BRIEF REPORT

Emergence of Ciprofloxacin-Resistant *Neisseria meningitidis* in North America

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SUMMARY

We report on three cases of meningococcal disease caused by ciprofloxacin-resistant *Neisseria meningitidis*, one in North Dakota and two in Minnesota. The cases were caused by the same serogroup B strain. To assess local carriage of resistant *N. meningitidis*, we conducted a pharyngeal-carriage survey and isolated the resistant strain from one asymptomatic carrier. Sequencing of the gene encoding subunit A of DNA gyrase (*gyrA*) revealed a mutation associated with fluoroquinolone resistance and suggests that the resistance was acquired by means of horizontal gene transfer with the commensal *N. lactamica*. In susceptibility testing of invasive *N. meningitidis* isolates from the Active Bacterial Core surveillance system between January 2007 and January 2008, an additional ciprofloxacin-resistant isolate was found, in this case from California. Ciprofloxacin-resistant *N. meningitidis* has emerged in North America.

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INCREASING ANTIMICROBIAL RESISTANCE AMONG BACTERIAL PATHOGENS is a public health threat.[1] *N. meningitidis*, the cause of meningococcal disease, has been an exception, rarely showing resistance to commonly used antibiotics in the United States.[2]

Nasopharyngeal carriage of *N. meningitidis* precedes meningococcal disease. Approximately 8 to 20% of the general population are asymptomatic carriers,[3] and the vast majority remain well. Close contacts of case patients are at increased risk for disease, and chemoprophylaxis is an urgent intervention for prevention of disease.[4] The currently recommended chemoprophylactic antibiotics are rifampin, ciprofloxacin, and ceftriaxone.[4] The fluoroquinolone ciprofloxacin is frequently prescribed, and ciprofloxacin-resistant *N. meningitidis* is rare.[5]

Between January 2007 and January 2008, three cases of infection with ciprofloxacin-resistant *N. meningitidis* were detected in the United States, one in North Dakota and two in Minnesota. We report on the epidemiologic investigation, the pharyngeal-carriage survey used to assess local circulation of the bacterium, and the molecular characterization of the strain. Recent *N. meningitidis* isolates from a population-based surveillance system were screened for resistance.

METHODS

CASE INVESTIGATION

Cases of meningococcal disease are reportable to state public health authorities. Confirmed cases were defined as those that were clinically compatible with isolation of *N. meningitidis* in a specimen from a normally sterile site, such as blood or cerebrospinal fluid.[6] Probable cases were defined as those that were clinically compatible with polymerase-chain-reaction (PCR), immunohistochemical, or latex-agglutination evidence of *N. meningitidis* at a normally sterile site. Sterile cerebrospinal fluid or blood cultures from clinically compatible cases in North Dakota and Minnesota were tested by the Minnesota Department of Health with the use of a PCR assay for *N. meningitidis* DNA performed according to published methods.[7] Cases were investigated by public health staff to identify close contacts for chemoprophylaxis.[4]

CARRIAGE SURVEY

A survey of *N. meningitidis* carriage was performed 2 to 3 weeks after the third case patient (Patient 3) presented with the disease. The survey included this patient's close contacts and a convenience sample of volunteers, 18 years of age or older, from three local universities and a pub. These sites were chosen after in-depth interviews of Patient 3 and associated close contacts, who identified social networks at these sites. The survey was conducted as part of a public health response and was not considered research; thus, institutional review for protection of human subjects was not required.

University students were invited to participate at designated locations and specific times, and passersby were also recruited. Nonstudents who were associated with the universities were also eligible to participate. The pub's owner organized a meeting during which employees and regular patrons were invited to participate. All participants provided written or oral informed consent and answered questions regarding demographic characteristics and risk factors for carriage (for details see the Supplementary Appendix, available with the full text of this article at NEJM.org). Specimens were obtained by swabbing the posterior pharynx and tonsils with a sterile Dacron or rayon swab. Specimens were inoculated directly onto modified Thayer Martin agar (Remel) and incubated in carbon dioxide–enriched, sealed containers at 37°C. The plates were inspected after 24, 48, and 72 hours, and suspected colonies underwent further characterization.

IDENTIFICATION OF ISOLATES AND ANTIMICROBIAL SUSCEPTIBILITY TESTING

N. meningitidis isolates were identified and characterized with the use of conventional microbiologic methods. Serogroup results were determined by slide agglutination and real-time PCR assays (for details on the latter, see the Supplementary Appendix). All isolates were tested for susceptibility to ciprofloxacin, azithromycin, penicillin G, rifampin, tetracycline, and ceftriaxone with the use of Etest (AB Biodisk) or broth-microdilution panels (PML Microbiologicals). The minimum inhibitory concentration (MIC) breakpoints used were those established by the Clinical and Laboratory Standards Institute (CLSI) for broth microdilution.[8] Nonsusceptible isolates were confirmed with the use of broth microdilution.

Susceptibility testing was performed with Etest on 155 *N. meningitidis* isolates received at the Centers for Disease Control and Prevention (CDC) from state and local health departments through the Active Bacterial Core surveillance system from January 2007 through January 2008. Active Bacterial Core surveillance is an active, laboratory, and population-based surveillance system in 10 states, including Minnesota, covering 13% of the U.S. population.[9]

MOLECULAR CHARACTERIZATION

Ciprofloxacin-resistant *N. meningitidis* isolates were compared by means of pulsed-field gel electrophoresis with the use of the restriction endonuclease *Nhe*I (Promega), as previously described.[10] Multilocus sequence typing and typing for the alleles for porin A (*porA*), porin B (*porB*), and the ferric enterochelin receptor (*fetA*) were performed as previously described[11-14] (for details see the Supplementary Appendix). Sequence types were assigned by querying the Neisseria Multi Locus Sequence Typing and neisseria.org Web sites.[14-17] The quinolone-resistance–determining region (QRDR) of the genes encoding subunit A of both DNA gyrase (*gyrA*) and DNA topoisomerase IV (*parC*) from neisseria species were amplified by means of PCR and were sequenced with the use of novel primers derived from identical regions in *N. meningitidis*, *N. gonorrhoeae*, and *N. lactamica*. The entire *gyrA* sequences from selected isolates were also determined by means of PCR with the

use of primers derived from identical regions in *N. meningitidis*, *N. gonorrhoeae*, and *N. lactamica*. (For details on sequencing, see the Supplementary Appendix.) Sequence data were analyzed with the use of the Genetics Computer Group package, version 10.3,[18] and Lasergene 7 (DNASTAR). Phylogenetic analysis was performed with the use of MEGA3.[19]

RESULTS

CASE ASCERTAINMENT

Three cases of meningococcal disease caused by ciprofloxacin-resistant *N. meningitidis* were detected in North Dakota and Minnesota from January 2007 through January 2008, constituting 9% of a total of 33 confirmed cases reported in these states during that period (Fig. 1). The first case patient (Patient 1) was a toddler attending a child-care center in eastern North Dakota, where in August 2006 an adult child-care worker died from probable meningococcal disease. A culture of cerebrospinal fluid from the child-care worker was sterile but was PCR-positive for *N. meningitidis*. Ciprofloxacin was administered to adults who were close contacts of the child-care worker and rifampin to children at the care center, including Patient 1. There were no secondary cases. In January 2007, Patient 1 was hospitalized with culture-confirmed meningococcal disease and recovered with ceftriaxone treatment.

In January 2008, two more cases were detected in the region. The second case patient (Patient 2) was an adult from western Minnesota who died of culture-confirmed meningococcal disease. Patient 3 was a college student from western Minnesota who was hospitalized with culture-confirmed meningococcal disease and recovered with ceftriaxone treatment. Because the results of antimicrobial-susceptibility testing were not initially available, close adult contacts of all three patients received ciprofloxacin. Five close contacts of Patient 3 were offered repeat chemoprophylaxis with azithromycin,[20] and four accepted. There were no secondary cases. No epidemiologic links were identified among Patients 1, 2, and 3, and none had a history of recent foreign travel.

CARRIAGE-SURVEY AND LABORATORY RESULTS

Pharyngeal swabs were obtained from 530 carriage-survey participants, including 5 close contacts of Patient 3 and a convenience sample of 525 volunteers from the universities and the pub. The median age was 21 years (range, 18 to 65). Other demographic and risk-factor data from the questionnaire are summarized in the Supplementary Appendix. *N. meningitidis* was isolated from 40 survey participants (7.5%), including 1 close contact of Patient 3. *N. lactamica* was isolated from nine participants.

Serogroup Identification and Antimicrobial Susceptibility Testing

The *N. meningitidis* isolates from all three patients belonged to serogroup B, and bacterial DNA amplified from the cerebrospinal fluid of the child-care worker was positive for the same serogroup (Table 1). Of the 40 *N. meningitidis* carriage isolates, 9 were serogroup B (including 1 close contact of Patient 3), 2 were serogroup Y, and 29 could not be grouped with slide agglutination methods. The isolates from Patients 1, 2, and 3 and the close contact of Patient 3 were resistant to ciprofloxacin (MIC, 0.25 μg per milliliter, with susceptibility defined as ≤0.03 μg per milliliter, intermediate susceptibility defined as 0.06 μg per milliliter, and resistance defined as ≥0.12 μg per milliliter) and susceptible to penicillin, ceftriaxone, rifampin, tetracycline, and azithromycin. All carriage isolates were susceptible to azithromycin; three (8%) had an MIC at the limit of susceptibility (2.0 μg per milliliter).

Among 155 Active Bacterial Core surveillance isolates tested, 3 were resistant to ciprofloxacin. Two were the isolates from Patients 2 and 3, and the third was a serogroup Y isolate cultured from the blood of an adult with pneumonia (MIC, 0.25 μg per milliliter) in California in January 2008. Before susceptibility results were available, close adult contacts were given ciprofloxacin or ceftriaxone; there were no secondary cases.

Molecular Characterization

The ciprofloxacin-resistant *N. meningitidis* isolates from all three case patients and the close contact of Patient 3 had indistinguishable patterns of pulsed-field gel electrophoresis, belonged to the same clonal complex (sequence type [ST]–162), and had the same multilocus sequence type (ST-162) and *porA*, *porB*, and *fetA* types (Table 1). *N. meningitidis* DNA from the child-care worker's cerebrospinal fluid matched these isolates for *porA*, *fetA*, and clonal complex; there was insufficient DNA to characterize *porB* and the shikimate dehydrogenase gene (*aroE*), the latter being necessary for multilocus sequence typing. The 40 carriage-study isolates had varied multilocus-sequence-typing

profiles, and the only ST-162 isolate was from the close contact of Patient 3.

Sequences of the *gyrA* gene from the North Dakota–Minnesota ciprofloxacin-resistant *N. meningitidis* isolates were identical. The QRDR of the *gyrA* gene had a nucleotide change leading to a threonine-to-isoleucine substitution at amino acid 91 (T91I), a mutation associated with fluoroquinolone resistance.[5] The *gyrA* QRDR amplified from the child-care worker's cerebrospinal fluid revealed no mutations associated with fluoroquinolone resistance. Sequencing of the *parC* QRDR from ciprofloxacin-resistant isolates did not reveal any resistance-associated mutations.

The first 1265 nucleotides of the *gyrA* gene from the North Dakota–Minnesota ciprofloxacin-resistant isolates had only 94% similarity to the same region amplified from the child-care worker's cerebrospinal fluid, whereas the final 1486 nucleotides were identical, suggesting horizontal gene transfer (Fig. 2A). The *gyrA* sequence of the isolate from the California patient also showed the T91I mutation; however, the gene was 99.7% similar to the *gyrA* amplified from the child-care worker's cerebrospinal fluid, which is consistent with intraspecies variation (Fig. 2B). The *gyrA* genes from three *N. lactamica* isolates in the North Dakota–Minnesota carriage survey were sequenced. As compared with the *gyrA* gene from the North Dakota–Minnesota ciprofloxacin-resistant *N. meningitidis* isolates, the *gyrA* gene from one of the *N. lactamica* isolates had a single nucleotide difference in the first 1265 nucleotides at the position encoding the T91I mutation in the *N. meningitidis* isolates (99.9% similarity). The next 1441 nucleotides had 90% similarity, suggesting that an *N. lactamica* strain was the donor for the horizontal gene transfer (Fig. 2C). Phylogenetic analysis of the *gyrA* QRDR from 132 *N. meningitidis*, *N. gonorrhoeae*, and *N. lactamica* strains indicated clustering by species, except for the North Dakota–Minnesota ciprofloxacin-resistant *N. meningitidis* isolates, which were grouped within the *N. lactamica* cluster (for details, see the Supplementary Appendix).

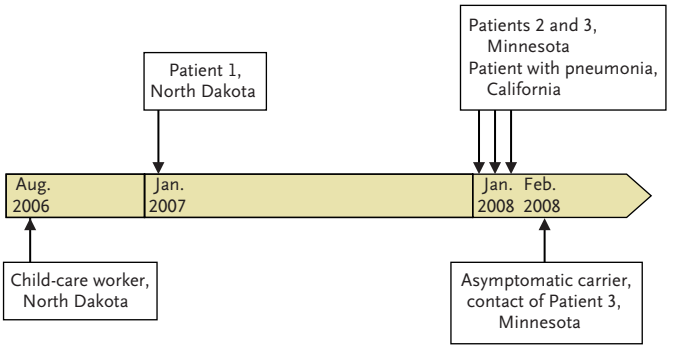

Figure 1. Timeline for Four Patients Infected with Ciprofloxacin-Resistant *Neisseria meningitidis*, a Patient with an Associated Infection, and an Asymptomatic Carrier.

In addition to the three cases of ciprofloxacin-resistant meningococcal disease identified in North Dakota and Minnesota between January 2007 and January 2008, there was a case involving a child-care worker associated with Patient 1 (suspected to have been infected with ciprofloxacin-susceptible *N. meningitidis*); an asymptomatic carrier of ciprofloxacin-resistant *N. meningitidis* (who had been in close contact with Patient 3), identified in the carriage survey; and a patient infected with ciprofloxacin-resistant *N. meningitidis* in California.

DISCUSSION

We report the emergence of ciprofloxacin-resistant *N. meningitidis* in North America. Since the case patients had no epidemiologic links and the duration of asymptomatic meningococcal carriage is often limited to a few months or less,[3,21] we suspect that the ciprofloxacin-resistant strain was probably maintained in the North Dakota–Minnesota area by multiple carriers. Although our carriage survey identified only one carrier of the ciprofloxacin-resistant strain, this low prevalence must be interpreted cautiously, since virulent *N. meningitidis* strains often represent a minority of carried strains.[21]

Fluoroquinolone antibiotics target bacterial DNA gyrase subunits A and B (encoded by *gyrA* and *gyrB*, respectively) and topoisomerase IV subunits A and B (encoded by *parC* and *parE*, respectively).[22] The *gyrA* T91I mutation in the ciprofloxacin-resistant isolates is analogous to *gyrA* mutations observed in fluoroquinolone-resistant *N. gonorrhoeae*[23] and has been described in ciprofloxacin-resistant *N. meningitidis* outside the United States.[5] The *gyrA* sequencing suggests that the ciprofloxacin-resistant strains from California and North Dakota–Minnesota acquired the mutation differently. The California strain most likely acquired resistance by means of point mutation. In contrast, the North Dakota–Minnesota strain probably acquired resistance through horizontal gene transfer from *N. lactamica*, since a large *gyrA* segment containing the T91I mutation closely matched the sequence from a locally carried *N. lactamica* strain. Although this *N. lactamica* sequence lacked the T91I mutation, we believe that horizontal gene

Table 1. Characterization of Ciprofloxacin-Resistant *Neisseria meningitidis* and Comparison Isolates.*

Source	State	Year	Sero-group	MLST	Clonal Complex	Ciprofloxacin MIC†	*gyrA*
						μg/ml	
Patient 1	ND	2007	B	ST-162	ST-162	0.25 (resistant)	T91I‡
Patient 2	MN	2008	B	ST-162	ST-162	0.25 (resistant)	T91I‡
Patient 3	MN	2008	B	ST-162	ST-162	0.25 (resistant)	T91I‡
Child-care worker§	ND	2006	B	NA¶	ST-162	Susceptible‖	Wild type
Contact of Patient 3	MN	2008	B	ST-162	ST-162	0.25 (resistant)	T91I‡
Patient in California	CA	2008	Y	ST-2533	ST-23/A3	0.25 (resistant)	T91I**

* MIC denotes minimum inhibitory concentration, MLST multilocus sequence type, and ST sequence type.
† MIC breakpoints established by the Clinical and Laboratory Standards Institute for broth microdilution were used, with susceptibility defined as ≤0.03 μg per milliliter, and resistance as ≥0.12 μg per milliliter.[8]
‡ In this threonine-to-isoleucine amino acid substitution at position 91, there is evidence that the mutation was introduced from an *N. lactamica* donor through horizontal gene transfer.
§ No isolate was cultured from the child-care worker, who worked in the day-care center attended by Patient 1.
¶ No isolate was obtained and there was not enough DNA to analyze the *aroE* gene.
‖ No isolate was cultured for susceptibility testing, but on the basis of the *gyrA* sequence, infection with ciprofloxacin-susceptible *N. meningitidis* was suspected.
** In this threonine-to-isoleucine amino acid substitution at position 91, there is no evidence that horizontal gene transfer introduced the mutation.

transfer remains the most likely mechanism of introduction (as opposed to mutation after transfer), since recombination events are at least 80 times as likely as mutation to introduce a change of any one nucleotide in a *N. meningitidis* housekeeping gene.[24] *N. lactamica* is a commensal of the human upper respiratory tract that is rarely pathogenic, and the horizontal gene transfer probably occurred in a person who simultaneously carried both neisseria species. *N. lactamica* carriage is common among infants,[25] and the child-care center might have been an ideal setting for interspecies genetic exchange.

Our findings add evidence to the theory that *N. meningitidis* can acquire resistance from the wider gene pool of related species. In all likelihood, horizontal gene transfers from other neisseria species introduced sulfonamide and β-lactam antibiotic resistance into some *N. meningitidis* strains.[26] *N. lactamica* with decreased susceptibility to ciprofloxacin has been suggested as a potential source for ciprofloxacin resistance in *N. meningitidis.*[27] Since most meningococci do not carry fluoroquinolone-resistance mutations, it seems unlikely that the mutation leads to any survival advantage in the absence of fluoroquinolone exposure. Therefore, use of fluoroquinolones, one of the most commonly prescribed classes of broad-spectrum antibiotics,[28] probably facilitated the emergence of fluoroquinolone-resistant strains.

Emerging fluoroquinolone resistance raises concern about current treatment and chemoprophylaxis recommendations for meningococcal disease. Practice guidelines from the Infectious Diseases Society of America consider fluoroquinolones an alternative therapy for presumptive or confirmed meningococcal meningitis.[29] Chemoprophylaxis recommendations evolved in the 1960s and 1970s in response to emerging resistance to sulfonamide and frequent adverse reactions to minocycline.[30] Cases of rifampin-resistant *N. meningitidis,* although rare, have been associated with chemoprophylaxis failures.[31] Among currently recommended agents, ciprofloxacin is often prescribed as chemoprophylaxis for adults (men and nonpregnant women) because the regimen is simple (a single oral dose) and because it is associated with a low rate of adverse events and relatively few drug–drug interactions. The isolation of the resistant strain from a close contact 2 weeks after receipt of ciprofloxacin might represent failure to clear carriage, although acquisition after chemoprophylaxis is possible.

Out of concern about the potential effect of resistance on the efficacy of chemoprophylaxis, a regional health advisory was issued for eastern North Dakota and western Minnesota, recommending that ciprofloxacin chemoprophylaxis not be used.[32] Rifampin or ceftriaxone is recommended in its place. A single dose of azithromycin is

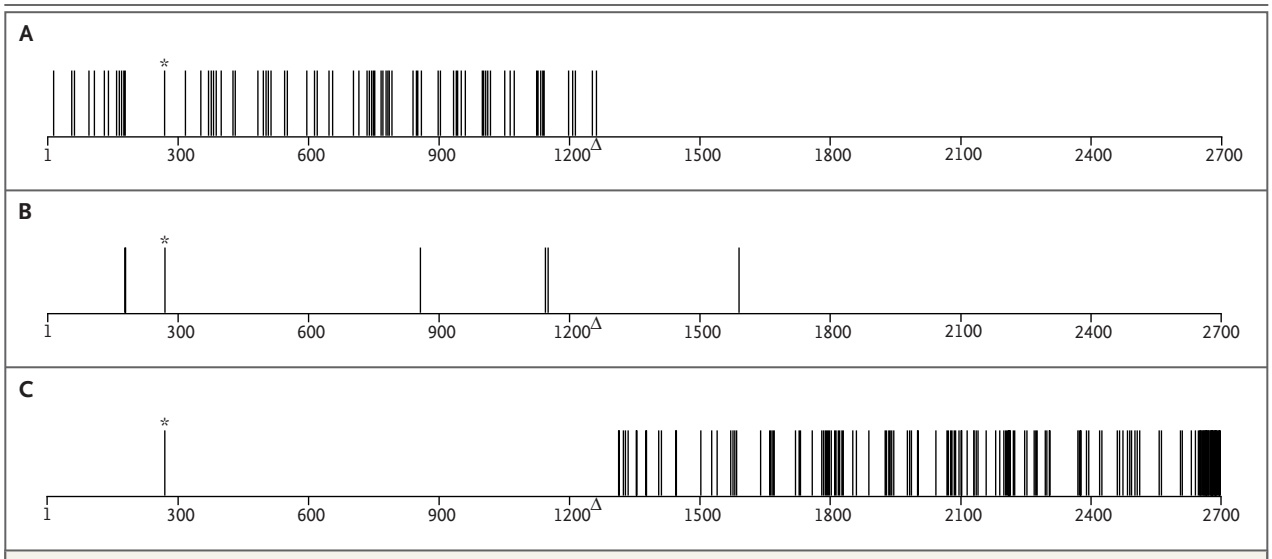

Figure 2. Distribution of Nucleotide Differences in Several *gyrA* Gene Sequences.

The 2751-nucleotide sequence of the *gyrA* gene is depicted to scale with the use of an isolate from Patient 1 (infected with ciprofloxacin-resistant *Neisseria meningitidis*) and amplified bacterial DNA from a child-care worker in North Dakota who was believed to have been infected with ciprofloxacin-susceptible *N. meningitidis* (Panel A), an isolate of ciprofloxacin-resistant *N. meningitidis* from a patient in California (2008) and the amplified bacterial DNA from the child-care worker (Panel B), and an isolate from Patient 1 and an *N. lactamica* isolate from a participant in the North Dakota–Minnesota carriage survey (the *N. lactamica gyrA* gene consists of 2739 nucleotides) (Panel C). Each vertical line represents a nucleotide change. The asterisk indicates the cytosine-to-thymine nucleotide change that leads to a mutation associated with ciprofloxacin resistance (T91I). Although the precise recombination breakpoint is unknown, it probably does not occur before nucleotide 1265 (indicated by delta [Δ]). The sequencing of the isolate from Patient 1 is representative of that from Patient 2, Patient 3, and the close contact of Patient 3.

effective in eradicating carriage[20] and can be considered in areas where the ciprofloxacin-resistant strain is established. However, our finding that 8% of *N. meningitidis* isolates from the carriage survey had MICs for azithromycin that were at the upper limit of susceptibility is a matter of concern. Therefore, areas with a single sporadic case of ciprofloxacin-resistant *N. meningitidis* should continue to follow current recommendations to use rifampin, ciprofloxacin, or ceftriaxone. Research is needed to evaluate the prophylactic efficacy of other antibiotics, including oral third-generation cephalosporins.[33]

Since antimicrobial susceptibility testing for *N. meningitidis* has not been routinely recommended in the United States, resistant cases may go undetected. At this time, widespread resistance to ciprofloxacin seems unlikely because secondary cases remain rare in the presence of routine ciprofloxacin use. It is unclear whether the ciprofloxacin resistance reported here represents a sporadic phenomenon or the early stage of wider dissemination. Previous experience with the widespread emergence of highly fluoroquinolone-resistant *N. gonorrhoeae*[34] raises concern. Health care providers should report suspected chemoprophylaxis failures to their local health departments. Wider surveillance for antimicrobial-resistant *N. meningitidis* would help inform future recommendations for chemoprophylaxis and treatment.

No potential conflict of interest relevant to this article was reported.

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