria (*5*). §One patient is included with 46-bp tandem-repeat resistance mechanism. ABPA, allergic bronchopulmonary aspergillosis; CPA, chronic pulmonary aspergillosis; R, resistant; S, susceptible; –, negative; +, positive.

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Table 1. Susceptibility for 3 antifungal medical azoles and 1 azole fungicide of resistant isolates (60 isolates) and control group (60 isolates) after species identification

Isolate	Median MIC, mg/L			
	Itraconazole	Voriconazole	Posaconazole	Tebuconazole
Azole-resistant *Aspergillus fumigatus*, n = 47	>8	2	1	8
A. fumigatus sibling species,* n = 13	1	2	0.25	>8
A. fumigatus controls, n = 60	0.25	0.5	0.06	2

**Aspergillus lentulus*, *Neosartorya pseudofischeri*, *N. udagawae*.

Table 2. Acquired resistance mechanisms from each country in c*yp51A* gene in 47 *Aspergillus fumigatus* isolates with an azole-resistant phenotype

Country	No. azole-resistant isolates, n = 47	TR_{34}/L98H or TR_{46}/Y121F/T289A mechanism (no. isolates)	Other mutations (no. isolates)	No. isolates without Cyp51A-mutations
Austria	2	TR_{34}/L98H (2)	0	0
Belgium	8	TR_{34}/L98H (7)	F46Y/M172G (1)	0
Denmark	6	TR_{34}/L98H (4)	0	2
France	4	TR_{34}/L98H (1)	G54W (1)	2
Italy	5	TR_{34}/L98H (5)	0	0
The Netherlands	7	TR_{34}/L98H (4), TR_{46}/Y121F/T289A (3)	0	0
Spain	1	No isolates	0	1
Sweden	1	No isolates	F46Y/M172G	0
United Kingdom	13	No isolates	P381R/D481E (1), L329V (1), M220K (1), L77V/L399I/D481E (1), M220I (3), M220R (1), G54R (1), G54E (1), G54W (1)	2
Resistant isolates, %	100	55.3	29.8	14.9

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Salmonella enterica Serotype Enteritidis in French Polynesia, South Pacific, 2008–2013

Simon Le Hello, Fiona Maillard, Henri-Pierre Mallet, Elise Daudens[1], Marc Levy, Valérie Roy, Philippe Branaa, Sophie Bertrand, Laetitia Fabre, François-Xavier Weill

Outbreaks of *Salmonella enterica* serotype Enteritidis infections associated with eggs occurred in French Polynesia during 2008–2013. Molecular analysis of isolates by using clustered regularly interspaced short palindromic repeat polymorphisms and multilocus variable-number tandem-repeat analysis was performed. This subtyping made defining the epidemic strain, finding the source, and decontaminating affected poultry flocks possible.

Over the past 2 decades, the incidence of *Salmonella enterica* serotype Enteritidis infections in humans has increased dramatically in all industrialized countries, with contaminated eggs the major source of infection (*1*,*2*). Despite a substantial decrease in outbreaks caused by this bacterium since the beginning of the 2000s, in particular in Europe due to the introduction of various control measures, *Salmonella* Enteritidis remains a major foodborne pathogen causing considerable human disease and high economic costs (*3*–*5*).

Different phenotypic and genotypic methods have been used to subtype *Salmonella* Enteritidis, including techniques such as phage typing and pulsed-field gel electrophoresis (PFGE). Results suggest the existence of major worldwide clones of *Salmonella* Enteritidis, of which most strains belong to phage type (PT) 4, followed by PT8 and PT1 (*1*,*6*). Recently, new methods such as standardized multilocus variable-number tandem-repeat analysis (MLVA) (*7*) and clustered regularly interspaced short palindromic repeats (CRISPR) typing (*8*,*9*) have been developed to subtype genetically homogeneous serotypes of *Salmonella*, in particular Enteritidis.

We report successive outbreaks of *Salmonella* Enteritidis in French Polynesia, South Pacific. To identify the source and determine the molecular subtypes of *Salmonella* Enteritidis strains that are circulating, we performed a comprehensive molecular and epidemiologic study on human and nonhuman strains isolated in Tahiti during 2008–2013.

The Study

Six cases of foodborne infection caused by *Salmonella* Enteritidis occurred on the island of Tahiti in October 2011, alerting public health authorities to an abnormal increase of these infections in humans. Epidemiologic and microbiological investigations confirmed that a tuna dish prepared with contaminated raw eggs was the food vehicle. Cases of *Salmonella* Enteritidis infection in Tahiti began to increase in July 2011, peaked in December 2011, and returned to baseline in April 2012; a total of 62 laboratory-confirmed cases occurred (Figure). A resurgence of 15 cases was registered during September–December 2012. Epidemiologic investigation by public health authorities revealed 20 clusters of cases (with a total of 54 cases) associated with the consumption of uncooked eggs produced by local layer farms. During November 2011–December 2012, a survey of 17 local poultry farms indicated the presence of *Salmonella* Enteritidis in 14 (1.9%) of 739 samples: 0 of 6 from drinking water sources, 0 of 15 from poultry feed, 3 (1.9%) of 155 from dust, 6 (1.5%) of 391 from feces, and 5 (2.9%) of 172 from eggs. The samples that tested positive were from 5 laying-hen houses on 2 farms that produce 3,000,000 eggs per year (70% of the local production).

A total of 112 *Salmonella* Enteritidis strains isolated in French Polynesia were sent to the Centre National de Référence des *Escherichia coli*, *Shigella*, et *Salmonella* for further analysis. During January 2008–August 2013, a total of 111 strains were isolated (96 from humans, 1 from the tuna dish, and 14 from laying hens); in November 2014, 1 strain was isolated from an imported chicken product from the United States. All but 3 *Salmonella* Enteritidis strains were susceptible to all antimicrobial drugs tested (*10*); the remaining 3 showed single-drug resistance to amoxicillin (data not shown).

Analysis by PulseNet (http://www.cdc.gov/pulsenet/pathogens/index.html) standardized *Xba*I PFGE showed a similar common profile, named JEGX01.0004 in a previous study (*11*), in 46 of 47 selected strains from Tahiti (Tables 1, 2). Phage typing revealed mostly 2 types, PT8 (n = 8) and PT13a (n = 4), for strains with the JEGX01.0004

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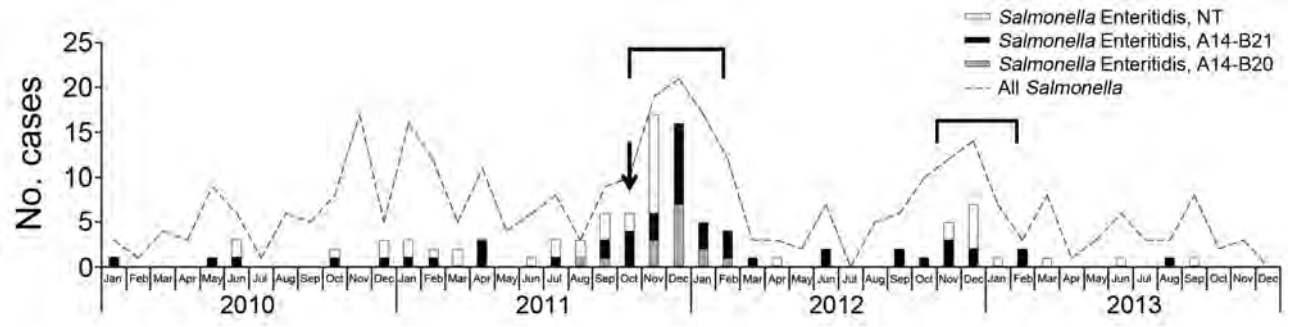

Figure. Number of confirmed cases of human infection with *Salmonella enterica* serotype Enteritidis per month and distribution of clustered regularly interspaced short palindromic repeats types, French Polynesia, 2010–2013. Arrow indicates when infections associated with tuna dish prepared with contaminated eggs occurred; brackets indicate periods of laying hen slaughters. NT, not typed.

profile. MLVA typing (*7*) on a subset of 60 strains showed main diversity in the SENTR4 and SENTR5 loci in isolates with the JEGX01.0004 PFGE profile. MLVA types 2-10-8-5-2 and 2-10-8-6-2 dominated in strains isolated from humans and laying hens. The CRISPR1 and CRISPR2 polymorphisms in 83 selected strains were studied by PCR amplification and sequencing as described elsewhere (*9*). The spacer content was determined by submitting the DNA sequences to the Institut Pasteur CRISPR database for *Salmonella* (http://www.pasteur.fr/recherche/genopole/PF8/crispr/CRISPRDB).

The 83 strains from French Polynesia had the same CRISPR1 allele (A14) but 2 different CRISPR2 alleles (B20 or B21), differing by the presence of a single spacer, EntB9 (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/21/6/14-1103-Techapp1.xlsx; online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/21/6/14-1103-Techapp2.xlsx). Both CRISPR2 alleles contained a triplication of the EntB8 spacer, which had not been observed in our database (194 *Salmonella* Enteritidis strains from France and Europe during 1920–2014) (*9*). However, this particular A14-B21 CRISPR profile is displayed by 37 *Salmonella* Enteritidis genomes deposited in the GenBank public database and originating in poultry or humans from North America (*8*,*11*,*12*) (online Technical Appendix 3, http://wwwnc.cdc.gov/EID/article/21/6/14-1103-Techapp3.xlsx).

Locally, in the month after the outbreak associated with consumption of the tuna dish, different control measures were implemented, depending on whether eggs were contaminated. Workers at farm A, where eggs were contaminated by both A14-B20 and A14-B21 strains, slaughtered laying hens. At farm B, where contamination was revealed only by sampling dust and feces (with only an A14-B21 CRISPR profile for *Salmonella* Enteritidis), minimal sanitary

Table 1. CRISPR-type characteristics of 67 *Salmonella enterica* serotype Enteritidis clinical isolates from French Polynesia, 2008–2013, compared with major examples from the Institut Pasteur database*

Country and period of isolation	No. isolates	Major PFGE types (no.)	Phage types available (no.)	CRISPR type allele1-allele2	MLVA type (no.)†
French Polynesia					
2008 Jan–2013 Aug	52	JEGX01.0004 (13)	PT8 (1), PT13a (2)	A14-B21	2-10-8-5-2 (20). 2-10-8-5-1 (1), 2-11-8-5-2 (6), 2-9-8-5-2 (5), 2-12-9-5-2 (1), 2-12-5-5-2 (1)
2011 Aug–2012 Feb	15	JEGX01.0004 (4)	PT8 (2)	A14-B20	2-10-8-6-2 (15)
France					
1957–2013	83	XEN-001 (57)	PT4 (45), PT1 (10), PT6 (6), PT21 (3), PT14b (1), PT22 (1), PT24 (1), PT34 (1), PT35 (1), PT44 (1)	A6-B7	3-11-5-4-1 (6), 3-11-5-6-1 (1), 3-10-5-4-2 (1), 3-10-5-4-1 (1)
2002	10	XEN-001 (10)	PT4 (6), PT35 (3), PT6a (1)	A8-B7	
1956–2014	8	XEN-001 (6)	PT4 (6)	A7-B7	2-9-4-5-1 (1), 1-8-9-4-1 (4)
1920–2001	7	XEN-001 (4) XEN-008 (2)	PT4 (6) PT6a (1)	A10-B7	
1956–2011	54	JEGX01.0004 (42)	PT8 (28), PT14b (12), PT13a (1), PT22 (1)	A14-B6	2-12-7-5-1 (1)

*All available CRISPR-types, and the spacer content of each, are described in online Technical Appendix 1 (http://wwwnc.cdc.gov/EID/article/21/6/14-1103-Techapp1.xlsx). CRISPR, clustered regularly interspaced short palindromic repeats. †SENTR7-SENTR5-SENTR6-SENTR4-SE3.

Table 2. Epidemiologic data, antimicrobial susceptibility patterns, *Xba*I PFGE types, phage types, MLVA types, and CRISPR types of nonhuman *Salmonella enterica* serotype Enteritidis isolates from French Polynesia, 2011–2014*

Period of isolation	Origin of sample	Sample type (no.)	No. isolates	Antimicrobial resistance profile (no.)	PFGE types (no.)	Phage types (no.)	CRISPR types, allele1-allele2 (no.)	MLVA type (no.)†
2011 Oct 25	Restaurant	Tuna dish with raw eggs	1	Susceptible	JEGX01.0004		A14-B20	2-10-8-6-2
2011 Nov–Jan 2012	Farm A	Egg (5), feces (1)	6	Susceptible (6)	JEGX01.0004 (5), XEN-033 (1)	8 (2), 23 (1)	A14-B21 (5), A14-B20 (1)	2-10-8-5-2 (4), 2-11-8-5-2 (1), 2-10-8-6-2 (1)
2011 Jan–2012 Dec	Farm B	Feces (5), dust (3)	8	Susceptible (8)	JEGX01.0004 (8)	8 (3), 13a (2)	A14-B21 (8)	2-10-8-5-2 (3), 2-11-8-5-2 (1), 2-9-8-5-2 (4)
2014 Nov	Imported chicken product	Legs–official control	1	NP	NP	NP	A14-B21	NP

*The spacer content of each CRISPR-type is described in online Technical Appendix 1 (http://wwwnc.cdc.gov/EID/article/21/6/14-1103-Techapp1.xls). CRISPR, clustered regularly interspaced short palindromic repeats; MLVA, multilocus variable-number tandem-repeat analysis; NP, not performed; PFGE, pulsed-field gel electrophoresis. †SENTR7-SENTR5-SENTR6-SENTR4-SE3.

policies were implemented (i.e., thermically treating eggs, disinfecting laying houses). Consequently, the incidence of human *Salmonella* Enteritidis infections has declined markedly in Tahiti. The reisolation of A14-B21 *Salmonella* Enteritidis strains from humans and farm B at the end of 2012 necessitated stronger measures, including slaughtering more laying hens. In total, 120,000 hens were slaughtered, representing 50% of the stock in Tahiti, which caused an egg-production deficit. After this outbreak ended in 2013, production levels returned to normal. Furthermore, controls on imported chicken products have begun in French Polynesia, and in November 2014, a frozen chicken product from the United States tested positive for *Salmonella* Enteritidis A14-B21. Given that the poultry sector has been importing eggs and laying hens from North America for decades, that the A14-B21 CRISPR profile is prevalent in *Salmonella* Enteritidis genomes from North America, and that a A14-B21 *Salmonella* Enteritidis strain has recently been isolated from imported poultry from the United States since the implementation of control on imported poultry products and animals, it is likely that the epidemic *Salmonella* Enteritidis strain that was circulating in French Polynesia was imported from North America before 2008.

Conclusions

When analyzed by classical subtyping methods, the *Salmonella* Enteritidis strains from French Polynesia displayed a very common and global profile, JEGX01.0004 PFGE type, PT8, and pansusceptibility to antimicrobial agents. Because of this, we used a combination of methods, such as CRISPR typing and MLVA, to more precisely define the epidemic strain and confirm that 2 local poultry farms were the source of the increase in human cases in Tahiti during July 2011–April 2012. By applying minimal to maximal control measures, depending on the CRISPR profile, and by sampling these flocks regularly, it became possible to follow and readjust the efficacy of the different control measures taken by the 2 layer farms. We also demonstrated that the epidemic strain has been circulating in French Polynesia since at least 2008 and was probably imported from North America but has not been associated with human cases since 2014.

Given the signatures offered by the polymorphism of the 2 CRISPR loci in our study and in previous works (*8*, *9*,*13*), we are convinced that CRISPR DNA targets might be very helpful for subtyping *Salmonella*, including serotype Enteritidis. Furthermore, because the CRISPR spacer content can be extracted easily from short-read DNA sequences, in contrast to MLVA loci, it could be used to define particular *Salmonella* Enteritidis strains together with, or as an alternative to, core genome single nucleotide polymorphisms when whole-genome sequencing for foodborne pathogen surveillance and investigation are implemented in public health and veterinary laboratories (*14*).

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Pneumonia Outbreak Caused by *Chlamydophila pneumoniae* among US Air Force Academy Cadets, Colorado, USA

Kevin A. Fajardo, Shauna C. Zorich, Jameson D. Voss, Jeffrey W. Thervil

During October 2013–May 2014, there were 102 cases of pneumonia diagnosed in US Air Force Academy cadets. A total of 73% of tested nasal washes contained *Chlamydophila pneumoniae*. This agent can be considered to be present on campus settings during outbreaks with numerous, seemingly disconnected cases of relatively mild pneumonia.

Chlamydophila pneumoniae is the most common *Chlamydophila* species that causes human infection (*1*). It responsible for up to 20% of community-acquired pneumonia cases in elderly adults (*1*). In recent years, *C. pneumoniae* has also been identified in outbreaks of pneumonia among younger age groups in a variety of close-quarters living environments, including military installations, prisons, universities, and single-family households (*2*–*6*).

We report the findings of our investigation into an outbreak of 102 cases of pneumonia at the US Air Force Academy, Colorado Springs, CO, USA. Laboratory testing identified *C. pneumoniae* as the likely causative pathogen.

The Study

The US Air Force Academy houses ≈4,000 cadets. The cadet population is composed of approximately equal-sized freshman, sophomore, junior, and senior classes. Members of each class year are randomly distributed to 1 of 40 cadet squadrons (numbered 1–40). Each squadron is composed of ≈100 cadets of both sexes. Approximately 80% of cadets are men. Ten squadrons are grouped together to form 1 of 4 cadet groups. All cadets receive their health care at the cadet clinic or other military installations.

In October 2013, a cluster of radiographic-confirmed cases of pneumonia was identified as part of routine medical surveillance by the preventive medicine staff at the US Air Force Academy. Nine cases of pneumonia were diagnosed in football team members in that month. In comparison, only 8 cases of pneumonia were diagnosed in the entire cadet population during the previous academic year. Although the incidence of mild upper respiratory infections was relatively high at the time of this cluster, cases of pneumonia other than in football players were not identified.

Laboratory testing ruled out *Streptococcus pyogenes*, influenza virus, and *Legionella pneumophila* as possible infectious etiologies in these cases. The following case definition was used to identify additional cases: any cadet receiving diagnostic codes from the International Classification of Diseases, 9th Revision, for bacterial pneumonia (482, 483, 484, 485, or 486) and who had radiographic confirmation of an acute pulmonary process.

In November 2013, the first case of pneumonia outside the football team was diagnosed. The primary care providers of the cadet medical clinic were encouraged to collect nasal wash samples from any cadets with upper respiratory infection symptoms, including those with pneumonia, for further laboratory testing. All samples were sent to the US Air Force School of Aerospace Medicine Epidemiology Laboratory (Wright-Patterson Air Force Base, OH, USA), which is a Department of Defense center for febrile respiratory illness surveillance.

Infection control guidance was developed by US Air Force Academy preventive medicine staff and disseminated to all cadets. Because all cadets reside in dormitory buildings, a close-quarters living environment, the recommended preventive measures focused on reinforcement of personal hygienic practices, social distancing, common contact surface decontamination, and wearing of surgical masks.

The outbreak lasted through May 2014, and a total of 102 cases of pneumonia were identified in US Air Force Academy cadets (Figure 1). Pneumonia was diagnosed in 74 male (73%) and 28 female (27%) cadets; cases were identified in members of every class year and cadet group (Table 1). Although no major differences in attack rate were noted among cadet groups, freshmen and juniors had higher attack rates than sophomores and seniors (Table 1). For freshmen, this finding is consistent with traditional military training risk factors because freshman undergo the most physically demanding training. It is unclear why the junior class had a similar incidence of pneumonia, although this finding might reflect a higher level of social contact outside the dormitory environment for this group.

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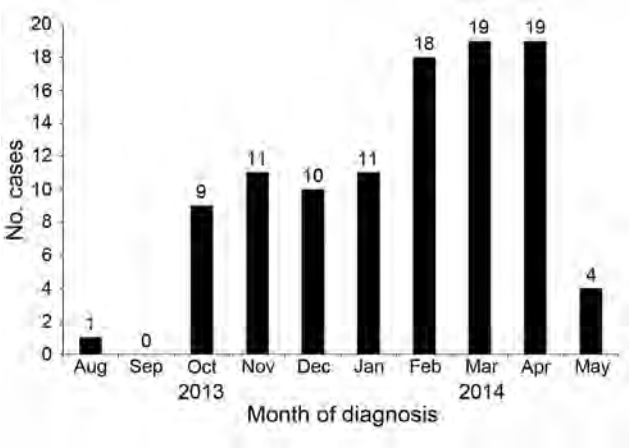

Figure 1. Chest radiograph–confirmed cases of pneumonia in cadets, US Air Force Academy, Colorado, USA, August 2013–May 2014.

Clinically, all cadets with pneumonia reported generalized, mild upper respiratory tract infection (URI) symptoms, such as cough, sore throat, and headache at the time of initial presentation. Chest radiographs showed acute, unilateral lobar consolidation for all patients. Only 4 cadets (5%) had documented evidence of fever at the time of presentation. Most cadets with pneumonia (101/102) required only a standard course of oral azithromycin therapy. One cadet, who had a pulmonary abscess, required hospitalization. He was given intravenous antimicrobial drugs and discharged from the hospital after 4 days.

Sixty-eight nasal wash samples from cadets were tested at US Air Force School of Aerospace Medicine Epidemiology Laboratory. Each specimen was tested by viral culture and real-time reverse transcription PCR for influenza virus. Influenza virus–negative specimens was then tested by using multiplex PCR testing; this PCR can identify many common respiratory pathogens, including *C. pneumoniae* (Table 2). Of the 68 samples, 15 were from cadets with pneumonia (Table 2). Of these 15 samples, 11 (73%) were positive for *C. pneumoniae*, 1 (7%) was positive for influenza A (H1N1) virus, and 3 (20%) were negative for both pathogens (Table 2).

Fifty-three nasal wash specimens were collected from cadets who had URI symptoms but were not given a diagnosis of pneumonia. Of these 53 specimens, 19 (36%) were positive for *C. pneumoniae* (Table 2). None of the 53 cadets from whom these samples were obtained received antimicrobial drug therapy, and all recovered without complications.

When charted on the basis of cadet squadron assigned, incident pneumonia cases were generally scattered throughout the cadet population (Figure 2). Only brief, self-limited clustering of cases was noted. For example, in February 2014, three squadrons had multiple cases diagnosed within a few weeks of each other (squadrons 24, 26, and 35). However, incident cases abruptly ended in these squadrons, which made it difficult to justify a large-scale antimicrobial drug prophylaxis campaign. Antimicrobial drug prophylaxis of close contacts of cadets with pneumonia was also of unclear benefit because we observed no evidence of roommate-to-roommate transmission.

The last case was diagnosed on May 15, 2014, although the surveillance period extended through July 31, 2014. Although aggressive reinforcement of infection control measures continued throughout the outbreak, the abrupt cessation of incident cases was more likely caused by the efflux of cadets off base after senior graduation and the start of underclassman summer activities.

Conclusions

Recent evidence supports *C. pneumoniae* as an increasingly common cause of outbreaks of community-acquired pneumonia, particularly in close-quarters living environments (*2*–*6*). The outbreak described supports this finding, and laboratory studies confirmed the presence of *C. pneumoniae* in 11 (73%) of 15 nasal wash samples from cadets given a diagnosis of pneumonia. Furthermore, 19 (36%) of 53 cadets with acute URI symptoms, but who were not given a diagnosis of pneumonia, were also positive for *C.*

Table 1. Pneumonia case distribution and attack rates for cadets, US Air Force Academy, Colorado, USA, August 2013–May 2014*

Group	Men, no. (%)	Women, no. (%)	No. cases	Total population	Attack rate, %	p value by χ^2 test of homogeneity
Class, y						
Senior, 2014	12 (71)	5 (29)	17	1,131	1.5	0.01†
Junior, 2015	23 (77)	7 (23)	30	884	3.4	
Sophomore, 2016	14 (70)	6 (30)	20	886	2.3	
Freshman, 2017	25 (71)	10 (29)	35	1,025	3.4	
Cadet group						
1	17 (77)	5 (23)	22	972	2.3	0.45‡
2	18 (78)	5 (22)	23	962	2.4	
3	26 (79)	7 (21)	33	1,002	3.3	
4	13 (54)	11 (46)	24	990	2.4	

*Group 1, squadron 1–10; group 2, squadron 11–20; group 3, squadron 21–30; group 4, squadron 31–40.
†Probability of random distribution among class years.
‡Probability of random distribution among cadet groups.

Table 2. Laboratory results for 68 cadet nasal wash specimens, US Air Force Academy, Colorado, USA, August 2013–May 2014

Bacteria or virus	No. (%) positive	
	Cadets with pneumonia, n = 15	Cadets without pneumonia, n = 53
Chlamydophila pneumonia	11 (73)	19 (36)
Influenza A(H1N1) virus	1 (7)	8 (15)
Adenovirus	0	2 (4)
Rhinovirus/enterovirus	0	1 (2)
Coronavirus	0	1 (2)
Human metapneumovirus	0	1 (2)
Parainfluenza viruses 1–4	0	0
Respiratory syncytial virus	0	0
Bordetella pertussis	0	0
Mycoplasma pneumoniae	0	0

pneumoniae. This finding might indicate that mild URI symptoms, rather than frank pneumonia, predominate in *C. pneumoniae* outbreaks. The long incubation period for infection with *C. pneumoniae*, estimated to be ≤4 weeks (*7*), also probably contributed to the difficulty in containing the outbreak because case diagnoses were generally made in a random pattern. Thus, the preventive intervention relied primarily on reinforcement of basic personal hygienic practices.

We suspect that routine testing for *C. pneumoniae* in outbreak situations is rare. Therefore, it is possible that *C. pneumoniae* commonly emerges on college campuses, in prisons, and other military training environments without any reporting. There are several reasons to be concerned about emergence of *C. pneumoniae* in these settings. First, unique transmission characteristics (incubation period, asymptomatic carriage) can lead to diagnostic uncertainty, which enables outbreaks to be sustained for long durations without a clear method for control. Second, if *C. pneumoniae* is not considered within the differential diagnosis, unnecessary testing for other pathogens might be conducted. Third, even if the acute illness is mild, *C. pneumoniae* has been linked with numerous chronic diseases (e.g., atherosclerosis [*8*] and asthma [*9*]).

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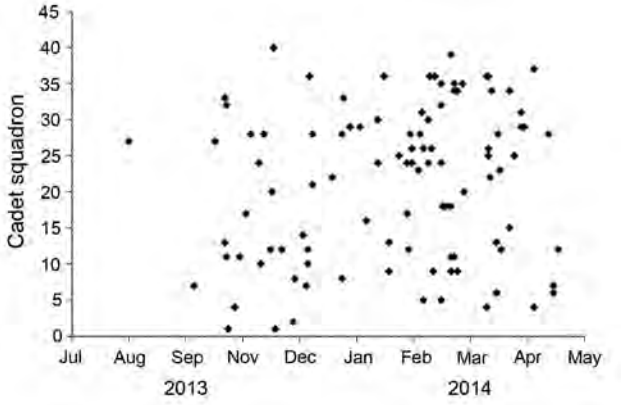

Figure 2. Scatter plot of pneumonia cases (diamonds) in cadets, by squadron, US Air Force Academy, Colorado, USA, August 2013–May 2014.

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Oligoarthritis Caused by *Borrelia bavariensis*, Austria, 2014

Mateusz Markowicz, Stefan Ladstätter, Anna M. Schötta, Michael Reiter, Gerhard Pomberger, Gerold Stanek

A case of Lyme oligoarthritis occurred in an 11-year-old boy in Vienna, Austria. DNA of *Borrelia bavariensis* was detected by PCR in 2 aspirates obtained from different joints. Complete recovery was achieved after a 4-week course with amoxicillin. Lyme arthritis must be considered in patients from Europe who have persisting joint effusions.

Lyme borreliosis is a tickborne disease caused by certain species of spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex. In Europe, several genospecies of *B. burgdorferi* s.l. cause the disease, whereas in North America, *B. burgdorferi* sensu stricto is the only agent of Lyme borreliosis. This difference causes variability in clinical manifestations (Figure). According to surveillance by the US Centers for Disease Control and Prevention, Lyme arthritis occurs in 30% of Lyme borreliosis patients in the United States (*1*), whereas in Europe, arthritis is reported in only 3%–7% of patients, as assessed in a few epidemiologic studies (*2*,*3*). Direct comparison of the frequencies of clinical manifestations is difficult because of possible differences in case definitions.

In most cases, diagnosis of Lyme arthritis is made on the basis of the clinical picture supported by serologic testing. PCR testing of synovial fluid or synovial tissue samples is the most reliable method for direct identification of the pathogen (*4*). Cultivation of the pathogen from these materials is difficult, and recovery has been reported only anecdotally.

Lyme arthritis usually affects 1 or several large joints, most commonly the knee (*3*). Several studies, mostly of serologic testing and clinical picture, have shown different patterns of joint involvement in children (*5*). Therefore, it is difficult to distinguish Lyme arthritis from other forms of arthritic diseases, particularly juvenile idiopathic arthritis, on the basis of clinical signs and symptoms. Both diseases may present with oligoarticular involvement with symmetrically or unilaterally occurring joint effusions. We report a case of Lyme oligoarthritis in an 11-year-old boy from Vienna, Austria.

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The Study

A signed consent form was obtained from the mother of the patient. The patient had reported recurrent joint pain, most prevalent in his left knee, since he was 6 years old. In December 2012, when he was 10 years old, his left knee became swollen, and he was treated locally with nonsteroidal antiinflammatory drugs.

In February 2014, the patient had effusions of both knees and the left ankle. The first joint aspiration of the left knee was performed in February 2014, but the patient was discharged without a diagnosis. Soon after that, he became febrile (temperature 39°C) and was referred to another hospital because of persistent effusions of all 3 joints. Clinical investigation revealed swelling of both knees, which were not warm or red, and a swollen, hot, red left ankle.

A routine blood test showed a normal leukocyte count, elevated C-reactive protein levels (79.4 mg/L, positive threshold 5 mg/L), and elevated erythrocyte sedimentation rates (85 mm/h, reference <7 mm/h, and 109 mm/2 h, reference <12 mm/2 h). Tests for rheumatoid factor and other autoantibodies (antinuclear antibodies, double-stranded DNA, proteinase 3, myeloperoxidase antibodies) showed negative results. The patient underwent needle aspiration of all 3 joints under general anesthetic to obtain synovial fluid: 18 mL from the right knee, 60 mL from the left knee, and 6 mL from the left ankle. The patient was given a preliminary diagnosis of juvenile idiopathic arthritis and treated with nonsteroidal antiinflammatory drugs. A few days

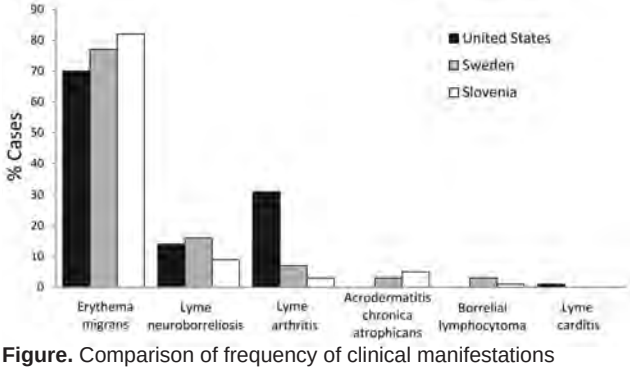

Figure. Comparison of frequency of clinical manifestations in Lyme borreliosis cases between the United States and 2 countries in Europe. Data from the United States are based on 154,405 patients identified during 2001–2010 by Centers for Disease Control and Prevention surveillance (*1*). Cases in Europe are represented by data from southern Sweden (1,471 patients, 1992–1993) (*2*) and Slovenia (1,020 patients, 2000) (*3*). The category Lyme neuroborreliosis includes all neurologic manifestations, such as radiculoneuropathy, facial palsy, and meningitis or encephalitis. Some patients had >1 manifestation.

Table 1. Primer and probe sequences used in identification of *Borrelia bavariensis* in 11-year-old patient with oligoarthritis, Vienna, Austria, February 2014*

Primer or probe	Target gene	Sequence, 5′ → 3′	Reference
Primer			
16SF	16S rDNA	GCT GTA AAC GAT GCA CAC TTG GT	(*6*)
16SR	16S rDNA	GGC GGC ACA CTT AAC ACG TTA G	(*6*)
BorF	Flagellin	GAA TTA GCA GTT CAA TCA GG	(*7*)
BorR	Flagellin	TTC GTC TGT AAG TTG CTC TAT	(*7*)
rrf-rrl IGS F	5S–23S IGS	CTG CGA GTT CGC GGG AGA	(*8*)
rrf-rrl IGS R	5S–23S IGS	TCC TAG GCA TTC ACC ATA	(*8*)
B5S-23S_Fn	5S–23S IGS	GAG TTC GCG GGA GAG TAA G	(*8*)
B5S-23S_Rn	5S–23S IGS	TAG GCA TTC ACC ATA GAC TCT T	(*8*)
V1a	*ospA*	GGG AAT AGG TCT AAT ATT AGC	(*10*)
V1b	*ospA*	GGG GAT AGG TCT AAT ATT AGC	(*10*)
V3a	*ospA*	GCC TTA ATA GCA TGT AAG C	(*10*)
V3b	*ospA*	GCC TTA ATA GCA TGC AAG C	(*10*)
R1	*ospA*	CAT AAA TTC TCC TTA TTT TAA AGC	(*10*)
R37	*ospA*	CCT TAT TTT AAA GCG GC	(*10*)
Probe			
LBTM	16S rDNA gene	FAM–TTC GGT ACT AAC TTT TAG TTA A–TAMRA	(*6*)
BorTM	Flagellin gene	FAM–AAC GGC ACA TAT TCA GAT GCA GAC–TAMRA	(*7*)

*IGS, intergenic spacer.

later, another aspiration of the left knee was performed, followed by an intraarticular injection of steroids.

Infection with pathogens associated with reactive arthritis was ruled out by negative serologic test results for *Chlamydia* spp. (IgG and IgA enzyme immunoassays), *Mycoplasma pneumoniae* (IgG and IgA enzyme immunoassays), *Salmonella* spp. (agglutination assay), and *Yersinia* spp. (agglutination assay). Antibodies against *B. burgdorferi* s.l. were detected by *Borrelia* ELISA (Medac, Hamburg, Germany); IgG levels were highly elevated (IgG ELISA >200 AU/mL, cutoff 10.8 AU/mL; IgM ELISA results were negative). Test results obtained by using the Anti-*Borrelia* Euroline Westernblot (Euroimmun, Lübeck, Germany) were positive for IgG with strong band intensities for VlsE, p83, p39, p30, p21, p19, and p17 and weak band intensity for p25 (OspC). Cytologic test results for synovial fluid showed an inflammatory infiltrate with lymphocytes and segmented neutrophils. Culture for bacterial pathogens was negative.

Synovial fluid samples from all 3 joints were tested by using PCR. DNA was extracted by using the PeqGOLD Tissue DNA Mini Kit (Peqlab, Erlangen, Germany). Two TaqMan-based real-time PCR assays targeting the 16S rDNA gene (*6*) and the flagellin gene (*7*) were performed; primer and probe sequences are listed in Table 1. The DNA of an in-house *B. burgdorferi* s.l. strain was used as a positive control, and PCR-grade water was used as a negative control. To check for PCR inhibition, we used samples spiked with borrelial DNA in an extra well. In 2 of the 3 joint aspirates (left knee and left ankle), borrelial DNA was detected by both assays. For genotype identification, samples were subjected to a previously described nested PCR targeting the 5S-23S intergenic spacer region (*8*).

Amplicons were purified by using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and sent to MWG Eurofins (Munich, Germany) for bidirectional sequencing by using primers B5S-23S_Fn and B5S-23–S_Rn (*8*). Sequencing revealed the same *Borrelia* strain in both joints. When compared with known sequences by using BLAST (http://www.ncbi.nlm.nih.gov/blast), the sequences showed 100% identity with that of the PBi strain.

Because the PBi strain and all PBi-like strains are now known as a distinct genospecies within the *B. burgdorferi* s.l. complex, namely *B. bavariensis* (*9*), and because *B. bavariensis* strains are associated with OspA serotype 4, another PCR for identifying the OspA serotype was carried out. To determine the OspA type of the *Borrelia* spp. found in the joint aspirates, we performed another previously described nested PCR targeting the *ospA* gene (*10*), then sequenced and identified a virtual restriction fragment polymorphism by using CLC Main Workbench version 7.0 (CLC bio, Aarhus, Denmark).

Table 2. Reports of Lyme arthritis and identified genospecies in patients in Europe*

Study	Year published	PCR target	Total no. cases	PCR-positive cases	*Borrelia* spp., no. (%) identified		
					B. burgdorferi sensu stricto	*B. afzelii*	*B. garinii*
Eiffert et al. (*11*)	1998	*ospA* gene	11	7	3 (43)	1 (14)	3 (43)
Vasiliu et al. (*12*)	1998	*ospA* gene	20	13	4 (31)	5 (38)	4 (31)
van der Heijden et al. (*13*)	1999	5S–23S IGS	4	3	3 (100)	0	0
Jaulhac et al. (*14*)	2000	Flagellin gene	12	10	9 (90)	0	1 (10)
Total			47	33	19 (58)	6 (18)	8 (24)

*IGS, intergenic spacer.

Because the obtained restriction fragment length polymorphism pattern matched OspA serotype 4, the presence of a *B. bavariensis* strain in both *Borrelia*-positive joints could be confirmed.

After the patient was treated with amoxicillin (500 mg 3×/d for 28 days) (*4*), all joint effusions resolved. At his 9-month follow-up visit, the patient did not report any symptoms. No erythema migrans was observed, and the patient's mother reported only 1 tick bite for the patient when he was 2 years old.

Conclusions

In Europe, Lyme arthritis can be caused by several genospecies of *B. burgdorferi* s.l. Published studies have most commonly identified *B. burgdorferi* sensu stricto in joints from Lyme arthritis patients in Europe (Table 2). *B. bavariensis*, the pathogen that caused the illness in the patient we describe, was formerly classified as *B. garinii* genospecies characterized by OspA serotype 4 (*9*). On the basis of multilocus sequence analysis of chromosomal housekeeping genes, this group was found to be genetically distinct from other *B. garinii* strains. Furthermore, the 2 genotypes differ in their hosts: *B. bavariensis* is a rodent-associated strain, whereas other *B. garinii* serotypes can be found in birds.

Most Lyme arthritis patients respond well to a single course of treatment with antimicrobial drugs, although in a small percentage of cases persistent synovitis can develop months or even years after treatment. For those patients whose synovial fluid PCR result is negative, intraarticular application of corticosteroids can be beneficial (*15*).

This case illustrates that Lyme arthritis must be taken into account in patients in Europe who have persisting joint effusions. Treatment with antimicrobial drugs is highly effective. We did not find any other report of cases in which the pathogen was detected in multiple joints by using a direct identification method. This case is further evidence for the systemic characteristics of Lyme borreliosis.

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European Rabbits as Reservoir for *Coxiella burnetii*

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We studied the role of European rabbits (*Oryctolagus cuniculus*) as a reservoir for *Coxiella burnetii* in the Iberian region. High individual and population seroprevalences observed in wild and farmed rabbits, evidence of systemic infections, and vaginal shedding support the reservoir role of the European rabbit for *C. burnetii*.

Wildlife play a major role in the maintenance and transmission of multihost pathogens (*1*,*2*). Understanding the role of host species involved in multihost zoonotic pathogen maintenance and transmission is essential to prevent disease caused by these pathogens.

Coxiella burnetii, which is the cause of Q fever, is a zoonotic pathogen that infects multiple hosts (*3*). The implication of wildlife in the life cycle of *C. burnetii* has been reported worldwide (*4*,*5*), and wildlife might act as a source for humans infections (*6*,*7*).

European rabbits (*Oryctolagus cuniculus*) are native to the Iberian Peninsula and have been introduced into Australia, New Zealand, Chile, and Argentina (*8*). Domestic varieties of European rabbits are farmed worldwide. Specific ecologic traits (high density, gregarious behavior, high reproductive rate) suggest that these rabbits might become a major reservoir of zoonotic pathogens. However, whether *C. burnetii* can infect, replicate in, and be shed by European rabbits and contaminate the environment is not known. In this study, we investigated the role of these rabbits in a region to which Q fever is endemic.

The Study

Serum samples were collected from European wild rabbits in 13 locations in Spain, Portugal, and the Chafarinas Islands during 2003–2013 (Figure 1). Wild rabbits from 1 of the study locations (LO; Figure 1) were obtained from 2 epidemiologic scenarios (*10*). The first scenario involved rabbits that coexisted with farmed red deer (*Cervus elaphus*) (sites A and B). The second scenario involved rabbits that had not been in contact with ruminants since 2002 (site C).

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In addition to serum samples, spleen, uterus, and mammary gland samples and vaginal and uterus swab specimens were collected from rabbits surveyed at location LO. Each rabbit from this location was weighed and sexed. Serum samples were also collected from farmed rabbits on 4 farms in Spain (Figure 1). Samples were stored at -20°C until tested.

Serum samples were analyzed by using the LSIVet Ruminant Q Fever Serum/Milk ELISA Kit (Life Technologies, Carlsbad, CA, USA) and horseradish peroxidase–conjugated protein G (Sigma-Aldrich, St. Louis, MO, USA) as secondary antibody (*10*). Results were interpreted according to manufacturer's recommendations.

DNA from tissues and swab specimens was extracted bu using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). Swabs were incubated at 56°C for 30 min in 200 μL of AL buffer containing 20 μL of proteinase K. Swabs specimens were then vortexed for 15 s and removed. The remaining solution was incubated at 56°C for 30 min. The manufacturer's blood extraction protocol was then used. DNA aliquots were frozen at –20°C. Negative controls (nuclease-free water; Promega, Madison, WI, USA) were included during DNA extraction.

DNA samples were analyzed by using a conventional PCR (*11*). PCR products were visualized by electrophoresis in 1.2% agarose gels containing 0.1 μL/mL of GelRed Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA).

Logistic regression models were used to test the effect of potential factors (Table) on the individual risk of exposure to *C. burnetii*. Individual ELISA results were included as response variables in the models and the location origin of rabbits was used as a random factor.

Logistic regression models were also used for individual exposure of rabbits from location LO to *C. burnetii* (ELISA), for the presence/absence of *C. burnetii* DNA in spleen (a proxy of systemic infection), and for the presence/absence of *C. burnetii* DNA in the reproductive tract (a proxy of shedding; including PCR results from uterus, and vaginal and uterus swab specimens). Location LO was included as a random factor, and sex, weight and ruminant presence/absence were also included as predictor variables (Table). Models were created by using a forward stepwise procedure. The model with the lowest Akaike information criterion (*12*) was selected.

Statistical analyses were performed in SPSS version 20.0 (IBM, Armonk, NY, USA). Prevalence-associated, Clopper-Pearson exact 95% CIs were estimated.

Serum samples from 572 rabbits (464 wild and 108 farmed) (Figure 1) were analyzed. Overall seroprevalence

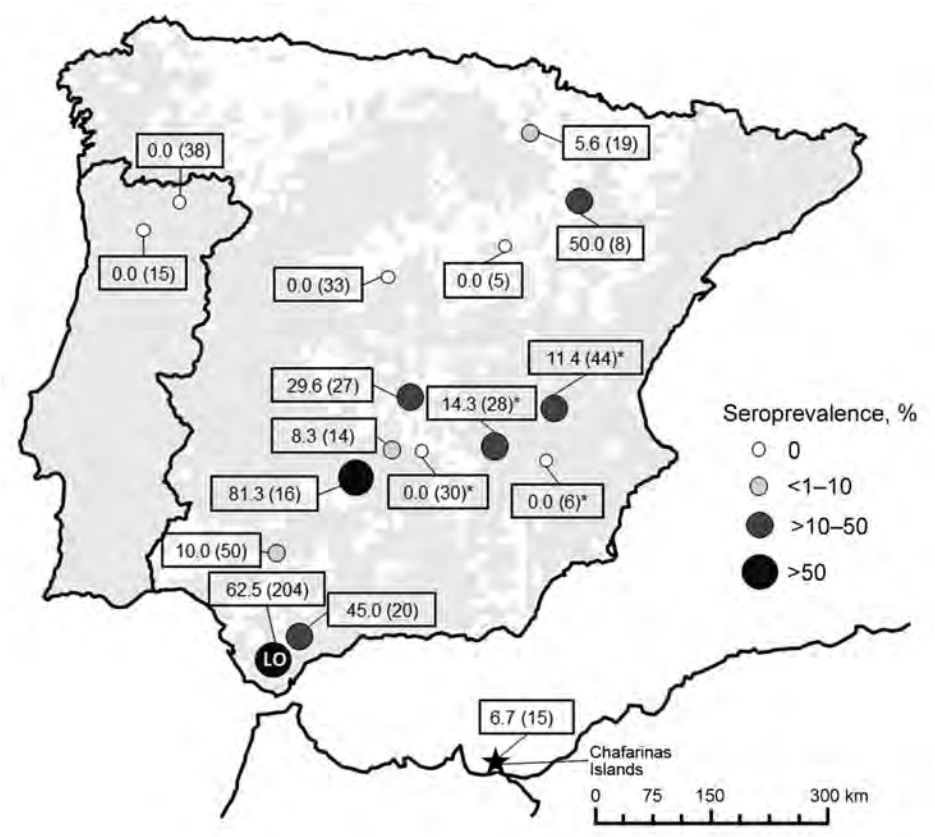

Figure 1. Seroprevalence of *Coxiella burnetii* (sample size) in wild and farmed European rabbits (*Oryctolagus cuniculus*), Iberian Peninsula and Chafarinas Islands. The distribution area of wild rabbits in the Iberian Peninsula (10 x 10 km Universal Transverse Mercator squares) is shown (gray shading) according to Mitchel-Jones et al. (*9*). LO sampling location is indicated. *Rabbit farm.

was 32.3% (95% CI 28.5%–36.4%) for wild and farmed rabbits, 37.9% (95% CI 33.5%–42.5%) for wild rabbits, and 8.3% (95% CI 3.8%–15.2) for farmed rabbits. Seroprevalence in wild rabbit populations ranged from 6.7% to 81.3%. Nine (64.3%) of 13 wild rabbit populations and 2 (50%) of 4 farms had ≥1 seropositive rabbit. The best model for *C. burnetii* exposure retained sampling year and season, and the risk for seropositivity was higher in summer (Table).

Seroprevalence at location LO was 65.2% (133/204; 95% CI 58.2%–71.7%); it was slightly lower at site C than at sites A and B (Figure 2, panel A). However, none of the considered factors were retained in the best model (Table). Six (4.4%; 95% CI 1.6%–9.4%) of 136 spleen samples analyzed at location LO were positive by PCR (4 male and 2 female rabbits). Five of the 6 spleen PCR–positive animals were seropositive. The 2 female rabbits were positive for *C. burnetii* DNA in vaginal swab specimens. Spleen PCR–positive rabbits were observed only at sites A and B (Figure 2, panel B).

The best model for the presence of *C. burnetii* DNA in spleen retained sampling year, season, presence of ruminants, and sex (Table). Results suggest expected higher systemic infection prevalence in rabbits coexisting with farmed red deer (Figure 2, panel B). *C. burnetii* DNA was detected in the reproductive tract of 9 (14.1%; 95% CI 6.6%–25.0%) of 64 female rabbits at sites A, B, and C (Figure 2, panel F). The presence of ruminants was retained in the best model for *C. burnetii* DNA in the reproductive tract (Table). None of the 13 mammary glands analyzed was positive for *C. burnetii* DNA.

Conclusions

This study provides 3 results that suggest that European rabbits might be reservoirs of *C. burnetii*. These 3 results are high seroprevalence of this bacteria; systemic infections; and bacterial shedding in vaginal secretions, which, in other host species, constitutes the main source for environmental contamination and transmission between species (*13*).

Host density is a major factor in *C. burnetii* prevalence in livestock (*14*). The highest seroprevalence values were observed at 2 locations where rabbit populations are managed for hunting purposes, which promotes high densities of rabbits. These findings suggest that rabbit density may be a major factor in the ecology of *C. burnetii*. In addition, the European rabbit is a gregarious species with a high reproductive rate. This rate favors transmission of *C. burnetii* from infected to susceptible animals, which is enhanced by replacement of *C. burnetii*–negative rabbits

Table. Variables considered as potential risk factors and outputs (coefficient/statistic) of best fitted risk factor models for *Coxiella burnetii* exposure in European rabbits (*Oryctolagus cuniculus*), Iberian Peninsula and Chafarinas Islands*

Variable code	Variable, units	Cb_{sp}	Cb_{spLO}	Cb_{splLO}	Cb_{rtLO}
Intercept	NA	67.776/4.98†	–037270.00‡	–2,942.687/1.15‡	2,925.025/0.49‡
X	Longitude, decimal degrees	§	¶	¶	¶
Y	Latitude, decimal degrees	§	¶	§	¶
Ye	Year	–0.033/0.20‡	§	1.464/0.42‡	–1.453/0.45‡
Se	Season	§	§	§	§
Sp	Spring	1.209/5.45‡	§	–1.583/2.78‡	§
Su	Summer	2.257/5.45†	§	Referent	§
Au	Autumn	0.043/5.45‡	§	§	§
Wi	Winter	Referent	§	§	§
Mg	Management, wild vs. farmed	§	¶	¶	¶
Rum	Ruminants, presence vs. absence	¶	§	0.059/0.0‡	2.004/1.08‡
Sex	Sex, M vs. F	¶	§	–0.383/0.27‡	2.004/0.22‡
Wg	Weight, g	¶	§	§	§

*Cb_{sp},overall seropositivity; Cb_{spLO}, seropositivity at location LO; Cb_{splLO}, systemic infection (*C. burnetii* DNA in spleen of wild rabbits); CB_{rtLO}, shedding (*C. burnetii* DNA in reproductive tracts of wild rabbits); NA, not applicable.
†p≤0.05.
‡p>0.05.
§Variable was included in each model but was not retained in the best model.
¶Variable not tested.

and can contribute further to spread of this bacterium in the environment.

The higher risk of exposure to *C. burnetii* observed during the summer might be related to increased indirect interaction with *C. burnetii* shed by coexisting ruminants, whose main shedding season is late spring–early summer (*3*). Inclusion of ruminants in the final models for systemic infection and vaginal shedding at location LO might support this hypothesis. However, further analyses, including molecular typing of circulating strains, would be needed to

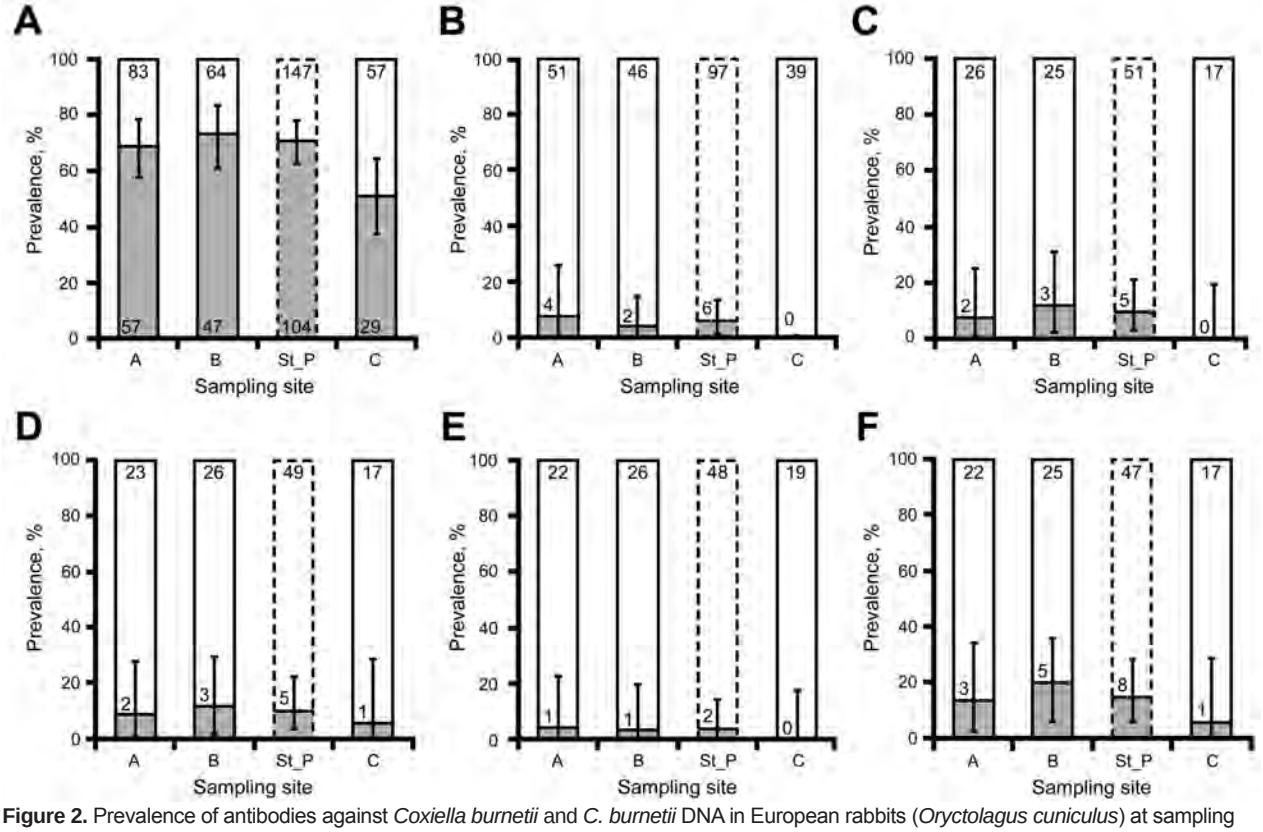

Figure 2. Prevalence of antibodies against *Coxiella burnetii* and *C. burnetii* DNA in European rabbits (*Oryctolagus cuniculus*) at sampling location LO, Iberian Peninsula. A) Antibodies; B) DNA in spleen; C) DNA in vaginal swab specimen; D) DNA in uterine swab specimen; E) DNA in uterus; F) DNA in reproductive tract (vaginal swab specimen, uterine swab specimen, uterus). Gray bars indicate seroprevalence. St_P indicates results for sites with ruminants (sites A and B); no ruminants were present at site C. Values at the top of bars indicate number of samples, and values at the bottom of bars indicate number of positive samples. Error bars indicate prevalence-associated exact 95% CIs.

confirm the direction, frequency, and magnitude of interspecies interactions favoring transmission of *C. burnetii*.

Indirect transmission of *C. burnetii* between rabbits, humans, livestock, and other wild species may be enhanced in regions with high-density rabbit populations and in regions in which the European rabbit is a major game or farm species. Hunters, game keepers, rabbit farmers, veterinarians, wildlife researchers, livestock producers and livestock might be exposed to *C. burnetii* from rabbits (*6*,*15*). The European rabbit shows a high potential as a reservoir of *C. burnetii* for infection of livestock and humans in Europe.

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Mycobacterium bovis in Panama, 2013

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Panama remains free of zoonotic tuberculosis caused by *Mycobacterium bovis*. However, DNA fingerprinting of 7 *M. bovis* isolates from a 2013 bovine tuberculosis outbreak indicated minimal homology with strains previously circulating in Panama. *M. bovis* dispersion into Panama highlights the need for enhanced genotype testing to track zoonotic infections.

Zoonotic tuberculosis (TB) is a chronic infectious disease of humans caused by transmission of *Mycobacterium bovis* from cattle (*1*). *M. bovis* infection in humans occurs after direct contact with infected cattle, ingestion of unpasteurized dairy products or raw or undercooked meat, or (rarely) person-to-person transmission (*2*). Despite the low incidence of zoonotic tuberculosis in the Americas, accumulating evidence confirms that death rates from *M. bovis* pulmonary infection in specific groups and settings, including in the United States and Mexico, are substantial (*3*,*4*). The risk for death is twice as high for children and persons with HIV co-infection and extrapulmonary TB than for HIV-negative persons with TB (*3*). *M. bovis* infection in cattle (bovine TB) has a major effect on meat and live animal export trade and dairy industry development and expansion (*1*). Thus, bovine TB eradication plans across the Americas are based on the elimination of any cattle with a positive tuberculin skin test (TST) result (*5*).

The most recently reported bovine TB outbreak in Panama occurred in 1997 in the western province of Bocas del Toro. The origin of this outbreak remains unclear. Since 2008 (after the slaughter of ≈7,000 cattle during the 1997 outbreak), Panama has not reported any bovine TB cases to the World Organisation for Animal Health (OIE) (Figure 1) (*6*). However, Panama has not received bovine TB-free accreditation. OIE data show that clinical bovine TB was continually reported from Colombia and Costa Rica during the same period (*6*).

In August 2013, despite active surveillance at country borders and in-country animal health controls, a new bovine TB outbreak in Panama was reported to OIE (*6*). Neither the neighboring countries of Colombia and Costa Rica nor Panama have reported zoonotic tuberculosis to OIE in the past 20 years (*4*,*7*). Among these countries, only Costa Rica does not test *M. tuberculosis* complex isolates to identify *M. bovis*. In contrast, Guatemala continually reports cases of zoonotic TB (*6*). Yet, the genetic biodiversity of *M. bovis* in Central America remains unexplored. Comparisons of mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) and single-nucleotide polymorphism (SNP) analyses based on whole-genome sequencing have proven to be helpful for identifying TB outbreaks elsewhere (*8*,*9*). We characterized and genotyped *M. bovis* isolates that reemerged in Panama during the 2013 outbreak of bovine TB.

The Study

In March 2013, as part of the Panamanian bovine TB control program, TSTs were administered to a dairy herd of 1,680 Jersey cows in Coclé Province, Panama. Animals with TST indurations ≥4 mm in diameter were considered positive. From animals with positive TST results, blood samples were collected for confirmation with an interferon-gamma release assay (BOVIGAM; Prionics, La Vista, NE, USA). Bovine TB was confirmed for 9 animals, 1 of which died naturally and 8 of which were sent to slaughter for postmortem examination and collection of samples for *M. bovis* culture. Epidemiologic investigation revealed that within the past 2 years, all 9 animals had been imported from Guatemala and Costa Rica according to controlled and legal commercial trade protocols. Visible lesions were found on all 8 slaughtered animals. Samples were obtained and sent for culture at the Laboratorio de Diagnóstico e Investigación Veterinaria de Salud Animal del Ministerio de Desarrollo Agropecuario.

Mycobacteria were isolated from 7 animals. Bacterial genomic DNA was extracted, and PCR identified the isolates as *M. bovis* (*10*). DNA from the *M. bovis* isolates was genotyped by 24-loci MIRU-VNTR (*11*). Whole-genome sequencing was performed by using the Illumina MiSeq

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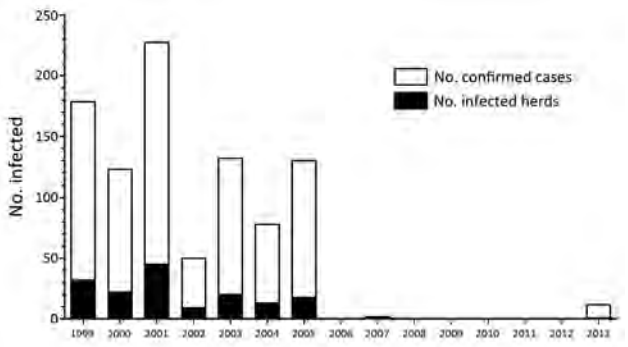

Figure 1. Bovine tuberculosis (TB) in Panama, 1999–2013. Data from the World Organisation for Animal Health (http://www.oie.int/animal-health-in-the-world/the-world-animal-health-information-system/the-oie-data-system/).

platform (*12*), and sequencing reads were aligned on reference genome by using the bowtie2 program (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) and for further SNP calling and variant cell format file processing by using a combination of SAMtools (http://samtools.sourceforge.net) and VCFtools (http://vcftools.source¬forge.net). *M. bovis* genome sequence NC_002945.3 was used as a reference for SNP/indel detection. Sequencing data for the *M. bovis* sequenced genomes were deposited in the National Center for Biotechnology Information Sequence Read Archive (accession no. PRJNA267480). SNP analysis revealed high homology among the 7 *M. bovis* isolates. However, the isolates were not directly related to 2 *M. bovis* isolates available from the 1997 bovine TB outbreak in Bocas del Toro (Figure 2).

Among the 7 isolates, a least four 24-loci MIRU-VNTR *M. bovis* genotypes were identified, including 5 distinct loci sequences (Table, http://wwwnc.cdc.gov/EID/article/21/6/14-1821-T1.htm). These 4 distinct genotypes suggest that bovine TB may have been introduced by at least 4 infected animals. The *M. bovis* strains obtained from animals imported from Guatemala were clustered according to MIRU-VNTR genotypes (Table). Similarly, MIRU-VNTR genotypes for *M. bovis* isolates obtained from 3 animals imported from Costa Rica were more closely related. The 2 *M. bovis* isolates from the 1997 Bocas del Toro outbreak shared the same MIRU-VNTR genotypes with those from the recent outbreak. The molecular clock speed for this genotyping tool may account for the lack of differentiation in this last comparison, but we cannot rule out endemic spread of the same strain in this herd.

SNP analysis revealed high genetic similarity among all 7 *M. bovis* isolates from the 2013 bovine TB outbreak. In contrast, we found no direct relation between *M. bovis* isolates from the 2013 and 1997 outbreaks. Because all 7 isolates were grouped in 2 branches, it is possible that they were derived from a common ancestor in Central America. A comparison of the SNPs from the 7 *M. bovis* isolates from the 2013 outbreak with 2 *M. bovis* isolates from the 1997 Bocas del Toro outbreak showed no close relationships. Adjacent countries have often reported *M. bovis* strains with highly similar genotypes, but geographically distant countries have not (*13*).

We hypothesize that similar genotypes are being distributed in Central America because of augmented commercial trade between neighboring countries. Coincidentally, since January 2013, importation of cattle from Nicaragua, the United States, and Costa Rica into Panama for dairy and meat production has increased by ≈300%. Unfortunately, whole-genome sequencing data for *M. bovis* isolates from Central America are not available for validation of this hypothesis. Determination of the genetic structure of all *M. bovis* strains circulating in Central America will require further multicenter studies.

Conclusions

The diagnosis of bovine TB in Panama requires urgent attention. Accumulated genetic changes in *M. bovis* isolates from 2 outbreaks occurred 15 years apart (*8*). Currently in Latin America, use of the TST remains the preferred method of identifying animals with bovine TB, despite its sensitivity range of 68% to 95% (*14*,*15*). In addition, several factors can lead to false-positive and false-negative TST results (e.g., purified protein derivative antigen quality and

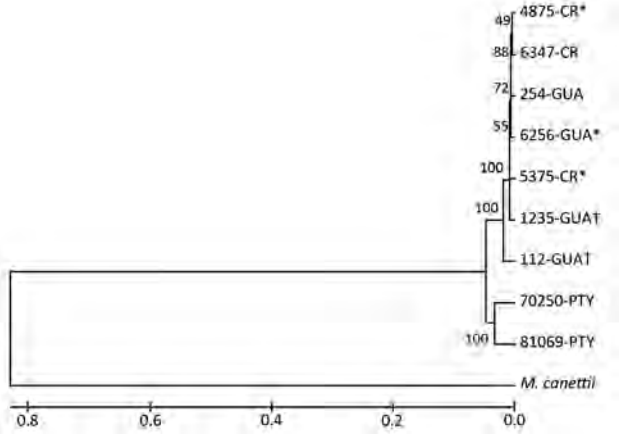

Figure 2. Phylogenic placement of the *Mycobacterium bovis* isolates. The *M. bovis* AF2122/97 sequence was used as a reference for single-nucleotide polymorphism analysis (GenBank accession no. NC_002945.3).The tree was derived from an unweighted pair-grouping method analysis algorithm by using DNA fragment sequence analysis. Numbers on branches represent bootstrap percentages from 500 replicates. *M. canettii* was used as the outgroup. Evolutionary analyses were performed by using MEGA6 software (http://www.megasoftware.net). Symbols indicate *M. bovis* isolates with identical genotypes according to mycobacterial interspersed repetitive units–variable number of tandem repeats. Scale bar indicates mean distances between strains according to base substitutions (%). CR, Costa Rica; GUA, Guatemala, PTY, Coclé, Panama.

manipulation, skin induration interpretation, and injection dose protocols) (*15*). In contrast, interferon-gamma release assays are expensive and cost-prohibitive for rural farmers. For detection and quantification of the disease, novel and low-cost biomarker-based tests are needed; they would enable proper identification and disposal of diseased animals to prevent new outbreaks of zoonotic TB. After bovine TB is diagnosed, whole-genome sequencing and MIRU-VNTR can differentiate *M. bovis* lineages and identify patterns of bovine TB transmission across Central America.

Our study provides the baseline genotypes and sequences of *M. bovis* strains involved in the 2013 outbreak of bovine TB in Panama. These data could serve as a reference to determine future sources of zoonotic *M. bovis* infection and help track the movement of *M. bovis* strains between Central American countries. Together these strategies will reinforce international bovine TB control and eradication efforts.

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Drug Resistance–Associated Mutations in *Mycoplasma genitalium* in Female Sex Workers, Japan

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Mycoplasma genitalium was detected in 21 (14.1%) of 149 vaginal swab samples and in 1 (0.7%) of 149 throat washing samples from female sex workers during 2013–2014 in Japan. Prevalences of *M. genitalium* with macrolide resistance–associated 23S rRNA mutations and fluoroquinolone resistance–associated *parC* alterations were 47.1% and 36.8%, respectively.

For *Mycoplasma genitalium* infections, azithromycin regimens have been considered first-line treatments, and fluoroquinolone regimens, including those of moxifloxacin and sitafloxacin, have been effective second-line treatments (*1*). However, the proportion of *M. genitalium* harboring macrolide or fluoroquinolone resistance–associated mutations has been increasing in male and female patients with *M. genitalium* infections (*2*–*5*), and treating *M. genitalium* infections with current antimicrobial chemotherapies is increasingly difficult (*5*,*6*).

The prevalence of *M. genitalium* infections in women at low risk for sexually transmitted infections (STIs) is reportedly 2.0%, with the range for most cohorts being <1%–5%. In high-risk populations, the prevalence is 0%–42% (*7*). For female sex workers (FSWs), the range of *M. genitalium* prevalence rates is reportedly 12%–26% (*7*–*10*). FSWs could be a reservoir of *M. genitalium* infections, but little is known about drug resistance in *M. genitalium* in FSWs.

In this study, vaginal swab and throat washing samples collected from 149 FSWs were examined for the presence of *M. genitalium*. Positive specimens were then tested for drug resistance–associated mutations in the *M. genitalium* DNA.

The Study

This cross-sectional prospective study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Japan (reference number 22–11). A total of 149 FSWs who attended Hoshina Clinic, Kyoto, Japan, for regular screening for STIs from August 2013 through January 2014 were enrolled in this study after informed consent was obtained. The women were 19–47 years of age (mean 29 years). All performed fellatio on their clients without use of condoms. Six (4.0%) had received antimicrobial drug treatment (i.e., azithromycin, clarithromycin, ceftriaxone, or amoxicillin) for gonococcal or chlamydial infections during the 3 months before visiting the clinic. Sixty-five (43.6%) had histories of STIs, including gonococcal infections, chlamydial infections, genital condyloma, genital herpes, and syphilis. Other sociodemographic information, sexual history, or HIV serologic status was not obtained from most participants. At clinic visits, all were asymptomatic. On genital examination, however, genital herpes was found in 1 (0.7%), and mucopurulent vaginal discharge was found in 3 (2.0%).

Vaginal swab and throat washing samples were collected from all 149 women, as previously recommended (*11*). These specimens were tested by using Cobas 4800 CT/NG (Roche Molecular Systems, Pleasanton, CA, USA) to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. The specimens were also tested for *M. genitalium*, *M. hominis*, *Ureaplasma urealyticum*, and *U. parvum*, as previously recommended (*12*). The DNA specimens were then stored at –80°C. The 1 throat washing positive for *M. genitalium* was tested by PCR with primers specific for the 23S rRNA genes of the genital mycoplasmas, which were used in the PCR-based assay. The PCR product was sequenced, and its sequence was compared to the 23S rRNA genes of *M. genitalium* and *M. pneumoniae* (*13*,*14*).

A total of 6 bacterial species were detected in the samples (Table 1). *M. genitalium* was detected in the vaginal swab samples from 21 FSWs (14.1%, 95% CI 8.5%–19.7%). *M. genitalium* was also detected in a throat washing sample from 1 FSW (0.7%, 95% CI 0%–2.0%), whose vaginal swab sample was negative for *M. genitalium*. The sequence of the PCR product amplified from the DNA from the throat washing specimens aligned with that of the 23S rRNA gene of *M. genitalium* but not with that of *M. pneumoniae*.

The prevalence of *M. genitalium* in vaginal swab samples from the asymptomatic FSWs in this study was similar to that reported in FSWs worldwide (*7*–*10*). The FSWs enrolled in this study were at high risk for pharyngeal STIs.

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Table 1. Bacterial species detected in vaginal swab and throat washing samples from 149 female sex workers, Japan, August 2013–January 2014

Bacterial species	No. (%) specimens positive	
	Vaginal swab	Throat washing
Neisseria gonorrhoeae	7 (4.7)	10 (6.7)
Chlamydia trachomatis	26 (17.4)	11 (7.4)
Mycoplasma genitalium	21 (14.1)	1 (0.7)
Mycoplasma hominis	52 (34.9)	2 (1.5)
Ureaplasma parvum	109 (73.2)	4 (2.7)
Ureaplasma urealyticum	52 (34.9)	5 (3.4)

However, *M. genitalium* was found in only 1 throat washing sample and was not detected in throat washing samples obtained from 403 FSWs in our previous study (*11*). The prevalence of *M. genitalium* in the genitalia of FSWs would be expected to be high, whereas the prevalence of mycoplasma in the pharynx has been extremely low.

For the 21 vaginal swab samples and 1 throat washing sample that were positive for *M. genitalium* from the 22 FSWs, the portion of the 23S rRNA gene, including A-2058 and A-2059 in the 23S rRNA gene of *Escherichia coli*, and the region corresponding to the quinolone resistance–determining regions of the *E. coli gyrA* and *parC* genes were amplified from the stored DNA specimens by PCR, and sequencing of the PCR products was performed, as reported previously (*4*). The 6 FSWs to whom antibiotics had been administered 3 months before this study were not included in these 22 FSWs.

Table 2 shows the results of analyses of these 22 specimens for the drug resistance–associated alterations. Five vaginal swab specimens and the 1 throat washing specimen could not be analyzed because their stored DNA yielded no reliable PCR products. The storage of the frozen specimens likely had degraded the quality of the DNA, or the specimens might have contained low bacterial loads of *M. genitalium*. However, for samples for which genes could be analyzed, 8 (47.1%, 95% CI 23.4%–70.8%) of 17 vaginal swab samples had macrolide resistance–associated 23S rRNA mutations, and 7 (36.8%, 95% CI 15.1%–58.5%) of 19 samples had fluoroquinolone resistance–associated *parC* alterations. For 21 vaginal swab samples, no fluoroquinolone resistance–associated *gyrA* alterations were found. Four of 16 vaginal swab samples that could be analyzed for the 23S rRNA *gyrA* and the *parC* genes showed drug resistance–associated alterations in both genes (25.0%, 95% CI 3.8%–46.2%).

In Australia and the United Kingdom, the proportions of *M. genitalium* harboring macrolide resistance–associated mutations in clinical specimens from male and female patients with *M. genitalium* infections ranged from 36.1% to 43.4% (*2*,*3*,*5*), but proportions of the mycoplasma harboring the fluoroquinolone resistance–associated amino acid changes in *gyrA* or *parC* ranged from 4.5% to 15.4% (*2*,*3*). For Japan, we reported that drug resistance–associated 23S rRNA mutations and *parC* alterations were observed in

Table 2. Mutations in the 23S rRNA gene and amino acid changes in GyrA and ParC in *Mycoplasma genitalium* detected in female sex workers, Japan*

Female sex worker	Specimen type	Mutations in the 23S rRNA gene	Amino acid changes in	
			GyrA	ParC
1	Vaginal swab	A-2058→G	–	–
2	Vaginal swab	NA	–	–
3	Vaginal swab	–	–	–
4	Vaginal swab	–	–	–
5	Vaginal swab	A-2058→G	–	–
6	Vaginal swab	A-2059→G	–	Ser-80→Ile
7	Vaginal swab	NA	–	NA
8	Vaginal swab	–	–	–
9	Vaginal swab	–	–	–
10	Vaginal swab	–	–	–
11	Throat washing	NA	NA	NA
12	Vaginal swab	–	–	–
13	Vaginal swab	NA	–	–
14	Vaginal swab	A-2059→G	–	Ser-80→Ile
15	Vaginal swab	A-2058→G	–	Ser-80→Asn
16	Vaginal swab	NA	–	Ser-80→Ile
17	Vaginal swab	–	–	Ser-80→Ile
18	Vaginal swab	–	–	–
19	Vaginal swab	–	–	Ser-80→Asn
20	Vaginal swab	A-2058→G	–	Ser-80→Asn
21	Vaginal swab	A-2058→G	–	–
22	Vaginal swab	A-2058→G	–	NA

5 (29.4%) and 8 (47.1%), respectively, of 17 first-voided urine specimens from men with *M. genitalium*–positive nongonococcal urethritis in 2013 (*4*). The present study suggests that macrolide- and fluoroquinolone-resistant strains of *M. genitalium* will be emerging and spreading in asymptomatic FSWs and other patients with *M. genitalium* infections in Japan.

Conclusions

This study has several limitations: the small number of enrolled FSWs, the inability to analyze all specimens for drug resistance–associated mutations, the lack of knowledge of most participants' HIV serologic status, and the lack of longitudinal observations for FSWs with *M. genitalium* infections. Nevertheless, this study suggests that, in addition to the high prevalence of *M. genitalium* in FSWs, the mycoplasmas might frequently harbor macrolide or fluoroquinolone resistance–associated alterations. Several studies have suggested that *M. genitalium* might increase the risk for HIV acquisition in FSWs (*15*). This growing evidence indicates that *M. genitalium* infections should be included in STI control strategies for FSWs.

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Lack of Protection Against Ebola Virus from Chloroquine in Mice and Hamsters

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The antimalarial drug chloroquine has been suggested as a treatment for Ebola virus infection. Chloroquine inhibited virus replication in vitro, but only at cytotoxic concentrations. In mouse and hamster models, treatment did not improve survival. Chloroquine is not a promising treatment for Ebola. Efforts should be directed toward other drug classes.

Chloroquine was first used as an antimalarial drug until widespread resistance in *Plasmodium falciparum* strains emerged. However, for >30 years this drug also has been recognized as having broad-spectrum antiviral properties (*1*), including activity against HIV-1 (*2*); the human coronaviruses, severe acute respiratory syndrome coronavirus (*3*) and OC43 (*4*); dengue virus (*5*); chikungunya virus (*6*); and influenza virus (*7*) in cell culture. Despite these data, chloroquine is not approved for use against any viral infections.

Previous in vitro data state a half maximal effective concentration (EC_{50}) and EC_{90} of 16 and 25 μmol/L for chloroquine against Ebola virus (EBOV), respectively (*8*). Twice daily dosing at 90 mg/kg intraperitoneally rapidly achieved a steady-state concentration of 2.5 μg/mL in the blood of mice. This dosing regimen resulted in survival of 85% of mice after infection with mouse-adapted (MA) EBOV (*8*). These data have led to the suggestion that chloroquine and its derivatives be used in persons with EBOV infection because this drug is approved for use in humans, has an extensive safety profile, and is inexpensive (*1,9*). To determine whether protection would extend to the EBOV hamster model, during 2013–2014 we investigated chloroquine treatment in this model and attempted to repeat previous in vitro findings and findings in the mouse model.

The Study

Vero E6 cells were infected with 100 focus-forming units of EBOV expressing enhanced green fluorescent protein. After a 1-h incubation, the inoculum was removed and replaced with media (Dulbecco's modified Eagle's medium with 2% fetal bovine serum, Penn/Strep, L-glutamine) containing chloroquine (Sigma, St. Louis, MO, USA). The supernatant was collected on days 1, 3, 5, 7, and 9 after infection and media replaced with fresh drug. Viral RNA was extracted from the supernatant and quantified by real-time quantitative reverse transcription PCR as previously described (*10*). Concurrently, cell viability was assayed by using Cell Titer96 Aqueous One Solution (Promega, Madison, WI, USA) according to the manufacturer's instructions. EC was determined by using Prism6 (GraphPad Software, San Diego, CA, USA).

When added 1 h after infection, chloroquine at 5 μg/mL and 25 μg/mL reduced the viral loads by 0.61 and 1.07 logs, respectively (peak reduction observed on day 5), without any significant cytotoxicity (Figure 1). Analysis of the data from day 5 resulted in an EC_{50} of 1.77 μg/mL and an EC_{90} of 23.34 μg/mL, concentrations that are comparable with previous data (*8*); however, reductions in viral loads at these concentrations at other time points were negligible. Although concentrations of ≥50 μg/mL of chloroquine reduced viral loads by 2–4 logs starting on day 3, this decrease was accompanied by a high level of cytotoxicity (≥50%) that was evident both in the cytotoxicity assay and microscopically resulting in poor selectivity of chloroquine.

Six-week-old BALB/c mice or Syrian hamsters (both from Harlan, Indianapolis, IN, USA) were inoculated intraperitoneally with 100 50% lethal dose of MA EBOV. The mouse (*11*) and the hamster (*12*) are well-established disease models of EBOV infection. Treatment was initiated 1 h after inoculation. Treatment groups (mice and hamsters) received 90 mg/kg of chloroquine alone (intraperitoneally). Vehicle groups received the equivalent volume of sterile saline (intraperitoneally). Mock-infected animals received sterile tissue culture media in place of MA EBOV. An additional group of hamsters received 50 mg/kg of chloroquine (intraperitoneally every 24 h) in combination with 2.5 mg/kg doxycycline (gavage every 12 h) and 50 mg/kg azithromycin (intraperitoneally every 24 h). After inoculation, animals were monitored at least twice daily and euthanized by using a humane endpoint scoring criteria as approved by the Animal Care and Use Committee at Rocky Mountain Laboratories (Hamilton, MT, USA). Analysis of survival was performed in Prism6 (GraphPad).

Two of 3 mock-challenged mice did not survive because of chloroquine (90 mg/kg) treatment alone (Figure 2, panel A). Only 2 of 9 mice infected with MA EBOV and

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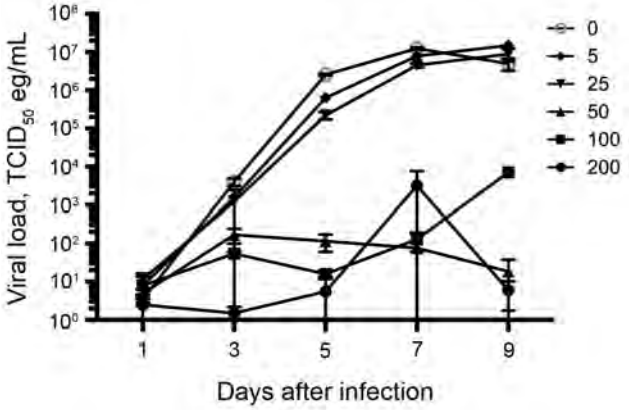

Figure 1. Viral loads from supernatants derived from Vero cells infected with Ebola virus expressing enhanced green fluorescent protein and treated with chloroquine at the indicated concentrations (0, 5, 25, 50, 100, and 200 μg/mL). $TCID_{50}$, 50% tissue culture infectious dose.

treated with chloroquine survived, and 1 of 9 mice infected with MA EBOV and treated with vehicle survived. With median survival of 7, 8, and 8 d for mock-challenged/chloroquine-treated mice, MA EBOV–infected/chloroquine-treated mice, and MA EBOV–infected/vehicle-treated mice, respectively, treatment had no significant effect on survival. This dose, although previously stated as the maximum tolerated dose in mice (*8*), was not well tolerated by the animals in this study and clearly did not improve survival in animals challenged with MA EBOV.

When the same dose (90 mg/kg) of chloroquine was given to hamsters challenged with MA EBOV, the study had to be terminated on day 2 after treatment. Nearly all the treated animals, in both the MA EBOV and the mock-challenged groups, died of acute toxicity after administration of chloroquine intraperitoneally, typically within 30 min after treatment (Figure 2, panel B).

In a separate study, hamsters were treated with chloroquine (50 mg/kg) in combination with doxycycline (2.5 mg/kg) and azithromycin (50 mg/kg) to additionally provide broad-spectrum antimicrobial drug coverage. Reperfusion injury of the gut after EBOV disease, which would subsequently result in bacterial sepsis, has been suspected as a possible cause of death. Thus, broad-spectrum antimicrobial drugs were proposed to help in this regard. In this study, no toxicity was observed in the mock-challenged group as a result of the combination treatment. This finding suggests that hamsters tolerate this dose of chloroquine. However, treatment had no effect on survival; no combination-treated or vehicle-treated groups survived, and median survival times were comparable (Figure 2, panel C).

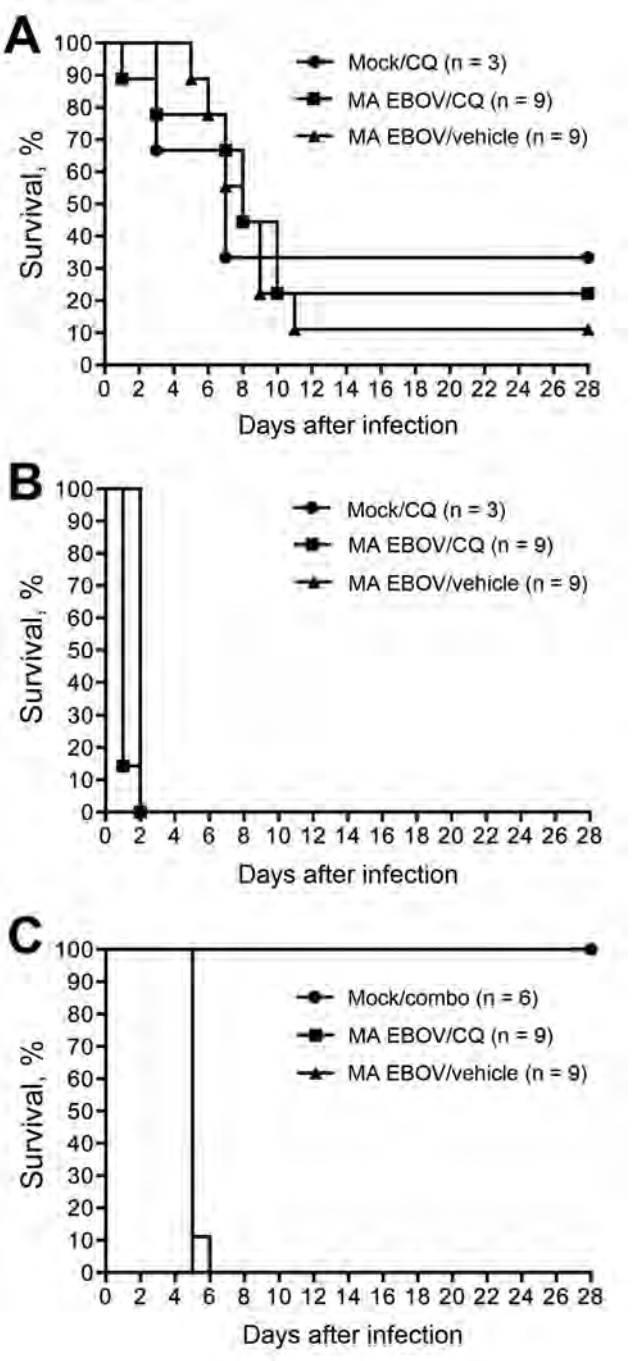

Figure 2. Survival of MA EBOV-inoculated mice (A) and hamsters (B) treated with CQ (90 mg/kg). C) Survival of MA EBOV–infected hamsters treated with a combination of CQ (50 mg/kg), doxycycline (2.5 mg/kg), and azithromycin (50 mg/kg). Combo, combination of chloroquine, doxycycline, and azithromycin; CQ, chloroquine; EBOV, Ebola virus; MA, mouse-adapted.

Conclusions

Despite some activity of chloroquine against EBOV in vitro, we observed no benefit to its administration in the mouse and hamster models. In the mouse model, a dose of 90 mg/kg resulted in toxicity but did not alter survival; therefore, higher concentrations of chloroquine in the mouse would not be expected to be possible. In the hamsters, this dose

was already lethal on its own. In the hamster model at a lower dose (50 mg/kg) combined with doxycycline and azithromycin—which together provide broad-spectrum antimicrobial coverage, in addition to doxycycline having a small antiviral effect against EBOV—survival did not change. Previous anecdotal reports of the incidental use of chloroquine in patients with filovirus infections also do not support any benefit from its use (*13*,*14*). Together, these data suggest that chloroquine is unlikely to provide any protection from EBOV infection in humans.

Given its in vitro activity against many different viruses and its longstanding use in humans, chloroquine has been put into multiple clinical trials. During dengue virus infection, viremia did not decrease (*15*), and chloroquine neither prevented influenza virus infection (*7*) nor improved outcome of chikungunya virus infection (*6*) despite promising in vitro activity against these viruses.

When taken together with previous findings for other less pathogenic viruses, the clinical use of chloroquine seems unlikely to provide any benefit for either prophylaxis or treatment of EBOV. Moreover, chloroquine has a small therapeutic window; dosing for treatment of acute malaria is ≈15 mg/kg, and lethality starts at 50 mg/kg. Thus, current preclinical data do not support the continued consideration of chloroquine for use against EBOV infections in humans.

Acknowledgments

We thank the animal care technicians for maintaining the animals used in this study.

This study was performed in accordance with the recommendations described in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, the Office of Animal Welfare, and the US Department of Agriculture. The Institutional Animal Care and Use Committee at Rocky Mountain Laboratories (RML) approved animal work. RML is an American Association for Accreditation of Laboratory Animal Care–approved facility. Trained personnel carried out all procedures with the animals under isoflurane anesthesia. The Institutional Biosafety Committee at RML approved all work with infectious EBOV under biosafety level 4 conditions.

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Dr. Falzarano, a research scientist at the University of Saskatchewan, performed this work while a visiting fellow at Rocky Mountain Laboratories. His research interests include determining post-translational modifications on EBOV glycoproteins and antiviral strategies for severe virus infections.

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Wohlfahrtiimonas chitiniclastica Bacteremia Associated with Myiasis, United Kingdom

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To the Editor: We report the identification of *Wohlfahrtiimonas chitiniclastica* bacteria in a sample of blood obtained from a patient in Surrey, United Kingdom. We highlight the importance of recognizing unusual bacteria that are associated with the larvae of parasitic flies as a potential causative agent of severe infection in patients with myiasis in the United Kingdom and possibly worldwide.

The patient, an 82-year-old woman, was found collapsed in her garden with maggots covering her body and hair. Skin excoriations over her neck, face, and head showed superficial tissue breakdown in keeping with proteolytic enzyme secretions of maggots. The woman may have been lying outside for 72–96 hours. She had a history of recurrent falls, hypertension, chronic kidney disease, ischemic heart disease, hypercholesterolemia, and osteoarthritis.

Blood analysis showed a marked inflammatory response. The patient had a C-reactive protein level of 157 mg/L (reference <10 mg/L); leukocyte count of 15.56 × 10^9 cells/L (reference 4.0–11.0 × 10^9 cells/L); predominant neutrophilia; and evidence of rhabdomyolysis. She had persistent acute kidney injury; her creatinine level was 131 μmol/L (reference 49–90 μmol/L), and her urea level was 23.3 mmol/L (reference 2.5–7.8 mmol/L). Her serum lactate level was 2.5 mmol/L (reference 0.6–2.2 mmol/L), suggesting sepsis.

Intravenous antimicrobial drug therapy with cefuroxime (750 mg 3×/d), metronidazole (500 mg 3×/d), and clarithromycin (500 mg 2×/d) was continued for 7 days, followed by oral flucloxacillin (500 mg 4×/d). Topical chloramphenicol and fusidic acid were applied to ear canals. Superficial maggots were manually removed; however, larvae continued to emerge from the patient's inflamed ear canals, requiring otoscopic removal and cleaning of her ears. Larvae were identified as the third instar of *Lucilia sericata*, the common green bottle fly. *W. chitiniclastica* bacteria, which were isolated from cultures of blood samples obtained on admission, were identified by using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Billerica, MA, USA), a tool for rapid identification of uncommon microorganisms, and confirmed by using 16S rRNA sequencing. Two *W. chitiniclastica* reference isolates were in the MALDI-TOF database at time of testing; scores matched to our isolate were 2.264 and 2.200, indicating a match to species level. We could not isolate *W. chitiniclastica* from swab specimens of superficial lesions or ear swab specimens.

Blood culture samples grew a mixture of *Proteus mirabilis, Providencia rettgeri*, and *Staphylococcus aureus*. The patient made good clinical recovery and was later discharged to a local rehabilitation unit.

Previous case reports from Argentina and the south of France of bacteremia caused by *Wohlfahrtiimonas* spp. involved homeless persons with histories of alcohol abuse, 1 of whom was infested with insect larvae (*1*,*2*). One of these patients died from sepsis. *W. chitiniclastica* is known to colonize at least 2 species of flies but is not reported in *Lucilia* sp. This bacterium has been isolated from larvae of the fly *Wohlfahrtia magnifica,* a serious parasite of livestock in eastern Europe, the Mediterranean, and Central Asia (*3*), but this fly is not usually seen in the United Kingdom. *W. chitiniclastica* has also been isolated in China from *Chrysomya megacephala* oriental latrine flies, a screwworm species common in tropical and subtropical regions that is a facultative cause of myiasis (*4*,*5*). A study from South Korea reported a new *Wohlfahrtiimonas* sp. isolated from the larval gut of *Hermetia illucens*, the black soldier fly, although this fly is not pathogenic (*6*).

L. sericata is a blowfly that is common across much of the world. Although it usually feeds on dead or necrotic tissue, it can invade healthy tissue and is the cause of sheep blowfly strike (i.e., cutaneous myiasis) in otherwise healthy livestock. This organism has a role in forensic investigations and is used in health care settings for larval debridement of necrotic tissue from wounds and ulcers (*5*). The woman in our study had myiasis (i.e., infestation) with some invasion of healthy tissue and tissue damage from enzymes secreted by the larvae.

In this case, use of MALDI-TOF mass spectrometry enabled rapid identification of a rare bacterial species (*7*) in a patient with myiasis; slower molecular methods were previously required for such diagnoses. Without local availability of this technology, considerably more time would have been required for the diagnosis. Previous lack of identification of this species may be due to the former shortage of *W. chitiniclastica* isolates in the MALDI-TOF database.

This case demonstrates association of *W. chitiniclastica* with myiasis, although the pathogenic role in this clinical situation is uncertain. It is difficult to ascribe the clinical symptoms solely to bacteremia caused by this organism because multiple organisms were isolated. The cultures might have been heavily contaminated, but this would still highlight an association between *L. sericata* and *W. chitiniclastica.* Although we did not test the extracted fly larvae

for *W. chitiniclastica,* we believe it is likely that the bacteremia originated from the patient's inner ear infestation. *L. sericata* may be a vector for this microorganism in the United Kingdom, and possibly worldwide, given this fly's widespread habitat.

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Response to Detection of New Delhi Metallo-β-Lactamase–Producing Bacteria, Brazil

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To the Editor: New Delhi metallo-β-lactamase (NDM) is an example of a successful antimicrobial drug resistance determinant and has become one of the most clinically significant carbapenemases. The gene bla_{NDM} was first described in India in 2009. Its dispersion is epidemiologically linked to the Indian subcontinent, from which increased international transmission has been detected in nosocomial, community, and environmental isolates (*1*). Currently, the main acquired carbapenemases around the world are *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and NDM. KPC is broadly detected and endemic to some areas; OXA-48 has been widely disseminated throughout European countries and has been reported in other regions. NDM is reported almost worldwide but did not successfully spread in most countries of Europe except the United Kingdom and recently, France, as has been found in *Enterobacteriaceae* (*2*) and in nonfermenting gram-negative bacilli, with progression toward rapid global prevalence.

NDM producers were detected most recently in South America (*3*). However, an increase in cases of NDM-producing bacteria has been noted. Carvalho-Assef et al. (*4*) described characterization of NDM in Brazil, in *Providencia rettgeri* isolated from a tissue sample excised from a patient in a hospital in Rio Grande do Sul state in southern Brazil, in 2013. Reports by Carvalho-Assef et al. (*5*) and Rozales et al. (*6*) have highlighted that *P. rettgeri* and isolates from the *Enterobacter cloacae* complex, clonally and nonclonally related, have been increasingly detected in the southern region of Brazil. In addition, retrospective studies have shown that NDM-1–producing *Enterobacter* have been present in Brazil since 2012 and have also been detected in Rio Grande do Sul (*5*). NDM-1–producing *Morganella morganii* (*6*), *Escherichia coli*, *Klebsiella pneumoniae* (*7*), *Acinetobacter baumannii* (*8*), and *Citrobacter freundii* (J. Campos, et al., https://www.escmid.org/escmid_library/online_lecture_library/?search=1&current_page=1&search_term=Citrobacter+freundii+NDM) have also been reported. Initial reports from Brazil also indicate that NDM producers have displayed characteristics such as co-resistance (*5*,*9*,*10*) and heteroresistance (*11*), but to date, occurrence in the community has not been reported. NDM producers were originally detected in the southern and southeastern regions of Brazil and have since moved into the northern states.

Brazil is a country of extremes that has industrialized and nonindustrialized regions, and this situation converges with social, economic, and infrastructure problems (e.g., sanitation and health care public services). This scenario is similar to the initial conditions that contributed to worldwide dissemination of NDM from the Indian subcontinent. Successful and widespread international high-risk clones and epidemic plasmids have been detected in Brazil and could have a critical role in rapid national expansion of NDM-encoding genes and NDM producers. Brazil is under imminent threat of national spread and prevalence of NDM.

Lessons learned from management of KPC-production bacteria will help infection prevention and control teams, clinicians, and microbiology laboratories in Brazil more effectively manage patients with infections caused by NDM producers. Moreover, because of knowledge gained associated with global KPC dissemination, including in Brazil, this country is better prepared to face the other β-lactamases emerging worldwide. Initiatives of regional agencies and the Brazilian Health Surveillance Agency include dissemination of risk alerts to health professionals and guidelines for technical standardization and management of multidrug-resistant bacterial infection. These alerts focus on NDM producers and specific prevention and control measures; therapeutic orientation; and interpretive criteria for the assessment of bacterial antimicrobial drug susceptibility. These efforts are aimed at rapid microbiology detection and clinical and epidemiologic measures to control infections caused by NDM producers and bla_{NDM} dissemination (e.g., using simple tests to detect carbapenemases, searching for colonized patients, and evaluating/monitoring hospital discharge of patients after NDM infection).

Another type of mobilization was promoted during the 27th Brazilian Congress of Microbiology, 29 September–3 October, 2013 in Natal, Brazil, in which a special symposium was dedicated to discussing and improving NDM counterattacks (http://www.sigeventos.com.br/sbmicrobiologia/admin/pro_lista_programa.asp?eveId=5&tipId=22 [in Portugese]). This symposium gathered a team of experts, including government representatives, to try to minimize the spread and effect of the entry of NDM into Brazil, as well as the future complications and consequences of national dissemination

These efforts are crucial and have strategic significance that affect major events in Brazil, such as the 2014 FIFA World Cup, which brought people from all around the world to all regions of Brazil, and the upcoming Games of the XXXI Olympiad, which will occur in Rio de Janeiro in August 2016. Also of importance to this issue are the geopolitical and economic characteristics of Brazil. Because of Brazil's global influence related to national and international movement of people and products, broad prevalence of NDM producers in Brazil could contribute to acceleration of the global spread of bla_{NDM}. Microbiologists, clinicians, and their respective organizations, as well as the government of Brazil, through its health and disease control agencies, should continue to strive to contain NDM dispersion and limit the possible global impact of the spread and prevalence of NDM producers in Brazil.

This letter was based on our observations and reflections regarding antibiotic-resistant bacteria during studies supported by funding agencies: research grant 2012/14740-3, São Paulo Research Foundation (FAPESP). L.N. Andrade was supported by a postdoctoral fellowship, grant 2011/08892-2 and grant 2013/26188-6, São Paulo Research Foundation (FAPESP).

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Histoplasmosis in Idaho and Montana, USA, 2012–2013

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To the Editor: Histoplasmosis occurs after infection with the dimorphic fungus *Histoplasma capsulatum* (*1*–*6*). Patients become ill after they inhale soil contaminated with *H. capsulatum* (*1*,*2*). Most infections are asymptomatic or result in mild illness not determined to be histoplasmosis (*1*,*2*). Symptoms usually develop 3–14 days after exposure and range from self-limited pneumonia to severe disseminated disease requiring antifungal therapy (*2*,*7*).

In the United States, *H. capsulatum* is endemic to the Mississippi and Ohio River Valleys (*1*,*2*,*5*,*8*) but is not known to be endemic to the Rocky Mountain region (*8*). During June 2012–November 2013, a total of 6 unrelated cases of histoplasmosis were reported in Idaho (n = 1) and Montana (n = 5) in patients who had no recent travel to recognized *H. capsulatum*–endemic regions. Public health authorities investigated the illnesses by reviewing medical records and collecting exposure and travel histories.

The median age of the patients (3 male, 3 female) was 68 (range 17–79) years (Table). Each case was diagnosed by a different physician; no known epidemiologic links existed among the patients. Five patients had ≥1 immunocompromising conditions (Table), and 2 had acute pneumonia; 1 each had left parotid gland enlargement, anterior cervical lymphadenopathy, tricuspid valve mass, and acute changes in mental status. Three patients were hospitalized: 2 required intensive care, and 1 died.

Histoplasmosis was diagnosed primarily on the basis of culture (n = 2), urine enzyme immunoassay (EIA) (n = 2), and histopathologic examination (n = 2) results; histopathologic examinations were conducted by 2 pathologists

Table. Characteristics of 6 persons with histoplasmosis, Idaho and Montana, USA, 2012–2013*

Characteristic	Value
Sex	
M	3 (50)
F	3 (50)
Median age, y (range)	68 (17–79)
Location of residence	
Idaho, southwestern	1 (17)
Montana	
Eastern	2 (33)
Southwestern	3 (50)
Immunocompromising condition, n = 5†	
Diabetes mellitus, type 2	3 (50)
Hepatitis C	1 (17)
Previous history of breast cancer	1 (17)
Acute mononucleosis	1 (17)
Previous history of colon cancer	1 (17)
Hospitalization	3 (50)
Death	1 (17)
Tests with positive results that contributed to histoplasmosis diagnosis	
Culture	2 (33)
Histopathology‡	2 (33)
Urine enzyme immunoassay‡	2 (33)
Diagnosis delayed >6 mo	3 (50)
At-risk activities	
Using potting soil containing bat guano	1 (17)
Exploring caves	1 (17)
Mowing grass in pasture	1 (17)
Cleaning pigeon cages	1 (17)
Traveling to an area where the disease is endemic <3 y of illness onset	0
None known	2 (33)

*Values are no. (%) patients except as indicated.
†One patient had diabetes mellitus type 2 and hepatitis C; 1 patient had diabetes mellitus type 2 and a previous history of colon cancer.
‡One patient with a culture positive for *Histoplasma capsulatum* also had histopathology and urine enzyme immunoassay results consistent with *H. capsulatum* infection (results not shown).

(online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/6/14-1367-Techapp1.pdf). One patient with *H. capsulatum*–positive cultures also had positive results by histopathology, serum antigen detection, and urine EIA. Another patient with positive urine EIA results for antigen detection also had low serum levels of *Histoplasma* antibodies measured by compliment fixation. No patient samples were tested by PCR.

The interval between a patient's initial visit to a health care provider and diagnosis ranged from 1 week to 20 months. Diagnosis was delayed >6 months for 3 patients. For 2 patients, a diagnosis was made on the basis of an *H. capsulatum*–positive urine EIA result <4 weeks from illness onset. Four (67%) patients underwent surgical procedures before histoplasmosis was diagnosed.

Each patient reported having traveled to *H. capsulatum*–endemic places, but none had traveled to these areas within 3 years of illness onset. Four patients reported exposures possibly related to infection (1 patient each): handling bat guano–containing potting soil manufactured in California, exploring caves, mowing pasture grass, and cleaning pigeon cages. The exposure to potting soil occurred in California; the other 3 exposures occurred in Montana. Two patients had no identifiable high-risk exposures to *H. capsulatum*.

These 6 patients with histoplasmosis represent potential acute infections and suggest that *H. capsulatum* might exist in Idaho and Montana, a geographic area farther west than areas where the fungus is known to be endemic. Areas of contaminated soils exist in microfoci outside recognized *H. capsulatum*–endemic areas and can be the source of infection for some persons (*6*). Previous studies suggest that the *H. capsulatum*–endemic area might extend into Montana and possibly other states in the Rocky Mountain region (*8*–*10*). Further environmental studies are needed to determine with certainty whether *H. capsulatum* fungi exist in natural environments in the Rocky Mountain region.

Delayed diagnosis of histoplasmosis increases the likelihood of delays in administering effective antifungal therapy. Histoplasmosis was diagnosed in 3 of these patients >6 months after they first sought care, probably because they had reported no recent travel to *H. capsulatum*–endemic areas. Among these 3 patients, none had urine EIA testing for the presence of *H. capsulatum* antigen. The 2 patients who received a diagnosis of histoplasmosis <4 weeks after they first sought care were assessed by using urine EIA. Urine EIA is a noninvasive and sensitive assay with high specificity but is subject to false-positive results in patients with other fungal infections, particularly blastomycosis (*6*), which is not known to be endemic in Montana or Idaho.

Investigation of these 6 histoplasmosis cases was limited because only 2 patients had cultures positive for *H. capsulatum*, and each patient had a remote travel history (≥3 years before infection) to an *H. capsulatum*–endemic area. These limitations raise the possibility that the cases represent reactivation of latent disease or delayed clinical manifestations following a low-inoculum exposure years earlier in an area where the fungus is endemic (*2*,*6*). However, data supporting the possibility that reactivation of latent *H. capsulatum* infection causes acute illness are inconclusive (*6*).

In summary, health care providers should consider a diagnosis of histoplasmosis for Idaho and Montana residents having symptoms consistent with the disease, regardless of whether they have a travel history to recognized *H. capsulatum*–endemic areas. When considering a diagnosis of histoplasmosis, providers should also consider testing with urine EIA, a noninvasive way to assess the presence of *H. capsulatum* infection.

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Seroconversions to Rickettsiae in US Military Personnel in South Korea

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To the Editor: Infections with typhus group rickettsiae (TGR), spotted fever group rickettsiae (SFGR), and scrub typhus group orientiae (STGO) have been reported among persons in South Korea in increasing numbers over the past decade (*1*,*2*). During 2001–2011 in South Korea, 51,825 orientiae group infections were reported (mean incidence 9.95 cases/100,000 residents/year) (*2*). TGR (*Rickettsia typhi*), SFGR (*R. akari, R. japonica, R. monacensis,* and *R. felis*), and STGO (*Orientia tsutsugamusi*) have been identified in their arthropod vectors and reservoirs in northern provinces and at US military training facilities in South Korea (*3*–*5*).

Currently, little data exist on the risk for rickettsioses and scrub typhus for US military deployed to South Korea. Thus, a retrospective serologic investigation to determine the level of exposure to rickettsiae among 9,303 military personnel deployed to South Korea was conducted. The study used de-identified predeployment and postdeployment serum samples made available from the Department of Defense Serum Repository (*6*). The study group consisted of men in combat-related jobs at US military training sites and military installations in South Korea during 1990–1995 while on active duty continuously for ≥1 year. This study protocol was reviewed and approved by the Naval Medical Research Command Institutional Review Board in compliance with all applicable federal regulations governing the protection of human subjects.

Age range of the 9,303 soldiers in the study group was 17–52 (median 24) years. Most (99.6%) were stationed in Dongducheon, Yongtaeri, and Seoul, located in the Gyeonggi and Gangwon provinces in northern South Korea. Primary military occupation specialties were infantryman (58.8%), fighting vehicle infantryman (22.4%), indirect fire infantryman (12.3%), and heavy anti-armor weapons infantryman (6.5%).

The soldiers' postdeployment serum samples (n = 9,303) were screened at a dilution of 1:100 for IgG against TGR, SFGR, and STGO by using group-specific ELISA whole-cell antigen preparations from *R. typhi* Wilmington, *R. conorii* Morrocan, and a mixture of *O. tsutsugamushi* Karp, Kato, and Gilliam, respectively (*7*,*8*). TGR, SFGR, and STGO IgG ELISA titers (range 100–≥6,400) were determined for screen-positive (net absorbance ≥0.500) postdeployment serum samples, and results were compared with matched predeployment serum samples. Samples with a net total absorbance ≥1.000 for serum dilutions 1:100, 1:400, 1:1,600, and 1:6,400 were considered titer positive. The inverse of the highest dilution of titer positive serum that produced a net absorbance ≥0.200 was determined to be the titer. Serum samples from laboratory animals infected with *R. felis* reacted specifically in the SFGR ELISA but not in the TGR ELISA (K.R. Macaluso and A.L. Richards, unpub. data); thus, any soldier infected with *R. felis* would have reacted in the SFGR but not the TGR ELISA.

The postdeployment seropositivity in US military personnel for antibodies against TGR, SFGR, and STGO at a titer ≥100 were 1.3% (117/9,249), 9.0% (805/8,918), and 0.5% (44/9,135), respectively (Figure). Seropositivity occurred for 10 (0.1%), 181 (2.0%), and 15 (0.2%) men who showed evidence of infection (seroconversion or 4-fold rise in antibody titer) with TGR, SFGR, and STGO, respectively, during their deployment to South Korea (Figure). The chance of a soldier having an infection with SFGR was significantly higher than the chance of having an infection with TGR or STGO (χ^2 test, $p<0.05$) (analysis performed in SAS version 9.4; SAS Institute Inc., Cary, NC, USA). For personnel who seroconverted or had a 4-fold rise in

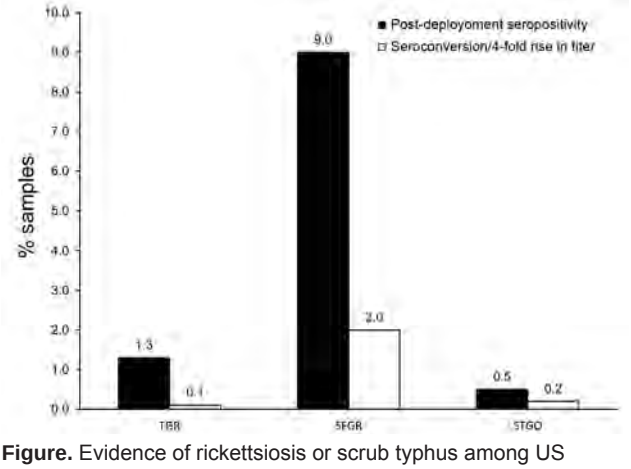

Figure. Evidence of rickettsiosis or scrub typhus among US military personnel deployed to South Korea. Black bars indicate postdeployment serum samples from US military personnel with a titer ≥1:100 (seropositive) to typhus group rickettsiae (TGR), spotted fever group rickettsiae (SFGR), or scrub typhus group orientia (STGO) IgG, as determined by ELISA. White bars indicate personnel determined by paired serum sample analyses to have seroconversion or 4-fold rise in titer between predeployment and postdeployment serum samples, indicating evidence of infection with the corresponding pathogen during deployment.

titer to TGR, SFGR, or STGO, the age range was 19–49 (median 25) years, and job specialties were infantrymen (63.5%), fighting vehicle infantrymen (16.4%), indirect fire infantrymen (14.2%), and heavy anti-armor weapons infantrymen (5.9%).

These results indicate that many US military personnel were exposed to rickettsiae and orientiae before their deployment to South Korea (Figure), perhaps because of previous deployments around the world or because of exposure to rickettsial agents at home (*8*–*10*). However, 206 (2.2%) of the men became infected with either a typhus group (n = 10) or spotted fever group (n = 181) rickettsia or a scrub typhus group orientia (n = 15) during their deployment to South Korea.

More SFGR infections occurred than TGR and STGO infections, although the pathogens for the latter infections (*R. typhi* and *O. tsutsugamushi*) are considered endemic to South Korea and are believed to affect the public and military health more than SFGR (*3*). The SFGR infections might correlate with recent observations of highly prevalent rickettsia-infected tick and *R. felis*–infected flea populations seen in South Korea (*4*,*5*). No evidence of co-infection was found in the men assessed during the deployment. These results suggest a risk for rickettsial disease, including scrub typhus and especially spotted fever, among US military personnel stationed in or visiting South Korea.

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MRSA *spa* t1081, a Highly Transmissible Strain Endemic to Hong Kong, China, in the Netherlands

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To the Editor: Control of methicillin-resistant *Staphylococcus aureus* (MRSA) is an international public health priority. The Netherlands is among countries in Europe that have a low prevalence of MRSA among humans, largely because of a national search and destroy policy (*1*). The overall prevalence in long-term care facilities (LTCFs) is low (*2*). However, this policy is challenged by an increase

in MRSA *S. aureus* protein A (*spa*) t1081, which specifically affects LTCFs. MRSA with the same *spa* type is endemic to Hong Kong, China, and affects hospitals and LTCFs (*3*–*5*). This finding prompted us to jointly explore epidemiologic and strain-related factors.

The low prevalence of MRSA enables the National Institute for Public Health and the Environment (Bilthoven, the Netherlands) to type all first MRSA isolates referred from clinical laboratories in the Netherlands. In 2007, *spa* typing replaced pulsed-field gel electrophoresis typing. The annual number of referred isolates ranged from 1,570 in 2008 to 2,439 in 2013, excluding livestock-associated strains. Numbers of MRSA *spa* t1081 isolates were low during 2007–2009 but increased to 127 isolates in 2011 and 218 isolates in 2013.

The search and destroy policy in the Netherlands requires that detection of MRSA infection is followed by screening of neighboring patients and personnel in successive circles until no new colonizations are found. Most reported t1081 isolates represent colonization. In 2013, there were 30 infection isolates, 19 unknown isolates, and 169 colonization isolates.

Severe illness caused by t1081 is rarely reported, and eradication therapy is usually successful. In LTCFs, MRSA t1081 was more prevalent, accounting for 27% (65/242) and 24% (72/299) of all MRSA isolates from LTCFs in 2011 and 2013 respectively. LTCF clusters were often small, but some became large. The t1081 strain was probably introduced into Amsterdam and subsequently spread eastward (Figure).

We sequenced 5 t1081 isolates, 3 from The Netherlands (NL2007, NL2011, and NL2013) and 2 from Hong Kong (HK2005 and HK2008), by using whole-genome sequencing (Illumina, San Diego, CA, USA). Sequence data showed the CC45/*agr* IV-MRSA V type (*3*,*6*). A total of 91.39% of reads of NL2007 aligned with the published CC45/USA600 sequence (*7*); NL2007 was more similar to HK2005 and

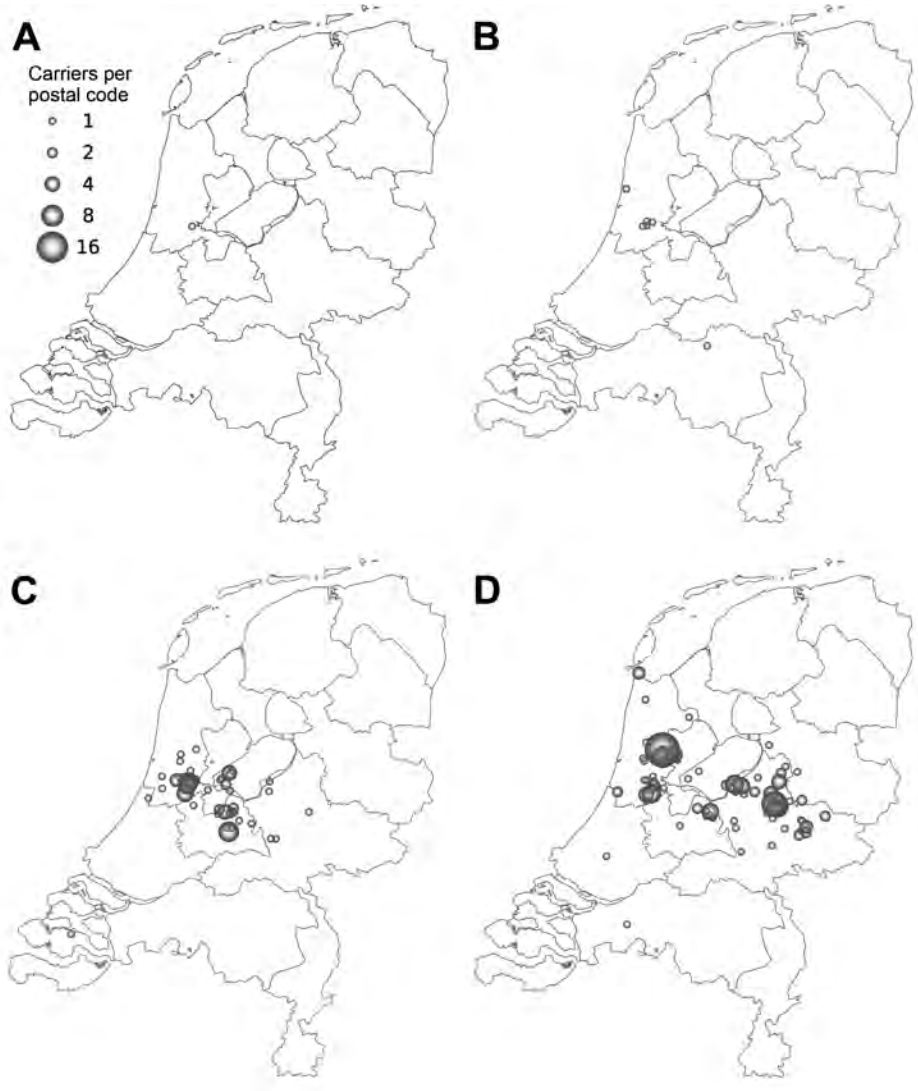

Figure. Spread of methicillin-resistant *Staphylococcus aureus spa* t1081 in the Netherlands, 2007–2013. A) 2007; B) 2009; C) 2011; D) 2013. Source: National Institute for Public Heath and the Environment (RIVM). A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/21/6/14-1597-F1.htm).

HK2008 (97.32% and 97.61% identity, respectively). NL2011 and NL2013 showed slightly decreasing similarity to HK2005 (96.86% and 96.59% identity, respectively) and HK2008 (96.97% and 96.69% identity, respectively). Staphylococcal cassette chromosome *mec* type V sequences in our isolates were more closely related to each other than to the closest reference sequence (GenBank accession no. AB505629), which originated from a CC398 isolate.

Phenotypic resistance to tetracycline and ciprofloxacin is common in t1081 and is often combined with gentamicin and macrolide resistance. *tet*K, a gene coding resistance to tetracycline that is located on the staphylococcal cassette chromosome *mec* element, was detected in reads of all sequenced isolates. The macrolide resistance gene *erm*C on plasmid pKH19 (GenBank accession no. NC_010685.1) was detected in HK2008, NL2011, and NL2013. Resistance to gentamicin (*aac*A/*aph*D genes) was detected in all isolates. A recent report on epidemic MRSA strain 15 based on many whole-genome sequenced strains highlights antimicrobial drug use as an evolutionary driving force (*8*). The *tet*K gene might benefit t1081 in LTCFs in the Netherlands, in which doxycycline is used more frequently than in hospitals (NethMap-MARAN 2014; http://www.swab.nl/nethmap).

None of our isolates was positive for Panton-Valentine leukocidin, and all isolates had the collagen-binding adhesion gene. In methicillin-sensitive *S. aureus*, this gene has been associated with carriage (*9*). The apparent high transmissibility of t1081 remains to be explained.

The present t1081 outbreak has elicited a debate on the policy in the Netherlands. Some elder-care physicians question benefits and costs of this policy for a strain that is weakly pathogenic. Residents in whom MRSA carriage cannot be eradicated face prolonged measures that some physicians say are unethical. Conversely, hospital infection control professionals emphasize that if MRSA can be controlled in hospitals, why not in LTCFs? The search and destroy policy in the Netherlands faced a major challenge in 2001 (*10*). Uncontrolled dissemination of MRSA had occurred throughout a large hospital in Rotterdam among patients and staff, as well as in neighboring institutions. This outbreak was eventually controlled (*10*). The Rotterdam area, which is southwest of Amsterdam, has not been affected by the current t1081 outbreak.

Concurrent with this professional debate, a public debate is ongoing on the quality of care in LTCFs. Residents have more illnesses than a decade ago because of increasingly stringent admission criteria. Skill levels of personnel have not kept pace in several LTCFs, as noted by the Health Care Inspectorate. Although virtually all LTCFs are publicly funded, quality differences are substantial. This situation is no longer acceptable, according to public opinion. A link between insufficient skill levels in specific LTCFs and spread of MRSA can be inferred.

The latest initiative to control multidrug-resistant organisms, including MRSA in LTCFs, is included in an existing program for rapid outbreak reporting and support for hospitals by the National Institute for Public Health and the Environment. This initiative is expected to begin early in 2015 and should facilitate control of MRSA in LTCFs.

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Multibacillary Leprosy in an Active Duty Military Member

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To the Editor: Leprosy (Hansen disease) is caused by *Mycobacterium leprae*, an extremely slow-growing, intracellular, acid-fast bacillus with a typical incubation period ≥2–5 years, ranging up to 20 years. Anesthetic, thickened skin lesions and granulomatous inflammation in biopsy specimens are typical findings because direct visualization of the organism from biopsy specimens is unreliable (*1*).

The worldwide prevalence of leprosy is estimated to be <181,000 cases (annual incidence ≈220,000 new cases in recent years), of which 96% (173,760) occur in 14 countries (*2*). In the United States, ≈150 cases are reported annually, two thirds of which are associated with overseas exposure; the remainder are believed to be domestically acquired (*3*).

We report a case of multibacillary leprosy in a 44-year-old man, an active member of the US military, residing in southern California, USA, who had a 2-week history of fatigue and large, erythematous plaques on the extremities. He was born in the Philippines and resided there until immigrating to the United States at 23 years of age. He subsequently joined the US military and served as an administrator in clinical and microbiology research laboratories. He resided in California, Maryland, Japan, Egypt, Guam, and Indonesia. He also was deployed to Afghanistan and had vacationed in Laos, Cambodia, and Thailand. Family history was unremarkable. No household or other ill contacts were identified.

Initial evaluation showed a weakly positive antinuclear antibody titer, which prompted consideration of cutaneous lupus. Annular skin lesions subsequently developed on his face, limbs, and trunk (Figure, panel A). His fatigue persisted, and further rheumatologic evaluation did not show any unusual results. Dermatologic evaluation showed madarosis, thickening of the glabella, and 8 large annular plaques. Light touch sensation was impaired, but all lesions were hypersensitive to trauma.

A skin biopsy specimen showed perineural lymphohistiocytic inflammation and nonnecrotizing granulomata (Figure, panel B). Results of acid-fast and Grocott's methenamine silver (fungal) staining were negative. A presumptive diagnosis of leprosy was made, and he was referred to the Division of Infectious Diseases at Naval Medical Center San Diego for subspecialty management. Dapsone and rifampin were given for multibacillary leprosy. Clofazamine was not available from the manufacturer at time of treatment. After 6 months of therapy, the patient's lesions were less prominent, and cutaneous sensation had improved. A 2-year treatment course was completed and resulted in total resolution of cutaneous lesions. Residual anesthesia remained only over the right pinna. His course was without complication by either reversal reaction or erythema nodosum leprosum.

Understanding of the transmission of *M. leprae* has been impeded by difficulty cultivating the organism in vitro. Transmission is believed to occur by prolonged exposure to nasal secretions of patients with high bacillary loads. Infection might also result from exposure to cutaneous lesions or animal reservoirs, such as 9-banded armadillos (*3*).

Leprosy has been linked to defects in cell-mediated immunity. Milder disease has been associated with human leukocyte antigen HLA-DR3, and more severe disease has

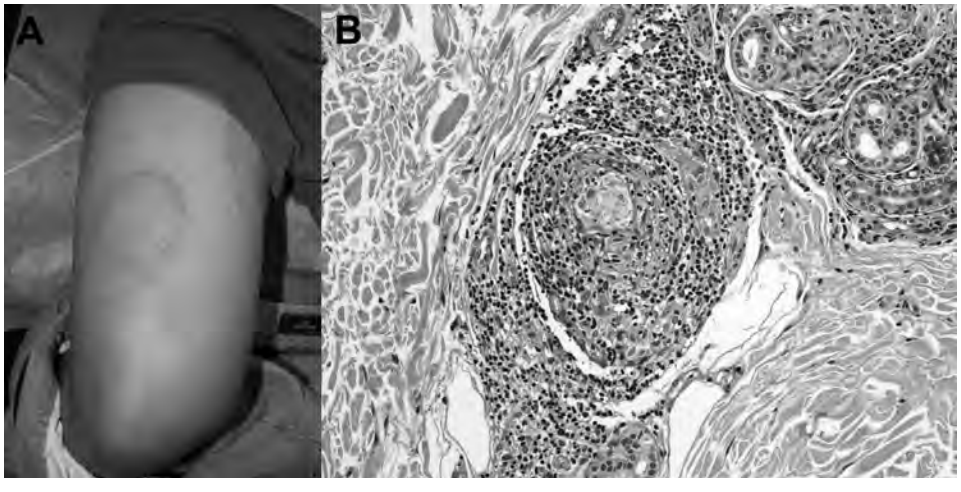

Figure. Physical examination and histopathologic manifestations of leprosy in a 44-year-old man in California, USA (active member of the US military). A) Large, annular, cutaneous plaque on the thigh. B) Skin biopsy specimen showing perineural lymphohistiocytic inflammation and non-necrotizing granulomas (hematoxylin and eosin stained, original magnification ×40). A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/21/6/14-1666-F1.htm).

been associated with HLA-DQ/DR variants (*4*). Although most persons lack susceptibility, high nasal carriage rates in disease-endemic areas and living conditions associated with poverty further increase infection risk for susceptible persons because acquisition is facilitated by malnutrition, overcrowding, and poor sanitation (*5*).

Leprosy treatment is determined according to disease severity. The Ridley-Jopling system assesses lesion quantity, neurologic involvement, and bacterial load, and the current World Health Organization system simplifies this system to facilitate clinical classification, defining paucibacillary leprosy as ≤5 skin lesions and multibacillary leprosy as ≥6 lesions (*6*).

Combination drug regimens for 6–24 months are highly effective. Together with efforts of the World Health Organization toward eradication, combination therapy has dramatically reduced the prevalence to current levels from previously stable levels of 10–12 million in the 1960s–1980s (*7*). Typical regimens include dapsone and rifampin, and clofazimine is available in the United States by investigational new drug application for multibacillary disease.

Patients undergoing treatment must be monitored for immunologic complications, such as cell-mediated reversal reaction (type 1 reaction) or interferon-α–mediated erythema nodosum leprosum (type 2 reaction). Reversal reactions may be especially severe and require urgent immunosuppression to avoid neurologic and vascular complications.

Leprosy is extremely rare in the United States (150 annual cases). Because transmission by prolonged close contact is more common than by casual contact, it is likely that the infection in this patient may have been acquired during childhood in a disease-endemic area, which represents the upper limit of incubation time. However, rare cases have been reported among military members, which makes it difficult to exclude the question of acquisition during military service in disease-endemic areas (*8–10*). Therefore, in patients with geographically appropriate foreign service or prolonged travel history, leprosy must be considered in the differential diagnosis of progressive skin lesions, particularly when lesional anesthesia is present.

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Tickborne Relapsing Fever in Southern Iran, 2011–2013

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To the Editor: Tickborne relapsing fever (TBRF) is endemic in Iran; >1,400 cases were confirmed in 19 provinces during 1997–2006 (*1*). In the western, northwestern, and foothill regions of the Alborz Mountains, the Argasid soft tick *Ornithodoros tholozani* is commonplace and accounts for ≈60% of TBRF cases attributed to *Borrelia persica*. However, in central and western Iran, *O. tholozani* and *B. microti*–infected *O. erraticus* ticks coexist (*1*,*2*). Two other *Borrelia* species, *B. latyschewii* and *B. baltazardi*, have also been described in northeastern and northwestern Iran (*3*,*4*), but no recent human infections with these species have been documented. Cases of TBRF occurring in southern Iran have presumably been caused by *B. microti* because its tick vector, *O. erraticus*, predominates in this region.

Relapsing fever infections in Hormozgan Province in southern Iran are commonly identified during routine checks for malaria. During 2011–2013, blood samples were obtained from 14 febrile patients referred to medical centers in Jask and Rodan in Hormozgan Province

(online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/21/6/14-1715-Techapp1.pdf). Informed verbal consent was obtained from all participants, and the ethical committee of Pasteur Institute of Iran approved the project. Patients seeking care had fever and >1 sign or symptom, such as headache, chills, sweating, or fatigue. Six patients reported recurrent fever and generalized muscle and joint pain. Each patient lived in a local tent, called a kapar, or in a brick or concrete-block house.

Thick and thin blood smears were prepared from blood samples, stained with Giemsa, and examined. None showed malaria parasites; however, spirochetes were observed in thick or thin smears from 3 patients (online Technical Appendix Table). Patients whose samples tested positive by microscopy were treated with 500 mg tetracycline every 6 hours for 10 days and became afebrile.

DNA was extracted from patients' serum samples by using the Miniprep DNA kit (QIAGEN, Hilden, Germany) and screened for borrelia DNA by using real-time PCR; negative and positive control DNA from *B. microti* or *B. persica* was also screened. *Borrelia* spp. DNA was detected in 5 (36%) of 14 serum samples (online Technical Appendix Table). Of these 5 samples, 2 were also positive by nested PCR that targeted the intergenic spacer (IGS) region (*5*). The 2 IGS regions were sequenced (ABI-3130XL sequencer; Applied Biosystems, Foster City, CA, USA) in both directions at the Pasteur Institute of Iran. The resulting 539- and 527-bp IGS sequences (GenBank accession nos. KM271987 and KM271988, respectively) were 97% homologous with *B. recurrentis* and *B. duttonii* from Africa (GenBank accession nos. CP000993 and DQ000280, respectively); 96% homologous with *B. microti* from Iran (GenBank accession no. JQ436580); and 92% homologous with *B. crocidurae* from Africa (GenBank accession no. GU350723). A neighbor-joining phylogenetic tree was constructed by using MEGA6 (http://www.megasoftware.net); the 2 IGS sequences clustered into a distinct group separate from *B. microti*, *B. duttonii*, and *B. recurrentis* genotypes (Figure).

B. microti was expected to be found because *O. tholozani* ticks that transmit *B. persica* are not seen in southern Iran, but *B. microti*–infected *O. erraticus* ticks have been frequently recovered from rodents' burrows in the region (*6*). Current molecular data from TBRF borreliae from Iran are limited to 2 isolates of *B. persica* and *B. microti* from *O. tholozani* and *O. erraticus* ticks, respectively (*5*,*7*,*8*). In situ IGS analysis revealed that spirochetes in our analysis had highest homology (97%) with relapsing fever agents from eastern Africa, *B. duttonii* and *B. recurrentis*, followed by *B. microti* (96%) from Iran (*8*). *B. microti* clustered with 1 strain (*B. duttonii*; GenBank accession no. GU350721) and apart from other *B. duttonii* IGS strains, suggesting that this strain may not be *B. duttonii*. The phylogenetic tree separated *B. duttonii* into 4 clades, 2 of which also contained *B. recurrentis*, confirming previous observations (*9*) and providing further support that *B. recurrentis* represents an ecotype of *B. duttonii* rather than a species (*10*). Furthermore, the high level of phylogenetic similarity among borreliae from eastern Africa and Iran indicates that the borreliae in our study might represent ecotype-adapted strains. More sequencing of different genomic markers is required to substantiate or refute this possibility. Lack of GenBank data for the remaining borreliae from Iran, *B. latyschewii* and *B. baltazardi*, prevent exclusion of these species.

Although relapsing fever spirochetes from southern Iran and those from borreliae in Africa have a close phylogenetic similarity, they have different virulence levels and abilities to infect vector and host species. Consequently, deciphering the evolutionary links for these *Borrelia* spp.

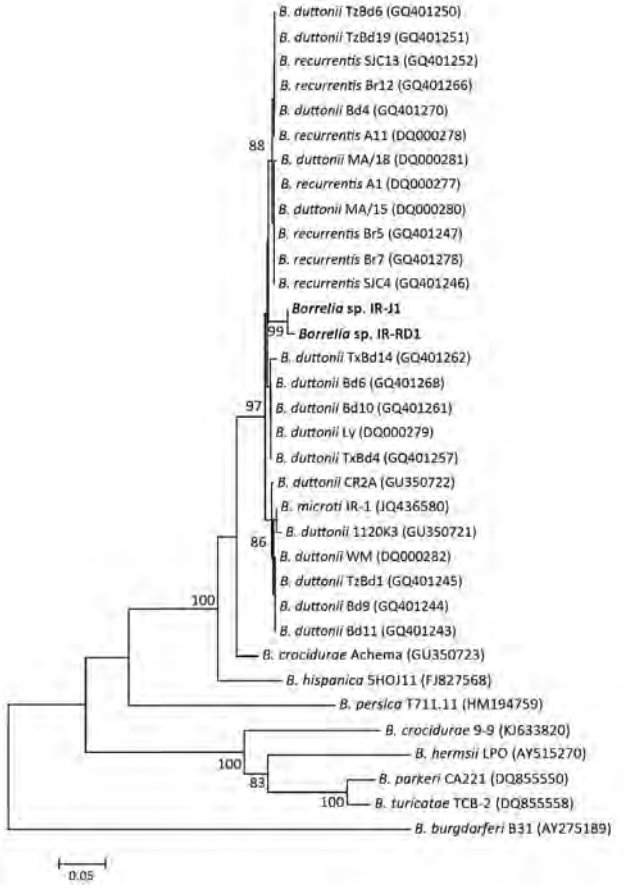

Figure. Phylogenetic tree of *Borrelia* spp. strains isolated in Iran, 2014. Constructed on the basis of intergenic spacer sequences, the tree is drawn to scale using evolutionary distance computed using the Jukes-Cantor method in which the units reflect substitutions per site. The final dataset used 587 bp. Numbers at nodes show the level of robustness in a bootstrap test performed with 2,000 replicates; numbers <85 were removed. Scale bar indicates nucleotide substitutions over length analyzed. GenBank accession nos. for nucleotide sequences of IGS from 2 patients (in bold) are KM271987 and KM271988.

is of paramount importance and might provide valued insights into host–microbe interactions.

Our report confirms a novel *Borrelia* IGS sequence type detected in situ from 2 relapsing fever patients. This species showed greatest homology with the relapsing fever borreliae from Africa, *B. recurrentis* and *B. duttonii*, but not with *B. microti*, which is transmitted by *O. erraticus* ticks, previously believed to be the only soft tick species in this region. These findings challenge the assumption that TBRF in Iran is attributed to only *B. persica* or *B. microti*.

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Reducing the Risk for Waterborne Nosocomial Neonatal Legionellosis

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To the Editor: I read with interest the report by Wei et al. (*1*) regarding 2 cases of neonatal legionellosis associated with infant formula prepared with hospital tap water. Two hospitals were involved, and water samples from both were positive for *Legionella pneumophila* bacteria that had molecular profiles indistinguishable from those for bacteria from the infected neonates. As Wei et al. (*1*) and others have established, control of waterborne pathogens, such as *Legionella* spp., in health care institutions remains a work in progress.

Recently, leading medical centers have recognized the efficacy and cost-effectiveness of performing certain measures to ensure the safety of hospital water. These measures include routine microbial analyses of tap water and use of waterborne pathogen prevention and control measures such as hot water flushing of plumbing; use of chlorination, chlorine dioxide, monochloramine, copper–silver ionization, or ultraviolet light; ozonation; and point-of-use water filtration. Each method has advantages and disadvantages related to ease of implementation, cost, maintenance issues, and short- and long-term effectiveness. Randomized controlled trials comparing the efficacy of these strategies are lacking, but the availability of guidance for using waterborne pathogen prevention and control strategies has resulted in substantial declines in health care–associated legionellosis (*2*). Efforts at waterborne pathogen detection and control are complicated by the role of biofilm, comprising microbes embedded in the polymeric matrix attached to internal plumbing surfaces, which protects waterborne pathogens from adverse environmental conditions, including antimicrobial agents and systemic controls (e.g., ultraviolet light, metals, acid pH) (*2*,*3*).

Prevention of legionnellosis in health care settings offers a clinically beneficial and cost-effective alternative to intermittent case detection and outbreak control. For example, it has been demonstrated that, even in the absence of a recognized outbreak, hospital units caring

for immunosuppressed patients can reduce infection rates by using water filtration at the point of use (*4*). Although further efforts are needed to systematically evaluate *Legionella* spp. control measures, a progressive approach to prevent health care–associated legionellosis includes routine microbial analysis of tap water in units for patients at high risk for infection, use of systemic water disinfection technology, and use of point-of-use water filtration in units where care is rendered for patients most vulnerable to infection with *Legionella* spp.

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Carnobacterium divergens Bacteremia in Woman

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To the Editor: *Carnobacterium* spp. are ubiquitous lactic acid bacteria isolated from cold and temperate environments (*1*). They are present in food including fish, meat, and dairy products. Only *C. divergens* and *C. maltaromaticum* (formerly *C. piscicola*) are found in dairy products (*2*). Carnobacteria are well known for their ability to produce bacteriocins that inhibit *Listeria monocytogenes* (*1*). Because *Carnobacterium* and *Listeria* bacteria are psychrotrophic and share the same ecologic niche, many studies have highlighted the potential use of carnobacteria as a biopreservative (*1*). These bacteria were previously believed to be nonpathogenic for humans. We report a case of *C. divergens* bacteremia in a woman.

In January 2013, a 57-year-old woman with a history of diabetes mellitus, severe undernutrition, and chronic alcoholism was admitted to the intensive care unit of the Avicenne Hospital, Bobigny, France, for diabetic ketoacidosis with altered level of consciousness. Physical examination revealed a low body temperature (30.1°C) and epigastric tenderness. At admission, a computed tomographic scan of the abdomen showed pneumoperitoneum with low-abundance ascites. Antimicrobial therapy with piperacillin/tazobactam and amikacin was empirically started. Exploratory laparotomy findings were within normal limits.

Three days after admission, acute necrotizing esophagitis ("black esophagus") with multiple gastroduodenal ulcerations was diagnosed by gastrointestinal endoscopy. By then, septic shock had developed. Antimicrobial drug therapy was empirically changed to imipenem/cilastatin and amikacin. A total esophagectomy with gastrostomy and esophagostomy was performed. No etiology for black esophagus could be established. Parenteral nutrition was begun 24 hours after surgery and relieved with enteral nutrition 72 hours after surgery. On hospitalization day 13, after having clinically improved, the patient consecutively experienced 2 episodes of hypoxemic cardiac arrest and resuscitation. Fever began 2.5 hours later and septic shock again developed. Exploratory laparotomy findings ruled out ischemic colitis.

Four sets of blood cultures collected on 3 days over a period of 5 days showed bacterial growth after 2 days of incubation in the BACTEC 9240 System (Becton Dickinson, Franklin Lakes, NJ, USA). Gram-positive *Listeria*-like rods were seen. Within 24 hours, the isolate grew on trypticase soy agar with 5% horse blood and chocolate PolyViteX agar (bioMérieux, Marcy l'Étoile, France). The colonies were gray, 1–2 mm in diameter, and nonhemolytic. The strain was facultative anaerobic. The catalase reaction was negative, and the esculin hydrolysis reaction was quickly positive. Results of testing with the API Coryne and API Listeria systems (bioMérieux) were unclear. The isolate seemed to be susceptible to penicillins, carbapenems, macrolides, and gentamicin and resistant to cephalosporins. MICs were as follows: penicillin 0.19 mg/L, amoxicillin 0.125 mg/L, amoxicillin/clavulanic acid 0.094 mg/L, cefotaxime >32 mg/L, ofloxacin 1 mg/L, ciprofloxacin 0.38 mg/L, imipenem 0.064 mg/L, vancomycin 2 mg/L, teicoplanin 1 mg/L, linezolid 0.50 mg/L, amikacin 16 mg/L, and rifampin 0.006 mg/L.

Because blood cultures were positive for gram-positive rods susceptible to amoxicillin, our initial diagnosis

[1]These authors contributed equally to this article.

was listeriosis. Empirically prescribed antimicrobial therapy (ceftazidime, colistin, amikacin, and metronidazole) was given for 96 hours and then replaced by gentamicin for 48 hours and amoxicillin for 3 weeks; clinical results were favorable.

The isolate strain was analyzed by the Division of Bacterial Identification (Pasteur Institute, Paris, France). The 16S rRNA gene was completely sequenced. A phylogenetic tree was generated by using the neighbor-joining algorithm (*3*). The isolate was found be *C. divergens*. Microbiological cultures and 16S rRNA testing results for another sample of enteral nutrition solution and a surgical specimen of the necrotic esophagus were negative.

Three reports of isolation of *Carnobacterium* sp. from humans have been published. The first report described isolation of *Carnobacterium* sp. from 1 set of blood cultures from a man who had prepared fish before onset of fever (*4*). The imputability of this diagnosis could not be clearly established because only 1 set of blood cultures had positive results. The second report described isolation of *C. piscicola* from pus after traumatic amputation of a hand by an industrial water sawmill (*5*). The third report described isolation from a child's hand with multibacterial synergistic gangrene (*6*).

For the case described here, the presence of *C. divergens* in blood cultures cannot be considered contamination because it was isolated from 4 sets of blood cultures collected over 5 days. We hypothesize that bacterial translocation was caused by low mesenteric flow after 2 episodes of cardiac arrest. Because the patient was receiving exclusively enteral nutrition, we presume that the origin of the infection was bacterial contamination of the solution or colonization of the feeding tube. Carnobacteria and lactobacilli (which are used as probiotic bacteria or fermented food products) are similar in that each is found in food, can be used as a biopreservative, and is considered nonpathogenic. The pathogenic relevance of lactobacilli is uncommon, but some clinical infections have been reported, including septicemia and meningitis (*7*). Because *C. divergens* seems to be able to cause life-threatening infection in immunocompromised patients, its safe use in such patients and in the food industry should be monitored.

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Fatal Nosocomial MDR TB Identified through Routine Genetic Analysis and Whole-Genome Sequencing

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To the Editor: In November 2012, a 44-year-old HIV-negative white man (patient 1) with fever, fatigue, and breathlessness sought care at a hospital in the United Kingdom. He had never traveled abroad but had biopsy-proven alcoholic cirrhosis. No acid-fast bacilli were seen on multiple samples, including ascitic fluid, and he received treatment for presumptive abdominal tuberculosis (TB). *Mycobacterium tuberculosis* was subsequently cultured after 12 days. His clinical condition deteriorated, and he died of multiorgan failure 44 days after admission. The cultured *M. tuberculosis* was subsequently

confirmed as multidrug resistant (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/6/14-1903-Techapp1.pdf).

Routine mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) testing was performed (*1*) (online Technical Appendix Table). A matching MIRU-VNTR profile was identified from a 42-year-old South African–born, HIV-positive health care worker (patient 2) who had died in 2008 after admission to the same hospital. She has been described previously in detail because she had worked at Tugela Ferry hospital in KwaZulu-Natal, South Africa, which was associated with a 2005 outbreak of multidrug-resistant TB (MDR TB) and extensively drug-resistant TB (*2*,*3*) (online Technical Appendix Figure 1). To ascertain whether these isolates could have matching MIRU-VNTR patterns by chance alone, we compared the MIRU-VNTR results with a national database of ≈11,745 isolates typed since the UK typing service began in 2010. Only 2 other isolates matched (from patients 3 and 4), originating from a UK hospital ≈100 miles away. Although both patients were HIV-positive health care workers from sub-Saharan Africa, no history of contact could be established with patients 1 or 2.

A review of admission records established that patients 1 and 2 were admitted to the same medical ward in 2008 for 8 days, suggesting a high probability of nosocomial transmission. The ward had a traditional "Nightingale" configuration with beds for male and female patients arranged dormitory-style. In 2009, patient 1 had been identified as a contact of patient 2 and was offered screening for latent infection but had failed to attend appointments and was not under regular medical follow-up. No other common contact was identified. The estimated time from known contact between patients 1 and 2 until the clinical presentation of patient 1 was 49 months.

Sequencing libraries from genomic DNA extracted from the 4 UK *M. tuberculosis* isolates that had matching MIRU-VNTR profiles were paired-end sequenced by using Illumina MiSeq (Illumina, San Diego, CA, USA). To investigate the origins of the infections, they were compared with 36 South Africa strains (including 1 from the Tugela Ferry outbreak [*4*]) sequenced by using Illumina HiSeq 2000 platforms.

For each sequenced strain, a random subset of reads was aligned at ≈100× coverage to the *M. tuberculosis* H37Rv reference genome by using BWA version 0.5.9-r16 (*5*). Pilon v1.5 (http://www.broadinstitute.org/software/pilon/) was run in variant discovery to generate a list of single-nucleotide polymorphisms (SNPs) and insertions and deletions. We estimated a phylogeny using RAxML v7.7.8 (*6*) using a general time reversible + gamma substitution model with 1,000 bootstrap replicates.

Pairwise comparison of whole-genome sequences from *M. tuberculosis* isolated from patients 1 and 2 found that the 2 sequences differed at only 4 SNPs (Table). Based on previous estimates of background mutations rates of 0.5 SNP/year (*7*), the pairwise distance between isolates from patient 1 and 2 increases confidence in the epidemiologic data implicating transmission >4 years earlier, although uncertainties exist around such estimates. Comparison between samples from patient pairs (1+2 vs. 3+4) found differences of 69–72 SNPs, which strongly argues against transmission between them.

In comparison with isolates sampled from KwaZulu-Natal (online Technical Appendix Figure 1), isolates from patients 1 and 2 were closely related to a strain associated with the Tugela Ferry outbreak (KZN605; online Technical Appendix Figure 2). Isolates from patients 3 and 4 were less closely related to isolates from the Tugela Ferry outbreak but were closely related to other isolates circulating within the region, consistent with the hypothesis that both infections originally occurred within South Africa.

This investigation illustrates the power of current technology to inform our understanding of the links in MDR TB transmission between low- and high-incidence areas. Whole-genome sequencing of pathogens is becoming part of routine practice for establishing transmission and resistance patterns (*8*). The greater certainty it brings to transmission data can provide evidence to justify more active policies of screening and isolation as part of infection control. The nosocomial transmission described here is consistent with the fact that a person with pulmonary TB (patient 2) was managed on an open ward before being put into respiratory isolation and had not been previously screened by occupational health services.

Recent data reviewing MDR TB transmission in the United Kingdom before 2007 did not identify cases of

Table. Pairwise distances between 2 pairs of *Mycobacterium tuberculosis* isolates from patients in the United Kingdom, an isolate from the 2005 Tugela Ferry outbreak in KwaZulu-Natal, South Africa (KZN605), and reference strain H37Rv

Isolate	Patient 1	Patient 2	KZN605	Patient 3	Patient 4	H37Rv
Patient 1	0					
Patient 2	4	0				
KZN605	21	24	0			
Patient 3	84	80	87	0		
Patient 4	87	83	90	2	0	
H37Rv	862	862	887	849	830	0

nosocomial transmission during that period (*9*). However, the emergence of MDR TB in regions of high HIV prevalence is relatively recent (*10*), and the cases described here suggest that increased vigilance for TB and MDR TB among migrating health care workers might be required.

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Fatal Bacteremia Caused by *Campylobacter gracilis*, United States

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To the Editor: *Campylobacter* species are well known to cause gastrointestinal infections in humans. However, extraintestinal illnesses caused by *Campylobacter* spp., including bacteremia, can also occur, primarily in immunocompromised persons (*1*). *Campylobacter gracilis* is a newly recognized species (*2*) that is commonly found in the oral flora and that has been associated with periodontal diseases and pleuropulmonary infections (*3*–*6*). Furthermore, a wide range of infectious etiologies caused by *C. gracilis* at different anatomic sites have been reported in the literature, suggesting its highly pathogenic potential (*7*,*8*). We describe a case of bacteremia due to *C. gracilis* complicated by pneumonia.

An 80-year-old man with a history of hypertension, hypertensive nephropathy, and chronic obstructive pulmonary disease (COPD) was in his usual health status when he began having worsening productive cough, fevers, and malaise; he sought health care 5 days later at Long Island College Hospital (Brooklyn, NY, USA). A heavy smoker who was noncompliant with his COPD treatment, he had frequent episodes of COPD exacerbation necessitating chronic maintenance with oral steroid therapy.

At physical examination, the patient appeared chronically ill and had mild respiratory distress. His temperature was 100.8°F, blood pressure 124/67 mm Hg, pulse 106 beats/min, respiration 22 breaths/min, and oxygen saturation 94% on room air. His heart sounds revealed tachycardia without murmurs, and his lung sounds disclosed scattered wheezing and rhonchi.

Laboratory studies revealed a leukocyte count of 14,400 cells/mm^3 (reference range 4,500–11,500) with 85% polymorphonuclear leukocytes, a hemoglobin level of 11.7 g/dL (reference range 14.0–18.0), and a platelet count of 174,000/mm^3 (reference range 150,000–450,000). His sodium level was 133 mmol/L (reference range 135–145), potassium 4.6 mmol/L (reference range 3.5–4.5), bicarbonate 30 mEq/L (reference range 22–28), urea nitrogen 118 mg/dL (reference range 9–23), and creatinine 5.2 mg/dL (reference range 0.7–1.3). A chest radiograph showed consolidation with large pleural effusion in the right lung.

He was empirically given vancomycin, cefepime, and azithromycin. Severe respiratory distress developed, and the patient died a few days later. Respiratory cultures at that time showed *Klebsiella pneumoniae* and *C. gracilis*. Blood cultures were positive for *C. gracilis*.

C. gracilis, originally known as *Bacteroides gracilis*, was transferred to the genus *Campylobacter* in 1995 after analysis of the cellular fatty acids, respiratory quinones, and proteins of *B. gracilis* and a comparison of them with the corresponding chemotaxonomic features of *Campylobacter* spp. (*2*). *C. gracilis* is a nonmotile, non–spore-forming, anaerobic gram-negative rod that uniquely requires formate and fumarate in its metabolism. *C. gracilis* primarily inhabits the gingival crevice and has been associated with a wide variety of periodontal diseases (*3*,*7*).

A study of 28 persons with chronic asymptomatic periradicular lesions showed *C. gracilis* in 6 (21.4%), including 2 (16.7%) of 12 who had acute apical periodontitis and 4 (23.5%) of 17 who had acute periradicular abscess (*4*). *C. gracilis* has also been isolated from other anatomic sites and has caused severe infections such as peritonitis, pneumonia, and bacteremia (*5*,*8*).

Our patient had *C. gracilis* bacteremia complicated by acute respiratory distress secondary to pneumonia. Although another gram-negative rod was isolated from the respiratory cultures, *C. gracilis* potentially played a major pathogenic role for this patient because of concomitant bacteremia that resulted in an unfavorable outcome. Pleuropulmonary infections with *C. gracilis* are not surprising because of the frequency of its detection in the human oral flora. In a study of 23 isolates of *C. gracilis* and their associated clinical diagnosis, 7 were from patients with lung abscess or empyema, and 2 were from those with aspiration pneumonia (*6*).

Campylobacter spp. are commonly associated with extraintestinal complications, including bacteremia, in immunocompromised hosts. In a study of 183 patients with *Campylobacter* bacteremia, the main underlying conditions were liver disease (39%) and cancer (38%). In that study, *C. fetus* was the most frequently identified species, found in 53% of the patients involved, followed by *C. jejuni*, *C. coli*, and *C. lari* (*1*). In another case report, *C. lari* bacteremia was described in a patient with multiple myeloma (*9*). Although uncommon, *C. gracilis* bacteremia has been reported in the literature (*8*).

Optimal antimicrobial drug treatment for *C. gracilis* remains to be established. Available antimicrobial susceptibility patterns in the literature have shown conflicting results (*5*,*10*). In 1 study, penicillin susceptibility was 67% and cephalosporin susceptibility was 67%–84% in 23 isolates of *C. gracilis* (*6*).

Further research is warranted to elucidate the mechanisms of pathogenicity and virulence of *C. gracilis*. Its pathogenic potential should not be underestimated because of the spectrum of disease, severity of infection, and its possible high frequency of antimicrobial drug resistance.

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Induction of Influenza (H5N8) Antibodies by Modified Vaccinia Virus Ankara H5N1 Vaccine

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To the Editor: Aquatic birds form a natural reservoir of avian influenza viruses from which new human and animal influenza viruses originate. After initial detection in 2010 in China, a new highly pathogenic avian influenza (HPAI) virus of the H5N8 subtype reemerged in ducks in South Korea in 2014 (*1*,*2*). The hemagglutinin gene of this virus was distantly related to those of H5N1 subtypes that have caused infections in humans since 1997 (*3*). The World Health Organization/World Organisation for Animal Health/Food and Agriculture Organization of the United Nations H5N1 Evolution Working Group has assigned this new H5 to clade 2.3.4.4. Several poultry farms in the Netherlands, Germany, United Kingdom, and Italy were recently affected by infection with H5N8 virus closely related to the strains circulating in Asia (*4*), leading to implementation of preventive measures to restrict viral spread. Human infections with this new HPAI subtype have not been reported.

Modified vaccinia virus Ankara (MVA) is a promising viral vector platform for the development of influenza vaccines (*5*). We previously conducted a randomized double-blind phase 1/2a trial in young healthy persons to evaluate an MVA-based H5 vaccine (registered in the Netherlands' trial register under NTR3401). Preclinical testing was conducted before this trial (*6*,*7*). Thirty-nine study participants received MVA-H5-serumfree Munich-Rotterdam (sfMR), which encoded hemagglutinin of influenza virus A/Vietnam/1194/2004 (H5N1), and 40 received vector control. Persons received 1 or 2 doses (with an interval of 4 weeks) of 10^7 or 10^8 PFU. Twenty-seven of the MVA-H5-sfMR–vaccinated persons received a booster vaccination 1 year later (again 10^7 or 10^8 PFU). The MVA-based vaccine was well tolerated and induced antibodies to both the homologous (A/Vietnam/1194/2004, clade 1) and a heterologous (A/Indonesia/5/2005, clade 2.1) H5N1 virus (*8*).

Although the newly emerged HPAI (H5N8) virus thus far has been detected only in birds, zoonotic transmission to humans exposed to large numbers of infected birds might occur (e.g., during culling operations). Therefore, shortly after the H5N8 outbreak in poultry in the Netherlands, we determined whether MVA-H5-sfMR–induced antibodies cross-react with the new H5N8 strain. Post-infection A/Vietnam/1194/2004 (clade 1) ferret serum (infected with a low pathogenic reverse genetics virus produced with hemagglutinin and

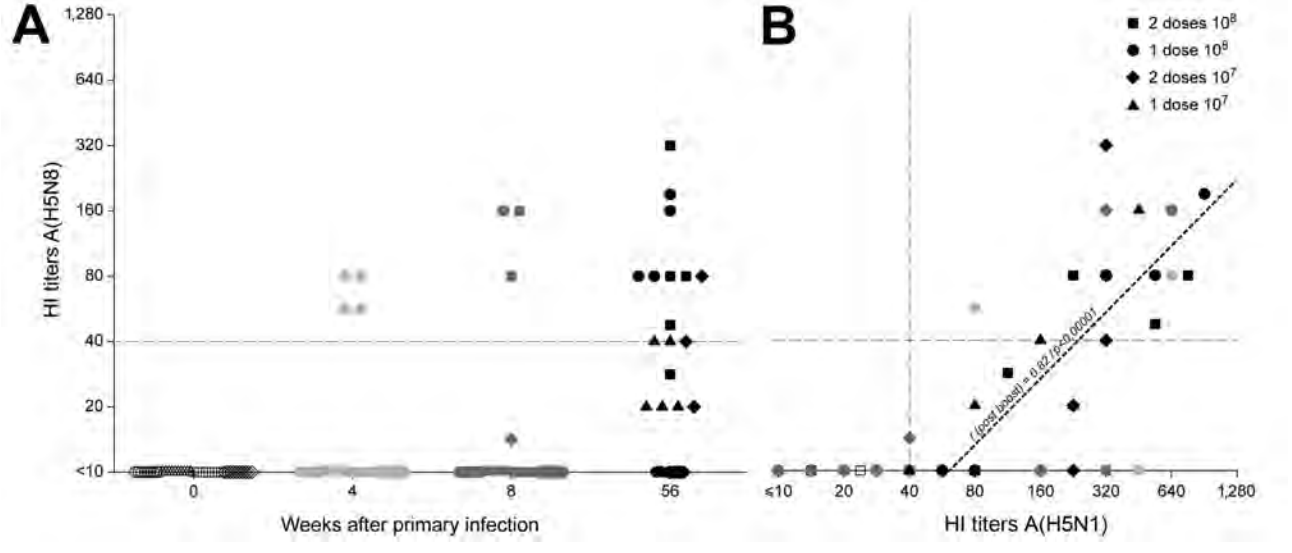

Figure. Results of hemagglutination-inhibition (HI) testing of modified vaccinia virus Ankara influenza vaccine cross-reactivity. Each symbol represents a person in the clinical trial; symbol shapes indicate different vaccination regimens. A) Timeline for development of HI titers against influenza virus A(H5N8) (A/chicken/Netherlands/EMC-3/2014). B) Correlation between HI titers against H5N8 and A/Vietnam/1194/2004 (H5N1) viruses. Linear regression for samples after booster vaccination is shown (r = 0.82, p<0.0001).

neuraminidase gene segments of A/Vietnam/1194/2004 and the remaining 6 gene segments of A/Puerto Rico/8/34) was tested by hemagglutination-inhibition (HI) for cross-reactivity with viruses belonging to clade 0 (A/Hong Kong/156/1997), 2.1 (A/Indonesia/5/2005), 2.2 (A/Turkey/turkey/1/2005), and 2.3 (A/Anhui/1/2005) and the emerging H5N8 strain A/chicken/Netherlands/EMC-3/2014. A/Vietnam/1194/2004-specific serum (homologous titer 80) displayed low cross-reactivity with the clade 0, 2.2, and 2.3 viruses and completely failed to react with H5N8 strain A/chicken/Netherlands/EMC-3/2014. Inversely, A/chicken/Netherlands/EMC-3/2014-specific ferret serum (homologous titer 160) completely failed to cross-react with A/Vietnam/1194/2004. This finding demonstrates an antigenic distance between these viruses. Furthermore, the World Health Organization Collaborating Centers have only found limited cross-reactivity of a panel of H5 vaccine candidates with subtype H5N8 (*9*).

The clinical trial serum samples were pretreated with receptor-destroying enzyme and horse erythrocytes and tested by HI assay for their reactivity with A/chicken/Netherlands/EMC-3/2014 according to standard procedures (*10*). HI antibodies were induced after MVA-H5-sfMR vaccination that displayed considerable reactivity with the antigenically distinct H5N8 strain A/chicken/Netherlands/EMC-3/2014 (Figure, panel A). The titers of cross-reactive antibodies correlated with those to the homologous strain A/Vietnam/1194/2004 (r = 0.82, $p<0.0001$; Figure, panel B).

As shown previously (*8*), the magnitude of the antibody response was dose-dependent. Also, the highest cross-reactive response to the H5N8 strain was observed after vaccination with 10^8 PFU (Figure, panel A) of MVA-H5. None of the study participants had prevaccination HI antibody titers ≥1:40 against A/Vietnam/1194/2004 or A/chicken/Netherlands/EMC-3/2014. Although most of the study participants had detectable HI antibody titers against the homologous virus 4 and 8 weeks after vaccination (*8*), antibodies against the H5N8 virus were barely detectable at these time points. HI antibody titers against the homologous virus increased in persons who received a booster vaccine at 52 weeks after primary vaccination. A large proportion (9 [82%] of 11 study participants; geometric mean titer 63) of participants who received a vaccine dose of 10^8 PFU (equally divided among groups that received 1 or 2 previous doses) also had detectable cross-clade titers against the H5N8 virus A/chicken/Netherlands/EMC-3/2014. Furthermore, virus neutralizing antibodies against H5N8 virus were detected in 10 of 27 persons and correlated with antibody titers measured by HI assay.

We showed that an MVA-based H5 (A/Vietnam/1194/2004) vaccine can elicit cross-clade antibodies against the newly emerging HPAI (H5N8) virus that is genetically and antigenically distinct from the clade 1 H5N1 virus A/Vietnam/1194/2004. The cross-reactive antibody response observed after the 1-year booster vaccination suggests that the use of MVA-H5-sfMR is an effective emergency vaccination strategy in case tailor-made vaccines are not yet available in an outbreak situation. Thus, such a strategy might also be effective against the newly emerging influenza A(H5N8) viruses, in case these viruses would cause human infections.

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Klebsiella pneumoniae Co-Producing NDM-5 and OXA-181 Carbapenemases, South Korea

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To the Editor: Carbapenemase-producing *Enterobacteriaceae* are being reported worldwide. Travel, medical tourism, and cross-border transfer of patients might play a role in the spread of these bacteria (*1,2*). *Klebsiella pneumoniae* co-producing New Delhi metallo-β-lactamase 5 (NDM-5) and oxacillinase 181 (OXA-181) carbapenemases was detected in South Korea in 2014.

On April 13, a 75-year-old man who had had a cerebral infarction was transferred from a tertiary care hospital in Abu Dhabi, United Arab Emirates (UAE), to Samsung Medical Center (Seoul, South Korea) for rehabilitation therapy. In Abu Dhabi, he had received broad-spectrum antimicrobial drugs for aspiration pneumonia. While at Samsung Medical Center, he experienced septic shock and acute respiratory failure due to pneumonia and was transferred to the medical intensive care unit (ICU). Carbapenem-resistant *K. pneumoniae* (strain CC1409-1) was isolated from a culture of bronchoalveolar lavage fluid. He was given meropenem and colistin for treatment of pneumonia, was discharged, and returned to the UAE.

Four months later, carbapenem-resistant *K. pneumoniae* (strain CC1410-1) was identified in the tracheal aspirate of a 74-year-old woman admitted to the surgical ICU at Samsung Medical Center for traumatic intracranial hemorrhage. She had no underlying disease or previous history of hospitalization or travel abroad. She was given colistin and piperacillin/tazobactam. Following the identification of colistin resistance, colistin was switched to tigecycline. However, her clinical condition worsened (aggravated pneumonia), and she died of refractory respiratory failure.

In vitro antimicrobial drug susceptibility tests of 2 isolates were performed by using broth microdilution. Results were interpreted following Clinical and Laboratory Standards Institute guidelines (*3*), except for those for colistin and tigecycline, for which European Committee on Antimicrobial Susceptibility Testing breakpoints were used (*4*). The first isolate was susceptible to colistin but none of the other antimicrobial agents tested (cefepime, ceftriaxone, ceftazidime, aztreonam, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole, ertapenem, imipenem, and meropenem)whereas the second isolate was susceptible only to tigecycline. Modified Hodge tests for both isolates showed positive results. Production of metallo-β-lactamase was detected by an imipenem-EDTA double-disk synergy test.

The presence of carbapenemase genes was determined by PCR and DNA sequencing (*2*). The bla_{NDM} and bla_{OXA-48} genes were detected in both isolates. The PCR product sequences were consistent with those of NDM-5 (GenBank accession no. JN104597.1) and OXA-181 (GenBank accession no. JN205800.1). Further analyses for other β-lactamases (TEM-type, SHV-type, and CTX-type) and 16S rRNA methylase aminoglycoside resistance determinants (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtF*, and *npmA*) revealed that both isolates carried bla_{TEM-1}, bla_{SHV-11}, $bla_{CTX-M-15}$, and *rmtB* genes.

Clonal relatedness was investigated by using multilocus sequence typing and pulsed-field gel electrophoresis (PFGE) (*5,6*). Multilocus sequence typing revealed that both isolates belonged to sequence type 147. PFGE showed that both isolates were the same strain (Figure).

The 2 patients were never hospitalized in the same ward and there was a substantial time lag between their hospitalizations. However, given sequence type and PFGE patterns between 2 isolates, we suspected nosocomial cross-transmission and performed infection control measures, including strict contact precautions and enhanced environmental cleaning with daily monitoring in the surgical ICU. In addition, environmental cultures and active surveillance cultures (rectal swabs and respiratory samples) on all patients in the units where these isolates were identified were performed to find asymptomatic carriers or contaminated environments as potential sources of transmission. All samples tested were negative for carbapenemase-producing *Enterobacteriaceae*. No further cases were reported in the hospital.

NDM-5 was first identified in a multidrug-resistant *Escherichia coli* sequence type 648 isolate from a patient in the United Kingdom who had a recent history of hospitalization in India (*7*). NDM-5 differs from existing enzymes due to substitutions at positions 88 (Val→Leu) and 154 (Met→Leu). OXA-181, a variant of OXA-48, was initially

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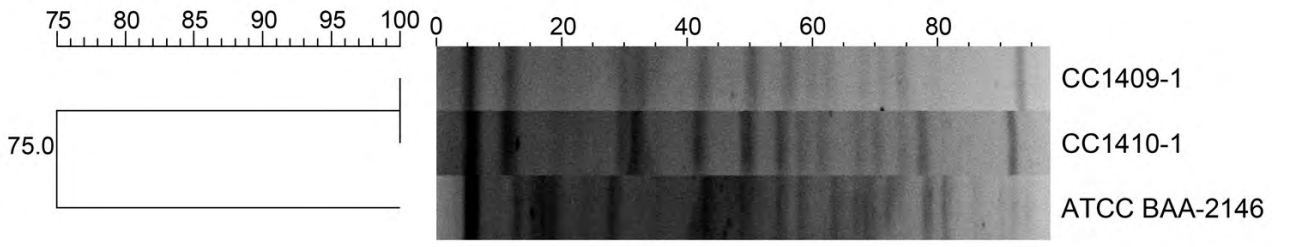

Figure. Dendrogram of pulsed-field gel electrophoresis patterns showing the genetic relationship between 2 *Klebsiella pneumoniae* isolates co-producing New Delhi metallo-β-lactamase 5 and oxacillinase 181 carbapenemases, South Korea, 2014. ATCC BAA-2146 indicates New Delhi metallo-β-lactamase 1 *K. pneumoniae* used as a reference strain. Scale bar indicates percentage genetic relatedness.

reported in India but has been sporadically detected in the United Kingdom, the Netherlands, France, New Zealand, Oman, and Singapore (*8*). It has also been found to be associated with other carbapenemase genes, such as the $bla_{\text{NDM-1}}$ and $bla_{\text{VIM-5}}$ genes, and particularly in isolates with a link to the Indian subcontinent.

In the cases we describe, the first *K. pneumonia*e isolate was recovered from a patient transferred from the UAE. Recent studies suggest that the Middle East, a region with close ties to the Indian subcontinent that hosts a large expatriate population, may act as another reservoir of OXA-48 and NDM producers (*9*,*10*). The emergence of extremely drug-resistant isolates carrying multiple carbapenemase genes is of concern because of limited treatment options and the possibility of global dissemination by means of cross-border transfer. A collaborative interdisciplinary strategy, including active surveillance for high-risk patients and adequate infection control measures against spread of such highly transmissible multidrug-resistant strains in health care settings, is necessary.

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Salmonella enterica Paratyphi A Infections in Travelers Returning from Cambodia, United States

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To the Editor: Health authorities from Cambodia and European Union member states recently described a pronounced increase in *Salmonella enterica* serotype Paratyphi A infections in Cambodia resulting from an ongoing outbreak

(*1*,*2*). To further characterize this outbreak, we analyzed 2013–2014 data on Paratyphi A infections associated with travel to Southeast Asia that were reported to the Centers for Disease Control and Prevention (CDC) National Typhoid and Paratyphoid Fever Surveillance (NTPFS) system and the CDC National Antimicrobial Monitoring System (NARMS).

NTPFS began tracking *Salmonella* Paratyphi A infections in 2008. During 2008–2012, ten cases were reported in patients who had traveled to Southeast Asia within 30 days before illness onset; only 1, who also reported travel to Sri Lanka, Nepal, and Nigeria, reported travel to Cambodia. During January 1, 2013–August 22, 2014, however, NTPFS received 19 reports of laboratory-confirmed Paratyphi A infection in travelers returning from Southeast Asia; 13 traveled to Cambodia, and 8 of them reported travel only to Cambodia (Table). Of the 7 patients who traveled only to Cambodia and reported reason for travel, all cited "visiting friends and relatives." Six (75%) of the 8 patients who traveled only to Cambodia were hospitalized (median duration 7 days, range 2–10 days), and all recovered. Cases occurring in 2014, especially later in the year, might not yet have been reported, so the 2014 data most likely are an underestimate. Although many cases reported to health authorities in Cambodia and the European Union clustered in the Phnom Penh region (*1*,*2*), we lack information about destinations within Cambodia for US patients.

Paratyphi A isolates from southern Asia (e.g., India, Pakistan, Bangladesh) often are resistant to the quinolone nalidixic acid or are multidrug resistant (i.e., resistant to ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole) (*3*), but little is known about antimicrobial drug resistance among Paratyphi A strains from Southeast Asia. However, most outbreak-associated isolates from Cambodia reported by others have been pansusceptible (*1*,*2*). CDC NARMS characterized the antimicrobial susceptibility of isolates from all patients who reported travel only to Cambodia; 7 (87.5%) were pansusceptible, and 1 (12.5%) was resistant to nalidixic acid and had reduced susceptibility to the fluoroquinolone ciprofloxacin. CDC NARMS also tested isolates from all patients who reported travel to Cambodia and other countries in Southeast Asia and from 2 patients who reported travel to other countries in Southeast Asia only; all were pansusceptible.

The Paratyphi A outbreak in Cambodia appears to be large and ongoing. To our knowledge, information about possible sources and risk factors that could help inform prevention activities is not yet available. This outbreak highlights the urgent need for a paratyphoid fever vaccine; although typhoid fever vaccines exist, persons living in and visiting regions of active Paratyphi A transmission have no alternative to relying exclusively on close attention to food and water safety to mitigate risk (*4*). Furthermore, although most isolates from this outbreak appear to have been pansusceptible, antimicrobial drug resistance has emerged quickly among Paratyphi A strains in southern Asia (*5*–*7*). More comprehensive surveillance of antimicrobial resistance among Paratyphi A strains is warranted in Southeast Asia to determine the extent of geographic expansion of resistant strains from southern Asia and to inform treatment options for management of patients. We recommend a systematic outbreak investigation to determine source and routes of transmission.

Table. Characteristics of patients with *Salmonella enterica* serotype Paratyphi A infection returning to the United States from Southeast Asia, NTPFS, 2013–2014*

Characteristic	Cambodia only, n = 8	Cambodia and other countries in Southeast. Asia, n = 5†	Other countries in Southeast. Asia only, n = 6‡
Travel history§			
Reason for travel, no (%)			
Business	1 (14)	0	3 (60)
Tourism	0	3 (60)	3 (60)
Visiting friends and relatives	7 (100)	3 (60)	0
Missionary work	0	1 (20)	0
Immigration	0	0	1 (17)
Unknown	1 (<1)	0	0
Demographics			
Age, y, median (range)	23 (9–50)	21 (18–59)	39 (25–52)
Female sex, no. (%)	5 (63)	4 (80)	4 (67)
Clinical			
Hospitalized, no. (%)	6 (75)	3 (60)	2 (33)
No. days, median (range)	7 (2–10)	6 (4–7)	3 (1–4)
Recovered, no. (%)	8 (100)	5 (100)	6 (100)
Specimen source, no. (%)			
Blood	7 (88)	4 (80)	4 (67)
Feces	1 (12)	1 (20)	2 (33)

*Cases occurring in 2014, especially later in the year, might not yet have been reported to NTPFS. NTPFS, National Typhoid and Paratyphoid Fever Surveillance system.
†In addition to Cambodia, patients also visited Vietnam (2 patients) and Laos (1 patient).
‡Other countries in Southeast Asia included Indonesia (4 patients) and Thailand (2 patients).
§Of patients with known reason for travel. Some patients listed multiple reasons.

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Candida auris Candidemia in Kuwait, 2014

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To the Editor: Recent reports from Asia (*1*–*4*) have highlighted the increasing incidence of the fungus *Candida auris* as a nosocomial bloodstream pathogen affecting persons of all age groups. We report a case of *C. auris* candidemia in a 27-year-old woman in Kuwait with a long history of chronic renal failure. On May 9, 2014, the patient was admitted to the intensive care unit with symptoms of septic shock secondary to lobar pneumonia and complicated by acute renal failure. The patient was known to have immotile cilia syndrome (primary ciliary dyskinesia) and bronchiectasis with recurrent episodes of sinusitis. Beginning on day 1, she received treatment with different courses of a wide range of broad-spectrum antimicrobial drugs. However, despite treatment, the patient's condition continued to deteriorate. On day 12 after admission, a blood culture yielded yeast growth that was identified with 99% probability as *C. haemulonii* by using the Vitek 2 yeast identification system (bioMérieux, Marcy l'Etoile, France). As part of routine patient care, we sent the isolate (Kw1732/14) to the Mycology Reference Laboratory at Kuwait University for further identification and antifungal susceptibility testing. The isolate was resistant to fluconazole (MIC of ≥256 μg/mL), but it appeared susceptible to amphotericin B (MIC of 0.064 μg/mL), voriconazole (MIC of 0.38 μg/mL), and caspofungin (MIC of 0.064 μg/mL) by using the Etest (bioMérieux, Marcy l'Etoile, France). The patient was started on liposomal amphotericin B (150 mg/day), but the next day, she died from multiorgan failure.

On MAST ID CHROMagar Candida medium (Mast Group Ltd., Bootle, UK), the isolate formed pink colonies, which grew well at 42°C but not at 45°C. The isolate did not grow on BBL Mycosel Agar (BD, Sparks, MD, USA) containing 0.4 g cycloheximide per liter of medium. As with *C. auris* isolates from India and South Africa, this isolate assimilated *N*-acetyl glucosamine (*2*,*5*). Because the isolate showed reduced susceptibility to fluconazole, it was further characterized by sequencing of internal transcribed spacer and D1/D2 domains of ribosomal DNA. Genomic sequences for the internal transcribed spacer and D1/D2 regions (EMBL accession nos. LN624638 and LN626311) shared 99%–100% identity with sequences for corresponding regions of several *C. auris* strains (identification nos. CBS12874, CBS12875, CBS12876, CBS12880, CBS12882, CBS12886, and CBS12887, and several isolates from India).

C. auris was isolated in 2009 from the ear canal of a woman in Japan (*6*). The species has attracted attention because of its reduced susceptibility to azoles and amphotericin B (*2*,*5*) and its misidentification as *C. haemulonii* or *Rhodotorula glutinis* by commercial yeast identification systems (*1*,*4*). Because there are no reliable phenotypic methods for the rapid identification of *C. auris* and because molecular methods are not yet widely available, it is reasonable to infer that *C. auris* may be a more frequent cause of candidemia than previously recognized, particularly in Asian countries. A recently published multicenter study from India supports this view (*7*). In that study, a significantly higher occurrence of *C. auris* candidemia was re-

ported among patients admitted in public sector hospitals compared with those in private hospitals (8.2 vs. 3.9%; p = 0.008) (*7*). The report reinforces the growing clinical implications of rare *Candida* spp. in the etiology of candidemia and highlights the role of molecular methods for their unequivocal identification.

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The Antibiotic Era: Reform, Resistance, and the Pursuit of a Rational Therapeutics

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There has been a flood of recent publications and media coverage on the current crisis relating to drug-resistant microorganisms. The Antibiotic Era: Reform, Resistance, and the Pursuit of a Rational Therapeutics takes a unique approach by presenting a well-documented history, starting in the earliest days with the introduction of antimicrobial drugs to the present time. It is built around those reformers who recognized problems relating to the irrational use of these agents and the difficulties these reformers experienced over many years.

Early chapters provide a firm foundation for the many issues that follow. They trace the development of the sulfa drugs and penicillin up to the period of World War II, and more important, the postwar introduction of the group of broad-spectrum "wonder-drug" antibiotics. The introduction of these drugs changed the practice of medicine but also stimulated the need for infectious disease experts to clarify their use and help counter the extreme and extensive marketing of these drugs by the pharmaceutical companies in the form of journal advertisements, supplements, and free physician samples.

As the author notes, experts like Maxwell Finland became "therapeutic rationalists" or "reformers" to help fill the vacuum left by the American Medical Association and the Food and Drug Administration because they only dealt with drug safety, not efficacy, and did not want to dictate therapeutic choices to practicing physicians. It was the introduction of the fixed-dose combination antibiotics in the 1950s, especially Panalba (tetracycline/novobiocin), that reinforced the need for real reform, a movement that dragged on for several decades before coming to fruition with the acceptance of the requirement for controlled clinical studies to prove the efficacy, as well as the safety, of antimicrobial agents.

Except for the last chapter, this well-researched book focuses exclusively on issues debated in the United States. This easy to read book is arranged in a logical chronologic order, and traces the complexity of newer antimicrobial drug classes and their related policy issues. The author provides 105 pages of references and documentation that makes this book a valuable source of information for historians of science, clinical microbiologists, and infectious disease physicians. If for no other reason, the extensive reference sections makes this book a wise investment for anyone researching or writing on these issues now or in the future, especially because many of the past issues are still being argued today.

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Joaquin Sorolla y Bastida (1863–1923). The Wounded Foot, 1909. Oil on canvas 43 × 39 in. /109.2 × 99.1 cm. Digital image courtesy of the Getty's Open Content Program, The J. Paul Getty Museum, Los Angeles, CA, USA.

Light, Reflection, Illumination

Byron Breedlove

Although expressions such as "shed some light" or "I saw the light" figure often in our everyday speech, we do not typically contemplate light. We may marvel at its seemingly mysterious expression through auroras, rainbows, lasers, or celestial objects or find comfort in its subtler forms such as sunbeams, candles, campfires, or fireflies. Artists and scientists, by contrast, do study and manipulate light, reflection, and illumination.

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Impressionistic painters strive to capture an impression of a fleeting moment, a particular scene. Many among their numbers are considered masters for their skill at capturing light as it shimmers across surfaces. Claude Monet once described Joaquín Sorolla y Bastida Sorolla as "the painter of light above all other."

Sorolla, was born in Valencia, Spain, in 1863. He and his infant sister Concha were orphaned when their parents died during a cholera epidemic 2 years later, and they were raised by their mother's relatives. Early on, Sorolla seemed destined to become an artist: "When Joaquin was of an age to go to school, he manifested little inclination for his studies

proper, though he revealed a stealthy and incorrigible craze for scrawling embryonic drawings in his copy books. . . ."

In 1878, he began studies at the Fine Arts School of San Carlos, and he soon won awards at the Academy of Valencia. During 1879, the young artist traveled to Paris, where he toured exhibitions and met painters who worked in the open air, a practice that accentuated attention to light, color, and movement. That experience proved pivotal. Sorolla developed a passion for painting outdoors, preferring natural light and settings. After his military service, Sorolla subsequently attended the Fine Arts Academy in Rome on a 4-year scholarship beginning when he was 21. By the time Sorolla was 30, his paintings had been displayed across Europe and in the United States, and by the turn of the century, he was acknowledged to be among the Western world's best living painters.

He created many memorable paintings portraying the sun-drenched Spanish Mediterranean beaches and seascapes; he often painted portraits outside, reinvigorating the form with his fresh perspective. Sorolla's mastery is largely accorded to his ability to depict tones and colors of sunlight, and he dedicated his life to chasing the sun as it played over the people and places of his beloved Spain. He himself acknowledged, "I hate darkness. Claude Monet once said that painting in general did not have light enough in it. I agree with him. We painters, however, can never reproduce sunlight as it really is. I can only approach the truth of it."

Sorolla cut a dashing figure, dressed in a suit, working from a table-sized palette dabbed with an array of colors squeezed from emptied tubes, and wielding yard-long brushes. The vigor with which he worked—he often finished a painting within a few days—did not diminish even as his reputation and wealth grew: he completed more than 500 paintings during an energetic 4-year spurt.

This months' cover image, *The Wounded Foot,* is among a series of paintings Sorolla made on the beach at Valencia. This painting focuses on 2 children, one of whom sits on the wet sand inspecting her foot, possibly injured from a jagged shell or broken shard of glass; her companion crouches, peering from under a wide-brimmed hat, perhaps offering comfort. That child's arm falls beyond the edge of the canvas; blurred figures bob, swim, and frolic in the ocean. Reflected light scatters and glistens across the wet sand, swirling water, and foamy edges of waves. The casual composition belies the artist's expertise in capturing transitory light and motion. The J. Paul Getty Museum notes that "The colored reflections of late afternoon light animate this beach scene and actively define the forms, from the injured child's shoulder to the liquid sea and the figures playing in the water. The sun's highlights on the hurt child's hand, the sand around her foot, and her companion's hat draw the viewer's attention to the injured limb."

The study of light, reflection, and illumination also seized the imagination of German scientist August Köhler, one of Sorolla's contemporaries. In 1893, Köhler developed the microscope illumination technique that both bears his name and remains in use: Köhler illumination. This technique uniformly illuminates specimens without background glare. When this means of illumination is used on Gram-stained specimens, the defining features and structures of bacteria are readily and vividly revealed.

Köhler illumination proved ground-breaking. Advances in detection and surveillance cannot come swiftly enough as researchers and public health professionals grapple with the increasing emergence of drug resistance in bacteria. Antimicrobial drug resistance diminishes the ability to treat bacterial infections, increases risks associated with many medical procedures, and threatens animal health and agriculture. In 2014, the United States government released *The National Action Plan for Combating Antibiotic-Resistant Bacteria*, acknowledging the global scope of the problem and supporting the One Health approach to disease surveillance for pathogens of humans and animals as being critical for combating resistance to antibiotics.

Light, reflection, and illumination led an artist to capture a fleeting incident on a sunny beach in *The Wounded Foot* and inspired a scientist to find a durable solution for a problem that had vexed researchers. The growing problem of antimicrobial resistance could make treating that child's wound today more complicated than we imagined when the widespread use of antibiotics began in the 1940s, reminding us that we must keep shedding new light on an old problem.

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EMERGING INFECTIOUS DISEASES®

Upcoming Issue

Disseminated Infections with *Talaromyces* (*Penicillium*) *marneffei* in Non-AIDS Patients Given Monoclonal Antibodies against CD20 and Kinase Inhibitors

Assessment of Arbovirus Surveillance in the United States 13 Years after Introduction of West Nile Virus

Lack of Transmission among Close Contacts of Patient with Imported Case of Middle East Respiratory Syndrome into the United States, 2014

MERS-CoV in Upper Respiratory Tract and Lungs of Dromedary Camels, Saudi Arabia, 2013–2014

Macacine Herpesvirus 1 in Long-Tailed Macaques, Malaysia, 2009–2011

Parechovirus Genotype 3 Outbreak among Infants, New South Wales, Australia, 2013–2014

Monitoring of Ebola Virus Makona Evolution through Establishment of Advanced Genomic Capability in Liberia

Transdermal Diagnosis of Malaria Using Vapor Nanobubbles

Malaria Prevalence among Young Infants in Different Transmission Settings, Africa

Seroprevalence of Hepatitis E and other Viral Hepatitides among Diverse Populations, Malawi

Hemagglutinin Receptor Binding of a Human Isolate of Influenza A(H10N8) Virus

Gastroenteritis Outbreaks Caused by Norovirus GII.17, Guangdong Province, China, 2014–2015

Geographic Range Expansion for Rat Lungworm in North America

Readability of Ebola Information on Websites of Public Health Agencies, United States, United Kingdom, Canada, Australia, and Europe

Evaluation of Patients under Investigation for MERS-CoV Infection, United States, January 2013–October 2014

Rapidly Expanding Range of Highly Pathogenic Avian Influenza Viruses

Wildlife Reservoir for Hepatitis E Virus, Southwestern France

Determination of Predominance of Influenza Virus Strains in the Americas

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

Upcoming Infectious Disease Activities

August 10–21, 2015
14th International Dengue Course
Havana, Cuba
http://instituciones.sld.cu/ipk/14thdenguecourse/

August 24–26, 2015
ICEID
International Conference on Emerging Infectious Diseases
Atlanta, GA, USA

August 29–September 2, 2015
IDBR
20th Annual Infectious Disease Board Review Course
McLean, VA, USA
http://smhs.gwu.edu/cehp/activities/courses/idbr

September 17–21, 2015
ICAAC
Interscience Conference on Antimicrobial Agents and Chemotherapy
San Diego, CA, USA

October 25–29, 2015
ASTMH
American Society of Tropical Medicine and Hygiene
64th Annual Meeting
Philadelphia, Pennsylvania, PA, USA
info@astmh.org

February 8–10, 2016
ASM Biodefense and Emerging Diseases Research Meeting
Arlington, VA, USA
biodefense@asmusa.org

March 2–5, 2016
ISID
17th International Congress on Infectious Diseases
Hyderabad, India

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

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

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 75% passing score) and earn continuing medical education (CME) credit, please go to http://www.medscape.org/journal/eid. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the "Register" link on the right hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/about-ama/awards/ama-physicians-recognition-award.page. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for AMA PRA Category 1 Credits™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Estimated Illnesses and Deaths Averted During Fungal Meningitis Outbreak Associated with Contaminated Steroid Injections, United States, 2013–2014

1. You are consulting for a public health department regarding lowering the risk for fungal infection outbreak associated with injectable drugs. According to the report by Smith and colleagues, which of the following statements about the 2012–2013 outbreak of fungal meningitis linked with contaminated steroid injections and the effects of the public health response is correct?

A. The responsible pathogen was *Exserohilum rostratum*, an environmental mold
B. The outbreak was linked with injections of preservative-containing methylprednisolone acetate (MPA)
C. Public health efforts led to a 12% reduction in case fatality rate
D. An estimated nearly 1000 injections were averted, along with nearly 50 cases of meningitis or stroke and 40 deaths

2. According to the report by Smith and colleagues, which of the following statements about the nature of the public health response to the 2012–2013 outbreak of fungal meningitis linked with contaminated steroid injections is correct?

A. Specific contaminated lots could not be identified
B. The implicated manufacturer voluntarily recalled all of its injectable steroids
C. Potentially exposed persons were notified indirectly by media reports
D. The Centers for Disease Control and Prevention and partners developed diagnostic and treatment guidelines, posted them on the outbreak website, and disseminated them through health advisory notices

3. According to the report by Smith and colleagues, which of the following factors would most likely be associated with the risks for meningitis and stroke during the 2012–2013 outbreak of fungal meningitis linked with contaminated steroid injections?

A. Earliest-produced lots
B. Youngest vials
C. Diagnosis on or before October 4 (the date when the outbreak was widely publicized)
D. Preservative-containing formulation

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 75% passing score) and earn continuing medical education (CME) credit, please go to http://www.medscape.org/journal/eid. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the "Register" link on the right hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/about-ama/awards/ama-physicians-recognition-award.page. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for AMA PRA Category 1 Credits™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Acquired Drug Resistance in *Mycobacterum tuberculosis* and Poor Outcomes among Patients with Multidrug-Resistant Tuberculosis

CME Questions

1. You are seeing a 55-year-old recent immigrant from the republic of Georgia. He has been treated for pulmonary tuberculosis (TB) for the past 8 months, but gaps appear in his treatment course. What should you consider regarding the epidemiology and definitions of complicated TB?

A. The overall control of multidrug-resistant TB (MDR TB) has exceeded goals set by the World Health Organization
B. The rate of new cases of MDR TB worldwide was 480,000 in 2013
C. MDR TB is defined by resistance to ethambutol and ofloxacin
D. TB resistant to fluoroquinolones but sensitive to injectable agents should still be labeled extensively drug-resistant TB (XDR TB)

2. You are justifiably concerned regarding resistance to anti-TB agents in this case. Which of the following statements regarding acquired resistance (AR) in TB in the current study is most accurate?

A. Almost 14% of patients were thought to have AR
B. The mean time to the development of AR was 51 days
C. AR to ofloxacin was far more common than injectable agents
D. AR was not associated with the development of XDR TB

3. Which baseline factor was most associated with the risk for incident AR?

A. Cavitary disease
B. Older age
C. Male gender
D. History of previous TB

4. What should you consider regarding patient outcomes in the current study?

A. Approximately 90% of patients had a poor outcome
B. The most common category of poor outcomes was death
C. A positive result on sputum smear or culture at 4 and 6 months was consistently the most important variable associated with a poor outcome
D. AR was associated with a higher risk for death or treatment failure specifically

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

Ticks and Lyme Disease

For more information about Lyme disease, visit http://www.cdc.gov/Lyme

How to prevent tick bites when hiking and camping

Ticks can spread disease, including Lyme disease. Protect yourself:

- Use insect repellent that contains 20 - 30% DEET.
- Wear clothing that has been treated with permethrin.
- Take a shower as soon as you can after coming indoors.
- Look for ticks on your body. Ticks can hide under the armpits, behind the knees, in the hair, and in the groin.
- Put your clothes in the dryer on high heat for 60 minutes to kill any remaining ticks.

How to remove a tick

1. If a tick is attached to you, use fine-tipped tweezers to grasp the tick at the surface of your skin.
2. Pull the tick straight up and out. Don't twist or jerk the tick—this can cause the mouth parts to break off and stay in the skin. If this happens, remove the mouth parts with tweezers if you can. If not, leave them alone and let your skin heal.
3. Clean the bite and your hands with rubbing alcohol, an iodine scrub, or soap and water.
4. You may get a small bump or redness that goes away in 1-2 days, like a mosquito bite. This is not a sign that you have Lyme disease.

Note: *Do not put hot matches, nail polish, or petroleum jelly on the tick to try to make it pull away from your skin.*

If you remove a tick quickly (within 24 hours) you can greatly reduce your chances of getting Lyme disease.

National Center for Emerging and Zoonotic Infectious Diseases
Division of Vector Borne Diseases | Bacterial Diseases Branch

CS232422D

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles, see below and visit http://wwwnc.cdc.gov/eid/pages/author-resource-center.htm.

Summary of Authors' Instructions

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

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

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .tif or .jpeg files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

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Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references (not to exceed 15) but no abstract, figures, or tables. Include biographical sketch.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.