Abstract

Objectives: to compare colonization rates and antimicrobial resistance of nasopharyngeal pneumococci in healthy carriers and children with pneumonia.

Methods: a cross-sectional study. Healthy subjects of this study were selected from randomly chosen immunization centers and day-care centers, and those with pneumonia were selected in pediatric emergency rooms. Flexible pernasal alginate swabs were employed to collect nasopharyngeal pneumococci specimens. Isolation and identification were performed according to standard procedures. Minimum Inhibitory Concentrations were assessed by microdilution techniques.

Results: we studied 911 children, 429 healthy controls (60% of carriers, 72% attending day care centers and 49% recruited in immunization centers) and 482 children with pneumonia (50% of carriers) (P=0.02). The Minimum Inhibitory Concentration of penicillin to 441 isolates detected 198 (45%) of intermediate and 16 (4%) fully resistant pneumococci. Antimicrobial resistance rates of isolates from healthy carriers and children with pneumonia were, respectively: penicillin 48% (37% for immunization centers and 55% for day-care centers) and 50% (P>0.05), erythromycin 28% and 19% (P=0.05); cotrimoxazole 81% and 76% (P>0.05), chloramphenicol 6% and 7% (P>0.05), rifampin 5 and 3% (P>0.05), ceftriaxone 2 and 4% (P>0.05) and vancomycin 0% in both groups. An association among pneumococcal resistance to penicillin, erythromycin and cotrimoxazole was detected.

Conclusions: pneumococcal carriage rate was higher in healthy children than in children with pneumonia. Penicillin and cotrimoxazole resistance rates were high, especially among those attending day-care centers.


Introduction

Acute respiratory infections caused by S. pneumoniae account for high morbidity and mortality among children in developing countries. Each year, 150,000 children younger than five years die in Americas; 90% of these deaths occur in the poorer countries or regions of the continent. The World Health Organization has underscored the necessity of early treatment of pneumonia in children younger than five years, using the diagnostic criteria of coughing and tachypnea (respiratory frequency greater than 50 per minute in infants younger than 12 months, and greater than 40 per minute in children older than one year), and use of penicillin...
and cotrimoxazole as the major drugs against mild and moderate pneumonias at the primary level of care. The control program of acute respiratory infections (ARI) has been used worldwide, especially in developing countries, and has saved many lives, since the program allows non-medical professionals to identify and treat pneumonia, thus favoring the globalization of health care.

On the other hand, strains of S. pneumoniae resistant to antibiotics, especially penicillin and cotrimoxazole (sulfamethoxazole + trimethoprim) have been reported worldwide. This causes great concern in developing countries, in which the high cost of alternative drugs may compromise ARI control programs. Several of these countries conducted surveillance studies on the resistance of S. pneumoniae to penicillin in order to assess the magnitude of the problem, and determine the main factors associated with the development of pneumococcal resistance and the major serotypes involved, thus contributing towards the development of a conjugate polysaccharide vaccine. In Latin America, SIREVA multicenter study analyzed pneumococci in sterile fluids (blood, cerebrospinal fluid, body fluid spills) of patients with systemic infection. In Brazil, 20% of the pneumococci presented low sensitivity to penicillin, and 1.4% showed total resistance.

Even though the pneumococci that colonize the nasopharynx cannot be considered direct causative agents of severe or even moderate infections, their study is useful to assess the prevalence of antimicrobial resistance in a given community. The surveillance of the pneumococci that colonize the nasopharynx in children is practical and has been used, for instance, in epidemics of resistant pneumococci in South Africa (32 to 45% of penicillin resistance), in Pakistan (7-14% of penicillin resistance), in groups of children with high resistance in Taiwan (71% of resistance). It has also been used in other occasions, such as outbreaks of resistant pneumococcal infections at day-care centers.

Comprehensive studies on S. pneumoniae usually lack clinical information about the patients. In addition, prevalence data and the resistance profile of strains in affected individuals are scarce in Brazil and have often been made available in the southern region of the country. No studies on the carrier status and on antibiotic resistance have added clinical data to the microbiological assay when children with pneumonia and healthy subjects were compared. Our aim was to determine the rate of colonization by S. pneumoniae, the profile of antimicrobial resistance, establishing a correlation between children with pneumonia and healthy children, and associating these data with epidemiological, clinical and individual characteristics of carriers.

Methods

**Patients**

The children included in the study lived in the city of Fortaleza and their ages ranged between two and 59 months. The selection was based on two categories: children with pneumonia and healthy controls, each group consisting of children younger and older than one year.

**Inclusion criteria for children with pneumonia**

Children with pneumonia were sequentially included in the study after they had been admitted at least three times at pediatric emergency rooms and been diagnosed according to WHO criteria for children with pneumonia: history of coughing and tachypnea (greater than 50 breaths per minute in infants aged between two and 12 months, and greater than 40 breaths per minute in children aged between one and five years), or subcostal retraction (sensitivity of 77-81% and specificity of 77-80%, according to studies conducted in the Philippines and Swaziland).

**Inclusion criteria for healthy children**

The control group consisted of children recruited during consultation at immunization centers (IC) and children attending public day-care centers (DCC). Immunization centers and municipal day-care centers were randomly selected by the stratification of eight health districts of Fortaleza. Between eight and 50 health units with regular immunization were selected, in addition to 11 to 150 day-care centers, state-owned or in covenant with FEBEM (Foundation for Child Welfare) of Ceará, with children younger than 12 months.

**Exclusion criteria**

The exclusion criteria were: hospital admission within the last three months, use of antibiotics for five or more days, in adequate doses, in the last 30 days, presence of previous moderate to severe organic or systemic disease. The healthy children had not had fever in the last seven days.

**Laboratory methods**

The nasopharyngeal specimen collected by pernasal, flexible, calcium alginate swab (Transwab™, Medical Wire & Equipment, Whiltshire, United Kingdom) was referred to the Laboratory of Microbiology of Universidade Federal do Ceará in Amies medium two or six hours after collection.

Once in the laboratory, the swabs were rolled along TSA plates with sheep blood, containing 5 mg/l of gentamycin (SBA-Gen), and incubated in candle jars at 37°C for 48 hours. The initial identification was made by the characteristic aspect of colonies and confirmed by the sensitivity to the 5µg disk of optochin and bile solubility test, as previously described.

The samples identified as S. pneumoniae were submitted to the screening test for beta-lactamic antibiotic sensitivity by the diffusion method of 1mg oxacillin disk (P-discs, Oxoid, Hampshire, United Kingdom), on Mueller-Hinton
Agar plates with sheep blood. After the tests, the samples were introduced into screw-cap cryogenic storage vials (Microbank™, Pro-Lab Diagnostics, Neston, United Kingdom), and preserved at -80 °C. After transfer to the Eijkman-Winkler Institute, Holland, the minimum inhibitory concentrations (MIC) of antibiotics were determined by the microdilution plating method (Sensititre HP Panel™, AccuMed Int, Sussex, United Kingdom); the susceptibility was interpreted according to the NCCLS criteria for MIC threshold values.14 The following antibiotics were tested: penicillin, ceftriaxone, erythromycin, clindamycin, cotrimoxazole, rifampicin, chloramphenicol and vancomycin. The used concentrations in mg/l were: 0.008 - 0.015 - 0.03 - 0.06 - 0.12 - 0.25 - 0.5 - 1.0 - 2.0 - 4.0 - 8.0 - 16.0. The Sensititer plating microdilution method is used with good results in international multicenter programs, such as the Alexander project, for the surveillance of resistant germs of the lower respiratory tract, and was considered adequate and comparable to the E-test by Centers for Disease Control.15

Statistical analysis

The sample calculation was based on a hypothetical rate of pneumococcal carriers consisting of 50% of the study population, and on a 25% general resistance of pneumococcal strains to penicillin, according to previous studies.22 For the two-tailed test, we adopted a 95%CI of +10%. An effect of the design = 2 (sampling per groups) was used. A safe margin of 10% was used, accounting for 450 children recruited in each clinical group.16

The clinical and microbiological data were collected on specific forms and typed into a computer. For the statistical analysis, the Epi-Info 6.0416 and Excel for Windows 2000 were used. After checking the consistency of data, parametric (means and standard deviations) and non-parametric (analysis of variance) methods were used to compare the different variables among groups. In all cases, a 5% first order error was used.

Mothers or guardians were previously informed about the objectives and procedures of the study, and verbally consented the collection of nasopharyngeal material and the clinical examination of their child. This study was approved by the Research and Ethics Committee of the Regional Medical Council of the state of Ceará and of the Universidade Federal de São Paulo.

Results

Participants and carrier status

911 children were recruited between March and December 1998. Forty-four percent of 907 children were younger than 12 months. 482 (53%) children were diagnosed with pneumonia, and were distributed according to their age in 66% younger and 34% older than 12 months. Among 429 healthy children, 216 were recruited from immunization centers (99% younger than 12 months) and 213 attended public day-care centers, of which 194 (91%) were at least one year old.

Strains of S. pneumoniae were isolated in 500 children, totaling a carrier status of 55%. [Confidence interval (95%CI): 50.2-59.5]. The rate of healthy children who carry these strains reached 60% (95%CI: 53.0-66.5) and was greater (50%) than that of children with pneumonia (95%CI: 43.5-56.5), (P < 0.002). In the healthy subgroup recruited from immunization centers, 49% (95%CI: 39.4-58.8) were carriers while in the subgroup recruited from day-care centers, the rate reached 72% (95%CI: 62.1-79.9), a rate significantly higher than the others (P < 0.001).

The previous use of antibiotics (up to 30 days) was referred by 182 participants (20%). Among the children who had used antibiotics previously, 86 (47%, 95%CI: 34.9-54.7) presented pneumococci, in comparison with 415 (57%, 95%CI: 53.2-60.5) of those who had not used antibiotics before (P = 0.02).

Screening test for sensitivity to beta-lactamic antibiotics

Of the 500 samples submitted to oxacillin disk testing, 64% (95%CI: 59.6-68.2) showed reduced sensitivity to beta-lactamic antibiotics (inhibition zone diameter less than 20 mm). Fifty-seven percent (95%CI: 52.0-61.9) of 400 children younger than 12 months showed low sensitivity, compared to 69% (95%CI: 64.8-73.0) of 507 children aged one year or older (P = 0.004). Children with pneumonia showed a sensitivity of 66%, compared to 62% in the healthy group (P > 0.05).

Susceptibility of the strains to antibiotics

During the preservation and transport of the material to the University of Utrecht, Holland, 59 strains (12%) were lost, 34% from children younger than one year and 70% from children with pneumonia. The MIC of antibiotics was determined for 441 strains.

The distribution of penicillin MIC, according to the cutoff limits was: 227 (51%, 95%CI: 46.7-56.2) sensitive samples, 198 (45%, 95%CI: 40.2-49.7) with intermediate resistance and 16 samples (4%, 95%CI: 2.2-5.9) with total resistance. By comparing the MIC with the oxacillin disk testing, no sample with total resistance presented an inhibition zone diameter ≥20 mm. Oxacillin disk testing compared to the MIC values showed a sensitivity of 88% and a specificity of 58%, including 34% of false-positives; the positive predictive value for the oxacillin disk reached 66.4% while the negative predictive value reached 83.5%.

In the group of children up to the age of 12 months, 42% of the pneumococci showed intermediate or total resistance to penicillin, compared to a 53% resistance in the group of children older than one year (P = 0.03). This was due to the predominance of the subgroup of children who attended day-care centers, with higher resistance to penicillin in children older than one year.
According to the recruitment site, there was increased resistance (intermediate and total) to penicillin among the strains collected from children who attended day-care centers than from those who had pneumonia and also from those recruited at immunization centers (54.6% compared to 49.8% among children with pneumonia and 37.4% of those recruited at immunization centers) (chi-square test 7.14, P=0.03). There was no difference between the different sites when these were analyzed separately (P=0.05) (Table 1).

The MIC analysis of the nasopharyngeal strains of 88 children who reported previous use of antibiotics showed more resistance to penicillin (62%) than 353 children without recent use of antibiotics (46%) (P=0.01).

The MIC of ceftriaxone revealed 2% of intermediate resistance and 1% of total resistance. When we compare the test for oxacillin sensitivity with the MIC of ceftriaxone, we observe that no sample resistant to ceftriaxone showed oxacillin inhibition zone diameters ≥20 mm, confirming that this test is efficient for the screening of ceftriaxone-resistant pneumococci. Twelve of 16 strains that were resistant to ceftriaxone also showed low sensitivity to penicillin.

The resistance to penicillin found in 141 strains from children who attended day-care centers varied significantly according to the considered unit (14% to 92%) (Table 2).

Among pneumococci with some resistance to erythromycin, 23% showed total resistance to the drug (Table 3). When erythromycin-resistant and clindamycin-resistant strains were compared, we observed 19 strains that were resistant to erythromycin and sensitive to clindamycin, which characterizes phenotype M, bacterial resistance to macrolides not shared by lincosamines.

The total resistance of strains to cotrimoxazole reached 79%, where only 21% of the pneumococci were sensitive to the drug. The resistance of strains to rifampicin and chloramphenicol ranged between 4 and 7%, revealing the potential use of these drugs in more severe infections. No strain was resistant to vancomycin (Table 3). There was a strong association between penicillin resistance and resistance to ceftriaxone, erythromycin, clindamycin and cotrimoxazole (P<0.001) (Table 4).

Children older than one year carried strains with higher resistance to penicillin than those younger than 12 months. However, this relationship was not present among the other antibiotics that were tested.

Pneumococci with total resistance to one or two drugs were identified. Among the 16 strains with high resistance to penicillin, 15 were also resistant to erythromycin, 13 to cotrimoxazole, nine to clindamycin, five to ceftriaxone, one to chloramphenicol and one to rifampicin.

Among the 16 strains with total resistance to penicillin, 14 were multi-resistant (with total resistance to three or more drugs). Multi-resistant strains were found in 11% of the samples: 15% in children who attended day-care centers, 10% in children with pneumonia and 5% in those recruited at immunization centers. The carriers of multi-resistant strains were younger than one year in 60% of the cases. Twenty-three percent of the children who carried multi-resistant germs had used antibiotics before, while among those who carried sensitive pneumococci, only 13% had received previous antibiotic therapy (P= 0.02).

Discussion

The percentage of children carrying pneumococci was high in Fortaleza, especially among children who were attending day-care centers; it was higher than the 35% rate observed in children attending day-care centers in São Paulo. In Salvador, Bahia, the rate of pneumococcal colonization in children from day-care centers or from the community was 69.7%, which is closer to our results.

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**Table 1** - Susceptibility of *S. pneumoniae* isolates to penicillin and clinical situation/samples recruitment site

<table>
<thead>
<tr>
<th>Category</th>
<th>Sensitivity (%)</th>
<th>Susceptibility to penicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Category</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Total</td>
</tr>
<tr>
<td>With pneumonia</td>
<td>100 (50)</td>
<td>92 (46.3)</td>
</tr>
<tr>
<td>Healthy</td>
<td>126 (52)</td>
<td>106 (44)</td>
</tr>
<tr>
<td>Day-care center</td>
<td>64 (45)</td>
<td>70 (49)</td>
</tr>
<tr>
<td>Immunization</td>
<td>62 (63)</td>
<td>36 (36)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>227 (51)</strong></td>
<td><strong>198 (45)</strong></td>
</tr>
</tbody>
</table>

Chi-square = 9.18 (P=0.05)  Interm.: intermediate; resist.: resistance
Table 2: *S. pneumoniae* isolates according to penicillin susceptibility, regarding the day-care centers involved in the study

<table>
<thead>
<tr>
<th>Day-care centers</th>
<th>Sensitivity (%)</th>
<th>Resistance</th>
<th>Total</th>
<th>R (%)</th>
<th>IR</th>
<th>TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alto Alegre</td>
<td>5 (45)</td>
<td>6 (55)</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aprisco</td>
<td>5 (38)</td>
<td>8 (62)</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAIC</td>
<td>7 (32)</td>
<td>15 (68)</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentil Barreira</td>
<td>9 (50)</td>
<td>9 (50)</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inês Brasil</td>
<td>12 (75)</td>
<td>4 (25)</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luiza Távora</td>
<td>4 (36)</td>
<td>7 (70)</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucuripe</td>
<td>4 (36)</td>
<td>7 (64)</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autran Nunes</td>
<td>6 (55)</td>
<td>5 (45)</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castelão</td>
<td>5 (56)</td>
<td>4 (44)</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>São Gabriel</td>
<td>1 (8)</td>
<td>12 (92)</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unidade</td>
<td>6 (86)</td>
<td>1 (14)</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>64 (45)</td>
<td>78 (50)</td>
<td>142</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R: resistance; IR: intermediate resistance; TR: total resistance

Sensitivity to penicillin: MIC < 0.1 mg/l; IR: 0.1 < MIC < 1 mg/l; TR: MIC ≥ 2 mg/l

Table 3: Susceptibility to cotrimoxazole, erythromycin, clindamycin, chloramphenicol, rifampicin and vancomycin according to the cutoff limits of MIC for *S. pneumoniae*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitivity</th>
<th>Pn Healthy</th>
<th>Total</th>
<th>Pn Healthy</th>
<th>Total</th>
<th>Pn Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriazone</td>
<td>427 (97)</td>
<td>191</td>
<td>236</td>
<td>9 (2)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>93 (21)</td>
<td>47</td>
<td>46</td>
<td>164 (37)</td>
<td>69</td>
<td>95</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>336 (76)</td>
<td>158</td>
<td>178</td>
<td>3 (1)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>357 (81)</td>
<td>166</td>
<td>191</td>
<td>3 (1)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>412 (93)</td>
<td>185</td>
<td>227</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>424 (96)</td>
<td>194</td>
<td>230</td>
<td>5 (1)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>441 (100)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Interm.: Intermediate; Resist.: resistance; Pn: children with pneumonia

Nasopharyngeal culture has been used for the surveillance of the level of resistance and of prevalent types of pneumococci, including the invasive ones, causing bacteremia and systemic infection. In studies on nasopharyngeal pneumococci conducted in the community and among children who attend day-care centers, a good correlation with systemic infection strains (e.g., bacteremia) or even infection by multi-resistant strains in the children who took part of the study.

In a study carried out in Bahia, the pneumococci isolated from 221 samples of cerebrospinal fluid presented intermediate resistance to penicillin in 13% of the cases, whereas nasopharyngeal isolates from healthy children in Salvador showed 10% of intermediate resistance, reaffirming the good bacteriological correlation of isolates in different materials.

Nevertheless, other studies show differences as to the profile of resistance between pneumococci, as in São Paulo, whose systemic infection strains in children younger than six years revealed a 31% penicillin resistance, which is a lot higher (15.6%) than that found in children from day-care centers. Several factors may contribute to these differences,
such as the size of the sample and the methodology used to determine antibiotic resistance (E-test and other methods used for the determination of MIC). In our study, strain resistance to penicillin varied a lot, especially due to the small number of children examined at each care unit. Unfortunately, no reliable data on penicillin resistance based on pneumococci isolated from patients with systemic infection are available in Fortaleza. Our study has a limited impact, since systemic pneumococci were not studied comparatively (e.g.: in blood or pleural fluid samples), using the same methodology applied to nasopharyngeal isolates.

The age of children seems to play an important role in the status of individuals who carry resistant strains according to population studies, as that conducted in New York City, where the highest resistance occurred in children younger than two years. Among our patients, penicillin resistance was associated with the group of children attending day-care centers (90% older than 12 months). Considering only children with pneumonia (one group with a balanced participation of children older and younger than two years), the analysis did not allow any significant association with penicillin resistance.

The high rate of pneumococcal carriers in public day-care centers and the highest prevalence of resistant and multi-resistant strains should alert us on the role of these institutions as a source of selection and respiratory dissemination of resistant strains. Independent studies conducted at public day-care centers in the states of Rio Grande do Sul and Ceará reported a strong association between day-care attendance and episodes of pneumonia (odds ratio between 5 and 11, according to the studies). Our findings show higher pneumococcal colonization in children who attend day-care centers. Measures to reduce pneumococcal colonization in these children could be designed through the surveillance of carriers.

Previous studies reported a strong association between use of antibiotics and the presence of resistant pneumococci in the nasopharynx. In our patients, we observed a significant association between previous use of antibiotics and penicillin resistance just by using the information provided by parents/guardians in the last 30 days. The increased resistance of pneumococci to antibiotics, especially indoors, should warn us against the indiscriminate use of these drugs.

In this cross-section study, we limited ourselves to the analysis of carrier status and the profile of resistance to drugs in the described situations. Prospective studies are required for the identification of factors involved in the eradication, persistence or recolonization of the nasopharynx by pneumococci, also including risk factors for the colonization by strains that are resistant to different antibiotics.

A relevant and unavoidable issue regarding the high resistance of nasopharyngeal strains of pneumococci to penicillin and cotrimoxazole is concerned with the
effectiveness of these drugs in routine outpatient services for the treatment of respiratory infections. The analysis of the evolution of patients colonized by sensitive or resistant strains was not the aim of our study. In Pakistan, a randomized and community-based study conducted by Qazi28 showed that cotrimoxazole, despite the high rates of resistance, was effective for the treatment of pneumonias in outpatient services, reaching a rate greater than 90%. Another multicenter study conducted in the USA showed that the treatment of patients with community-acquired pneumonias caused by penicillin-resistant pneumococci with standardized beta-lactamic antibiotics did not have a different prognosis when compared to patients with penicillin-sensitive strains.29 Very likely, the cutoff limits for considering the resistance of pneumococci to beta-lactamic antibiotics and cotrimoxazole in the treatment of pneumonias are underestimated. Some authors gathered by the Center for Disease Control discussed alternatives for the treatment of pneumonias caused by resistant pneumococci in older children and adults and proposed higher limits for penicillin MIC (sensitivity: MIC ≤ 1 mg/l, intermediate resistance MIC= 2 mg/l and total resistance MIC ≥ 4 mg/l).30 However, the treatment of other respiratory infections such as acute otitis media caused by penicillin-resistant germs (current MIC limits) may require higher doses (in the case of amoxicillin) or use of alternative drugs.31 Further studies are therefore necessary, especially on pediatric respiratory infections in regions with a high rate of antibiotic-resistant pneumococci.

References


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