Acquisition of ciprofloxacin resistance among an expanding clade of β-lactamase positive, serogroup Y *Neisseria meningitidis* in the United States

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Abstract

Background: Penicillin and ciprofloxacin are important for invasive meningococcal disease (IMD) management and prevention. IMD cases caused by penicillin- and ciprofloxacin-resistant *Neisseria meningitidis* containing a ROB-1 β-lactamase gene (bla_{ROB-1}) and a mutated DNA gyrase gene (*gyrA*), have been recently reported in the United States.

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Methods: We examined 2097 meningococcal genomes collected through US population-based surveillance from January 2021 to February 2020 to identify IMD cases caused by strains with bla_{ROB-1} or $gyrA$-mediated resistance. Antimicrobial resistance was confirmed phenotypically. The US isolate genomes were compared to non-US isolate genomes containing bla_{ROB-1}. Interspecies transfer of ciprofloxacin resistance was assessed by comparing $gyrA$ among *Neisseria* species.

Results: Eleven penicillin- and ciprofloxacin-resistant isolates were identified after December 2018; all were serogroup Y, sequence type 3587, clonal complex (CC) 23, and contained bla_{ROB-1} and a T91I-containing $gyrA$ allele. An additional 22 penicillin-resistant, bla_{ROB-1}-containing US isolates with wild-type $gyrA$ were identified from 2013–2020. All 33 bla_{ROB-1}-containing isolates formed a single clade, along with 12 bla_{ROB-1}-containing isolates from six other countries. Two-thirds of bla_{ROB-1}-containing US isolates were from Hispanic individuals. Twelve additional ciprofloxacin-resistant isolates with $gyrA$ T91 mutations were identified. Ciprofloxacin-resistant isolates belonged to six CCs and contained 10 unique $gyrA$ alleles; seven were similar or identical to alleles from *N. lactamica* or *N. gonorrhoeae*.

Conclusions: Recent IMD cases caused by a dual resistant serogroup Y suggest changing antimicrobial resistance patterns in the United States. The emerging dual-resistance is due to acquisition of ciprofloxacin resistance by β-lactamase-containing *N. meningitidis*. Routine antimicrobial resistance surveillance will effectively monitor resistance changes and spread.

INTRODUCTION

Neisseria meningitidis causes life-threatening invasive meningococcal disease (IMD). Suspected meningococcal disease is treated empirically with cefotaxime or ceftriaxone, while penicillin and ampicillin have historically been treatment options only after meningococcal disease is confirmed [1]. Penicillin is also sometimes used for long-term meningococcal disease chemoprophylaxis among patients using complement inhibitors such as eculizumab or ravulizumab [2–5]. Ciprofloxacin is commonly used for antibiotic prophylaxis for close contacts of meningococcal disease patients; rifampin, or ceftriaxone can also be used for prophylaxis [1].

Antimicrobial resistance has historically been rare among *N. meningitidis*. While isolates with reduced susceptibility to penicillin have been reported in multiple countries [6–10], only intermediate susceptibility phenotypes are commonly detected; penicillin-resistant isolates remain rare [11, 12]. Resistance to ciprofloxacin is relatively uncommon globally, with the notable exception in China, where the majority of invasive and carriage isolates were ciprofloxacin-resistant [6, 7, 13–18]. A novel ciprofloxacin- and penicillin-resistant *N. meningitidis* strain was recently identified in several US cases [19, 20]; all isolates were serogroup Y (NmY) and belonged to the sequence type (ST)-23 clonal complex (CC23). Six meningococcal isolates, collected during 2017–2019, with similar characteristics (dual-resistance, belonging to CC23, and serogroup Y) were also identified in El Salvador [21].

Nearly all previously characterized ciprofloxacin-resistant *N. meningitidis* isolates have isoleucine (I) or phenylalanine (F) mutations at position 91 in the quinolone resistance determining region (QRDR) of *gyrA* [6, 13, 14, 16, 17, 21–26]. Additional mutations in *gyrA* (D95N and T193A) and also in *parC* (D86N, S87R, and E91G) have also been associated with higher ciprofloxacin minimum inhibitory concentrations (MICs) in *N. meningitidis* [17, 22, 25–27]. These mutations are frequently introduced by homologous recombination from other *Neisseria* species, as reported for three US ciprofloxacin-resistant *N. meningitidis* serogroup B (NmB) isolates [13] and several ciprofloxacin-resistant isolates from China [25].

Recently, penicillin resistance due to the β-lactamase gene bla_{ROB-1} has been observed in *N. meningitidis* despite being rare historically. The β-lactamase-containing isolates were identified in Canada, France, the United States and El Salvador [11, 12, 19, 21, 28]. β-lactamase-independent mechanisms of penicillin resistance are poorly understood in *N. meningitidis*; intermediate susceptibility can be caused by mosaic *penA* alleles, in which inter-species recombination introduces four to five amino acid substitutions within penicillin-binding protein 2 [10, 29].

Here, we evaluated penicillin and/or ciprofloxacin resistance among IMD cases reported through meningococcal disease surveillance in the United States and explored the origins of the resistance mechanisms observed in the dual-resistant CC23 isolates.

METHODS

Invasive meningococcal disease surveillance and strain collection

Epidemiologic information on all US IMD cases was submitted to the Centers for Disease Control and Prevention (CDC) through the National Notifiable Diseases Surveillance System; state health departments provided supplemental epidemiologic data for cases with isolates containing bla_{ROB-1}. Meningococcal isolates from cases reported from January 1st 2011 to February 14th 2020 were submitted by jurisdiction health departments through three surveillance programs, including the Active Bacterial Core Surveillance during 2011–20, which covers ~14% of the US population [30], expanded surveillance sites in 2013–14 [31] and Enhanced Meningococcal Disease Surveillance 2015–20, with isolates submitted for over 75% of US IMD cases by 2018 [32]. If two or more isolates were submitted for a single meningococcal case, only one was included in the analysis.

Collection of meningococcal case information is still on-going; total meningococcal case counts during 2019–20 are not currently available.

Whole Genome Sequencing (WGS) and Identification of Antimicrobial Resistance Determinants

All submitted meningococcal isolates were sequenced as described previously [33]. Each isolate genome was characterized using standard molecular typing methods (multilocus sequence typing [MLST] and typing of PorA and FetA) [33]. Genes involved in antibiotic resistance were identified by a BLAST search of genome assemblies using reference sequences for bla_{ROB-1} from the *Haemophilus influenzae* plasmid pB1000 (NC_019178)

and the full-length *gyrA* (NEIS1320), *parC* (NEIS1525), *penA* (NEIS1753), and *bla*_{TEM-1} (NEIS2357) genes from the PubMLST *Neisseria* database [34]. Amino-acid mutations in *gyrA, parC,* and *penA* were identified by aligning the translated sequence to allele 1 from PubMLST using BioPython [35]. Alleles for *gyrA* were identified according to the PubMLST locus definition for a 525bp fragment that contains the QRDR [22, 34] (defined as *gyrA*-QRDR). Isolates with *bla*_{ROB-1} or T91 mutations in *gyrA* are listed in Supplemental Table 1 and genome assemblies were submitted to PubMLST.

The PubMLST database was queried on April 21, 2020 to identify genomes containing *bla*_{ROB-1} or *bla*_{TEM-1} using the BLAST function and to identify isolates with *gyrA* T91 mutations using the API [22, 34].

Phenotypic antimicrobial susceptibility testing

Isolates containing *bla*_{ROB-1} or a mutation at position T91 of *gyrA* were assessed for β-lactamase activity by nitrocefin testing (BD BBL DrySlide; Franklin Lakes, NJ). All isolates with a mutation at position T91 of *gyrA* and a subset of *bla*_{ROB-1} -containing isolates were tested by reference broth microdilution (BMD) in accordance with the Clinical and Laboratory Standards (CLSI) guidelines; susceptibility interpretations were assigned according to M100, 30th Edition [36]. Frozen reference microdilution panels were prepared at CDC [37] and contained the following antimicrobial dilution series (μg/mL): ampicillin (0.03–16), azithromycin (0.06–4), cefotaxime (0.015–8), ceftriaxone (0.015–8), chloramphenicol (1–16), ciprofloxacin (0.015–4), levofloxacin (0.03–8), meropenem (0.06–4), minocycline (0.25–2), penicillin (0.015–8), rifampin (0.06–4), and trimethoprim-sulfamethoxazole (0.06/1.14 – 4/76).

Whole genome sequence comparisons

Whole-genome phylogenetic analysis was performed with *bla*_{ROB-1}-containing isolates and closely related isolates with matching STs (Supplemental Table 1). Assemblies were aligned to the complete genome sequence of a ST-23 isolate collected in 2003 from the United States (M10868) using Snippy v4.3.8 [38]. Recombinant regions were removed from the 1,706,760 bp core-genome alignment using Gubbins v1.4.1 [39]. Single nucleotide polymorphisms (SNPs) were counted as the number of nucleotide differences between each pair of genomes in the alignment, both before and after running Gubbins. Finally, a maximum likelihood phylogenetic tree rooted on M10868 was created using RAxML-NG v0.9 [40] with GTR+G substitution model, a minimum branch length of 10^{-8} substitutions/site, Stamatakis ascertainment correction, and autoMRE bootstopping, which determined that bootstrap support values converged after 1000 replicates.

gyrA-QRDR sequence comparisons

Neighbor-net analysis of *gyrA*-QRDR was performed with SplitsTree4 [41], including the seven *Neisseria spp.* in the PubMLST database [34] that had *gyrA*-QRDR alleles documented for at least 10 isolates. Alleles from the US invasive meningococcal disease isolate collection were included if they contained either the T91I or T91F mutations.

RESULTS

Characterization of US penicillin-and/or ciprofloxacin-resistant meningococcal isolates

A total of 2,097 isolates were available from US IMD cases occurring between January 2011 and February 2020, including 773 NmB (36.9%), 580 NmC (27.7%), 374 NmY (17.8%), 207 NmW (9.9%), 153 nongroupable (7.3%), 9 NmE and 1 NmZ.

Whole genome analysis identified 45 isolates that have bla_{ROB-1} encoding penicillin resistance and/or a $gyrA$ mutation causing ciprofloxacin resistance (T91), with at least one of these isolates detected in every year except 2012 (Figure 1A). Eleven isolates collected from 2019–2020 contained both bla_{ROB-1} and $gyrA$ T91I ($gyrA$-QRDR allele 242). Twenty-two isolates collected from 2013–2020 contained bla_{ROB-1} and a wild type $gyrA$ ($gyrA$-QRDR allele 2). Twelve isolates from 2011–2019 without β-lactamase genes contained a $gyrA$ T91 mutation (9 with T91I and 3 with T91F); nine different $gyrA$-QRDR alleles with T91 mutations were identified.

To confirm the genomic susceptibility predictions, phenotypic testing was completed. Of the 19 bla_{ROB-1}-containing isolates tested by reference BMD (Table 1), all were resistant to penicillin and ampicillin. Eleven of the 19 isolates had $gyrA$-QRDR allele 242 and where resistant to ciprofloxacin and levofloxacin; the remaining 8 isolates had a wildtype $gyrA$-QRDR allele and were fluoroquinolone susceptible. All 19 isolates were resistant to trimethoprim-sulfamethoxazole but susceptible to all other antibiotics tested, including cefotaxime and ceftriaxone. β-lactamase activity was confirmed by nitrocefin testing for these 19 isolates plus an additional 14 bla_{ROB-1}-containing isolates that were not assessed by BMD. Twelve isolates with a $gyrA$-QRDR T91 mutation lacked β-lactamase activity and were resistant to ciprofloxacin and levofloxacin. One of these isolates contained four previously characterized mutations ($gyrA$ T91I, D95N, T173A; $parC$ S87I) and had an MIC of 4 μg/mL for both fluoroquinolones. All other isolates with $gyrA$ T91I or T9IF mutations had MICs ranging from 0.12–0.5 μg/mL (Table 2).

All 33 bla_{ROB-1}-containing isolates had an intact NmY capsule locus, but one was nongroupable (NmNG) phenotypically. One molecular profile was shared by 30 of the 33 bla_{ROB-1}-containing isolates (PorA P1.5–2,10–2: FetA F4–1: ST-3587: CC23), including all 11 dual-resistant isolates. The remaining three isolates were ST-15379 or ST-13034, each differing from ST-3587 at a single typing locus. The ST-13034 isolate had the same PorA and FetA types as the ST-3587 isolates, while the two ST-15379 isolates contained P1.5–2,10–25. The 12 β-lactamase-negative, ciprofloxacin-resistant isolates were genetically diverse (Table 2); five serogroups and six CCs were detected. Four NmY CC23 isolates were detected across four separate years; all contained a novel $gyrA$-QRDR allele (348) and the molecular profile (P1.5–2,10–1: F4–1: ST-2533: CC23), which differed from the bla_{ROB-1}-containing isolates at three MLST loci and the PorA locus.

Invasive Meningococcal Disease caused by penicillin- and/or ciprofloxacin-resistant isolates

The 33 IMD cases caused by bla_{ROB-1}-containing isolates were reported from 11 US states (Figure 1B) across several age groups. The highest proportions of cases were in persons

aged ≥45 years (45%) and aged <1 year (18%); the age distribution was similar for cases caused by bla_{ROB-1}-containing isolates that were ciprofloxacin resistant and ciprofloxacin-susceptible. Of the 33 cases, 22 (67%) occurred in Hispanic individuals, including 8 of the 11 cases with ciprofloxacin-resistant isolates. There were no known epidemiologic links among cases. One patient, with IMD associated with a ciprofloxacin-susceptible isolate, died (case-fatality ratio: 3.0%).

Twenty-one of 33 IMD patients with bla_{ROB-1}-containing isolates had additional epidemiologic data available. Six (29%) had resided in or traveled to Mexico shortly before meningococcal disease onset (all had isolates containing bla_{ROB-1} and a wild type $gyrA$-QRDR). Among 18/33 cases with treatment information, none were treated with penicillin or ampicillin.

The antibiotic used for prophylaxis of close contacts was reported for five of the 11 cases with dual-resistant isolates. Ciprofloxacin was initially used as the primary prophylaxis antibiotic for contacts of four cases; however, prophylaxis was repeated for the contacts of two cases with an alternate antibiotic once susceptibility results were available. No prophylaxis failures were reported.

The 12 IMD cases caused by β-lactamase negative, ciprofloxacin-resistant isolates were reported from eight states (Figure 1B) and occurred across several age groups, with the highest proportion in adults aged ≥45 years (33%) and 22–44 years (33%). Only one case (8%) occurred in a Hispanic individual. One case was fatal.

Comparison between US and non-US meningococcal isolates

The PubMLST database included 12 additional bla_{ROB-1}-containing *N. meningitidis* genomes (11 NmY and 1 NmNG) [11, 12]. All twelve isolates were ST-3587 (CC23) with a wild type $gyrA$-QRDR allele 2 and were collected during 2016–19 from six countries: 7 from Mexico, 4 from four European countries, and 1 from Canada (Supplemental Table 2). A screen for a second β-lactamase encoding gene identified six bla_{TEM-1}-containing meningococcal genomes in PubMLST, collected during 2013–18 from five countries. The bla_{TEM-1} isolates were diverse, belonging to six CCs (Supplemental Table 3); bla_{TEM-1} was not identified in the US collection.

The genomes of the bla_{ROB-1}-containing isolates (33 US, 12 non-US) were compared to invasive ST-3587 isolates lacking bla_{ROB-1} (10 US, 1 non-US). Phylogenetic analysis based on 23,286 polymorphic positions (Figure 2, Supplemental Tables 4 & 5) showed that the 11 US isolates with the bla_{ROB-1} gene and the $gyrA$ T91I mutation (allele 242) formed a single subclade (bootstrap = 99%) within a larger clade of bla_{ROB-1}-containing isolates (bootstrap = 100%). Outside of the dual-resistant subclade, the US isolates were intermixed with isolates from Mexico, Canada, and Europe (Figure 2). One isolate in the bla_{ROB-1}-containing clade did not contain the bla_{ROB-1} gene; this US isolate was β-lactamase-negative and collected in 2017. All 45 bla_{ROB-1} genes were identical to the *H. influenzae* reference sequence and located between the NEIS0803 and NEIS0807 loci, which are both present in the β-lactamase negative ST-3587 genomes, indicating chromosomal integration. Additionally, all isolates within this bla_{ROB-1}-containing clade

contained a mosaic *penA* gene with 5 amino acid mutations (A510V, N512Y, I515V, H541N, I566V), which are associated with intermediate penicillin susceptibility [10], as did five other ST-3587 isolates outside of the clade.

The phylogenetic analysis of *bla*$_{ROB-1}$-containing isolates identified a 2,115 bp recombination event in the ancestor of the dual-resistant subclade that included the entire *gyrA* gene and introduced the T91I and T193A mutations associated with reduced susceptibility to ciprofloxacin. The recombination introduced 120 single-nucleotide polymorphisms, replacing *gyrA*-QRDR allele 2 with allele 242. In the PubMLST database, allele 242 was only present in three *N. lactamica* genomes.

PubMLST contained 63 *N. meningitidis* genomes with a *gyrA* T91 mutation and MLST results; these were collected in 2011–2020 from 14 countries (Supplemental Table 6). Only one genome had a T91F mutation [26]. Similar to the US isolates, the 62 T91I genomes in PubMLST were diverse, with 11 different CCs and all six common invasive serogroups plus NmNG detected (Supplemental Table 6). Nearly half of the T91I genomes belonged to CC4281 with diverse serogroups (NmB, NmC, NmW, NmY) and were collected predominantly from China. The one CC23 isolate was NmY with a different molecular profile than the β-lactamase positive strains (P1.5–1,2–2: F5–8: ST-23: CC23).

Tracing the genetic origin of *gyrA* alleles

The T91-mutant *gyrA*-QRDR allele sequences from invasive US isolates, including allele 242 from the dual-resistant isolates, were compared to all *gyrA*-QRDR sequences from seven *Neisseria* species in the PubMLST database (Figure 3). For each *Neisseria* species examined, the most common *gyrA*-QRDR alleles clustered together based on sequence similarity; *N. meningitidis* alleles containing T91I or T91F clustered with four other species: *N. lactamica, N. gonorrhoeae, N. cinerea,* and *N. subflava* (Figure 3; colored open circles). The 2011–20 US isolates included 7 unique T91I *gyrA*-QRDR alleles; three clustered with the most common *N. meningitidis* alleles and four clustered with the most common *N. lactamica* alleles (Figure 3; filled red circles). The two T91F/D95G alleles from US isolates belonged to the *N. gonorrhoeae* cluster: allele 140 was found in 1,552 of the 4,354 *N. gonorrhoeae* isolates in PubMLST and allele 363 was similar but not identical to the *N. gonorrhoeae* alleles.

DISCUSSION

Widespread resistance to either penicillin or ciprofloxacin could have important implications for treatment and prevention of meningococcal disease. Ciprofloxacin or penicillin resistance in *N. meningitidis* was uncommon in the United States prior to 2012 [6]. However, reports of ciprofloxacin resistance and intermediate susceptibility to penicillin around the world demonstrate that reduced susceptibility can develop in *N. meningitidis* populations. Here, we report 45 US IMD cases during 2011–20 that were caused by penicillin-resistant and/or ciprofloxacin-resistant *N. meningitidis*, including 11 cases due to penicillin- and ciprofloxacin-resistant *N. meningitidis* isolates; all dual-resistant meningococcal isolates were serogroup Y, a serogroup that is vaccine preventable and accounted for 14.6% of U.S. IMD cases in 2018 [32]. While single resistant IMD isolates

were detected in almost every year assessed, the 11 dual-resistant isolates were only detected in a 14-month period beginning in January 2019, from a diverse array of states with no known epidemiological links. Altogether, the data indicate that the recent increase in cases caused by ciprofloxacin- and penicillin-resistant *N. meningitidis* is due to the emergence of a *bla*$_{ROB-1}$-containing meningococcal strain within the United States.

Other than the 11 dual-resistant isolates, ciprofloxacin resistance in meningococcal cases remains uncommon in the United States. Consistent with previous US trends, only a few ciprofloxacin-resistant isolates that lacked a β-lactamase were identified each year of this study, indicating that acquisition of ciprofloxacin resistance remains an uncommon event in *N. meningitidis* [6, 13]. In contrast to the closely related dual-resistant isolates, the ciprofloxacin-resistant isolates were from diverse genetic lineages, suggesting that the increased ciprofloxacin resistance observed in 2019–20 was specifically caused by the expansion of the β-lactamase positive ST-3587 (CC23) strain that acquired ciprofloxacin resistance from *N. lactamica*. Notably, dual-resistant ST-3587 isolates were reported from El Salvador, in Central America, while this manuscript was under review [21]; a phylogenetic comparison demonstrated that they belong to a single dual-resistant subclade with the US isolates and are likely part of this emerging strain (data not shown). In addition to ST-3587, expansion of ciprofloxacin resistance within a specific CC had only been previously observed in serogroup B and C strains of CC4821 [17, 42]. Overall, the ciprofloxacin-resistant isolates analyzed in this study developed resistance from either mutation events or interspecies recombination from other *Neisseria* species, which is consistent with previous reports [13, 25, 26].

Even though β-lactamase acquisition by *N. meningitidis* has been rare historically, this analysis identified 45 phylogenetically-related isolates with the *bla*$_{ROB-1}$ gene that were collected from a total of 7 countries, including the United States. However, this analysis is limited to the data released through PubMLST, which may not receive a representative dataset from submitter countries. All *bla*$_{ROB-1}$-containing isolates also contained an identical *bla*$_{ROB-1}$ sequence, indicating expansion of a single strain that possibly acquired the gene from *H. influenzae*. However, novel acquisition of β-lactamases remains rare in *N. meningitidis*; no *bla*$_{ROB-1}$ sequences were detected outside ST-3587 and the *bla*$_{TEM-1}$ gene was rare among *N. meningitidis* isolates.

By screening the genomes of invasive US isolates for the *bla*$_{ROB-1}$ gene or a T91 mutation in *gyrA*, this analysis accurately predicted penicillin- and ciprofloxacin-resistant isolates. The expanding genomic surveillance programs in many countries provide an opportunity for rapid identification of isolates with known mechanisms of antimicrobial resistance. However, genomic screens will be unable to detect novel resistance mechanisms and may have reduced accuracy when predicting susceptibility phenotypes from complex resistance mechanisms such as the mosaic *penA* alleles. Thus, a combination of phenotypic susceptibility studies in addition to genomic screens will be necessary to effectively monitor future trends.

Acquisition of *bla*$_{ROB-1}$ and ciprofloxacin resistance within ST-3587 in US IMD cases has emphasized the importance of antimicrobial resistance surveillance for *N meningitidis*,

particularly with cases detected in both Central and North America. Susceptibility to penicillins should be confirmed prior to use of penicillin or ampicillin for meningococcal disease treatment [20]. In addition, clinicians and public health staff in areas that have experienced IMD cases caused by ciprofloxacin-resistant strains within the past 1–2 years should consider performing AST on meningococcal isolates to inform prophylaxis decisions; however, AST should not delay initiation of prophylaxis [20]. Accurate and timely antimicrobial resistance surveillance, using AST and/or WGS, is essential to understanding the prevalence and spread of resistance and informing future meningococcal disease treatment and prophylaxis recommendations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
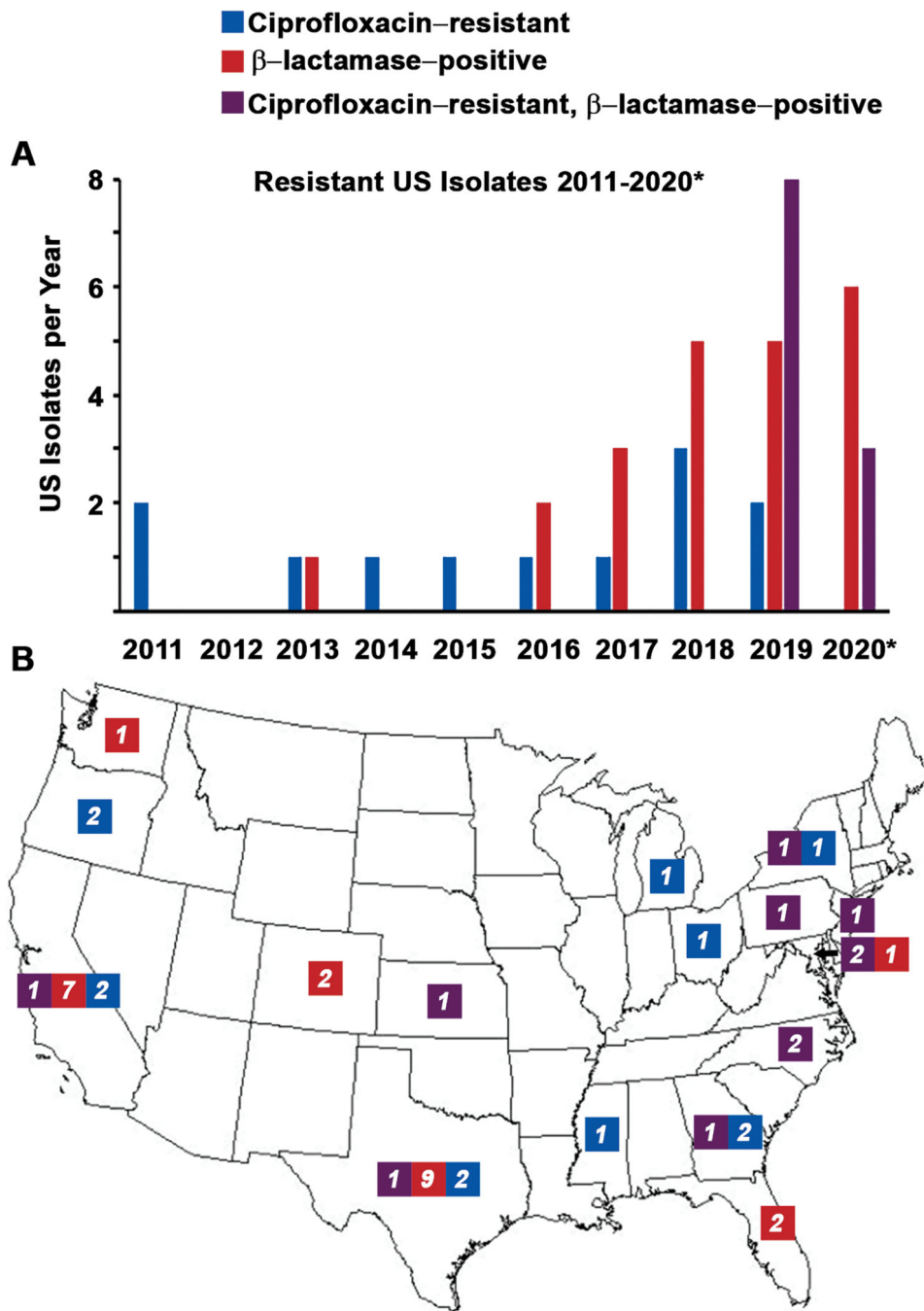
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Key Points

Ciprofloxacin- and penicillin-resistant *N. meningitidis* caused 11 cases of invasive meningococcal disease in the US during a 14-month period. The dual-resistant *N. meningitidis* were not detected before 2019 and belong to a single genetic lineage, suggesting changing antimicrobial resistance patterns.

Figure 1.
Temporal (A) and geographic (B) distribution of *Neisseria meningitidis* isolates with β-lactamase activity (red: contain the *bla*$_{ROB-1}$ gene), ciprofloxacin resistance (blue: T91I or T91F mutations in *gyrA*), or both (purple) in the United States. States are labeled with the number of isolates identified with each resistance profile. *2020 only includes isolates collected through February 14th, 2020.

Figure 2.
Phylogeny of *Neisseria meningitidis* isolates containing *bla*ROB-1 and others with the same sequence types (ST-3587, 15379 or 13034) available in the PubMLST database as of April 21, 2020, based on a core genome alignment with recombinant regions removed. Isolates are labeled with the year and country of collection. Colored bars to the right of the phylogeny indicate the presence of antimicrobial resistance markers: *penA* mosaic allele (yellow), *bla*ROB-1 (red), and *gyrA* T91I (blue). The scale bar is 10^{-5} substitutions per site. Branches

with bootstrap percentages over 70% are labeled. The tree is rooted on a ST-23 isolate collected in the USA in 2003.
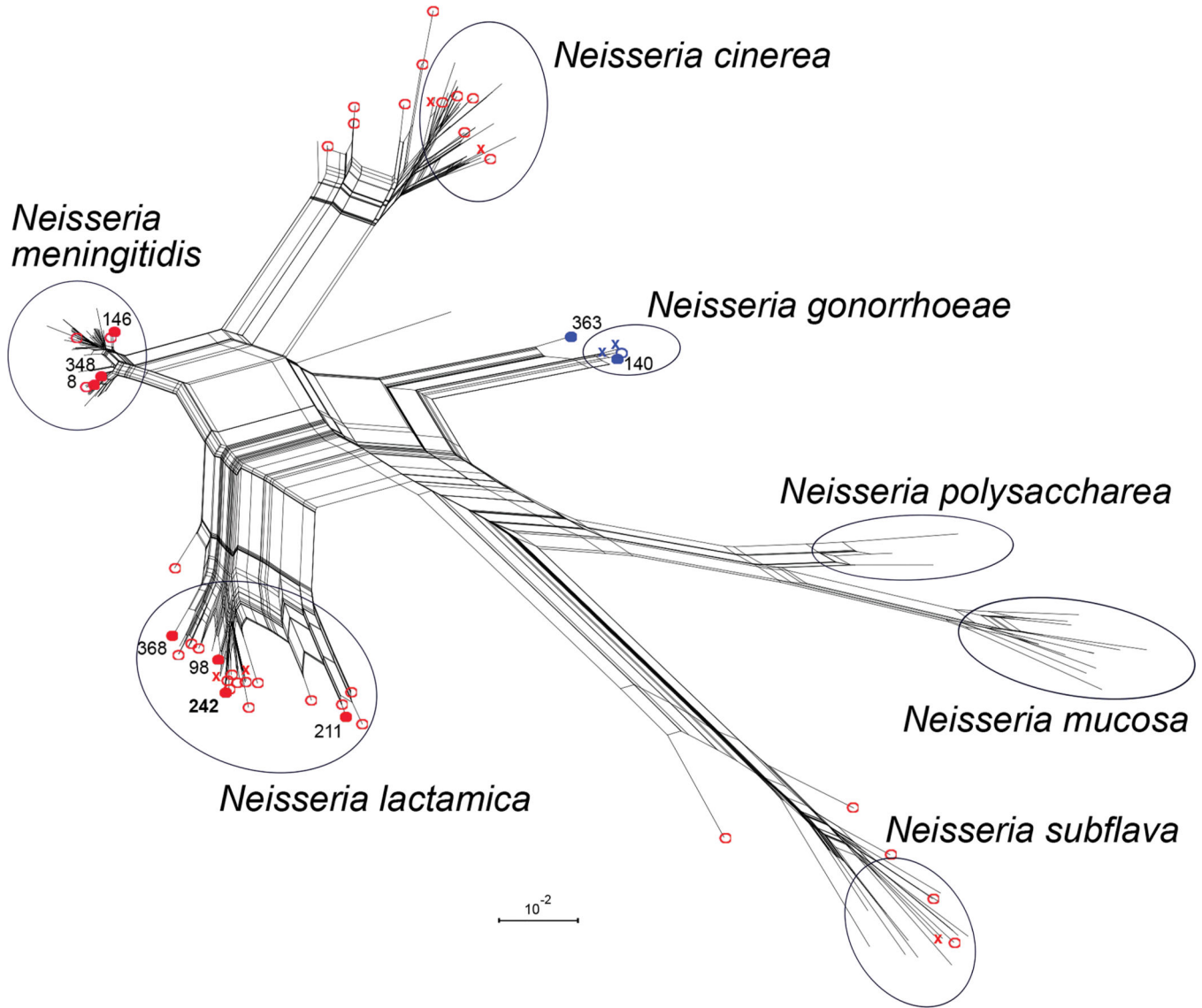
Figure 3.

Neighbor-net of 177 *gyrA* gene fragments containing the QRDR. Alleles with ciprofloxacin resistance-associated mutations are marked in blue (T91F) or red (T91I). Filled circles indicate T91F and T91I alleles that were present among the 2,097 invasive meningococcal isolates collected in the United States between January 1st, 2011 and February 14th, 2020. Open circles indicate additional T91F and T91I alleles from 18,249 *N. meningitidis* isolates in the PubMLST database. Colored "X"'s indicate T91F and T91I alleles that were only identified in isolates from other species in the PubMLST database: 4,354 *N. gonorrhoeae*, 363 *N. lactamica,* 39 *N. cinerea,* 33 *N. subflava,* 22 *N. mucosa,* and 21 *N. polysaccharea.* Allele numbers detected in US isolates are written adjacent to filled circles, with allele 242 from *bla*$_{ROB-1}$-containing isolates in bold. Black circles indicate clusters in the network that contain all moderate frequency alleles (>1%) for the named species. Scale bar represents 1%

sequence divergence and only sequences present in the PubMLST database, as of April 21, 2020, are shown.

Table 1:

Antimicrobial susceptibility testing results for the isolates containing the bla_{ROB-1} gene

Antibiotic	CLSI Breakpoints			bla_{ROB-1}-containing and T91I gyrA mutation (n=11)				bla_{ROB-1}-containing and wild type gyrA-QRDR (n=8‡)				T91 gyrA mutation without bla_{ROB-1} (n=12)			
					No. of Isolates				No. of Isolates				No. of Isolates		
	S	I	R	MIC Range	S	I	R	MIC Range	S	I	R	MIC Range	S	I	R
Penicillin *	0.06	0.12–0.25	0.5	8 - >8	0	0	11	8 - >8	0	0	8	0.016 – 0.5	6	5	1
Ampicillin *	0.12	0.25–1	2	8 - >16	0	0	11	16 - >16	0	0	8	0.03 – 1	7	5	0
Cefotaxime *	0.12	--	--	0.015	11	--	0	0.015	8	--	0	0.015 – 0.03	12	--	0
Ceftriaxone *δ	0.12	--	--	0.015	11	--	0	0.015	8	--	0	0.015	12	--	0
Meropenem *	0.25	--	--	0.06	11	--	0	0.06	8	--	0	0.06	12	--	0
Ciprofloxacin δ	0.03	0.06	0.12	0.12–0.25	0	0	11	0.015	8	0	0	0.12 – 4	0	0	12
Levofloxacin	0.03	0.06	0.12	0.25	0	0	11	0.03	8	0	0	0.12 – 4	0	0	12
Chloramphenicol	2	4	8	1	11	0	0	1 – 2	8	0	0	1 – 2	12	0	0
Azithromycin	2	--	--	0.25–0.5	11	--	0	0.5–1.0	8	--	0	0.06 – 1	12	--	0
Minocycline	2	--	--	0.25	11	--	0	0.25	8	--	0	0.25 – 0.5	12	--	0
Rifampin δ	0.5	1	2	0.06 – 0.5	11	0	0	0.06	8	0	0	0.06 – 0.25	12	0	0
Trimethoprim-sulfamethoxazole	0.12/2.4	0.25/4.75	0.5/9.5	2/38 - >4/76	0	0	11	4/76 - >4/76	0	0	8	1/19 - >4/76	0	0	12

*
The antibiotics recommended for patient treatment and for chemoprophylaxis

δ
are denoted in the table [1]. MICs were determined using reference broth microdilution according to CLSI guidelines and interpretive criteria were applied according to the M100. S = susceptible, I = Intermediate (a dash indicates that an intermediate breakpoint is not defined for that antibiotic), and R = resistant.

‡
Eight bla_{ROB-1}-containing isolates, collected in seven different years from six states, were selected for reference broth microdilution.

Table 2.

Invasive Ciprofloxacin-Resistant, β-lactamase-negative *Neisseria meningitidis* Isolates with *gyrA* Mutations at Position T91 in the United States, 2011–19

ST, CC	No. of Isolates	Collection Years	*gyrA* Mutations (Allele No.)	*parC* Mutations	MIC Range (µg/mL)		
					Ciprofloxacin	Levofloxacin	
NmB							
ST-6595, CC4821	2	2014, 2019	T91F, D95G (363)	--	0.12–0.25	0.25	
ST-5662, unassigned	1	2017	T91I (146)	--	0.25	0.5	
ST-13904, CC41/44	1	2018	T91I, T173A (98)	--	0.12	0.12	
NmC							
ST-273, CC231	1	2018	T91I, T173A (368)	--	0.25	0.25	
NmW							
ST-11, CC11	1	2016	T91I, D95N, T173A (211)	S87I	4	4	
NmY							
ST-767, CC167	1	2011	T91I (8)	--	0.25	0.25	
ST-2533, CC23	4	2011, 2013, 2015, 2018	T91I (348)	--	0.12–0.25	0.12–0.25	
NmNG							
ST-11, CC11	1	2019	T91F, D95G (140)	--	0.5	0.25	

MICs were determined using reference broth microdilution according to CLSI guidelines and interpretive criteria were applied according to the M100.