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

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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 3544 *N. meningitidis* isolates collected throughout Brazil from 2009 to 2016 were evaluated for antimicrobial resistance. Meningococcal isolates showing minimal inhibitory concentrations, MICs≥0.125µg 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 insatisfactory 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

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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 VR1/VR2	MIC‡ μg ml^{-1}					IZ§ mm	*gyrA*‖
						CIP	PEN	CLO	RIF	CRO	MIN	AZM	
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$\geq$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, Asn103 Asp, 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, North Americaand 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.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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