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Original article

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, Institute 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.

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

Palabras clave:
Vigilancia de meningococo
Neisseria meningitidis
Penicilina
Susceptibilidad a los antimicrobianos

RESUMEN

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.

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 *pen*A 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, Centers 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 μg/mL (Susceptible $\leq$0.06 μg/mL, Intermediate = 0.125-0.500 μg/mL, Resistant $\geq$1 μg/mL); ampicillin $\leq$0.12/$\geq$2 μg/mL (Susceptible $\leq$0.125 μg/mL, Intermediate = 0.250-1 μg/mL, Resistant $\geq$2 μg/mL); chloramphenicol $\leq$2/$\geq$8 μg/mL (Susceptible $\leq$2 μg/mL, Intermediate = 4 μg/mL, Resistant $\geq$8 μg/mL); ciprofloxacin $\leq$0.03/$\geq$0.5 μg/mL (Susceptible $\leq$0.03 μg/mL, Intermediate = 0.06-0.25 μg/mL, Resistant $\geq$0.5 μg/mL); ceftriaxone $\leq$0.12 μg/mL (Susceptible $\leq$0.12 μg/mL, $\geq$0.12 μg/mL not described), and rifampicin $\leq$0.25/$\geq$2 μg/mL (Susceptible $\leq$0.25 μg/mL, Intermediate = 0.5-1 μg/mL, Resistant $\geq$2 μg/mL).

Analysis of the molecular mechanism of resistance to rifampicin

A fragment of the *rpo*B gene (encoding amino acids 435–644) was amplified using primers *rpo*B-F1 and rpoB-R1as previously described.[16]

Quality control

An external Quality Assurance Program for characterization (QAP), introduced by the SIREVA network in Latin American countries[17] 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 <.05 were considered to be statistically significant.

Results

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