

Population-Based Surveillance of *Neisseria meningitidis* Antimicrobial Resistance in the United States

Brian H. Harcourt,^{1,a} Raydel D. Anderson,^{1,a} Henry M. Wu,¹ Amanda C. Cohn,¹ Jessica R. MacNeil,¹ Thomas H. Taylor,¹ Xin Wang,¹ Thomas A. Clark,¹ Nancy E. Messonnier,¹ and Leonard W. Mayer¹; Active Bacterial Core Surveillance Team²

¹Meningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, Centers for Disease Control and Prevention, and ²Emerging Infections Program, Atlanta, Georgia

Background. Antimicrobial treatment and chemoprophylaxis of patients and their close contacts is critical to reduce the morbidity and mortality and prevent secondary cases of meningococcal disease. Through the 1990's, the prevalence of antimicrobial resistance to commonly used antimicrobials among *Neisseria meningitidis* was low in the United States. Susceptibility testing was performed to ascertain whether the proportions of isolates with reduced susceptibility to antimicrobials commonly used for *N meningitidis* have increased since 2004 in the United States.

Methods. Antimicrobial susceptibility testing was performed by broth microdilution on 466 isolates of *N meningitidis* collected in 2004, 2008, 2010, and 2011 from an active, population-based surveillance system for susceptibility to ceftriaxone, ciprofloxacin, penicillin G, rifampin, and azithromycin. The molecular mechanism of reduced susceptibility was investigated for isolates with intermediate or resistant phenotypes.

Results. All isolates were susceptible to ceftriaxone and azithromycin, 10.3% were penicillin G intermediate (range, 8% in 2008–16.7% in 2010), and <1% were ciprofloxacin, rifampin, or penicillin G resistant. Of the penicillin G intermediate or resistant isolates, 63% contained mutations in the *penA* gene associated with reduced susceptibility to penicillin G. All ciprofloxacin-resistant isolates contained mutations in the *gyrA* gene associated with reduced susceptibility.

Conclusions. Resistance of *N meningitidis* to antimicrobials used for empirical treatment of meningitis in the United States has not been detected, and resistance to penicillin G and chemoprophylaxis agents remains uncommon. Therapeutic agent recommendations remain valid. Although periodic surveillance is warranted to monitor trends in susceptibility, routine clinical testing may be of little use.

Keywords. broth microdilution; ciprofloxacin; *Neisseria meningitidis*; penicillin G; susceptibility testing.

Neisseria meningitidis is a Gram-negative human pathogen that can cause severe meningitis and septicemia with symptoms that can worsen rapidly; approximately 10% of infected persons die, and 10%–15% of survivors suffer serious sequelae [1]. Antimicrobial treatment and chemoprophylaxis for patients with meningococcal

disease and their close contacts is critical to reduce morbidity and mortality and to prevent secondary cases. Although an extended-spectrum cephalosporin such as ceftriaxone is recommended for empirical treatment of meningitis [2], some treatment guidelines recommend switching to penicillin G when *N meningitidis* is confirmed [3]. Ceftriaxone, ciprofloxacin, and rifampin are the currently recommended chemoprophylactic antimicrobials [4]. Azithromycin was recommended as an alternative chemoprophylactic antimicrobial in eastern North Dakota and western Minnesota when ciprofloxacin resistance was first reported in North America (serogroup B) [5]. In the 1990's, the prevalence of antimicrobial resistance among *N meningitidis* was low in the United States [6, 7]. Since that time, increases in the number of penicillin G-intermediate and -resistant isolates [8, 9] and

Received 6 May 2015; accepted 3 August 2015.

^aR. D. A. and B. H. H. contributed equally to the project and are joint first authors.

Correspondence: Brian H. Harcourt, PhD, Meningitis Laboratory, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, MS D-11, Atlanta, Georgia 30329 (beh0@cdc.gov).

Open Forum Infectious Diseases

Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2015. This work is written by (a) US Government employee(s) and is in the public domain in the US.

DOI: 10.1093/ofid/ofv117

sporadic strains that are resistant to ciprofloxacin or rifampin have been reported in the United States [10, 11]. The clinical significance of these changes in susceptibility is unclear, although a single case in the United States has been reported where resistance to rifampin may have resulted in a child contracting meningococcal disease from a sibling [10].

Reduced susceptibility to penicillin G in *N meningitidis* has been most closely linked to alterations in the *penA* gene leading to 5 amino acid changes, F504L, A510V, I515V, G541N, I566V, in the penicillin binding protein 2 (PBP2) [12, 13]. The *penA* genes of susceptible isolates are highly similar in sequence, but isolates with reduced susceptibility to penicillin G have high sequence variability in the *penA* gene. These variable regions are mosaic due to transformation with commensal *Neisseria* spp resistant to penicillin G [12–14]. Resistance to ciprofloxacin in *N meningitidis* has been linked to mutations in the *gyrA* gene [15–17], and resistance to rifampin has been linked to mutations in the central region of the *rpoB* gene [18, 19]. Understanding the molecular mechanism responsible for a reduced susceptibility phenotype can provide insight into population biology and changes in susceptibility patterns of circulating strains with the limitation that not all reduced susceptibility is caused by a single common mechanism.

We tested isolates of *N meningitidis* collected from a population-based surveillance system in 2004, 2008, 2010, and 2011 for susceptibility to ceftriaxone, ciprofloxacin, penicillin G, rifampin, and azithromycin using broth microdilution to assess whether the proportions of isolates with reduced susceptibility to the antimicrobials most commonly used in the United States for treatment and chemoprophylaxis have changed since the mid-1990s. The molecular mechanism of reduced susceptibility was investigated for all isolates with intermediate or resistant phenotypes.

METHODS

Test Isolates

Antimicrobial susceptibility testing was performed on all 466 isolates of *N meningitidis* collected in 2004 (N = 142), 2008 (N = 150), 2010 (N = 78), and 2011 (N = 96) from the Active Bacterial Core Surveillance system (ABCs). The Active Bacterial Core Surveillance system is an active, laboratory, and population-based surveillance system in 10 states or metropolitan areas, and it covers approximately 13% of the US population [20]. All isolates were cultured from normally sterile sites such as cerebrospinal fluid, blood, or joint fluid. Isolates were confirmed as *N meningitidis* and further characterized using standard biochemical testing, slide agglutination serogrouping, and real-time polymerase chain reaction (PCR) methods [21, 22].

Susceptibility Testing

Broth microdilution testing was performed for penicillin G, ceftriaxone, ciprofloxacin, rifampin, and azithromycin. Panels were

either commercially prepared (PML, Wilsonville, OR) or prepared in-house by using cation-adjusted Mueller Hinton broth (CAMHB) (BD, Franklin Lakes, NJ) containing 5% lysed horse blood following Clinical and Laboratory Standards Institute guidelines [23]. Inoculum preparation and minimum inhibitory concentration (MIC) interpretations were performed according to previously reported guidelines [23, 24] (see [Supplementary Table 1](#) for interpretive breakpoints used). Antimicrobial concentrations ranged from 0.0039 µg/mL to 8.0 µg/mL in log₂ dilutions for all antimicrobials tested using the PML panels. Antimicrobial concentrations ranged from 0.015 µg/mL to 2.0 µg/mL in log₂ dilutions for ceftriaxone and penicillin G, 0.008 µg/mL to 1.0 µg/mL in log₂ dilutions for ciprofloxacin, and 0.03 µg/mL to 4.0 µg/mL in log₂ dilutions for rifampin and azithromycin using in-house panels. Cultures grown for 20–24 hours on chocolate agar containing hemoglobin and IsoVitaleX (BD, Franklin Lakes, NJ) at 37°C in 5% CO₂ were used to prepare an inoculum adjusted to a 0.5 McFarland standard equivalent using CAMHB. *Streptococcus pneumoniae* ATCC 49 619 and *Escherichia coli* ATCC 25 922 were included in each batch as quality control organisms. All isolates resistant to penicillin G were tested for β-lactamase activity using nitrocefin disks, following manufacturer's instructions (Remel, Lenexa, KS).

DNA Amplification and Sequencing

All gene amplifications were performed using the Expand High Fidelity PCR System (Roche Diagnostics, Indianapolis, IN). DNA sequencing was performed using the BigDye Terminator version 3.1 Cycle Sequencing Kit and analyzed on a 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA).

To identify amino acid alterations in the PBP2 protein associated with reduced susceptibility to penicillin G, the highly variably region of the *penA* gene (402 bps) was amplified and sequenced from isolates with a penicillin G-intermediate or -resistant phenotype as previously described [13]. The full *penA* gene of the isolates that had both a penicillin G-intermediate or -resistant phenotype and none of the 5 PBP2 amino acid changes commonly associated with reduced susceptibility to penicillin G was amplified and sequenced using the primers listed in [Supplementary Table 2](#). Polymerase chain reaction amplification conditions for the full-length *penA* gene were as follows: 95°C for 5 minutes followed by 35 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes, followed by 72°C for 7 minutes. Full-length *penA* gene sequences are available in GenBank (see [Supplementary Table 3](#) for accession numbers). The *penA* gene nucleotide and PBP2 amino acid positions are given relative to the serogroup B MC58 reference strain of *N meningitidis* (GenBank accession number AE002098).

To determine the molecular mechanism of reduced susceptibility in ciprofloxacin resistant isolates, the first 539 nucleotides of the *gyrA* gene and the first 520 nucleotides of the *parC* genes were amplified, sequenced, and analyzed as previously

described [11]. Reduced susceptibility to rifampin has been shown to be associated with a 717 nucleotide region of the *rpoB* gene between nucleotides 1221 and 1937 [18]. This region was amplified and sequenced from any isolate categorized as rifampin intermediate or resistant using the primers listed in Supplementary Table 2 and PCR conditions previously described [11].

Data Analysis

Nucleotide sequence was assembled and trimmed using DNAs-tar LaserGene 9 (Madison, WI). Further analysis of DNA sequence data was performed using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) [25]. The assignment of *penA* allele types was performed by querying <http://pubmlst.org/neisseria/> [26]. Pearson's χ^2 and Fisher's exact tests were used to compare proportions with a value of 0.05 set as cutoff for significance. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

Among the 466 isolates, 406 (87.1%) were susceptible to all antimicrobials tested. All isolates tested were ceftriaxone and azithromycin susceptible. The most reduced susceptibility observed was to penicillin G (Table 1). Four (0.9%) isolates were ciprofloxacin resistant (all with MICs of 0.25 $\mu\text{g}/\text{mL}$), and 1 (0.2%) isolate was rifampin resistant (MIC of 2.0 $\mu\text{g}/\text{mL}$).

Table 1. Antimicrobial Susceptibility Testing Surveillance for *Neisseria meningitidis* in United States in 2004, 2008, 2010, and 2011 by Broth Microdilution

Antimicrobial	2004 N (%) ^a	2008 N (%)	2010 N (%)	2011 N (%)
Ceftriaxone				
Susceptible	142 (100)	150 (100)	78 (100)	96 (100)
Ciprofloxacin^b				
Susceptible	142 (100)	147 (98.0)	78 (100)	95 (99.0)
Intermediate	0	0	0	0
Resistant	0	3 (2.0)	0	1 (1.0)
Penicillin G^b				
Susceptible	129 (90.8)	137 (91.3)	64 (82.1)	85 (88.5)
Intermediate	12 (8.5)	12 (8.0)	13 (16.7)	11 (11.5)
Resistant	1 (0.7)	1 (0.7)	1 (1.3)	0
Rifampin^b				
Susceptible	141 (99.3)	149 (99.3)	78 (100)	96 (100)
Intermediate	0	1 (0.7)	0	0
Resistant	1 (0.7)	0	0	0
Azithromycin				
Susceptible	142 (100)	150 (100)	78 (100)	96 (100)

^a Percentages may not add up to 100 due to rounding.

^b Relative rates of susceptibility, intermediate, or resistant not significantly different between years (all Pearson's χ^2 $P > .16$).

One (0.2%) isolate was rifampin intermediate (MIC of 1.0 $\mu\text{g}/\text{mL}$). There were no significant differences, year to year, between the proportion of isolates with antibiotic nonsusceptibility (all Pearson χ^2 , $P_s > .16$). There were no meaningful differences in the MIC₅₀s or MIC₉₀s for any of the antimicrobials from year to year (Table 2).

The overall proportion of isolates that were penicillin nonsusceptible was 10.9% (51 of 466). The proportion of penicillin G intermediate isolates ranged from 8.0% (12 of 150) in 2008 to 16.7% (13 of 78) in 2010 (Table 1). The range does not represent a statistically significant difference across years (Pearson's χ^2 , $P = .41$), although there was a higher percentage of resistant and intermediate specimens in 2010 and 2011 than in 2004 and 2008. Three isolates were penicillin G resistant, and none had detectable β -lactamase activity. For 2004, 2008, 2010, and 2011 combined, penicillin G nonsusceptibility, including both intermediate and resistant, was observed in 19.5% of serogroup Y isolates, including all 3 penicillin G-resistant isolates (26 of 133), 7.0% of serogroup B isolates (13 of 185), 7.7% of serogroup C isolates (9 of 117), 11.1% of serogroup W isolates (2 of 18), and 7.7% of nongroupable isolates (1 of 13). These proportions represent a significant difference across serogroups (Pearson's χ^2 , $P = .006$). A higher percentage of serogroup Y isolates were penicillin G nonsusceptible than the other serogroups combined (19.5% [26 of 133] vs 7.5% [25 of 333]; Pearson's χ^2 , $P < .001$). Isolates from 3 of 52 (5.8%) fatal cases of meningococcal disease were penicillin G intermediate, and each isolate belonged to a different serogroup. There was no significant difference for any antimicrobial (all Pearson χ^2 , $P_s > .21$; all Fisher's exact table probability, $P_s > .13$) or with regard to reduced susceptibility to penicillin G between isolates cultured from cerebrospinal fluid (13 of 108) vs blood (37 of 348). Ten isolates from 4 other sources were not included in this comparison.

Of the 51 penicillin G-intermediate or -resistant isolates, 30 had mosaic *penA* alleles, all of which contained the 5 amino acid changes closely associated with reduced susceptibility to penicillin G [13, 27]. Fifteen different mosaic *penA* alleles were detected among these 30 isolates, with allele 9 being the most common, which was detected in 7 isolates. No other allele was represented by more than 3 isolates. Among these 30 isolates, 12 (40%) were serogroup B, 10 (33.3%) were serogroup Y, 5 (16.7%) were serogroup C, 2 (6.7%) were serogroup W, and 1 (3.3%) was nongroupable. Fifteen of the 20 isolates that were penicillin G intermediate and had nonmosaic *penA* alleles were serogroup Y, and 12 of those had *penA* allele 22. Sequencing of the entire *penA* gene from these 20 isolates did not reveal any mosaic DNA structures, and all had the same amino acid sequence as the *N meningitidis* type strain MC58, which has no known amino acid changes associated with reduced susceptibility to penicillin G. Mosaic *penA* alleles were detected in 2 of 3 penicillin G-resistant isolates (alleles 8 and 318). The penicillin G-resistant isolate without a mosaic *penA* allele had allele 22.

Table 2. Minimum Inhibitory Concentration (MIC)₅₀, MIC₉₀, and Ranges for Antimicrobial Susceptibility Testing Surveillance for *Neisseria meningitidis* in United States in 2004, 2008, 2010, and 2011 by Year by Broth Microdilution

Antimicrobial	2004			2008			2010			2011		
	MIC ₅₀ µg/mL	MIC ₉₀ µg/mL	Range µg/mL	MIC ₅₀ µg/mL	MIC ₉₀ µg/mL	Range µg/mL	MIC ₅₀ µg/mL	MIC ₉₀ µg/mL	Range µg/mL	MIC ₅₀ µg/mL	MIC ₉₀ µg/mL	Range µg/mL
Ceftriaxone	≤0.003	≤0.015	≤0.003 – ≤0.015	≤0.003	≤0.015	≤0.003–0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015
Ciprofloxacin	≤0.003	≤0.008	≤0.003–0.03	≤0.003	≤0.008	≤0.003–0.25	≤0.008	≤0.008	≤0.008–0.015	≤0.008	≤0.008	≤0.008–0.12
Penicillin G	0.03	0.06	≤0.015–0.5	0.03	0.06	≤0.015–1.0	0.06	0.12	≤0.015–>2	0.03	0.12	≤0.015–0.25
Rifampin	0.03	0.12	≤0.003–2	0.015	0.12	≤0.003–1	0.06	0.25	≤0.015–0.5	≤0.03	0.12	≤0.03–0.25
Azithromycin	0.5	0.5	≤0.03–2	0.12	0.5	0.03–2	0.185	0.5	≤0.015–2	0.25	0.5	≤0.03–1

Sequencing of the entire *penA* gene of this isolate revealed a single adenine to threonine amino acid change at position 434 relative to the PBP2 protein from *N meningitidis* strain MC58. It is unlikely that this amino acid change is responsible for the penicillin G-resistant phenotype because the amino acid sequence of the PBP2 protein is identical to a known penicillin G-susceptible isolate (GenBank accession number AY127633).

Ciprofloxacin resistance was observed in 4 (0.9%) isolates, all with an MIC of 0.25 µg/mL; 2 serogroup B isolates and 1 serogroup Y isolate in 2008 and 1 serogroup Y isolate in 2011 (Table 2). Two of the 2008 isolates were from a cluster of cases in Minnesota and have been previously described [5, 11]. A threonine to isoleucine change at amino acid 91 of the GyrA protein closely associated with ciprofloxacin resistance was found in the resistant isolates from 2008 and 2011. One rifampin-resistant serogroup Y isolate in 2004 with an MIC of 2.0 µg/mL and 1 intermediate serogroup B isolate in 2008 were detected (Table 2), but no amino acid changes in the RpoB protein associated with reduced susceptibility to rifampin were found.

DISCUSSION

Antimicrobial susceptibility surveillance of isolates of *N meningitidis* collected in 2004, 2008, 2010, and 2011 provides reassuring data that nonsusceptibility to antimicrobials commonly used for treatment and chemoprophylaxis of meningococcal disease is stable and low in the United States. The clinical significance of the increase in the proportion of penicillin G-intermediate isolates is unknown; however, the proportion of penicillin G-intermediate isolates among fatal cases was low. Active population-based surveillance is useful for evaluating antimicrobial susceptibility trends, but nonsusceptible isolates may have been missed in intervening years or in cases or clusters of cases outside of the surveillance area. There was no evidence of any clusters of penicillin G nonsusceptible cases in this study. Although penicillin G is not recommended for empirical treatment of suspected bacterial meningitis in the United States [2], it may be used as the definitive treatment when meningococcal disease is confirmed [3, 28]. Before treatment with penicillin, susceptibility to penicillin G should be ascertained.

Over the past 2 decades, an increase in the proportion of isolates penicillin G intermediate has been observed in many parts of the world, with the highest rates in Europe [13, 29–32]. A similar trend occurred in the United States with percentages of penicillin G-intermediate isolates increasing from 3.0% in 1997 [7] to a peak of 16.7% in 2010. This increase is not as pronounced as that observed in Belgium from 2000 to 2010 where the penicillin G-nonsusceptibility rate rose from 4.8% in 2000 to 40.9% in 2010, with fluctuations during intervening years [33]. In Ontario, Canada, 21.7% of isolates tested from 2000 to 2006 were penicillin G intermediate, although the rates remained stable over the years surveyed. We observed a strong association

between reduced susceptibility to penicillin G and serogroup Y that was not present when susceptibility testing was performed on ABCs isolates in 1997 [7]. Elevated MICs to penicillin G were also recently reported to be common in serogroup Y in Ontario, Canada, although that group observed a significant correlation with increased MICs to penicillin G and serogroup W [34]. The low number of serogroup W isolates in our surveillance set makes it difficult to render conclusions about overall susceptibility to penicillin G in that serogroup, although other groups have reported correlations between serogroup W strains and reduced susceptibility to penicillin G [13, 33, 35].

The absence of a mosaic *penA* allele encoding the 5 amino acid changes associated with reduced susceptibility to penicillin G is not necessarily indicative of a penicillin G-susceptible phenotype. Approximately 40% of penicillin G-intermediate or -resistant isolates did not have a mosaic *penA* allele and did not contain amino acid substitutions in the PBP2 protein commonly associated with reduced susceptibility to penicillin G. In 1 study of approximately 1600 isolates collected mostly from Europe, 65% showed reduced susceptibility to penicillin G, but only 38% had mosaic *penA* alleles [13]. du Plessis et al [36] identified mosaic *penA* genes in only 25 of 87 isolates determined to be penicillin G intermediate. Thus, relying solely on *penA* genotyping to screen for reduced susceptibility to penicillin G is likely not sufficient. These observations suggest that reduced susceptibility to penicillin G can arise from other as yet unidentified mechanisms besides mutations in the *penA* gene. Reduced affinity of PBP-1 for penicillin G and decreased expression of the class 3 porin have been shown experimentally to increase resistance to penicillin G in *N meningitidis* [37].

Resistance to ciprofloxacin or rifampin remains sporadic in the United States and around the world [10, 15, 16, 38–40]. The threonine to isoleucine amino acid change in the 2 ciprofloxacin resistant isolates from Minnesota arose from a horizontal gene transfer event with *Neisseria lactamica* and have previously been described [11, 17]. The resistant isolate from 2011 contained the threonine to isoleucine change at amino acid 91 with no evidence of a horizontal gene transfer event, but not the recently described threonine to alanine change at amino acid 173 [17]. In a recent study, all isolates but 1 with an MIC of >1 µg/mL to rifampin had 1 or more specific amino acid changes in the RpoB protein [19]. The RpoB protein of the 1 rifampin-resistant isolate in this study, which had an MIC at the cutoff point for resistance, did not contain amino acid changes associated with resistance to rifampin and may be utilizing a different mechanism of resistance.

CONCLUSIONS

As of 2011, resistance of *N meningitidis* to the antimicrobials used for empirical treatment of meningitis in the United States had not been detected, and resistance to chemoprophylaxis agents

remained uncommon and sporadic, thus therapeutic agent recommendations remain valid. A sporadic cluster of resistance is concerning because antimicrobials are such an important component of treatment and prevention of secondary cases of meningococcal disease; therefore, clinicians should report suspected treatment and/or chemoprophylaxis failures. Periodic surveillance of antimicrobial susceptibility patterns in circulating isolates of *N meningitidis* to monitor emergent trends is warranted.

Acknowledgments

We thank Anita Glennen (Minnesota Department of Health, St. Paul, MN); Joel Mortensen (Cincinnati Children's Hospital, Cincinnati, OH); Karen Anderson and David Lonsway (Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention [CDC], Atlanta, GA) for recommendations, technical guidance, and preparing panels; and Brian Plikaytis (Division of Bacterial Disease, CDC) for providing statistical support.

Financial support. This study was funded by a grant from the Antimicrobial Resistance Working Group, CDC.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

References

1. Rosenstein NE, Perkins BA, Stephens DS, et al. Meningococcal disease. *N Engl J Med* **2001**; 344:1378–88.
2. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. *Clin Infect Dis* **2004**; 39:1267–84.
3. American Academy of Pediatrics. Meningococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, eds. *Red Book: 2012 Report of the Committee on Infectious Diseases*. 28th ed. Grove Village, IL: American Academy of Pediatrics, **2012**:500–9.
4. Bilukha OO, Rosenstein N, National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). Prevention and control of meningococcal disease. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* **2005**; 54:1–21.
5. Centers for Disease Control and Prevention (CDC). Emergence of fluoroquinolone-resistant *Neisseria meningitidis*—Minnesota and North Dakota, 2007–2008. *MMWR Morb Mortal Wkly Rep* **2008**; 57:173–5.
6. Jackson LA, Tenover FC, Baker C, et al. Prevalence of *Neisseria meningitidis* relatively resistant to penicillin in the United States, 1991. Meningococcal Disease Study Group. *J Infect Dis* **1994**; 169:438–41.
7. Rosenstein NE, Stocker SA, Popovic T, et al. Antimicrobial resistance of *Neisseria meningitidis* in the United States, 1997. The Active Bacterial Core Surveillance (ABCs) Team. *Clin Infect Dis* **2000**; 30:212–3.
8. Jorgensen JH, Crawford SA, Rosenstein NE. Antimicrobial resistance among U.S. surveillance isolates of *Neisseria meningitidis* from 1997–2002. In: 43rd Annual Meeting of the Infectious Diseases Society of America. **2005**; San Francisco, CA.
9. Mortensen JE, Gerrety MJ, Gray LD. Surveillance of antimicrobial resistance in *Neisseria meningitidis* from patients in the Cincinnati tristate region (Ohio, Kentucky, and Indiana). *J Clin Microbiol* **2006**; 44:1592–3.
10. Rainbow J, Cebelinski E, Bartkus J, et al. Rifampin-resistant meningococcal disease. *Emerg Infect Dis* **2005**; 11:977–9.

11. Wu HM, Harcourt BH, Hatcher CP, et al. Emergence of ciprofloxacin-resistant *Neisseria meningitidis* in North America. *N Engl J Med* **2009**; 360:886–92.
12. Spratt BG, Zhang QY, Jones DM, et al. Recruitment of a penicillin-binding protein gene from *Neisseria flavescens* during the emergence of penicillin resistance in *Neisseria meningitidis*. *Proc Natl Acad Sci U S A* **1989**; 86:8988–92.
13. Taha MK, Vazquez JA, Hong E, et al. Target gene sequencing to characterize the penicillin G susceptibility of *Neisseria meningitidis*. *Antimicrob Agents Chemother* **2007**; 51:2784–92.
14. Zhang QY, Jones DM, Saez Nieto JA, et al. Genetic diversity of penicillin-binding protein 2 genes of penicillin-resistant strains of *Neisseria meningitidis* revealed by fingerprinting of amplified DNA. *Antimicrob Agents Chemother* **1990**; 34:1523–8.
15. Shultz TR, Tapsall JW, White PA, Newton PJ. An invasive isolate of *Neisseria meningitidis* showing decreased susceptibility to quinolones. *Antimicrob Agents Chemother* **2000**; 44:1116.
16. Singhal S, Purnapatre KP, Kalia V, et al. Ciprofloxacin-resistant *Neisseria meningitidis*, Delhi, India. *Emerg Infect Dis* **2007**; 13:1614–6.
17. Castanheira M, Deshpande LM, Jones RN, Farrell DJ. Evaluation of quinolone resistance-determining region mutations and efflux pump expression in *Neisseria meningitidis* resistant to fluoroquinolones. *Diagn Microbiol Infect Dis* **2012**; 72:263–6.
18. Carter PE, Abadi FJ, Yakubu DE, Pennington TH. Molecular characterization of rifampin-resistant *Neisseria meningitidis*. *Antimicrob Agents Chemother* **1994**; 38:1256–61.
19. Taha MK, Hedberg ST, Szatanik M, et al. Multicenter study for defining the breakpoint for rifampin resistance in *Neisseria meningitidis* by *rpoB* sequencing. *Antimicrob Agents Chemother* **2010**; 54:3651–8.
20. Schuchat A, Hilger T, Zell E, et al. Active bacterial core surveillance of the emerging infections program network. *Emerg Infect Dis* **2001**; 7:92–9.
21. Mothershed EA, Sacchi CT, Whitney AM, et al. Use of real-time PCR to resolve slide agglutination discrepancies in serogroup identification of *Neisseria meningitidis*. *J Clin Microbiol* **2004**; 42:320–8.
22. Murray PR, Baron EJ, Jorgensen JH, et al. eds. *Manual of Clinical Microbiology*. 9th ed. Washington, DC. ASM Press; **2007**.
23. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth ed.* Wayne, PA; Clinical and Laboratory Standards Institute; **2009**.
24. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement*. CLSI Document M100-S25 ed. Wayne, PA; Clinical and Laboratory Standards Institute; **2015**.
25. Altschul SF, Madden TL, Schaffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **1997**; 25:3389–402.
26. Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinform* **2010**; 11:595.
27. Stefanelli P, Carattoli A, Neri A, et al. Prediction of decreased susceptibility to penicillin of *Neisseria meningitidis* strains by real-time PCR. *J Clin Microbiol* **2003**; 41:4666–70.
28. Terranella A, Beekmann SE, Polgreen PM, et al. Practice patterns of infectious disease physicians for management of meningococcal disease. *Pediatr Infect Dis J* **2012**; 31: e208–12.
29. Arreaza L, de La Fuente L, Vazquez JA. Antibiotic susceptibility patterns of *Neisseria meningitidis* isolates from patients and asymptomatic carriers. *Antimicrob Agents Chemother* **2000**; 44:1705–7.
30. Campos J, Fuste MC, Trujillo G, et al. Genetic diversity of penicillin-resistant *Neisseria meningitidis*. *J Infect Dis* **1992**; 166:173–7.
31. Canica M, Dias R, Ferreira E. *Neisseria meningitidis* C:2b:P1.2,5 with intermediate resistance to penicillin, Portugal. *Emerg Infect Dis* **2004**; 10:526–9.
32. Bijlsma MW, Bekker V, Brouwer MC, et al. Epidemiology of invasive meningococcal disease in the Netherlands, 1960–2012: an analysis of national surveillance data. *Lancet Infect Dis* **2014**; 14:805–12.
33. Bertrand S, Carion F, Wintjens R, et al. Evolutionary changes in antimicrobial resistance of invasive *Neisseria meningitidis* isolates in Belgium from 2000 to 2010: increasing prevalence of penicillin nonsusceptibility. *Antimicrob Agents Chemother* **2012**; 56:2268–72.
34. Brown EM, Fisman DN, Drews SJ, et al. Epidemiology of invasive meningococcal disease with decreased susceptibility to penicillin in Ontario, Canada, 2000 to 2006. *Antimicrob Agents Chemother* **2010**; 54:1016–21.
35. Hedberg ST, Olcen P, Fredlund H, Unemo M. Antibiotic susceptibility of invasive *Neisseria meningitidis* isolates from 1995 to 2008 in Sweden—the meningococcal population remains susceptible. *Scand J Infect Dis* **2010**; 42:61–4.
36. du Plessis M, von Gottberg A, Cohen C, et al. *Neisseria meningitidis* intermediately resistant to penicillin and causing invasive disease in South Africa in 2001 to 2005. *J Clin Microbiol* **2008**; 46:3208–14.
37. Orus P, Vinas M. Mechanisms other than penicillin-binding protein-2 alterations may contribute to moderate penicillin resistance in *Neisseria meningitidis*. *Int J Antimicrob Agents* **2001**; 18:113–9.
38. Corso A, Faccione D, Miranda M, et al. Emergence of *Neisseria meningitidis* with decreased susceptibility to ciprofloxacin in Argentina. *J Antimicrob Chemother* **2005**; 55:596–7.
39. Skoczynska A, Alonso JM, Taha MK. Ciprofloxacin resistance in *Neisseria meningitidis*, France. *Emerg Infect Dis* **2008**; 14:1322–3.
40. Sorhouet-Pereira C, Efron A, Galetti P, et al. Phenotypic and genotypic characteristics of *Neisseria meningitidis* disease-causing strains in Argentina, 2010. *PLoS One* **2013**; 8:e58065.