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High prevalence of children colonized with penicillin-resistant *Streptococcus pneumoniae* in public day-care centers

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Abstract

Objectives: To investigate the prevalence of *Streptococcus pneumoniae* (pneumococci) in the nasopharynx of healthy children enrolled in public day-care centers of the municipality of Umuarama, state of Paraná, Brazil. The susceptibility of the pneumococcal strains to antimicrobial agents was also studied.

Methods: Nasopharyngeal specimens from 212 children were collected from April to October 2008. After the specimens were seeded onto blood agar and incubated at 37 °C for 24-48 hours, the colonies suspected of belonging to *S. pneumoniae* were identified using α-hemolysis, optochin sensitivity, and bile solubility test. Penicillin susceptibility was investigated using the disk diffusion and dilution tests. Susceptibility to the other antimicrobial agents indicated for the treatment of pneumococcal infections was investigated using the disk diffusion test.

Results: The prevalence of nasopharyngeal pneumococci was 43.4% (92/212), with higher rates in children between 2 and 5 years old (p = 0.0005). There was no significant difference between sexes. Intermediate and full resistance to penicillin were found in 34.8 (32/92) and 22.8% (21/92) isolates, respectively. Sixty-seven strains (72.8%) were resistant to sulfamethoxazole-trimethoprim, eight (8.7%) were resistant to erythromycin, and six (6.5%) to tetracycline. One strain was resistant to clindamycin (1.1%) and another was resistant to chloramphenicol (1.1%). All strains were sensitive to levofloxacin, ofloxacin, rifampicin, telithromycin, linezolid, and vancomycin. Nine strains were considered multiresistant because they were resistant to three or more classes of antimicrobial agents.

Conclusions: The present study detected a high prevalence of healthy children colonized with penicillin-resistant *S. pneumoniae* strains who may be important reservoirs of this pathogen in the community.

J Pediatr (Rio J). 2009;85(6):516-522: Streptococcus pneumoniae, nasopharynx, healthy carriers, penicillin resistance.

Introduction

Streptococcus pneumoniae bacteria, also known as pneumococci, are Gram-positive cocci present in the microbiota of the upper respiratory tract of a variable number of individuals, and these bacteria may colonize the nasopharynx of human beings since the first day of life.[1] Most of the time, pneumococci colonize the mucosal surface, so individuals become healthy carriers of the bacteria. However, when the host has some predisposing factors, these bacteria may invade adjacent sterile sites or the bloodstream causing localized or systemic infection.[2-4]

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The acquisition of pneumococcus and the carrier status among individuals of a healthy population depend on age, ethnicity, geographical area, and socioeconomic and environmental conditions.[2,3,5] The colonization rate is higher during childhood and is strongly influenced by crowding, as in the case of children who attend day care centers or schools or those who live in orphanages.[2,6]

For many years, pneumococci were sensitive to penicillin. However, since the first case of penicillin-resistant *S. pneumoniae* isolation reported in Australia in 1967,[7] increased resistance the increase in the resistance of this microorganism to beta-lactam antibiotics, macrolides, tetracyclines, lincosamides, chloramphenicol, and sulfamethoxazole-trimethoprim has been reported in different countries, including isolates from both clinical cases and carriers.[8,9]

Differently from most pathogens, *S. pneumoniae* is not primarily disseminated by individuals who are ill, but by healthy carriers who shelter the bacterium in their nasopharynx.[6] Since they have high colonization rates, children play an important role in the horizontal dissemination of this pathogen in the community.[3] Because of the frequent use of antimicrobial drugs during childhood, this group of carriers constitutes an important reservoir of resistant strains.[2,4,10,11]

Studies on the prevalence of pneumococcal carriers, mainly regarding antimicrobial drug-resistant strains, especially in healthy children, are important because they can monitor the occurrence of resistance to this bacterium in the community, as well as indirectly guide therapeutic approaches to pneumococcal diseases. In Brazil, the incidence of resistant pneumococci isolated from clinical cases has been increasing.[12] Nevertheless, because of the country's large territory and the geographical and climatic differences among the several states, the studies on pneumococcal carriers conducted so far in different municipalities provide only local or regional data and cannot be extrapolated to the entire Brazilian population.

The lack of data on healthy carriers of *S. pneumoniae* in the pediatric population in the state of Paraná motivated us to investigate the prevalence of pneumococcal carriers among children enrolled in public day-care centers in the municipality of Umuarama, Brazil, and to study the susceptibility of the pneumococcal isolates to antimicrobial agents.

Methods

Selection of volunteers

The municipality of Umuarama, located in the state of Paraná, has 23 public day-care centers with 1,276 children enrolled. For this study, the prevalence of healthy carriers of nasopharyngeal pneumococci was estimated in 20% based on a study conducted in the South region of the country[13] that found a prevalence rate of 18.45%. The sample size was calculated using general equation for small populations, with a 95% confidence level and a 5% confidence interval, resulting in the need of a sample including 207 children.[14] We randomly selected nine day-care centers that had 570 children enrolled. Nasopharyngeal specimens were collected upon authorization from the directors of the institutions and after the children's parents or guardians signed a written consent form.

After signing the written consent form, guardians or parents received a social and epidemiological questionnaire with the purpose of collecting the following data: child's age and sex, number of people living with the child, number and age of siblings, diseases during the last 30 days, use of antimicrobial drugs in the previous 30 days and agent used, fever in the week before collection, and immunization with pneumococcal vaccine.

Specimen collection

Specimen collection was carried out from April to October 2008. Two hundred and twelve children aged from 3 months to 6 years participated in the study. For nasopharyngeal specimen collection, we used sterile flexible swabs (Copan Diagnostics Inc., Italy), which were inserted into the children's nostrils until touching the posterior wall of the nasopharynx.[15] After performing rotatory movements for 5 seconds so that secretion could be absorbed, the swab was removed and submerged in Amies medium without charcoal and sent to the microbiology laboratory at room temperature. A single collection was performed for each child.

Specimen culture and identification of bacterial isolates

Immediately after being delivered at the microbiology laboratory, the swab was seeded onto one third of a Petri dish surface containing blood agar prepared with tryptose blood agar base (Difco, Becton Dickinson and Company, Sparks, MD, USA) supplemented with 5% of defibrinated sheep blood. Next, with the help of a bacteriological seeding loop, the material was streaked onto the remaining parts of the plate and incubated at 35-37 °C in carbon dioxide (CO_2) atmosphere for 24-48 hours. We used one blood agar plate for each specimen. After incubation, the plates were observed under reflected light and all α-hemolytic colonies showing central depression, mucoid or grayish appearance were considered as possibly belonging to *S. pneumoniae*. Morphology and staining characteristics were confirmed by means of Gram staining, with the presence of Gram-positive, lanceolate cocci, arranged in pairs or in short chains. Identification of isolates was carried out using optochin sensitivity and bile solubility tests.[16]

Test of susceptibility to antimicrobial agents

The susceptibility test of *S. pneumoniae* strains to the antimicrobial agents clindamycin, tetracycline, chloramphenicol, erythromycin, levofloxacin, ofloxacin, sulfamethoxazole-trimethoprim, vancomycin (Laborclin, Pinhais, PR, Brazil), rifampicin, telithromycin, linezolid, and oxacillin (Oxoid Ltd., Basingstoke, Hampshire, England) was carried out using the disk diffusion method.[17] In addition, the minimum inhibitory concentration (MIC) of penicillin for all pneumococcal strains was determined using the Etest (AB Biodisk, Solna, Sweden). Strips embedded with penicillin G at a concentration gradient ranging from 0.002 µg/mL to 32 µg/mL were applied to the surface of the plate containing Müeller Hinton agar (Difco) supplemented with 5% of sheep blood previously seeded with inoculate adjusted at 10^8 UFC of pneumococci per millimeter. The plates were incubated at CO_2 atmosphere at 35-37 ºC for 20-24 hours. The MIC reading was considered as the concentration of the strip that intersected with the edge of the inhibition ellipse of bacterial growth. Test results were interpreted according to the criterion adopted by the Clinical and Laboratory Standards Institute (CLSI)[18] for oral penicillin V (i.e., sensitivity, MIC ≤ 0.06 µg/mL; intermediate resistance, 0.12 µg/mL to 1.0 µg/mL; full resistance, MIC ≥ 2.0 µg/mL). The standard strain of *S. pneumoniae* of the American Type Culture Collection (ATCC 49619) was used as control for the tests. Pneumococcal strains that were resistant to three or more classes of antimicrobial agents were considered to be multiresistant.[12]

Statistical analysis of data

We used the computer program Epi-Info version 3.5.1 to perform the chi-square test and Fischer's exact test with a significance level of 5%. We investigated which variables related to the children were correlated with isolation of pneumococci from their nasopharynx and with resistance to antimicrobial agents and with the presence of any resistance relationship between the antimicrobial agents.

Ethical aspects and biosafety

The present study was approved by the Research Ethics Committee (CEPEH) of Universidade Paranaense (UNIPAR) during a meeting held on February 9, 2008 (CAAE – 0001.0.375.093-07, Protocol no. 1012/2007).

Results

As shown in Table 1, the prevalence rate of *S. pneumoniae* carriers among the children studied was 43.4% (92/212). Forty-seven out of 108 male children (43.5%) and 45 of of 104 female children (43.3%) had positive cultures for *S. pneumoniae* (p = 0.9187). A significant difference was found when the positive result of pneumococcal isolation was arranged according to age group (p = 0.0005), with a higher rate of isolation in the children aged from 2 to 5 years (51%; 74/145). Among 33 children less than 2 years old, 13 (39.4%) had positive culture for pneumococcus. Positivity among the children more than 5 years old was 14.7% (5/34).

With regard to the social and epidemiological questionnaire, only the report of regarding rhinitis in the month previous to the collection was associated with isolation of nasopharyngeal pneumococcus (Table 1). Eighty-two children (38.7%) were treated with antimicrobial drugs in the month previous to the study and, of these, 53 (64.6%) reported the name of the antimicrobial agent, with amoxicillin was most often used (30/53; 56.6%). Among the children investigated, only one had been immunized with pneumococcal vaccine and had negative result for pneumococcus.

The results of the penicillin susceptibility tests of pneumococci, carried out using both disk diffusion and dilution technique, determined that 39 (42.4%) of the 92 specimens were sensitive and 53 (57.6%) were resistant. Penicillin MIC for pneumococcal isolates ranged from 0.006 µg/mL and > 32 µg/mL.

The distribution of the 92 pneumococcal strains regarding penicillin susceptibility and its association with resistance to the antimicrobial drugs tested are shown in Table 2. Penicillin intermediate resistance and full resistance were found, respectively, in 34.8 (32/92) and 22.8% (21/92) of the isolates. Sixty-seven strains (72.8%) were resistant to sulfamethoxazole-trimethoprim, eight (8.7%) were resistant to erythromycin, and six (6.5%) to tetracycline. One pneumococcal strain was resistant to clindamycin (1.1%) and another was resistant to chloramphenicol (1.1%). All tetracycline-, clindamycin- and chloramphenicol-resistant isolates were sensitive to penicillin. The great majority (96.2%; 51/53) of the penicillin-resistant strains were resistant to sulfamethoxazole-trimethoprim. All isolated strains were sensitive to vancomycin, telithromycin, levofloxacin, ofloxacin, rifampicin, and linezolid. Nine strains (9.8%) were considered to be multiresistant, of with four were susceptible and five resistant to. The multiresistant profile of these microorganisms is shown in Table 3.

Discussion

In the present study, the prevalence of children colonized with nasopharyngeal *S. pneumoniae* was 43.4%, and we believe that this is the first epidemiologic investigation conducted with healthy children enrolled in public day-care centers in the state of Paraná, Brazil. Similar studies conducted in different Brazilian regions showed different isolation rates, such as, for instance, 72% in children from public day care centers in Fortaleza, state of Ceará,[19] 41.4% in Goiânia, state of Goiás,[20] and 18.5% in the Northern

Table 1 - Association of social and epidemiological data with the isolation of nasopharyngeal *Streptococcus pneumoniae* of the 212 children studied

Variable	***S. pneumoniae* isolation**		p
	Negative, n (%)	**Positive, n (%)**	
Population	120 (56.6)	92 (43.4)	
Sex			0.9187
Female	59 (56.7)	45 (43.3)	
Male	61 (56.5)	47 (43.5)	
Age			0.0005
< 2 years old	20 (60.6)	13 (39.4)	
2 to 5 years old	71 (49.0)	74 (51.0)	
> 5 years old	29 (85.3)	5 (14.7)	
Number of people living in the house			0.6514
Up to three	45 (58.4)	32 (41.6)	
From four to five	60 (55.0)	49 (45.0)	
More than five	9 (69.2)	4 (30.8)	
Not reported	6 (46.2)	7 (53.8)	
Number of siblings			0.6078
None	37 (53.6)	32 (46.4)	
One	49 (62.0)	30 (38.0)	
≥ two	28 (54.9)	23 (45.1)	
Not reported	6 (46.2)	7 (53.8)	
Siblings ≤ 5 years old			0.7348
No	82 (57.3)	61 (42.7)	
Yes	32 (57.1)	24 (42.9)	
Not reported	6 (46.2)	7 (53.8)	
Use of antibiotics (last month)			0.9175
No	65 (57.5)	48 (42.5)	
Yes	45 (54.9)	37 (45.1)	
Not reported	10 (58.8)	7 (41.2)	
Fever (last week)			0.9348
No	78 (55.7)	62 (44.3)	
Yes	32 (58.2)	23 (41.8)	
Not reported	10 (58.8)	7 (41.2)	
Rhinitis (last month)			0.0479
No	113 (58.9)	79 (41.1)	
Yes	1 (14.3)	6 (85.7)	
Not reported	6 (46.2)	7 (53.8)	

Table 2 - Number and percentage of pneumococcal strains resistant to different antimicrobial agents distributed according to penicillin susceptibility

Antimicrobial agent	**Penicillin-resistant strains, n (%)**		
	Susceptible (n = 39)	**Intermediate resistance (n = 32)**	**Full resistance (n = 21)**
Erythromycin	3 (7.7)	–	5 (23.8)
Chloramphenicol	1 (2.6)	–	–
Tetracycline	6 (15.4)	–	–
Sulfamethoxazole-trimethoprim	16 (41)	31 (96.9)	20 (95.2)
Clindamycin	1 (2.6)	–	–
Multiple agents	4 (10.3)	–	5 (23.8)

All strains were sensitive to vancomycin, telithromycin, levofloxacin, ofloxacin, rifampicin, and linezolid.

Table 3 - Multiresistant profile of nine strains of *Streptococcus pneumoniae* isolated from nasopharynx

Antimicrobial agents	Resistant strains, n (%)
Sulfamethoxazole-trimethoprim, penicillin, erythromycin	5 (55.6)
Sulfamethoxazole-trimethoprim, erythromycin, tetracycline	2 (22.2)
Sulfamethoxazole-trimethoprim, tetracycline, chloramphenicol	1 (11.1)
Sulfamethoxazole-trimethoprim, erythromycin, tetracycline, clindamycin	1 (11.1)

region of the country.[13] The variation of these rates can be caused by known risk factors for pneumococcal colonization in the nasopharynx, such as age group, geographical and climatic variations at the time of collection.

In addition, other variables, including genetic differences and socioeconomic conditions of the population, such as access to the health system, personal hygiene, number of individuals in the family, parents' educational level, children's attendance frequency of day-care centers or schools, among others, are mentioned as variables that can be related to positivity of pneumococcal isolation in healthy carriers.[5,21]

According to our expectations and the previous description by Lo et al.,[22] our study confirmed that child's age is an important factor for pneumococcal isolation, with high prevalence of isolates in the age group between 2 and 5 years old. On the other hand, there was no correlation between pneumococcal isolation and presence of siblings. This can be explained by the fact that 33% of the children investigated did not have siblings, 37% had only one, and only 26% had siblings ≤ 5 years old, which reduces the number of the people living in the same house and decreases the risk of colonization.

The occurrence of rhinitis during the 30 days prior to the collection contributed to pneumococcal isolation, since 85% (6/7) of the children with rhinitis had positive culture from the nasopharynx. However, the small number of children with rhinitis included in our study makes it difficult to assess this variable. In addition, for a better understanding of the role played by respiratory alterations as a risk factor for pneumococcal colonization, it is necessary to know the causes of each one of the alterations.

According to Borer et al.,[23] the alterations in the mucosa of the respiratory tract of allergic patients can result in impaired mucociliary activity, predisposing to bacterial colonization in this site. Viral infections of the upper respiratory tract are common in children and they often precede episodes of acute otitis media and pneumococcal pneumonia, both triggered by the increase in the adherence of the pneumococcus to the epithelial cells of the respiratory tract induced by the virus.[24]

The susceptibility interpretation pattern of the CLSI for oral penicillin V used in the present study showed that the pneumococcal resistance rate was higher than those found in studies involving children from other Brazilian states whose authors adopted the same criterion of interpretation.[13,19,25] Two studies conducted in 2001 with healthy children and children with respiratory diseases showed intermediate resistance rates to penicillin ranging from 1.4 to 16% and absence of isolation of strains fully resistant to penicillin.[13,25] In 2002, Rey et al.[19] found resistance to penicillin in 49% of the isolated pneumococcal strains, and 4% of them were fully resistant.

Since the present study examined the nasopharyngeal pneumococci of healthy carriers, we believe that the criterion recommended by the CLSI for oral penicillin is the most adequate. However, strains from asymptomatic carriers may occasionally cause infectious in different sites. In such cases, the CLSI recommends other criteria based on the parenteral use of penicillin in meningitis (sensitive, ≤ 0.06 μg/mL, and resistant, ≥ 0.12 μg/mL) and other infections (sensitive, ≤ 2 μg/mL, intermediate, 4 μg/mL, and resistant, ≥ 8 μg/mL). If our results were interpreted using the criterion adopted for meningitis, we would find the same percentage of sensitivity (42.4%). On the other hand, sensitivity would have a significant increase (94.6%) if we adopted the criterion for infection in other sites.

Previous antibiotic therapy may increase the rate of isolation of penicillin-resistant nasopharyngeal pneumococci.[10] This fact may explain the high prevalence of penicillin-resistant pneumococcal strains found in the present study, since 82 (39%) of the 212 children had been treated with antimicrobial drugs in the month prior to the study.

The high rate of resistance to sulfamethoxazole-trimethoprim found in our study (72.8%) is similar to that

described by other studies, as well as the association between penicillin resistance and sulfamethoxazole-trimethoprim resistance.[13,19,26]

With regard to the resistance rates of 8.7 and 6.5% for erythromycin and tetracycline found in our study, similar results were found with strains isolated in different Brazilian studies, with results ranging from 1 to 10% for erythromycin and from 12.6 to 24% for tetracycline.[13,19,26] In Europe, these rates are higher, and they may be higher than 50% for macrolides and higher than 80% for tetracycline.[27,28] These differences might result from medical prescription habits, dose of antibiotic used, as well as differences in the bacterial resistance genes between different regions.[28]

It is important to highlight the large number of children who are enrolled in public day-care centers in the city of Umuarama and the need to monitor the rates of *S. pneumoniae* carriers in this population, since we found a high rate of this microorganism, as well as significant rates of penicillin resistance among the strains isolated.

Considering that the study of nasopharyngeal isolates may be a useful indicator of the prevalence of resistant strains in the community and that these children live in small houses occupied by many individuals, it is worth mentioning that the dissemination of resistant and multiresistant isolates may occur, contributing to the occurrence of diseases that are difficult to treat.

Nesti & Goldbaum[29] recommend the following measures for the control of transmissible diseases in day-care centers: rules establishing the maximum number of children per room, hand washing routine, cleaning routine for surfaces and toys, among others. In order to implement these measures, it is very important to train the employees of day-care centers and preschools, as well as to involve health professionals and public health managers.[30] We also believe that increasing parental awareness about using of antibiotics only when they are prescribed by a doctor is an important measure to prevent the dissemination of pneumococcal-resistant strains.

In short, the present study detected a high prevalence of healthy children colonized with penicillin-resistant *S. pneumonia* strains in the region of Umuarama. These children may constitute important reservoirs or sources of transmission of resistant pneumococci in the community.

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