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Antimicrobial susceptibility of *Neisseria meningitidis* strains isolated from meningitis cases in Brazil from 2006 to 2008

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ABSTRACT

Objective: To analyze the profile of antimicrobial susceptibility of meningococcal disease isolates collected throughout Brazil from 2006 to 2008 and forwarded to the National Reference Laboratory for Meningitis, Instituto Adolfo Lutz – São Paulo.

Materials and methods: The MIC to penicillin, ampicillin, chloramphenicol, ceftriaxone, ciprofloxacin and rifampicin was determined in a sample of 1096 (55% of the total isolates received) randomly chosen using the broth microdilution procedure. The breakpoints used were those recommended by the European Monitoring Group on Meningococci (EMGM).

Results: Decreased susceptibility to penicillin and ampicillin was detected in 13% and 12.9% respectively. All isolates were susceptible to chloramphenicol, ceftriaxone, and ciprofloxacin. Two strains (0.2%) showed high resistance to rifampicin and 0.5% of the isolates displayed intermediate resistance to rifampicin.

Conclusions: The meningococcal strains isolated in Brazil during 2006–2008 were globally susceptible to all antibiotics currently used in treatment or chemoprophylaxis of meningococcal disease in Brazil.

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Sensibilidad a antimicrobianos de las cepas de *Neisseria meningitidis* aisladas de casos de meningitis en Brasil desde 2006 a 2008

RESUMEN

Palabras clave:

Vigilancia de meningococo

Neisseria meningitidis

Penicilina

Susceptibilidad a los antimicrobianos

Objetivo: Analizar el perfil de susceptibilidad a los antimicrobianos de las cepas de meningococos aisladas de casos de enfermedad meningocócica en Brasil entre 2006 y 2008 y enviadas al Laboratorio Nacional de Referencia para Meningitis, Instituto Adolfo Lutz, São Paulo.

Material y métodos: Se determinó la CIM a penicilina, ampicilina, cloranfenicol, ceftriaxona, ciprofloxacino y rifampicina, mediante el procedimiento de microdilución seriada en caldo en una muestra de 1.096 aislados (55% de los aislados recibidos) escogida al azar. Los puntos de corte utilizados fueron los recomendados por el European Monitoring Group on Meningococci (EMGM).

Resultados: Se detectó disminución de la susceptibilidad a la penicilina y la ampicilina en el 13 y el 12,9% respectivamente. Todos los aislados fueron susceptibles a cloranfenicol, ceftriaxona y ciprofloxacino. Dos cepas (0,2%) mostraron alta resistencia a la rifampicina y el 0,5% de los aislados presentaron resistencia intermedia a la rifampicina.

Conclusiones: Las cepas de meningococos aisladas en Brasil en el periodo 2006–2008 fueron globalmente susceptibles a los antibióticos actualmente utilizados en el tratamiento o quimioprofilaxis de enfermedad meningocócica en Brasil.

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Introduction

Neisseria meningitidis is a pathogen of great public health importance for causing periodic epidemics of meningococcal disease

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with high case-fatality rate even with appropriate treatment and intensive care, affecting particularly infants, adolescents and young adults. *Neisseria meningitidis* is a leading cause of bacterial meningitis in Brazil, presenting, in the last ten years, a case-fatality rate of about 11% in its clinical form of meningitis, 16% in meningitis and septicemia, rising to 38% in septicemia. The clinical forms of meningitis, meningitis and septicemia and septicemia alone accounted for 39.2%, 33% and 27.8%, respectively, of the confirmed cases of meningococcal disease in Brazil, during this period. (<http://www.saude.gov.br/sinanweb>)

In view of the rapid progression and high lethality of the meningococcal disease, prompt treatment seems to be crucial to reduce the mortality and to improve the meningococcal disease outcome.

Penicillin remains as suitable treatment for meningococcal disease; however, since 1985 the increase of the meningococcal strains with reduced susceptibility to penicillin has been described in some countries, reaching percentages as 4% in the United States, 23% in Sweden, 30% in France and 70% in Turkey.¹ Given the empiric therapy of patients with suspected bacterial meningitis and the high levels of resistance of *Streptococcus pneumoniae* to penicillin (about 30% in Brazil),² initial therapy is based on the third-generation cephalosporin, such as ceftriaxone and cefotaxime in industrialized countries.^{3–5} Meningococcal strains with reduced susceptibility to ceftriaxone have not been described. However, the description of *Neisseria gonorrhoeae* strains resistant to cefixime, also a third-generation cephalosporin, associated with alterations in *penA* gene, alerts for the possibility of this resistance to be extended to meningococci in the future.⁴ Chloramphenicol has not been used for treatment of meningococcal disease in many countries for the description of its toxic effect, as the marrow aplasia.⁶ High-level chloramphenicol resistance was described in Vietnam and in France.⁷ The rifampicin and ciprofloxacin are widely used as chemoprophylaxis of contacts of patients with meningococcal disease. Rifampicin resistance is not widely present and it is usually limited to sporadic cases. However, some reports on meningococcal resistance to ciprofloxacin might cause concern because the potential for spreading the resistance.^{8–13}

The worldwide problem of resistance in *N. meningitidis* emphasizes the urgent need of careful surveillance on antibiotic susceptibilities for better control and prevention of meningococcal disease.

The aim of the present study was to describe the susceptibility profile to antimicrobials of a large collection of meningococcal isolated in Brazil during the period of 2006–2008.

Material and methods

Bacterial strains

The Institute Adolfo Lutz (IAL), located in the State of São Paulo, is the Brazilian National Reference Laboratory for Bacterial Meningitis, to where clinical isolates are forwarded through a national epidemiologic system. The IAL receives an annual average of 660 meningococcal invasive isolates from all Brazilian regions for full phenotypic characterization. Between 2006 and 2008, 7 720 cases of meningococcal disease were reported in Brazil of which 2 567 (33.3%) were laboratory-confirmed by culture. Of the 2 567 cases confirmed by culture, 1 988 strains (77.4%) were sent to the IAL by 27 Public Health Laboratories and hospitals located in the Southeast (59.2%), Northeast (17.6%), Central-West (8.8%), South (11.9%) and North (2.5%) regions of Brazil. From this collection, a statistical representative random sampling of 1 096 (55.1%) strains was submitted to antimicrobial susceptibility tests, calculated by using the Statcalc Program (Epi Info software version 6.04, Cen-

ters for Diseases Control and Prevention, Atlanta, GA). The isolates were recovered from cerebrospinal fluid (n=817; 74.5%) or blood (n=279; 25.5%).

The selected sample was comprised by serogroups B (n=344 [31.4%]), C (n=677 [61.8%]), W135 (n=60 [5.4%]), and Y (n=15 [1.4%]). Serogroup B isolates included 50 phenotypes being the most prevalent 4,7:P1.19,15 (220/344; 63.9%) and 4,7:P1.7,1 (24/344; 7%) with the remaining serogroup B phenotypes representing less than 2%. Serogroup C included 27 different phenotypes, being the most prevalent 23:P1.14-6 (560/677; 82.7%), and 2a:P1.5 (31/677; 4.6%) with the remaining serogroup C phenotypes representing less than 3%. Serogroup W135 included 14 phenotypes being the most prevalent 2a:P1.2 (19/60; 31.7%), 2a:P1.5 (10/60; 16.7%) and 2b:P1.2 (6/60; 10%) with the remaining serogroup W135 phenotypes representing less than 5%. Serogroup Y included 8 different phenotypes being the most prevalent 17,7:P1.5 (7/15; 46.7%) followed by 4,14:P1.7 (2/15; 13.3%) with the remaining serogroup Y phenotypes representing less than 7%.

Phenotypic antimicrobial susceptibility testing

The susceptibility of meningococcal strains to penicillin, ampicillin, chloramphenicol, ciprofloxacin, ceftriaxone and rifampicin was analyzed by determining the minimal inhibitory concentration (MIC) using the broth microdilution procedure described in CLSI document M7-A7.^{14,15} The susceptibility/resistance breakpoints were those recommended by the European Monitoring Group on Meningococci (EMGM),⁵ as follows: penicillin G $\leq 0.06/\geq 1$ $\mu\text{g/mL}$ (Susceptible ≤ 0.06 $\mu\text{g/mL}$, Intermediate = 0.125–0.500 $\mu\text{g/mL}$, Resistant ≥ 1 $\mu\text{g/mL}$); ampicillin $\leq 0.12/\geq 2$ $\mu\text{g/mL}$ (Susceptible ≤ 0.125 $\mu\text{g/mL}$, Intermediate = 0.250–1 $\mu\text{g/mL}$, Resistant ≥ 2 $\mu\text{g/mL}$); chloramphenicol $\leq 2/\geq 8$ $\mu\text{g/mL}$ (Susceptible ≤ 2 $\mu\text{g/mL}$, Intermediate = 4 $\mu\text{g/mL}$, Resistant ≥ 8 $\mu\text{g/mL}$); ciprofloxacin $\leq 0.03/\geq 0.5$ $\mu\text{g/mL}$ (Susceptible ≤ 0.03 $\mu\text{g/mL}$, Intermediate = 0.06–0.25 $\mu\text{g/mL}$, Resistant ≥ 0.5 $\mu\text{g/mL}$); ceftriaxone ≤ 0.12 $\mu\text{g/mL}$ (Susceptible ≤ 0.12 $\mu\text{g/mL}$, ≥ 0.12 $\mu\text{g/mL}$ not described), and rifampicin $\leq 0.25/\geq 2$ $\mu\text{g/mL}$ (Susceptible ≤ 0.25 $\mu\text{g/mL}$, Intermediate = 0.5–1 $\mu\text{g/mL}$, Resistant ≥ 2 $\mu\text{g/mL}$).

Analysis of the molecular mechanism of resistance to rifampicin

A fragment of the *rpoB* gene (encoding amino acids 435–644) was amplified using primers *rpoB*-F1 and *rpoB*-R1 as previously described.¹⁶

Quality control

An external Quality Assurance Program for characterization (QAP), introduced by the SIREVA network in Latin American countries¹⁷ has validated the performance of the *N. meningitidis* typing and antimicrobial susceptibility tests. The QAP is coordinated by the Reference Laboratory for Neisserias, National Center for Microbiology, Institute of Health Carlos III, Majadahonda, Madrid, Spain.

Statistical analysis

Association between antimicrobial resistance and variables such as age and gender of patients, *N. meningitidis* serogroup and the geographic distribution of isolates were assessed by the χ^2 test; P values <0.05 were considered to be statistically significant.

Results

The results of antimicrobial susceptibility testing are summarized in table 1. All isolates were susceptible to ciprofloxacin,

Table 1Susceptibility to six different antimicrobials of *Neisseria meningitidis* strains (n = 1 096) isolated from invasive disease in Brazil from 2006 to 2008.

Antibiotic	Breakpoints ^a (susceptible/resistant), µg/mL	MIC range obtained, µg/mL	MIC ₅₀	MIC ₉₀	No. (%) of isolates		
					Susceptible	Intermediate	Resistant
Penicillin G	≤0.06/≥1	0.003–0.5	0.03	0.125	953 (87)	143 (13.0)	0.0 (0.0)
Ampicillin	<0.12/≥2	0.003–0.5	0.06	0.25	954 (87.1)	142 (12.9)	0.0 (0.0)
Ciprofloxacin	≤0.03/≥0.5	0.0007–0.015	0.003	0.007	1 096 (100)	0.0 (0.0)	0.0 (0.0)
Chloramphenicol	≤2/≥8	0.5–2	1	1	1 096 (100)	0.0 (0.0)	0.0 (0.0)
Ceftriaxone	≤0.12/	0.0003–0.015	0.0007	0.0015	1 096 (100)	0.0 (0.0)	0.0 (0.0)
Rifampicin	≤0.25/≥2	0.003–>256	0.03	0.125	1 088 (99.3)	6.0 (0.5)	2.0 (0.2)

MIC: minimum inhibitory concentration; MIC₅₀ and MIC₉₀: minimum inhibitory concentration at which respectively 50% and 90% of isolates were inhibited.^a Breakpoints proposed by the European Monitoring Group on Meningococci (EMGM)⁵.

ceftriaxone, and chloramphenicol. Ciprofloxacin and ceftriaxone were the antibiotics with higher inhibitory activities showing minimal MIC₉₀ at the very low concentration range. MIC of 1 µg/mL to chloramphenicol, a concentration very near to the intermediate category (4 µg/mL) was detected in most strains (78%). There was a broad range of MICs to rifampicin, from 0.007 µg/mL to 0.125 µg/mL for most isolates. A total of 143 (13%) and 142 (12.9%) strains presented reduced susceptibility to penicillin and ampicillin, respectively. Although most of the isolates were susceptible to penicillin (63.9%) or to ampicillin (77.8%) far from the breakpoint values, 253 (23.1%) of them were only inhibited by 0.06 µg/mL, a value close to the penicillin intermediate category (data not shown), suggesting a tendency to a raise in MICs to penicillin.

Two isolates belonging to phenotypes B:4,7:P1.19,15 and C:23:P1.14-6 presented high resistance (MIC>256 µg/mL) to rifampicin (0.2%), and six (0.5%) isolates showed intermediate resistance to rifampicin. The analysis of the molecular mechanism of rifampicin resistance of the B:4,7:P1.19,15 and C:23:P1.14-6 isolates showed only one amino acid substitution: a Hys>Tyr, in position 552 of the *rpoB* gene. Both strains showed not only the same amino acid substitution but also the same *rpoB* allele based in the DNA sequence.

In order to better understand the distribution of the strains with reduced susceptibility to penicillin in the studied sample, we correlated the susceptibility with some variables as shown in table 2. There was no statistically significant association between the diminished susceptibility to penicillin and gender ($P = .279$), age ($P = .076$), geographic region ($P = .091$), or serogroup of *N. meningitidis* ($P = .742$).

The isolates with reduced susceptibility to penicillin belonged to a large variety of serosubtypes as shown in table 3. The phenotypes 4,7:P1.19,15 and 23:P1.14-6 were the most prevalent among serogroup B and C isolates, respectively; however, the prevalence of isolates with reduced sensibility to penicillin was not particularly associated with any serosubtype (data not submitted to statistical analysis by serosubtype diversity).

Discussion

The data show that meningococcal strains isolated in Brazil during 2006–2008 were globally susceptible to all antibiotics currently used both in treatment or chemoprophylaxis of meningococcal disease in the country.

Although fluoroquinolones have been recommended for chemoprophylaxis especially in adults for their efficacy in eradicating the meningococcus from the nasopharynx in 97% of the individuals for up to 33 days after the end of therapy, in a single-dose regimen¹⁸ and resistance has been already described in different geographical locations including Latin American region.^{12,13,19–22}

The reduced susceptibility to ceftriaxone has not been described around the world, and was not observed in Brazilian meningococcal strains to which ceftriaxone showed very high inhibitory activities, with MIC₉₀ within the very low concentration range.

No resistance to chloramphenicol was observed, but MIC of 1 µg/mL, which is very near to the intermediate category (4 µg/mL), was obtained for most of the isolates. This drug therefore can be an effective antibiotic therapy substitute, particularly in penicillin-allergic patients.

In spite of the fact that rifampicin has been widely used in Brazil for preventing secondary cases, in the present study *N. meningitidis* isolates remained highly susceptible to this antimicrobial drug (only two resistant isolates, 0.2%), as it has been described by others.^{16,23–26} The analysis of the molecular mechanism conferring resistance to rifampicin of both isolates showed a point mutation in a specific region of the *rpoB* gene as was already found in two strains from Spain.⁸ Our results support the use of rifampicin prophylaxis of meningococcal infections in Brazil, paralleled with a monitoring susceptibility program. A data base with alleles of the *rpoB* fragment analyzed in this study is under construction on the European Meningococcal Disease Society (EMGM) hosted on the web page <http://www.neisseria.org>

Table 2Susceptibility of *Neisseria meningitidis* strains (n = 1 096) to penicillin, according to patient characteristics, geographic regions and serogroups, Brazil from 2006 to 2008.

Variable	Susceptible, n (%)	Intermediate, n (%)	Total, n (%)	P
Gender				.279
Female	420 (85.7)	70 (14.3)	490 (44.7)	
Male	533 (88)	73 (12)	606 (55.3)	
Total	953 (87)	143 (13)	1 096	
Age (years)				.076
< 1	129 (88.4)	17 (11.6)	146 (13.3)	
1–4	214 (87.7)	30 (12.3)	244 (22.3)	
5–9	123 (82.6)	26 (17.4)	149 (13.6)	
10–14	87 (79.1)	23 (20.9)	110 (10)	
> 15	318 (88.1)	43 (11.9)	361 (33)	
Unknown	82 (95.3)	4 (4.7)	86 (7.8)	
Total	953 (87)	143 (13)	1 096	
Region^a				.091
CO	83 (91.2)	8 (8.8)	91 (8.3)	
N	25 (89.2)	3 (10.7)	28 (2.6)	
NE	194 (91.1)	19 (8.9)	213 (19.4)	
S	112 (88.2)	15 (11.8)	127 (11.6)	
SE	539 (84.6)	98 (15.4)	637 (58.1)	
Total	953 (87)	143 (13)	1 096	
Serogroup				.742
B	298 (86.6)	46 (13.4)	344 (31.4)	
C	587 (86.7)	90 (13.3)	677 (61.8)	
W135	55 (91.7)	5 (8.3)	60 (5.4)	
Y	13 (86.7)	2 (13.3)	15 (1.4)	
Total	953 (87)	143 (13)	1 096	

^a Geographic regions: Central-West (CO), North (N), Northeast (NE), South (S), and Southeast (SE).

Table 3

Serosubtype distribution among *Neisseria meningitidis* strains displaying reduced susceptibility to penicillin, Brazil from 2006 to 2008.

Serosubtype	Susceptible, n (%)	Intermediate, n (%)	Total
2a:P1.2	44 (97.8)	1 (2.2)	45
2a:P1.5	48 (94.1)	3 (5.9)	51
2a:P1.14-6	9 (90)	1 (10)	10
2a:NST ^a	24 (88.9)	3 (11.1)	27
2b:P1.2	13 (68.4)	6 (31.6)	19
2b:NST	5 (55.6)	4 (44.4)	9
4,7:P1.5	4 (80)	1 (20)	5
4,7:P1.7,1	25 (86.2)	4 (13.8)	29
4,7:NST	8 (72.7)	2 (18.2)	11
4,7:P1.19,15	209 (92.1)	15 (6.6)	227
4,7:P1-22-1,14	1 (33.3)	2 (66.7)	3
4,14:NST	2 (50)	2 (50)	4
15:P1.7,16	5 (62.5)	3 (37.5)	8
17,7:P1.5	5 (83.3)	1 (16.7)	6
19,1:NST	1 (14.3)	6 (85.7)	7
19,7.1:NST	2 (25)	6 (75)	8
19,10:P1.16	3 (75)	1 (25)	4
19,10:P1.19,15	2 (66.7)	1 (33.3)	3
19,14:P1.9	1 (50)	1 (50)	2
19,14:P1-22-1,14	4 (80)	1 (20)	5
21:NST	0	1 (100)	1
23:P1.5	18 (94.7)	1 (5.3)	19
23:P1.14-6	485 (86.6)	75 (13.4)	560
NT ^b :P1.2	5 (83.3)	1 (16.7)	6
NT:NST	2 (66.7)	1 (33.3)	3

^a Non-serosubtypable.

^b Non-serotypable.

which will allow the comparison among strains showing different level of rifampicin resistance and will be helpful to define proper breakpoints to be used for this antimicrobial drug.

In our study, penicillin-intermediate susceptibility isolates displayed a uniform distribution regarding to age, gender or geographic region. The prevalence of meningococcal strains with reduced susceptibility to penicillin remained stable over the analyzed period (2006–2008), ranging from 10.3% to 15.1% (data not shown). However, when these data are compared to the previous period of 2000–2005,²⁷ the prevalence of meningococci with reduced susceptibility to penicillin is shown to be clearly increasing from 0 to 15.1% over the 9-years period, ranging from 0% of penicillin-intermediate susceptibility isolates in 2000, to 4.1% in 2001, 3.1% in 2002, 4.1% in 2003, 6.2% in 2004, 8.9% in 2005, 13.5% in 2006, 10.3% in 2007, and 15.1% in 2008. The increase of the prevalence of meningococci with reduced susceptibility to penicillin seen in the period 2000–2008 was paralleled by a shift in the serogroup distribution. Serogroup B meningococci, B:4,7:P1.19,15-ST-5 complex ET-5 was hyperendemic in Brazil since 1988, accounting for 80% of all meningococcal disease between 1988 and 1989.^{28,29} However, there was a huge change in the prevalence of the serogroups with the increasing prevalence of a single serogroup C clone, C:23:P1.14-6, ST-103 Clonal Complex. This clone, which emerged in Brazil in 1990, has been gradually increasing, becoming the most prevalent phenotype in 2003, and accounting for 67.7% of *N. meningitidis* isolates in 2008.³⁰

These data are in agreement with those from other countries such as Italy where the prevalence of penicillin-intermediate susceptibility isolates has significantly risen from 7.5% before 2002 to 27.4% in 2004 being directly associated with an increase in serogroup C (C:2b:P1.5) strains 31, or Spain where an increase of penicillin-intermediate susceptibility isolates from 0.4% to 43% in only 5 years (1985–1990) was reported and associated with a shift between meningococcal serogroups B (B:4:P1.15) and C (C:2b:P1.5,2) isolates.^{9,31,32} However, the penicillin-intermediate susceptibility isolates distribution has not been associated to meningococcal serogroups or serosubtypes in this study and the

prevalence increase of isolates with reduced susceptibility to penicillin can not be associated with the emergence of clone C:23:P1.14-6, ST-103 complex in Brazil. These results suggest that the rising prevalence of meningococcal penicillin-intermediate susceptibility strains is most probably due to a selective pressure created by the wide use of antimicrobial drugs, resulting in novel alleles that are mosaic genes encoding proteins with decreased affinity for penicillin, instead of the emergence and spread of a single clone exhibiting reduced susceptibility to penicillin. These results are in agreement with those of South Africa, where penicillin resistance do not appear to be due to the spread of a single clone.¹

Although the prevalence of strains with reduced penicillin susceptibility in Brazil is not so high to be an alarming question, since the alteration of only one of the *N. meningitidis* PBPs (PBP2) has been described until now, strains with decreased susceptibility might be still evolving around the world. Therefore, the emergence of penicillin-resistant strains may be expected in the future indicating the need of a continued surveillance for monitoring the trends on antimicrobial susceptibility in *N. meningitidis*.

Conflict of interest

The authors have no conflicts of interest to declare.

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