OBSERVATION



Penicillin- and Ciprofloxacin-Resistant Invasive Neisseria meningitidis Isolates from Japan

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ABSTRACT Neisseria meningitidis causes a life-threatening invasive meningococcal disease (IMD). Isolates resistant to antibiotics, such as penicillin, ceftriaxone, and ciprofloxacin that are recommended for the treatment of IMD patients and their close contacts have been serious public health concerns globally. However, susceptibility profiles to critically important antibiotics and the genetic characteristics of isolates possessing antibiotic resistance are extremely limited as IMD incidence is low in Japan. We assessed the susceptibility profiles of 87 randomly selected, sterile site-derived N. meningitidis strains isolated from hospitals nationwide, recovered between April 1998 and March 2018 in Japan, to seven antibiotics. As a result, we demonstrated, for the first time, that the isolates remained highly susceptible to ceftriaxone, meropenem, azithromycin, ciprofloxacin, chloramphenicol, and rifampin, but not to penicillin. We then characterized the genetic relatedness of six penicillin- and/or ciprofloxacin-resistant isolates obtained in this study with global 112 genomes using core-genome phylogenetic analysis. These results provide the first evidence that invasive lineages such as a penicillin-resistant serogroup W, sequence type (ST)-11 clonal complex (CC), and a ciprofloxacin-resistant serogroup B/C, ST-4821 CC that is considered as a global threat, have been sporadically identified in Japan. Our findings highlight the need to monitor antibiotic resistance in clinical isolates of N. meningitidis, thereby preventing the spread of antibiotic-resistant invasive lineages and maintaining effective treatment for IMD patients and their close contacts.

Microbiology Spectrum

AMERICAN SOCIETY FOR MICROBIOLOGY

IMPORTANCE Although antibiotics such as penicillin and ceftriaxone can treat invasive meningococcal disease (IMD), the emergence and spread of antibiotic-resistant *Neisseria meningitidis* have become a global concern. To provide effective treatment, including chemoprophylaxis to IMD patients and their close contacts, we highlighted the importance of recognizing the antibiotic resistance and genetic features of *N. meningitidis* isolates.

KEYWORDS *Neisseria meningitidis*, chemoprophylaxis, ciprofloxacin resistance, invasive lineage, invasive meningococcal disease, penicillin resistance

nvasive meningococcal disease (IMD) is a well-known life-threatening illness caused by the bacterium *Neisseria meningitidis*. *N. meningitidis* strains with resistance to antibiotics, such as penicillin, ciprofloxacin, and ceftriaxone, that are recommended for the treatment and chemoprophylaxis of IMD patients and their close contacts, are reported globally and raise serious public health concerns worldwide owing to limited options for treatment (1–9). However, data such as the resistance rate to critically important antibiotics and the genetic relationship between isolates collected are extremely scarce in Japan, as IMD incidence is low (0.028 cases/100,000 persons per year in 2014) (10). This situation **Editor** William Lainhart, University of Arizona/ Banner Health

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The authors declare no conflict of interest.

Received 18 February 2022 Accepted 1 April 2022 Published 25 April 2022

		MIC (mg/L)			Interpr catego	etative ry ^a	
Antibiotic	No.	Range	MIC ₅₀	MIC ₉₀	%S	%I	%R (% non-S)
Penicillin	87	0.031–0.5	0.063	0.125	52.9	42.5	4.6
Ceftriaxone	87	≤0.004	≤0.004	≤0.004	100.0	(0.0)	(0.0)
Meropenem	87	≤0.004-0.063	0.008	0.016	100.0	(0.0)	(0.0)
Azithromycin	87	0.125-4	1	2	96.6	(0.0)	(3.4)
Ciprofloxacin	87	≤0.004-0.25	≤0.004	0.008	90.8	3.4	5.7
Chloramphenicol	87	0.5–2	1	2	100.0	0.0	0.0
Rifampicin	87	≤0.004-0.25	0.008	0.063	100.0	0.0	0.0

TABLE 1 Antibiotic susceptibility profiles of the 87 N. meningitidis isolates used in this study

^aS, susceptible; I, intermediate; R, resistant. The category was interpreted by the Clinical and Laboratory Standards Institute guideline M100-ED30.

may cause inadequate treatment of IMD patients and make management of antibiotic-resistant strains more difficult based on genetic relatedness in Japan when those strains are disseminated. Here, we described the antibiotic susceptibility profiles of 87 *N. meningitidis* isolates recovered during a national surveillance conducted for approximately 20 years in Japan and provided insights on the genetic features of penicillin- and/or ciprofloxacinresistant isolates.

A total of 87 nonduplicate N. meningitidis isolates (blood, n = 62; cerebrospinal fluid, n = 22; synovial fluid, n = 2; aqueous humor, n = 1) were randomly selected from over 200 sterile site-derived isolates obtained between April 1998 and March 2018 at the National Institute of Infectious Diseases, Japan (Table S1 in the supplemental material). Microbial identification was performed using biochemical profiling (ID-test HN-20 Rapid System; Nissui Pharmaceutical, Tokyo, Japan) and the MALDI Biotyper (Bruker Daltonics, Karlsruhe, Germany). Serogroup determination and multilocus sequence typing (MLST) were performed as previously described (11). The MICs of penicillin, ceftriaxone, meropenem, azithromycin, ciprofloxacin, chloramphenicol, and rifampin were determined using an Etest (bioMérieux, Marcy-l'Étoile, France) on Mueller-Hinton agar with 5% sheep blood (Becton, Dickinson and Company, Franklin Lakes, NJ) according to the manufacturer's instructions. Quality control was performed using Streptococcus pneumoniae ATCC 49619 and Escherichia coli ATCC 25922. Results were interpreted according to the Clinical and Laboratory Standards Institute guideline M100-ED30 (12). β -lactamase production among strains that were nonsusceptible to penicillin was determined using BBL Cefinase paper discs (Becton, Dickinson and Company).

Among the 87 isolates, 41 (47.1%) were nonsusceptible to penicillin, including four resistant isolates (4.6%), eight (9.2%) were nonsusceptible to ciprofloxacin, including five resistant isolates (5.7%), and three (3.4%) were nonsusceptible to azithromycin (Table 1). Isolates NIID576, NIID614, and NIID620 were resistant to both penicillin and ciprofloxacin (Table S1). β -lactamase production was negative in all penicillin-nonsusceptible isolates. The geometric mean MICs tended to increase for penicillin and ciprofloxacin every seven years, due to the increasing rate of antibiotic-intermediate and -resistant isolates between 2012 and 2018 (Fig. S1), whereas the geometric mean MIC for azithromycin tended to decrease. No isolate was found nonsusceptible or resistant to ceftriaxone, meropenem, chloramphenicol, or rifampin. These results agree with previous studies that showed reduced susceptibility to penicillin among invasive isolates (7–9, 13, 14), suggesting that penicillin might not be suitable to treat IMD cases in Japan. However, we could not clarify the reason for the observation in this study, and it might have been associated with the antibiotic choice for the treatment of bacterial meningitis in Japan. Furthermore, we provide the first evidence that clinical isolates from Japan for approximately 20 years remain highly susceptible to critically important antibiotics, except for penicillin, that are currently used for treatment and chemoprophylaxis of IMD patients and their close contacts.

Next, to assess the genetic features of our six penicillin- and/or ciprofloxacin-resistant isolates defined using the CLSI guideline (12), we conducted whole-genome sequencing. Genomic DNA from the six isolates was extracted using the NucleoSpin tissue kit (TaKaRa Bio, Shiga, Japan). The DNA library was prepared using the Nextera XT DNA library preparation kit and sequenced on MiSeq (Illumina, San Diego, CA) generating a 2 × 300-bp paired-end read. The genome coverages were 77.1× to 186.1× among the six isolates. Genome assembly was performed using the SPAdes, v3.13.1 with default parameters (15). Subsequently, gene annotation was performed using Prokka v1.11 (16), and the sequence data were submitted to the PubMLST *Neisseria* genome database (17) and the DDBJ Sequence Read Archive. Moreover, to determine genetic relatedness among the 6 genomes obtained in this study and 112 genomes from global *N. meningitidis* strains categorized as penicillin- and ciprofloxacin-resistant (MICs \geq 0.5 and \geq 0.12 mg/L, respectively) (12) deposited in the PubMLST *Neisseria* genome database (Table S2; assessed June 11, 2021), a core-genome based phylogeny with 1,000 bootstrap replicates was constructed using IQ-TREE v1.6.12 (18). The tree was visualized and annotated using iTOL v6 (19). Most of the strains were isolated from usually sterile sites between 1999 and 2020.

A penicillin-resistant isolate NIID669 was classified into serogroup W, ST-11 clonal complex (CC) harboring penA9, a mosaic penA allele that has been reported in strains with reduced penicillin susceptibility (6, 9, 14), which clustered to a clade of the same serogroup and CC containing penicillin-resistant strains from New Zealand (penA9) (Fig. 1). This indicates, for the first time, that the penicillin-resistant invasive serogroup W, ST-11 CC lineage circulating in several countries (5, 6) has already emerged in Japan. Three penicillin and ciprofloxacin-resistant isolates, NIID576, NIID614, and NIID620, recovered from individuals in different prefectures, were classified as the same nontypeable serogroup ST-32 CC strains harboring both penA33 (identified previously in penicillin-nonsusceptible strains) (14) and gyrA376 with a T911 substitution responsible for ciprofloxacin resistance (Fig. 1). These isolates were clustered with the penicillin-resistant and ciprofloxacin-susceptible serogroup B, ST-32 CC strains identified in Malta and New Zealand. This highlights the necessity to monitor this lineage containing nontypeable serogroup strains due to the possible difficulty in preventing disease with vaccines available worldwide. A ciprofloxacin-resistant isolate NIID417 classified into serogroup A, ST-5 CC, harboring gyrA13 with a T911 substitution, had a close phylogenetic distance with a strain from New Zealand that also shared the same ciprofloxacin resistance mechanism but had a different serogroup identity (Fig. 1). The remaining ciprofloxacin-resistant isolate NIID652 was classified into serogroup C, ST-4821 CC, harboring gyrA71 with a T911 substitution, and clustered to a clade composed of serogroup B/C and the same CC with ciprofloxacin resistance, obtained from China and New Zealand (Fig. 1). Invasive strains with ciprofloxacin resistance, which belong to ST-4821 CC, ST-23 CC, and ST-175 CC with alleles mostly containing a T911 substitution, have been observed in several countries, including Japan (1-4). Although our five ciprofloxacin-resistant isolates, including the ST-32 CC strains, could have sporadically emerged, they are not widespread in Japan as of now; however, considering the current global concerns, these findings emphasize the need for a continuous and wider surveillance to prevent the circulation of ciprofloxacin-resistant clones in Japan.

Azithromycin is thought of as an alternative chemoprophylaxis in case of ciprofloxacin resistance (20). As three isolates nonsusceptible to azithromycin have already sporadically emerged in Japan, as shown in this study, the monitoring of resistance to azithromycin will be required for maintaining the effective chemoprophylaxis strategy for close contact individuals of IMD patients.

A major limitation of our study is that our results may not reflect the entire IMD scenario in Japan during the study period since the collection of strains from meningococcal meningitis cases is still not regulated by the Infectious Diseases Control Law in Japan although the patients' records of meningococcal meningitis were collected as a notifiable disease as per the law from 1999. We investigated the phylogenetic relationship of our penicillin- and ciprofloxacin-resistant isolates with international strains derived only from the PubMLST database, and these data sets extracted may be prone

Characteristics of Invasive N. meningitidis Isolates

	PubMLST ID 71654	Isolate NZ18MI0007	Country New Zealand	Year 2018	PEN	penA	QP	gyrA 13 (T91)	Serogroup	Clonal complex ST-5 complex
	106319	NIID417	Japan	2003	s	4	R	13 (T91I)	А	ST-5 comple
	52223 52222	Nm075 Nm064	China	2013 2011	s s	405	R	103 (T910) 113 (T910)	B	ST-4821 compl ST-4821 compl
_	52204	Nm025	China	2007	s	1	R	71 (T911)	c	ST-4821 compl
	52208	Nm039 Nm014	China China	2007 2006	S S	1	R	71 (T91) 71 (T91)	c c	ST-4821 compl ST-4821 compl
	52201 52242	Nm014 Nm374	China	2006	s	1	R	71 (1911)	c	ST-4821 compl ST-4821 compl
	52213	Nm047	China	2008	s	1	R	71 (T91I)	с	ST-4821 comp
	52206 71650	Nm032 NZ18MI0003	China New Zealand	2007 2018	S S	1	R	71 (T911) 71 (T911)	C C	ST-4821 compl ST-4821 compl
	52210	Nm044	China	2008	s	1	R	71 (T91I)	с	ST-4821 comp
	106323 52202	NIID652 Nm015	Japan China	2017 2005	s s	1	R	71 (T911) 71 (T911)	c c	ST-4821 comp ST-4821 comp
	52205	Nm029	China	2007	s	1	R	71 (T91I)	с	ST-4821 compl
	52212 52200	Nm046 Nm002	China	2008	S S	1	R	71 (T91I) 71 (T91I)	c c	ST-4821 comp ST-4821 comp
	52211	Nm045	China	2008	s	1	R	71 (T91I)	c	ST-4821 comp
	52207	Nm033 Nm119	China	2007	s	1	R	71 (791)	C B	ST-4821 comp ST-4821 comp
	61265 52217	Nm055	China China	2011 2010	S S	1	R R	71 (T91I) 71 (T91I)	c	ST-4821 comp
	35285	10.2352.E	UK	2010	s	3	R	12	В	ST-269 compl
	106321 88942	NID614 NID620	Japan Japan	2016 2016	R	33	R	376 (T911) 376 (T911)	NT NT	ST-32 compl ST-32 compl
1	106320	NID576	Japan	2014	R	33	R S	376 (1911)	NT	ST-32 comp
	89367 89272	M99 241997 M00 240824	Maita Maita	1999 2000	R	52 52	s	2	B	ST-32 compl ST-32 compl
	71723	NZ18MI0082	New Zealand	2018	R	10	s	2	В	ST-82 compl
	71706	NZ18MI0065 NZ19MI0026	New Zealand New Zealand	2018	R	414	S S	2	8 8	ST-32 compl ST-32 compl
	35287	10.2422.K	UK	2010	s	157	R	- 4	в	ST-41/44 com
	82038 71735	DE14667 NZ18MI0097	Germany New Zealand	2020 2018	R	19	S S	2	B	ST-41/44 com ST-41/44 com
	71506	NZ15MI0038	New Zealand	2015	R	782	s	3	8	ST-213 compl
	102408	NZ19MI0016 NZ19MI0040	New Zealand	2019	R	12	s	3	B B	ST-213 compl ST-213 compl
	102429 35307	NZ19MI0040 11.1709.8	New Zealand UK	2019 2011	s	14 5	R	3	B	ST-213 compl
L	92758	Nm_6-18	Portugal	2018	R	295	s	3	В	ST-213 compl
Ч С	92855 58827	Nm_53-17 W-6921_Str	Portugal Greece	2017 2018	R	295	S S	3	8 8	ST-213 compl ST-213 compl
	61349	Nm-771	Russia	2016	R	25	s	20	NA	ST-53 comple
	52876 20563	16.8708601 Nm3127	UK Canada	2016	R	33 331	S S	2	B Y	ST-461 compl ST-167 compl
	102464	NZ19MI0083	New Zealand	2019	R	19	s	12	Y	ST-167 compl
	71509 51612	NZ15MI0041 14.8703919	New Zealand UK	2015 2014	<u>R</u>	ND 722	S S	2	Y Y	ST-23 comple ST-23 comple
	93831	Nm_3-12	Portugal	2012	R	9	s	ND	в	NA
	85360 71736	AK-2037_Str NZ18MI0098	Greece New Zealand	2018	R	ND 12	S S	3	B	ST-60 comple ST-1572 comp
Ч	102425	NZ19MI0036	New Zealand	2019	R	12	s	11	8	ST-1572 comp
	102409 102437	NZ19MI0017 NZ19MI0048	New Zealand New Zealand	2019 2019	R	12 ND	S S	11	B	ST-1572 comp ST-1572 comp
	71598	NZ19MI0048 NZ17MI0029	New Zealand	2019	R	12	s	11	в	ST-1572 comp
	102466	NZ19MI0085	New Zealand	2019	R	12	s	11	в	ST-1572 comp NA
	92854 82430	Nm_51-17 20149	Portugal Vietnam	2017 2019	R	21 316	S R	382 (T91I)	B	ST-11 comple
L	81897	20153	Vietnam	2019	s	316	R	381 (T91F)	c	ST-11 comple
	71613 71799	NZ17MI0047 NZNMI142	New Zealand New Zealand	2017 2014	R	3	s	4	c c	ST-11 compl ST-11 compl
	71757	NZNMI1327	New Zealand	2013	R	7	s	4	с	ST-11 compl
	71520 71533	NZ16MI0012 NZ16MI0027	New Zealand New Zealand	2016	R	7	S S	4	c c	ST-11 compl ST-11 compl
L	71531	NZ16MI0025	New Zealand	2016	R	7	s	4	с	ST-11 comp
	71587 71593	NZ16MI0034 NZ17MI0024	New Zealand New Zealand	2016 2017	R	7	S S	4	C C	ST-11 compl ST-11 compl
	71539	NZ16MI0036	New Zealand	2016	R	7	s	4	с	ST-11 compl
	71639 71604	NZ17MI0075 NZ17MI0036	New Zealand New Zealand	2017 2017	R	7	S S	4	c c	ST-11 compl ST-11 compl
	71690	NZ18MI0047	New Zealand	2017	R	7	s	4	c	ST-11 compl
	71597 71668	NZ17MI0028 NZ18MI0022	New Zealand New Zealand	2017 2018	R	ND	s s	4	c c	ST-11 compl ST-11 compl
	102473	NZ19MI0092	New Zealand	2019	R	7	s	4	c	ST-11 compl
	102471 71714	NZ19MI0090 NZ18MI0074	New Zealand New Zealand	2019 2018	R R	7	S S	4	c c	ST-11 comple ST-11 comple
	102401	NZ19MI0007	NewZealand	2019	R	7	s	4	с	ST-11 comple
	102399	NZ19MI0004	New Zealand	2019	R	7	S	4	c c	ST-11 comple ST-11 comple
	93982 89322	IE19Nm25 M08 240537	ire land Malta	2019 2008	S R	1	s	4	с	ST-11 comple
Ц нііі	61352 61351	Nm-146 Nm-105	Russia Russia	2018 2016	R	59	s	1	w	ST-11 compl ST-11 compl
	71705	NZ18MI0064	New Zealand	2018	R	9	s	4	w	ST-11 comple
	71716 88953	NZ18MI0076	New Zealand Japan	2018	R R	9 9	s s	4	w	ST-11 compl
	71720	NZ18MI0080	New Zealand	2018	R	9	s	4	w	ST-11 comple
	71656	NZ18MI0020 NZ19MI0082	New Zealand New Zealand	2018 2019	R	9	S S	4	w	ST-11 comple ST-11 comple
	71708	NZ18MI0067	New Zealand	2018	R	9	s	4	w	ST-11 comple
	102411 71703	NZ19MI0019 NZ18MI0061	New Zealand New Zealand	2019 2018	R	9	s s	4	w	ST-11 comple ST-11 comple
	102441	NZ19MI0053	New Zealand	2019	R	9	s	4	w	ST-11 comple
• •	71724	NZ18MI0084 NZ18MI0024	New Zealand New Zealand	2018 2018	R R	9	S S	4	w	ST-11 compl ST-11 compl
	71719	NZ18MI0079	New Zealand	2018	R	9	s	4	w	ST-11 compl
	102484 102482	NZ19MI0105 NZ19MI0103	New Zealand New Zealand	2019 2019	R	9	s s	4	w	ST-11 compl ST-11 compl
	71734	NZ18MI0096	New Zealand	2018	R	9	s	4	w	ST-11 compl
	102457 102417	NZ19MI0075 NZ19MI0025	New Zealand New Zealand	2019 2019	R R	9	s s	4	w	ST-11 compl ST-11 compl
	102460	NZ19MI0079	New Zealand	2019	R	9	s	4	w	ST-11 compl
• •	71626	NZ17MI0062 NZ18MI0077	New Zealand	2017	R	9	s s	4	w	ST-11 compl ST-11 compl
	71717 71609	NZ18MI0077 NZ17MI0041	New Zealand New Zealand	2018 2017	R	9	s	4	w	ST-11 compl ST-11 compl
	102476	NZ19MI0095	New Zealand	2019	R	9	s	4	w	ST-11 compl
· · · ·	71629	NZ17MI0065 NZ19MI0035	New Zealand New Zealand	2017 2019	R R	ND 9	S S	4	NA W	ST-11 compl ST-11 compl
••	102421	NZ19MI0029	New Zealand	2019	R	9	s	4	w	ST-11 compl
· · ·	102487 102445	NZ19MI0108 NZ19MI0057	New Zealand New Zealand	2019 2019	R R	9	S S	4	w	ST-11 compl ST-11 compl
	102454	NZ19MI0069	New Zealand	2019	R	9	s	4	w	ST-11 comp
	102455	NZ19MI0071 NZ18MI0057	New Zealand New Zealand	2019 2018	8	9	S S	4	w	ST-11 compl ST-11 compl
				2018	R	9	5	4	w	ST-11 comp
	71699 102442	NZ19MI0054	New Zealand							
	102442 102426	NZ19MI0037	New Zealand	2019	R	9	s	4	w	
	102442				R R R	9 9 9	S S S	4 4 4	w w w	ST-11 comple
	102442 102426 102415	NZ19MI0037 NZ19MI0023	New Zealand New Zealand	2019 2019	R R R R	9 9 9 9	S S	4 4 4 4 4	w	ST-11 comple ST-11 comple ST-11 comple ST-11 comple ST-11 comple

FIG 1 A core-genome based phylogeny and data of six (labeled in red from this study) and 112 (global collection) penicillin- and/or ciprofloxacin-resistant *N. meningitidis* genomes. The maximum-likelihood phylogenetic tree with 1,000 bootstrap replicates was generated. Data on penicillin and ciprofloxacin categories, serogroup, and clonal complex are shown with the year and place of isolation and the *penA* and *gyrA* alleles. A T911 substitution responsible for ciprofloxacin resistance is also included alongside *gyrA*. ND, not determined; NA, not available.

to biases for regions. Analysis with a higher number of global strains would have helped to better appreciate the global distribution and genetic relatedness among them. Moreover, this study focused on the phenotype and genotype of *N. meningitidis* isolates in Japan but not the mechanisms underlying antibiotic resistance.

In conclusion, we discovered that *N. meningitidis* isolates recovered nationwide in Japan remained highly susceptible to antibiotics typically recommended for IMD patients and their close contacts, except for penicillin. However, invasive lineages with penicillin and/or ciprofloxacin resistance considered as public threats in several countries have been sporadically identified in Japan. Therefore, our findings highlight the importance of monitoring antibiotic resistance in preventing its spread, maintaining effective treatment and management of IMD patients and their close contacts, and improving infection control.

Data availability. The genomes have been deposited in GenBank under the BioSample accession numbers SAMD00391203 (NIID417), SAMD00320028 (NIID576), SAMD00320050 (NIID614), SAMD00320056 (NIID620), SAMD00286455 (NIID652), and SAMD00286469 (NIID669).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only. **SUPPLEMENTAL FILE 1**, PDF file, 0.3 MB.

ACKNOWLEDGMENTS

This work was supported by the Japan Agency for Medical Research and Development (AMED) under Grant Number JP20fk0108071 (R.S., H.K., H.T.) and the JSPS KAKENHI Grant Number JP20K08818 (R.S.). These funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We declare that we have no conflicts of interest.

REFERENCES

- Willerton L, Lucidarme J, Campbell H, Caugant DA, Claus H, Jacobsson S, Ladhani SN, Molling P, Neri A, Stefanelli P, Taha MK, Vogel U, Borrow R. 2020. Geographically widespread invasive meningococcal disease caused by a ciprofloxacin resistant non-groupable strain of the ST-175 clonal complex. J Infect 81:575–584. https://doi.org/10.1016/j.jinf.2020.08.030.
- Kawasaki Y, Matsubara K, Takahashi H, Morita M, Ohnishi M, Hori M, Isome K, Iwata A, Nigami H, Ikemachi M, Yamamoto G, Ohkusu K. 2018. Invasive meningococcal disease due to ciprofloxacin-resistant *Neisseria meningitidis* sequence type 4821: the first case in Japan. J Infect Chemother 24: 305–308. https://doi.org/10.1016/j.jiac.2017.11.001.
- Zhu B, Fan Y, Xu Z, Xu L, Du P, Gao Y, Shao Z. 2014. Genetic diversity and clonal characteristics of ciprofloxacin-resistant meningococcal strains in China. J Med Microbiol 63:1411–1418. https://doi.org/10.1099/jmm.0.078600-0.
- Tsang RS, Law DK, Deng S, Hoang L. 2017. Ciprofloxacin-resistant *Neisseria* meningitidis in Canada: likely imported strains. Can J Microbiol 63: 265–268. https://doi.org/10.1139/cjm-2016-0716.
- Mowlaboccus S, Jolley KA, Bray JE, Pang S, Lee YT, Bew JD, Speers DJ, Keil AD, Coombs GW, Kahler CM. 2017. Clonal expansion of new penicillin-resistant clade of *Neisseria meningitidis* serogroup W clonal complex 11, Australia. Emerg Infect Dis 23:1364–1367. https://doi.org/10.3201/eid2308.170259.
- Willerton L, Lucidarme J, Walker A, Lekshmi A, Clark SA, Gray SJ, Borrow R. 2021. Increase in penicillin-resistant invasive meningococcal serogroup W ST-11 complex isolates in England. Vaccine 39:2719–2729. https://doi .org/10.1016/j.vaccine.2021.03.002.
- Hedberg ST, Fredlund H, Nicolas P, Caugant DA, Olcen P, Unemo M. 2009. Antibiotic susceptibility and characteristics of *Neisseria meningitidis* isolates from the African meningitis belt, 2000 to 2006: phenotypic and genotypic perspectives. Antimicrob Agents Chemother 53:1561–1566. https://doi.org/ 10.1128/AAC.00994-08.
- Harcourt BH, Anderson RD, Wu HM, Cohn AC, MacNeil JR, Taylor TH, Wang X, Clark TA, Messonnier NE, Mayer LW. 2015. Population-based surveillance of *Neisseria meningitidis* antimicrobial resistance in the United States. Open Forum Infect Dis 2:ofv117. https://doi.org/10.1093/ofid/ofv117.

- Bertrand S, Carion F, Wintjens R, Mathys V, Vanhoof R. 2012. Evolutionary changes in antimicrobial resistance of invasive *Neisseria meningitidis* isolates in Belgium from 2000 to 2010: increasing prevalence of penicillin nonsusceptibility. Antimicrob Agents Chemother 56:2268–2272. https:// doi.org/10.1128/AAC.06310-11.
- Aye AMM, Bai X, Borrow R, Bory S, Carlos J, Caugant DA, Chiou CS, Dai VTT, Dinleyici EC, Ghimire P, Handryastuti S, Heo JY, Jennison A, Kamiya H, Tonnii Sia L, Lucidarme J, Marshall H, Putri ND, Saha S, Shao Z, Sim JHC, Smith V, Taha MK, Van Thanh P, Thisyakorn U, Tshering K, Vazquez J, Veeraraghavan B, Yezli S, Zhu B. 2020. Meningococcal disease surveillance in the Asia-Pacific region (2020): the global meningococcal initiative. J Infect 81:698–711. https://doi.org/10.1016/j.jinf.2020.07.025.
- Takahashi H, Kuroki T, Watanabe Y, Tanaka H, Inouye H, Yamai S, Watanabe H. 2004. Characterization of *Neisseria meningitidis* isolates collected from 1974 to 2003 in Japan by multilocus sequence typing. J Med Microbiol 53: 657–662. https://doi.org/10.1099/jmm.0.45541-0.
- 12. Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI, Wayne, PA, USA.
- Gorla MC, Pinhata JMW, Dias UJ, de Moraes C, Lemos AP. 2018. Surveillance of antimicrobial resistance in *Neisseria meningitidis* strains isolated from invasive cases in Brazil from 2009 to 2016. J Med Microbiol 67: 750–756. https://doi.org/10.1099/jmm.0.000743.
- Willerton L, Lucidarme J, Walker A, Lekshmi A, Clark SA, Walsh L, Bai X, Lee-Jones L, Borrow R. 2021. Antibiotic resistance among invasive *Neisseria meningitidis* isolates in England, Wales and Northern Ireland (2010/11 to 2018/19). PLoS One 16:e0260677. https://doi.org/10.1371/journal.pone .0260677.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

- Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3:124. https://doi.org/10.12688/wellcomeopenres.14826.1.
- Prah I, Ayibieke A, Mahazu S, Sassa CT, Hayashi T, Yamaoka S, Suzuki T, Iwanaga S, Ablordey A, Saito R. 2021. Emergence of oxacillinase-181 carbapenemase-producing diarrheagenic *Escherichia coli* in Ghana. Emerg Microbes Infect 10:865–873. https://doi.org/10.1080/22221751.2021.1920342.
- 19. Letunic I, Bork P. 2019. Interactive Tree of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res 47:W256–W259. https://doi .org/10.1093/nar/gkz239.
- Krone M, Lam TT, Vogel U, Claus H. 2020. Susceptibility of invasive Neisseria meningitidis strains isolated in Germany to azithromycin, an alternative agent for post-exposure prophylaxis. J Antimicrob Chemother 75:984–987. https:// doi.org/10.1093/jac/dkz535.