Abstract

Objective: describe the antimicrobial resistance and serotype distribution of pneumococcal strains.

Methods: in a 57-month period, a laboratory-based surveillance of invasive pneumococcal strains from patients aged < 20 years was conducted. Pneumococcus was identified by means of tests for solubility in bile and optochin. Pneumococcal resistance to penicillin was screened by 1 µg oxacillin disc and minimal inhibitory concentration was determined for the strains not susceptible to penicillin. Disc diffusion and broth microdilution methods were used for surveillance of resistance to other antimicrobials. Pneumococci were serotyped by means of the Neufeld-Quellung reactions.

Results: of 70 patients, 57.1% were males. The mean age was 1.92 yrs (mean 3.19 + 3.66 yrs, range 1 month to 19.5 yrs); 52.9% and 81.4% were < 2 yrs and < 5 yrs, respectively. The strains were isolated from blood (91.4%), CSF (2.9%), pleural (2.9%), peritoneal (1.4%) and abscess (1.4%) fluids from patients with pneumonia (77.1%), fever without localizing signs (10.0%), meningitis (4.3%), others (8.6%). Resistance was detected to penicillin (20.0%), trimethoprim-sulfamethoxazole (65.7%), tetracycline (21.4%), ofloxacin (6.3%), erythromycin (5.7%), clindamycin (2.9%). All tested strains were susceptible to chloramphenicol and vancomycin. Among penicillin-resistant strains, high resistance was detected in one, the same that showed intermediate resistance to cefotaxime. The most frequent serotypes were: 14 (22.9%), 6B and 19F (10.0% each), 6A and 19F (6.3% each), 9V and 18C (5.7% each). Resistance to penicillin was detected in serotypes 14 (71.4%), 6B and 19F (14.3% each).

Conclusions: of 70 strains, 67.2% were classified as serotypes included in the heptavalent conjugate pneumococcal vaccine as well as were all penicillin-resistant strains.


Introduction

Streptococcus pneumoniae is an important etiologic agent, especially of pneumonia, meningitis, and sepsis. The emphasis is on the pediatric age group in which it can cause serious illness. The World Health Organization estimates that between 1 and 2 million deaths a year occur worldwide among children younger than five years old as a result of pneumococcal infections and the majority of these deaths are registered in developing countries.
The rate of mortality per pneumococcal infection, including pneumococcal pneumonia, underwent a massive decrease after the introduction of sulfa drugs and penicillin, between 1930 and 1940.\textsuperscript{3} The first sulfa-resistant strains of pneumococcus had already been reported in 1943.\textsuperscript{4} In 1965, for the first time, the occurrence of a strain of \textit{S. pneumoniae} which was resistant to penicillin was described.\textsuperscript{5} Since then, and particularly during the last ten years, in a large range of parts of the world, reports have become ever more frequent of cases of infection caused by pneumococcus with diminished susceptibility to, or even totally resistant to penicillin and/or other antimicrobials used in the treatment of such infections;\textsuperscript{6-11} Brazil is not excluded.\textsuperscript{12-14}

In virtue of the data above, the use of a conjugated pneumococcal vaccine which is immunogenic for children from two months of age onward has been considered as a potential strategy for the control of pneumococcal infections.\textsuperscript{15,16} Nevertheless, more than 90 different pneumococcus serotypes have already been identified and many of them are serotypes which cause disease.\textsuperscript{17} The profile of which serotypes are most relevant varies from one region to another\textsuperscript{18} and induced immunity is apparently serotype-specific.\textsuperscript{15} Therefore, widespread conjugated pneumococcal vaccine use requires both adequate knowledge of the distribution of the most prevalent serotypes within each region and also the susceptibility of each pneumococcus to antimicrobials in order to allow the choice of the correct therapeutic system to be made for the treatment of this infection.\textsuperscript{19}

The objective of this investigation was to describe the pattern of antimicrobial resistance and the distribution of serotypes of invasive pneumococcus strains as isolated from a sample of children and adolescents in Salvador, Bahia.

\section*{Methods}

\textbf{Population and design of study}

Between September 1997 and May 2002, active vigilance was maintained of invasive pneumococcus strains. The Bacteriology Laboratories of the Hospital Complex which includes the Professor Edgard Santos Teaching Hospital - Professor Hosannah de Oliveira Pediatric Center (HUPES-CPPHO) and the Hospital Aliança (HA), during the entire period, of the Hospital Central Roberto Santos (HCRS) during 1999, and the Cerebrospinal Fluid Laboratory of the Division of Pediatric Infectology and Cerebrospinal Fluid Analysis of Bahia (or State of Bahia)- Fundação José Silveira (SINPEL-FIS), during 2001 and 2002, in Salvador, Bahia. Clinical and demographic data was collected on those patients from whose normally sterile fluids pneumococcus strains had been isolated. Data came from information recorded on the culture requests or, when necessary, based on an interview of the assisting doctor or through consultation of the patient’s medical record, immediately after the isolation of each invasive pneumococcus strain. In this study strains from patients less than twenty years old were included.

\textbf{Bacteriological data}

At the HUPES-CPPHO laboratory, until 1999, and at the SINPEL, the biological fluid to be cultivated was immediately inoculated (1.0 - 3.0 ml) in 30 ml of Brain Heart Infusion (BHI) with SPS (0.025%) and incubated at 35 °C. All cultures underwent subcultures in agar-blood (lamb) at 5% and agar-chocolate, at 35 °C, after 24 hrs, 48 hrs and seven days’ incubation in the BHI. At the HUPES-CPPHO laboratory (from 2000 onwards), the HA and the HCRS laboratories, 0.5-4.0 ml of the specimens collected were immediately inoculated in 20 ml of supplemented BHI and incubated in Organon Bact/Alert equipment at 35 °C, for seven days. Whenever the equipment signaled a positive result, the medium was subjected to sub-culture in Columbia agar with 5% lamb’s blood and in agar-chocolate, incubated at 35 oC with 5% CO₂ for 18-24 hours. \textit{S. pneumoniae} was distinguished from other alpha-hemolytic streptococcus by means of tests for solubility in bile and optochin. Pneumococcus strains were sent to the Bahia Central Laboratory (LACEN - BA- Laboratório Central da Bahia) and then to the Adolfo Lutz Institute, in São Paulo, where bacteriological identification was confirmed and serotyping and tests for antimicrobial susceptibility were carried out. Resistance of the pneumococcus to penicillin was initially identified through the use of an oxacillin 1 µg disc. The disc diffusion method was used to identify resistance to other antimicrobials including chloramphenicol, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, ofloxacin, vancomycin and tetracycline. When a strain was considered to have a diminished susceptibility to oxacillin (the bacterial growth inhibition zone around the oxacillin disc was smaller than 20 mm) it was submitted for minimum inhibitory concentration (MIC) measurement for penicillin and for cefotaxime, by means of the plate microdilution method (Mueller-Hinton broth supplemented with 2-5% lysed horse blood) \textsuperscript{(20)} The following MIC values were used to determine susceptibility to penicillin: MIC \(\leq 0.06\) µg/ml - susceptible, 0.12 µg \(\leq\) MIC \(\leq 1.00\) µg - intermediate resistance, MIC \(\geq 2.00\) µg/ml - absolute resistance.\textsuperscript{20} The pneumococci were serotyped by means of the Neufeld-Quellung reactions using antiserum produced by Statens Seruminstitut, Copenhagen, Denmark.

\textbf{Study location}

The bacteriological laboratories at the HUPES-CPPHO, HA and HCRS perform examinations for patients cared for at their respective hospitals. O HUPES-CPPHO is a public university hospital, located in the central part of Salvador and cares for patients of a predominantly low socio-economic status who come from Salvador and neighboring towns. The HCRS is public, located in a
lower-middle and lower class residential district and primarily cares for patients of low socio-economic status. The HA is located in a high and medium socio-economic class residential zone and cares for patients of these classes. The SINPEL-FJS performs cerebrospinal fluid tests for patients cared for at seventeen hospitals throughout the city, from low, medium and high socio-economic classes who come from Salvador and other towns in the state of Bahia. According to a study of community pneumonia in children and adolescents, conducted at the CPPHO and the HA, between 1997 and 1999, 7.4% of the children hospitalized with pneumonia in Salvador are interned in one of these two hospitals, where blood cultures are collected from 65.5% of this same group of patients and pneumococcus is isolated in 0.8% of samples.

**Data analysis**

The statistical analysis was descriptive and employed the *Statistical Package for Social Sciences* (SPSS 9.0). Since the primary objective was to describe the pattern of antimicrobial resistance of invasive pneumococcus, with an emphasis on penicillin, the sample size calculation was performed based on a resistance level of 20%, in agreement with De Cunto Brandileone MC et al., a confidence interval of 95%, width of 20% giving a minimum sample size of 62 strains. Consent was obtained from the directorate of each hospital or service to which each of the respective satellite laboratories is attached. This study is an integrant of the Pan American Health Organization Epidemiological Surveillance Network for Streptococcus pneumoniae (SIREVA-VIGIA), in Bahia.

**Results**

Seventy strains of pneumococcus were isolated: 55.7% at the HUPES-CPPHO, 41.5% at the HA, 1.4% at the SINPEL and 1.4% at the HCRS. The ages of the 70 patients varied from 1 month to 19.5 years, with a median of 1.92 years (mean average 3.19 ± 3.66 years); 52.9% and 81.4% of the patients presented ages < 2 years and < 5 years respectively; 57.1% of the patients were male. The strains were isolated from blood (91.4%), cerebrospinal fluid (2.9%), pleural fluid (2.9%), peritoneal fluid (1.4%) and fluid from an abscess (1.4%). The diagnoses of the patients were pneumonia (77.1%), fever without localized symptoms (10.0%), meningitis (4.3%), cellulitis (2.9%), acute otitis media (2.9%), sinusitis (1.4%) and peritonitis (1.4%). Of the 64 patients from whose blood pneumococcus were isolated, one presented with meningitis. Of the 70 strains, penicillin resistance was detected in 20.0%, resistance to sulfamethoxazole-trimethoprim in 65.7%, to tetracycline in 21.4%, to erythromycin in 5.7%, to clindamycin in 2.9%; among the 64 strains which were tested for susceptibility to ofloxacin, four (6.3%) proved themselves to be resistant, while among the 17 tested for susceptibility to cefotaxime, one (5.9%) was considered resistant. All of the seventy strains were susceptible to chloramphenicol as were the 44 strains tested for vancomycin susceptibility. Among the strains which were not susceptible to penicillin, absolute resistance was detected in one, the same which presented intermediate resistance to cefotaxime. The median of the ages of the patients whose strains where penicillin resistant was 2.75 years (mean average 4.22 ± 4.74 years), with 35.7% being less than 2 years old and 78.6% less than 5. Table 1 presents serotype distribution and the respective penicillin resistance frequencies. The strain which had absolute resistance was classified as serotype 14.

**Table 1 - Distribution of serotypes of invasive pneumococcal strains and penicillin resistance, Salvador, 1997-2002**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Frequency (%)</th>
<th>Resistance to Penicillin (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valid (n/N)</td>
<td>Cumulative</td>
</tr>
<tr>
<td>14</td>
<td>22.9 (16/70)</td>
<td>71.4 (10/14)</td>
</tr>
<tr>
<td>5</td>
<td>10.0 (7/70)</td>
<td>32.9</td>
</tr>
<tr>
<td>6A</td>
<td>10.0 (7/70)</td>
<td>42.9</td>
</tr>
<tr>
<td>6B</td>
<td>8.6 (6/70)</td>
<td>51.5</td>
</tr>
<tr>
<td>19F</td>
<td>8.6 (6/70)</td>
<td>60.1</td>
</tr>
<tr>
<td>9V</td>
<td>5.7 (4/70)</td>
<td>65.8</td>
</tr>
<tr>
<td>18C</td>
<td>5.7 (4/70)</td>
<td>71.5</td>
</tr>
<tr>
<td>23F</td>
<td>5.7 (4/70)</td>
<td>77.2</td>
</tr>
<tr>
<td>3</td>
<td>4.3 (3/70)</td>
<td>81.5</td>
</tr>
<tr>
<td>10A</td>
<td>4.3 (3/70)</td>
<td>85.8</td>
</tr>
<tr>
<td>15 †</td>
<td>4.3 (3/70)</td>
<td>90.1</td>
</tr>
<tr>
<td>19A</td>
<td>4.3 (3/70)</td>
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<td>1</td>
<td>1.4 (1/70)</td>
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<tr>
<td>9N</td>
<td>1.4 (1/70)</td>
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<tr>
<td>18A</td>
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<td>34</td>
<td>1.4 (1/70)</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 1 - Distribution of serotypes of invasive pneumococcal strains and penicillin resistance, Salvador, 1997-2002

* (n/N)
† Serotypes in the serogroup 15 were not possible to type.

**Discussion**

The elevated frequency pneumococcus strains isolated from blood (91.4%) calls attention to itself when compared with the frequency of strains isolated from other corporeal fluids which are normally sterile (8.6%). This finding indicates the practice of blood culture collection for patients with suspected bacteremic disease at the hospitals which took part in this study. The frequency with which this
procedure was performed is not known. It is known that for two of the hospitals (CPPHO and HA), between 1997 and 1999, blood cultures were collected from 65.5% of patients with pneumonia aged less than 15 years.22 It is important to point out that among the varied situations in which pneumococcus may be the cause of bacteremic disease, pneumonia, sepsis and occult bacteremia deserve to be highlighted and are more frequent occurrences than meningitis.1 Despite the elevated morbidity and lethality of pneumococcal meningitis,25 many of which represent subsequent stages of bacteremic disease, bacteremic diseases without meningitis are more prevalent.26 Furthermore, there are reports that the distribution of serotypes which cause bacteremia without meningitis may be different from the distribution of serotypes which cause bacteremia and meningitis.27,28 and that certain serotypes present higher frequencies of antimicrobial resistance than others.5,29,30 It therefore follows that information about the antimicrobial susceptibility and of the distribution of the serotypes of strains isolated from blood is fundamentally important to the choice of epidemiological and clinical measures such as the use of antimicrobials and vaccines.31 The number of tests that were performed in order to obtain the strains isolated here is not a known variable.

Within the sample presented in this work, 87.1% of the strains were isolated from the blood of patients with pneumonia or fever without localized symptoms. The predominance of invasive pneumococcal infections among children less than five years old attracts attention. The majority were less than two years old (median 1.92 years), and deserving to be highlighted, is the use of the conjugated vaccine which is immunogenic from an age of two months onwards.16 It has been estimated that between 5% and 10% of children with fever present fever without localized symptoms.34 While the great majority of these children are suffering from some sort of acute, auto-limiting and benign infectious disease,35 5% are suffering from occult bacteremia,35-38 of which S. pneumoniae is the most frequent cause in such situations.39 Furthermore, a study conducted by Baron and Fink demonstrated that children cared for at private surgeries had the same chance of having occult bacteremia as indigenous children.36 Therefore the importance of collecting blood cultures is further highlighted for children with fever without localized symptoms and risk factors for occult bacteremia such as: age less than 3 years, temperature at axilla > 39 °C, leukocytes > 15,000/mm³ or with toxic granulations or vacuolization, thrombocytopenia, chronic debilitating disease, a history of contact with diseases caused by Neisseria meningitidis or Haemophilus influenzae and somnolence or irritability.39,40 In addition to being able to provide information helpful to the correct handling of the patient, this procedure permits the study of the bacterial serotypes of the pneumococcus.

The rate of penicillin resistance reported here is similar to the rates reported by other studies carried out in large Brazilian cities.13,41 As this rate of absolute resistance is low, according to Friedland and McCracken, crystalline penicillin remains the first choice of treatment for pneumococcal infections with no central nervous system involvement and which require hospital treatment, just as amoxycillin remains the first choice for treatment, at clinics and outpatients units which do not require hospital treatment.42

Approximately 85.0% of the pneumococcal infections which occur globally within the pediatric age group are caused by seven serogroups: 4, 6A/B, 9V, 14, 18C, 19F and 23F,43 the same ones which are included in the heptavalent conjugated vaccine which has been licensed for use.44 Of the strains presented here, 67.2% belonged to the serotypes which are included in the vaccine.16 Of the remaining serotypes, the most frequent in this sample was serotype 5 (10.0%). The importance of the frequency of serotype 5 has been demonstrated before in investigations carried out in South America and published previously.9,10,11,13,41 However, these same publications observed an important frequency of serotype 1,13,41 a fact which was not observed in the current study nor in an investigation conducted here in Bahia, by Ko et al., in which patients with meningitis were studied.14 Despite the small sample size of this study, the consonance of the distribution with the results reported by other investigators reinforced the urgency of making available a vaccine which includes serotype 5, for use with the population at large, and, as such offering a wider protection.41 All of the strains which presented penicillin resistance in this study belong to the serotypes contained in the conjugated heptavalent vaccine, which thus proves to capable of offering protection against potentially more serious infections. As the greatest advantage of this vaccine is the fact that it is immunogenic for patients who are two or more months old and the greatest frequency of pneumococcal infections occurs in individuals younger than 2,15 the data obtained through this research suggest that the currently available vaccine can bring benefits, within the region studied, to individuals who present the greatest risk of pneumococcal infections, such as asplenic patients,45 or of more serious pneumococcal diseases, such as patients with the Acquired Immunodeficiency Syndrome.46

References


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