

Emergence of resistance to ciprofloxacin in *Neisseria meningitidis* in Brazil

Maria Cecília Gorla,^{1,*} Ana Paula Cassiolato,¹ Juliana Maira Watanabe Pinhata,¹ Camile de Moraes,² Alejandra Corso,³ Paula Gagetti³ and Ana Paula Lemos¹

Abstract

To prevent secondary invasive meningococcal disease (IMD) cases and outbreaks, antimicrobial prophylaxis of high-risk contacts is indicated. This study reports two ciprofloxacin-resistant *Neisseria meningitidis* strains in Brazil. The 3523 *N. meningitidis* isolates collected throughout Brazil from 2009 to 2016 were evaluated for antimicrobial resistance. Meningococcal isolates showing minimal inhibitory concentrations, MICs $\geq 0.125 \mu\text{g ml}^{-1}$ to ciprofloxacin, were analysed to determine the presence of mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes. Two ciprofloxacin-resistant *N. meningitidis* isolates were found, both presenting a single mutation in the quinolone resistance-determining region of the *gyrA* gene. These results confirmed that ciprofloxacin is still a first-line drug for chemoprophylaxis. However, we highlight the importance of continued surveillance to monitor the trends of *N. meningitidis* susceptibility profiles to the antimicrobials recommended for chemoprophylaxis and IMD treatment.

Meningococcus is the leading cause of bacterial meningitis in Brazil, although the incidence rates have slightly decreased in recent years from 1.57 cases per 100 000 inhabitants in 2010 to 0.67 cases in 2015 [1]. In Brazil the fatality rates for invasive meningococcal disease (IMD) have been about 20 % in cases of meningitis and about 50 % in cases of septicaemia [2], which are much higher than those described in the literature [3]. The timing of treatment and diagnosis is an important factor in satisfactory IMD prognosis. Close contacts of persons with IMD are at increased risk of contracting the disease. To prevent secondary cases and outbreaks, antimicrobial prophylaxis of high-risk contacts is indicated. The goal of chemoprophylaxis is the eradication of nasopharyngeal carriage of *Neisseria meningitidis* in contacts of the index case in order to interrupt the spread of the disease. The drugs of choice currently recommended by the Brazilian Ministry of Health for chemoprophylaxis are rifampin, ciprofloxacin and ceftriaxone [2]. The emergence of bacterial isolates with varying degrees of antimicrobial resistance is the most alarming aspect concerning the therapy of infectious diseases. The aim of the present study was

to report two ciprofloxacin-resistant *N. meningitidis* strains in Brazil in the period 2009–2016.

During this period, the Adolfo Lutz Institute in São Paulo, which is the Brazilian National Reference Laboratory for Meningitis, received 3544 *N. meningitidis* isolates from all Brazilian regions, comprising 27 states. These isolates represent about 78 % of the IMD cases confirmed by culture in the country. A total of 3523 (99.4 %) out of the 3544 isolates were evaluated for antimicrobial resistance. All *N. meningitidis* isolates were confirmed and serogrouped using conventional methods [4]. Serotyping was performed by dot-blotting using whole cell suspensions as previously described [5].

Multilocus sequence typing (MLST) was performed according to Maiden *et al.* [6]. Primers, determination of sequence alleles and designation of sequence types are described on the MLST website (<http://neisseria.org/nm/typing/mlst>).

Antimicrobial susceptibility testing was performed in accordance with the standards established by the Clinical and Laboratory Standards Institute (CLSI). The isolates were

Received 12 December 2017; Accepted 11 January 2018

Author affiliations: ¹Bacteriology Department, Adolfo Lutz Institute, Av. Dr. Arnaldo 351, São Paulo, CEP 01246-902, SP, Brazil; ²Secretariat of Health Surveillance, Ministry of Health, Esplanada dos Ministérios, Bloco G, Brasília, Federal District CEP 70058-900, Brazil; ³Servicio Antimicrobianos, Dpto Bacteriología, Instituto Nacional de Enfermedades Infecciosas “Dr Carlos G. Malbran”, A. Velez Sarsfield, 563 (1281), Buenos Aires, Argentina.

***Correspondence:** Maria Cecília Gorla, mcgorla@ial.sp.gov.br

Keywords: meningococcal surveillance; Ciprofloxacin; antimicrobial susceptibility.

Abbreviations: Asn, Asparagine; Asp, Aspartic acid; AZM, Azithromycin; CC, clonal complex; CIP, Ciprofloxacin; CLO, Chloramphenicol; CLSI, Clinical and Laboratory Standards Institute; CRO, Ceftriaxone; Gly, Glycine; Ile, Isoleucine; IMD, invasive meningococcal disease; IZ, inhibition zone; MIC, minimal inhibitory concentration; MIN, Minocycline; MLST, Multilocus sequence typing; PE, Pernambuco; PEN, Penicillin; QRDR, Quinolone Resistance Determining Region; R, Resistant; RIF, Rifampin; RS, Rio Grande do Sul; S, Susceptible; SIREVA II, Sistema Regional de Vacunas II; ST, sequence type; Thr, Threonine; Val, Valine.

Table 1. Characterization of ciprofloxacin-resistant *Neisseria meningitidis* isolates in Brazil

Strain number	State*	Sero-group	ST(CC)†	PorB sero-type	PorA VRI/VR2	MIC‡ µg ml ⁻¹						IZ§ mm	gyrA
						CIP	PEN	CLO	RIF	CRO	MIN		
N623/12	PE	C	3780 (ST-103)	23	22/14–6	0.125(R)	0.03(S)	1.0(S)	0.06(S)	0.0003(S)	0.125(S)	32(S)	Thr91Ile
N157/13	RS	W	11 (ST-11)	2a	5/2	0.250(R)	0.03(S)	1.0(S)	0.03(S)	0.0007(S)	0.125(S)	30(S)	Thr91Ile

*PE: Pernambuco; RS: Rio Grande do Sul.

†ST: Sequence type; CC: clonal complex.

‡MIC: Minimum inhibitory concentration; CIP: ciprofloxacin; PEN: penicillin; CLO: chloramphenicol; RIF: rifampin; CRO: ceftriaxone; MIN: minocycline; AZM: azithromycin; S: susceptible; R: resistant; §Inhibition zone.

||Threonine → isoleucine amino acid substitution at position 91 located in the fluoroquinolone resistance determining region (QRDR) of *gyrA* gene.

tested for susceptibility to ciprofloxacin, penicillin G, rifampin, chloramphenicol, ceftriaxone and minocycline by the broth microdilution method for determination of minimum inhibitory concentrations (MICs) [7], and to azithromycin by the agar disc diffusion method [8]. The results were interpreted according to the CLSI document M100-S27 (2017) [9].

Meningococcal isolates showing MICs ≥ 0.125 µg ml⁻¹ to ciprofloxacin were analysed to determine the presence of mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* (DNA gyrase, 847 bp) and *parC* genes (Topoisomerase IV, 822 bp) [10–13] as described by Wu *et al.* [14]. Nucleic acid sequencing was performed by the Sanger method, using BigDye terminators (Applied Biosystem, FSTC, CA). Analysis of nucleotide sequences was performed using the Chromas and Clustal X computer software packages, and the online Internet programs BLAST and PROSITE on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>).

Two of the 3523 *N. meningitidis* isolates analysed displayed resistance to ciprofloxacin. Neither of these isolates presented resistance to penicillin G, chloramphenicol, rifampin, ceftriaxone, minocycline or azithromycin. One of the isolates (N623/2012) was recovered in 2012 from the cerebrospinal fluid of a teenager in the state of Pernambuco, Northeast Region of Brazil. It was characterized as *N. meningitidis* C:23:P1.14–6:ST-103 complex and showed resistance to ciprofloxacin (MIC=0.125 µg ml⁻¹). The other isolate was obtained in 2013 (N157/2013) from the cerebrospinal fluid of a 6-month-old baby in the state of Rio Grande do Sul, South Region of Brazil, and was characterized as *N. meningitidis* W:2a:P1.5,2:ST-11complex/ET-37 complex. This isolate was resistant to ciprofloxacin (MIC=0.250 µg ml⁻¹). Both isolates presented a threonine (Thr) → isoleucine (Ile) mutation at amino acid 91 located in the QRDR of the *gyrA* gene. No additional mutations were found in the QRDRs of *gyrA* or *parC* genes (Table 1).

The isolates reported herein belong to two important and well-described hyper-virulent clones: serogroup C/ST-103 complex and W/ST-11 complex. Serogroup W:2a:P1.5,2:ST11 is frequently found in several Latin American countries, circulating also in Brazil [15, 16]. Serogroup C:23:P1.14–6:ST-103 represented 75.4 % of IMD cases in 2009 in Brazil [17]. Since these clones are well established in our region and are responsible for most IMD cases by serogroups C and W, we therefore discarded a potential foreign source of such isolates.

Mutations within the QRDR of *gyrA*, *gyrB*, *parC* and *parE* genes, and in the *mtrR* gene have been analysed to characterize fluoroquinolone resistance in *N. meningitidis* isolates [12, 13]. However, the main mechanism for ciprofloxacin resistance in these organisms appears to involve single mutations in the *gyrA* gene [13], resulting in amino acid substitutions Asp95Gly, Asp95Asn, Asn103Asp,

Ile111Val, Thr91Ile and Val120Ile and causing increases in ciprofloxacin MICs.

Although meningococcal resistance to ciprofloxacin remains uncommon, strains not susceptible (intermediate or resistant) to ciprofloxacin, showing MICs between 0.060 and 0.250 µg ml⁻¹, have been reported occasionally by Argentina, Australia, Canada, France, USA and Spain [11–14, 16, 18–20]. In our study, both isolates presented a threonine (Thr) → isoleucine (Ile) mutation at amino acid 91 located in the QRDR of the *gyrA* gene, resulting in increased MICs (0.125 and 0.250 µg ml⁻¹). Additionally, Hong *et al.* [21] showed in animal tests that the Thr91Ile mutation in *gyrA* was associated with the persistence of bacteria in infected mice despite ciprofloxacin treatment.

According to data from the SIREVA II network, a laboratory-based regional surveillance system and to the authors' knowledge, there is no report on the circulation of ciprofloxacin-non-susceptible (intermediate or resistant) *N. meningitidis* isolates in Latin American countries, except in Argentina, where Y:NT:P1.5 and B:1:P1. (non-serosubtypeable) meningococcal isolates not susceptible to ciprofloxacin (MICs=0.060 and 0.125 µg ml⁻¹) were detected in 2002 and 2003, respectively [12]. Sorhouet-Pereira *et al.* [16], also in Argentina, reported two isolates resistant to ciprofloxacin (MIC=0.125 µg ml⁻¹) belonging to the same W:2a:P1.5.2:ST-11 complex, and with the same mechanism of resistance as that described herein (*gyrA* Thr91Ile mutation)

The results clearly confirm that ciprofloxacin is still a first-line drug for chemoprophylaxis given the very small number of resistant isolates. However, we highlight the importance of continued surveillance to monitor the trends of *N. meningitidis* susceptibility profiles to the antimicrobials recommended for chemoprophylaxis and IMD treatment.

Funding information

The authors received no specific grant from any funding agency.

Acknowledgements

We thank Maria Vaneide de Paiva Batista and Ueslei José Dias for assistance in microbiological tests. We thank the central laboratories of Brazilian states for providing the meningococcal isolates; we also thank the surveillance team at the Secretariat of Health of Pernambuco and Rio Grande do Sul states. We thank Dr Carlos Henrique Camargo for his suggestions on this manuscript and Angela Pires Brandão for the thorough reading of it. This publication made use of the *Neisseria* Multi Locus Sequence Typing website (<https://pubmlst.org/neisseria/>) developed by Keith Jolley and sited at the University of Oxford (Jolley & Maiden 2010, *BMC Bioinformatics*, **11**:595). The development of this site has been funded by the Wellcome Trust and European Union.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Ministry of Health. 2016. Meningococcal disease in Brazil. http://portalarquivos.saude.gov.br/images/pdf/2016/outubro/33/tabela-2000-2015_meningite.pdf. (accessed 3 March 2017).
2. Doença Meningocócica. 2016. In: Health Surveillance Guide / Ministry of Health, Health Surveillance Secretary. Brasília: Ministry of

- Health Brazil. http://bvsms.saude.gov.br/bvs/publicacoes/guia_vigilancia_saude_1ed_atual.pdf.
3. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. *N Engl J Med* 2001;344:1378–1388.
4. Popovic T, Ajello GW, Facklam RR. *Laboratory Methods for the Diagnosis of Meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae*. Atlanta,GA: Centers for Disease Control, World Health Organization; 1998.
5. Wedege E, Høiby EA, Rosenqvist E, Frøholm LO. Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, dot-blotting and ELISA. *J Med Microbiol* 1990;31:195–201.
6. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE *et al.* Multi-locus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA* 1998;95:3140–3145.
7. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard - seventh informational supplement. In: *CLSI Document M7-A10*. Wayne, PA. Clinical and Laboratory Standards Institute; 2015.
8. Performance Standards for Antimicrobial Disk Susceptibility Tests: Twelfth Edition. In: *CLSI Document M02-A12*; 2015.
9. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth informational supplement. In: *CLSI Document M100-S27*. Wayne, PA: Clinical and Laboratory Standards Institute, 2017.
10. Lindbäck E, Rahman M, Jalal S, Wretling B. Mutations in *gyrA*, *gyrB*, *parC*, and *parE* in quinolone-resistant strains of *Neisseria gonorrhoeae*. *APMIS* 2002;110:651–657.
11. Alcalá B, Salcedo C, de La Fuente L, Arreaza L, Uría MJ *et al.* *Neisseria meningitidis* showing decreased susceptibility to ciprofloxacin: first report in Spain. *J Antimicrob Chemother* 2004;53:409.
12. Corso A, Faccione D, Miranda M, Rodriguez M, Regueira M *et al.* Emergence of *Neisseria meningitidis* with decreased susceptibility to ciprofloxacin in Argentina. *J Antimicrob Chemother* 2005;55:596–597.
13. Enríquez R, Abad R, Salcedo C, Pérez S, Vázquez JA. Fluoroquinolone resistance in *Neisseria meningitidis* in Spain. *J Antimicrob Chemother* 2008;61:286–290.
14. Wu HM, Harcourt BH, Hatcher CP, Wei SC, Novak RT *et al.* Emergence of ciprofloxacin-resistant *Neisseria meningitidis* in North America. *N Engl J Med* 2009;360:886–892.
15. Weidlich L, Baethgen LF, Mayer LW, Moraes C, Klein CC *et al.* High prevalence of *Neisseria meningitidis* hypervirulent lineages and emergence of W135:P1.5.2:ST-11 clone in Southern Brazil. *J Infect* 2008;57:324–331.
16. Sorhouet PC, Efron A, Gaget P, Faccione D, Regueira M *et al.* Phenotypic and genotypic characteristics of *Neisseria meningitidis* disease-causing strains in Argentina, 2010. *PlosOne* 2013;8:1–7. e58065.
17. Gorla MC, de Lemos AP, Quaresma M, Vilasboas R, Marques O *et al.* Phenotypic and molecular characterization of serogroup C *Neisseria meningitidis* associated with an outbreak in Bahia, Brazil. *Enferm Infecc Microbiol Clin* 2012;30:56–59.
18. 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.
19. Tsang RS, Law DK, Deng S, Hoang L. Ciprofloxacin-resistant *Neisseria meningitidis* in Canada: likely imported strains. *Can J Microbiol* 2017;63:265–268.
20. Skoczynska A, Alonso JM, Taha MK. Ciprofloxacin resistance in *Neisseria meningitidis*, France. *Emerg Infect Dis* 2008;14:1322–1323.
21. Hong E, Thulin Hedberg S, Abad R, Fazio C, Enríquez R *et al.* Target gene sequencing to define the susceptibility of *Neisseria meningitidis* to ciprofloxacin. *Antimicrob Agents Chemother* 2013;57:1961–1964.