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Antimicrobial Resistance and Serotypes of Nasopharyngeal Strains of *Streptococcus pneumoniae* in Brazilian Adolescents

DÉA MASCARENHAS CARDOZO,[1] CRISTIANA M. NASCIMENTO-CARVALHO,[1] MARIA ANGÉLICA BRANDÃO,[2] GILDETE MARIA SANTOS AZEVEDO,[2] FABIANE RIBEIRO DE SOUZA,[3] NÍVEA MARIA SILVEIRA SILVA,[3] ANGELA PIRES BRANDÃO,[4–6] ANA LÚCIA S. SGAMBATTI DE ANDRADE,[7] and MARIA CRISTINA C. BRANDILEONE[4]

ABSTRACT

The aim of this study was to describe the frequency of antimicrobial-resistance and serotypes of nasopharyngeal pneumococcal isolates from adolescents. Clinical data and nasopharyngeal specimens for culture were collected from 1,013 adolescents as a part of a population-based study. A total of 83 isolates of *Streptococcus pneumoniae* were identified (8.2%). Seventy-four of the 83 isolates were serotyped. The median age of the 83 adolescents colonized by pneumococci was 14 years (mean 14 ± 2.2 yrs); 55.4% were males. Intermediate resistance to penicillin was detected in 7.2% (6/83). No strain showed high resistance to penicillin. All isolates were susceptible to clindamycin, chloramphenicol, rifampin, and vancomycin; 37.3%, 18.1%, and 4.8% were resistant to trimethoprim-sulfamethoxazole, tetracycline, and erythromycin, respectively. The most frequent serotypes (5–10% of strains each) were 6B, 6A, 23F, and 18C among 28 serotypes/serogroups identified; 18.9% of the strains were nontypeable (NT). Intermediate resistance to penicillin was detected in serotypes 6B, 14, and NT. The rate of resistance to penicillin of nasopharyngeal isolates is low considering data from other studies about invasive strains recovered from children in Brazil. Serotype patterns are similar, except for type 14, which was unusually infrequent.

INTRODUCTION

STREPTOCOCCUS PNEUMONIAE is the leading bacterial cause of infection worldwide, ranging from common infections such as otitis media to life-threatening invasive infections such as sepsis, meningitis, and pneumonia.[6,17] *S. pneumoniae* belongs to the normal upper respiratory flora of humans,[15] and infection usually occurs after nasopharyngeal acquisition.[8] Of concern is the increasing frequency and rapid spread of drug-resistant pneumococcal strains worldwide,[12] which have complicated treatment of pneumococcal infections.[9] The surveillance of pneumococcal resistance in nasopharyngeal isolates is a practical way to determine the prevalence of resistant strains and is a reasonable predictor of resistance in systemic isolates.[10] Such information could be used to guide empiric therapy of pneumococcal infections.[7] Information regarding frequency of antimicrobial resistance and serotypes among pneumococcal strains recovered from adolescents is scarce and the aims of this study were to provide this information.

MATERIALS AND METHODS

Study design and population

This was a cross-sectional, population-based, descriptive study carried out between November, 2002, and July, 2003. Each participant was randomly recruited from public schools located in each District of Salvador, a city of 325 km^2, located in northeastern Brazil, with an estimated population of

[1]Department of Pediatrics, Faculty of Medicine, Federal University of Bahia (UFBA), Salvador, Bahia, Brazil.
[2]Central Laboratory of Bahia (LACEN), Salvador, Bahia, Brazil.
[3]Faculty of Biochemistry (UFBA), Salvador, Bahia, Brazil.
[4]Bacteriology Division, Adolfo Lutz Institute, São Paulo, Brazil.
[5]National Foundation of Health, São Paulo, Brazil.
[6]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.
[7]Institute of Tropical Pathology and Public Health, Federal University of Goiás, Goiânia, Brazil.

2,443,107 inhabitants,[3] of which 20.6% are adolescents (age 10–19 years).[16] Demographic data were collected on a standardized questionnaire. Each participant denied previous use of pneumococcal vaccine and antibiotics 72 hr or 10 days before collection of nasopharyngeal specimens for this study. Each participant gave written informed consent, and the study protocol was approved by the Ethical Committees of the School Hospital of the Faculty of Medicine, Federal University of Bahia, of Central Laboratory of Bahia (LACEN-BA) and of Adolfo Lutz Institute (IAL).

Bacteriologic data

Nasopharyngeal specimens were collected on cotton-tipped swabs (Transwab, pernasal, charcoal medium, MW 173C, Medical Wire & Equipment Co., Wiltshire, UK) through the nose and transported in Amies medium within 4 hr to the laboratory (LACEN-BA), where they were inoculated onto blood agar plates containing 5% sheep blood with 5 μg/ml of gentamicin. Plates were incubated at 37°C in 5% CO_2 for 18–24 hr. Each adolescent had just one collection of nasopharyngeal specimens. Pneumococcus strains were identified by Gram-staining, using bile solubility and optochin disc tests.[23] Pneumococcal resistance to penicillin was screened by using a 1-μg oxacillin disc. Penicillin resistance was considered probable with an oxacillin zone size less than 20 mm when MIC values of penicillin and ceftriaxone was determined using the E-test (AB Biodisk). The MIC intermediate and resistant limits accepted for these antibiotics were determined by taking into consideration the values determined by the Clinical and Laboratory Standards Institute[14] for penicillin G (MIC 0.1–1.0 μg/ml; intermediate and $\geq$2.0 μg/ml; resistant) and ceftriaxone (MIC $\geq$ 2 μg/ml; resistant). Disc diffusion was also used for determining resistance to trimethoprim-sulfamethoxazole, tetracycline, erythromycin, clindamycin, chloramphenicol, rifampin, and vancomycin.[14] Identification of pneumococcal strains was rechecked at IAL, where serotyping was done by the Neufeld–Quellung reaction using sera produced by the Statens Seruminstitut (Copenhagen, Denmark).

Database and statistical analysis

A database was created by using the EPI INFO 6 program. The analysis was carried out using SPSS, version 9.0. Differences in proportions were assessed by the Pearson chi square test or Fisher's exact test, and means of continuous variables were compared by Student's *t*-test or Mann–Whitney U, as appropriate. The statistical tests were two tailed, with a significance level of 0.05.

RESULTS

During a 9-month period, 83 strains of pneumococcus were isolated from 1,013 adolescents. The median age of the colonized adolescents was 14 years (mean 14 $\pm$ 2.2 years) and there were 55.4% males. Age stratum distribution was 37.3% (10–13 years), 49.4% (14–16 years), and 13.3% (17–19 years). The median number of individuals in the house was 4 (mean 5 $\pm$ 2) and in the same sleeping room 2 (mean 2 $\pm$ 1). Overall frequencies of several conditions were found: cigarette smokers (1.2%), having a smoker in the household (48.2%), having an upper respiratory infection (URI) during recruitment (47.0%), having a history of immunodeficiency (2.4%), sickle cell disease (1.3%), chronic lung disease (1.2%), other underlying illness (20.5%); occurrence, during the last year, of URI (85.5%), otitis (20.5%), rhinitis (14.6%), sinusitis (7.3%), asthma (8.4%), pneumonia (2.4%), bronchitis (1.2%), hospitalization (3.6%); children under 5 years of age in the household (14.5%), in the same sleeping room (3.6%), URI in household contacts (18.1%), nasal medicine use during previous 24 hr (1.2%), antimicrobial use during previous 21 days (1.2%), 3 months (5.5%). Each one of the participant denied having diabetes mellitus and chronic liver disease or the occurrence of meningitis during the previous year.

Intermediate penicillin resistance was detected in 7.2%

Table 1. Frequency of Serotypes and Antimicrobial Resistance to Penicillin of *S. Pneumoniae* Isolates Recovered in Nasopharyngeal Swabs from Brazilian Adolescents

	Resistance to penicillin[a] *(all at intermediate level)*		
Serotype	*Yes*	*No*	*Total (%)*
NT	3 (21.4)	11	14 (18.9)
6B	2 (33.3)	4	6 (8.1)
6A	0	5	5 (6.8)
23F	0	5	5 (6.8)
18C	0	5	5 (6.8)
16[b]/36/37	0	4	4 (5.4)
13/28[b]	0	3	3 (4.0)
9N	0	3	3 (4.0)
11A	0	3	3 (4.0)
10A	0	2	2 (2.6)
15B	0	2	2 (2.6)
17	0	2	2 (2.6)
19F	0	2	2 (2.6)
4	0	2	2 (2.6)
9	0	2	2 (2.6)
10F	0	1	1 (1.4)
14	1 (100.0)	0	1 (1.4)
15A	0	1	1 (1.4)
15C	0	1	1 (1.4)
18A	0	1	1 (1.4)
19A	0	1	1 (1.4)
22F	0	1	1 (1.4)
23B	0	1	1 (1.4)
24	0	1	1 (1.4)
7	0	1	1 (1.4)
8	0	1	1 (1.4)
9V	0	1	1 (1.4)
7B	0	1	1 (1.4)
29/34/35[b]/42/47[b]	0	1	1 (1.4)
Total	6 (8.1)	68 (91.9)	74 (100.0)

NT, nontypeable.
Results are presented in *n* (%).
[a]Evaluated E-test.
[b]Serogroup.

(6/83) of the strains. No strain showed high resistance to penicillin. No significant differences were found in all searched variables when compared with resistance to penicillin. All isolates were susceptible to clindamycin, chloramphenicol, rifampin, and vancomycin, but 37.3% (31/83), 18.1% (15/83), and 4.8% (4/83) were resistant to trimethoprim-sulfamethoxazole, tetracycline, and erythromycin, respectively. Resistance to ceftriaxone was not detected in 18 strains that had an oxacillin zone size less than 20 mm.

The frequency of serotypes and antimicrobial resistance to penicillin among 74 serotyped strains is presented in Table 1. Out of all strains, 38 (45.8%) were susceptible to all the studied antimicrobials, 35 (42.2%), 9 (10.8%), and 1 (1.2%) strains were resistant to one, two, or three antimicrobials, respectively. Out of the 6 penicillin nonsusceptible strains, 3 (50.0%) and 1 (16.7%) were resistant to another one or two antimicrobials, respectively.

DISCUSSION

Pneumococcal infections account for a significant proportion of bacterial infections in adolescents.[20] The last 15 years have been characterized by the increase in antibiotic resistance among respiratory bacterial pathogens, including *S. pneumoniae*.[4] The surveillance of pneumococcal antibiotic resistance and serotype distribution is hampered by the relatively low number of invasive pneumococcal infections detected.[12] However, it was demonstrated that serotypes causing disease were similar to commonly carried types (serotypes 6, 14, 19, 23).[8] It is logical to assume that, as the prevalence of nasopharyngeal carriage of resistant strains increases, more and more invasive infections will be caused by antibiotic-resistant pneumococci in the future.[21] To the authors' knowledge, this is the first study that evaluated antimicrobial resistance and serotype distribution of pneumococcal nasopharyngeal isolates specifically from adolescents.

The rate of penicillin-resistance (7.2%) was low in regard to data reported by studies about invasive strains recovered from children in Brazil (20%).[5,13] The absence of resistance to chloramphenicol and vancomycin was also reported in those two previous studies.[5,13] It is noteworthy the similarity between data about resistance to macrolide: herein it was 4.8%, whereas Tomasz[22] and Nascimento-Carvalho[13] reported 6.4% and 5.7%, respectively. Resistance to trimethoprim-sulfamethoxazole (37.3%) was the highest rate of antimicrobial resistance detected in this study. Co-resistance to penicillin and trimethoprim-sulfamethoxazole has been described by other authors studying patients with pneumococcal meningitis, in Salvador, northeastern Brazil.[11] Recent exposure to antibiotics and hospitalization for more than 24 hr were shown to be risk factors for penicillin resistance.[19]

Although pneumococci are classified into over 90 different serotypes, approximately 85% of pediatric infections are caused by eight pneumococcal types: 4, 6A, 6B, 9V, 14, 18C, 19F, and 23F.[1] These serotypes comprise 36.5% of the 74 strains serotyped in this study, and penicillin intermediate resistance was detected in isolates serotyped as 6B and 14 (Table 1). It is important to emphasize the low frequency of serotype 14 and the high frequency of nontypeable strains. We speculate that this finding may be due to loss of the capsule in strains recovered from nasopharynx. Serotype 14 ranks as the most common cause of meningitis and pneumonia in children but is less common among adults, including elderly adults, in Brazil.[5]

Limitations must be considered. The impact of antibiotics in the nasopharyngeal flora persists up to 4 weeks; the exclusion of patients referring previous antibiotic exposure up to only 10 days before sampling the nasopharynx may have lessened the number of strains recovered. Pneumococcal carriage studies conducted early in the 20th century used laboratory techniques, such as mouse inoculation assays, with high sensitivity for detecting carriage of multiple serotypes.[2] These assays are very labor intensive, expensive, and not practical for the use in large nasopharyngeal studies.[15] Although the nasopharyngeal swab seems optimal for the detection of *S. pneumoniae* among children over 13 months of age,[18] this strategy is an insensitive method for detecting multiple serotype carriage.[2] It means that this study may have underestimated the identified serotypes.

From the foregoing data, it is possible to conclude that the rates of resistance to antimicrobials and the distribution of serotypes of pneumococcal nasopharyngeal strains isolated from adolescents are similar to data reported regarding invasive strains from children, except for the low frequency of resistance to penicillin and of serotype 14. In addition to that, the low prevalence of pneumococcal colonization is much more similar to data from studies conducted among adults. Because adolescence is a transitional period between childhood and adulthood, adolescents share characteristics of both age groups.

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Address reprint requests to:
Dra. Cristiana Nascimento-Carvalho
Rua Professor Aristides Novis
N^o. 105, apto. 1201B
Salvador, Bahia, Brazil CEP 40.210-630

E-mail: nascimentocarvalho@hotmail.com