Active surveillance of Streptococcus pneumoniae bacteremia in Italian children

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Abstract

There are few data published regarding the incidence of Streptococcus pneumoniae bacteremia in Italian children. A 14-month surveillance study was conducted in 10 paediatric hospitals to investigate the rate of Sp bacteremia in children aged less than 5 years. The serotype prevalence and antimicrobial susceptibility of isolates were determined. A total of 55 Sp isolates were obtained from 4576 blood cultures (incidence rate, 1.2%). In order of frequency, the most common serotypes were 14, 23F, 19F, 9V, 1. Serotypes in the 7-valent conjugate pneumococcal vaccine (4, 6B, 9V, 14, 18C, 19F, 23F) accounted for 70% of isolates under 2 years of age, and 58% in the interval between 2 and 5 years of age.

Keywords: Streptococcus pneumoniae; Bacteremia; Pneumococcal vaccine; Children; Italy

1. Introduction

Streptococcus pneumoniae (Sp) is a major cause of morbidity and mortality in young children throughout the world, causing both invasive (meningitis, sepsis, bacteraemia) and non-invasive (pneumonia, acute otitis media, sinusitis) infections. The serotypes causing both invasive and non invasive diseases are quite similar [1,2].

Sp accounts for the vast majority of bacterial pathogens in children with occult bacteremia. The emergence of widespread resistance to penicillin and other antibiotics raises alarm about the possible impact of this pathogen, highlighting the importance of vaccine development [3,4].

Although morbidity and mortality associated with invasive pneumococcal disease (IPD) are known to be elevated in many epidemiological settings, the situation may vary by areas. In Western countries, occult bacteremia and sepsis in the paediatric age group represent most cases of IPD. Accurate ascertainment and sound epidemiological data are essential for the rational development of effective programmes for prevention and treatment. Most of the data regarding the epidemiology of pneumococcal infections in paediatric age have been collected in the U.S.A. or in other industrialized countries different from Italy [5]. In Italy only studies concerning the carrier state of Sp, community-acquired pneumonia and meningitis are available [6–8]. No precise assessments exist of the importance of Sp in bacteremia in Italy: the real total impact of invasive infections on the paediatric community is...
unknown [9]. Epidemiological studies of IPD, and in particular of bacteremia, associated or not with sepsis or pneumonia, must necessarily be based on aetiological diagnosis, by means of routine performance of blood cultures, whereas, due to reasons of budget and organization, blood cultures in children in Italy are less frequently performed than in other Western countries [9]. Better detection is fundamental to assess paediatric rates [10].

The study, promoted by the Italian Paediatric Society (SIP) and the Italian Association for the Study of Antimicrobials and Resistances (AISAR), started in November 2002; a “network” was set up to monitor Sp bacteremia in paediatric age, based on an appropriate sample of the population. Objectives of the study were: to create an active prospective monitoring system based on 10 reference children’s hospitals distributed all over Italy, including all the cases between 0 and 60 months of age referred to each centre; to perform blood culture tests in all the eligible patients, possibly before an antibiotic treatment was started; to assess the cumulative rate of Sp bacteremia in the recruited subjects, to evaluate the importance of risk factors, the incidence and spectrum of clinical manifestations, and the outcome of IPD in children; to serotype all isolated Sp strains, to assess the potential coverage by the new heptavalent pneumococcal conjugate vaccine, as well as obtaining the antibiotic-resistance profile.

2. Materials and methods

2.1. Study programme

The 10 children’s hospitals involved (7 teaching hospitals and 3 city hospitals) are distributed all over Italy: four in the north (Regina Margherita Paediatric Hospital, Turin; G. Gaslini Hospital, Genoa; De Marchi Paediatric Clinic, Milan; S. Orsola Polyclinic Paediatric Clinic, Bologna), three in the Centre (A. Mayer Paediatric Hospital, Florence; G. Salesi Paediatric Hospital, Ancona; Bambin Gesù Paediatric Hospital, Rome), and three in the South of the Country (S. Annunziata Hospital, Naples (Naples 1); Santobono Hospital, Naples (Naples 2); Giovanni XXIII Paediatric Hospital, Bari).

The surveillance started in November 2002 and ended in December 2003; it included all children between 0 and 60 months of age admitted to the hospital paediatric units with at least one of the following clinical conditions: fever (rectal temperature) ≥38.5°C, or two febrile episodes ≥38.0°C in a 48 h time, with neutrophilia (>15,000/mm³), with or without focalized infection; clinically detected pneumonia or bronchopneumonic focus; post-surgery infective complication (within 1 week after surgery); febrile episode in neutropenia condition. Patients entered into study at any time during hospitalization. All consecutive eligible patients were enrolled; without further exclusion criteria. As all hospitals recover cases of general Paediatrics, no risk of selection due to the specializations of hospitals was present.

The survey included the monthly monitoring for each centre of the number of the eligible subjects, the number of subjects actually recruited in whom blood cultures were performed, the rate of antibiotic treatment prior to blood cultures, and the number of blood cultures positive for Sp. Only for the positive cases an additional group of clinical data was later collected: the diagnosis at enrolment and at discharge, the detailed history of antibiotic treatment prior to blood testing, and the main clinical parameters.

No confirmed consent for study participation was obtained, as no medical or technical procedure was performed in addition to standard care.

Data were entered in a relational database developed in MSAccess 2000 under the surveillance of the Centre Naples 1, and sent to the reference statistician for merging datasets and statistical analysis. Incidence of Sp positive blood cultures is indicated as “95% Exact Poisson Confidence Intervals”. Proportions were compared using the Chi-square test.

2.2. Microbiological procedures

Isolation and identification of Sp strains were performed using methods standard to each participating laboratory. Five hospitals (Ancona, Florence, Rome, Naples 1, Naples 2) used BacT/Alert 9000 (Becton Dickinson Italia S.P.A.), and five hospitals (Turin, Genoa, Milan, Bologna, Bari) used BacT/Alert (bioMérieux France) as blood cultures systems. Isolates were sent to the central laboratory (Institute of Microbiology, University of Genoa), using heavily inoculated swabs maintained in Amies transport medium, to confirm their identity by Gram stain morphology, catalase reaction, optochin susceptibility and bile solubility [11] and to test their antimicrobial susceptibility. Serotyping was done by the capsular reaction test (Quellung test) with serotype and serogroup serum reagents available from the Statens Seruminstitut, Copenhagen, Denmark, as previously described [12].

Antibiotics tested were: Penicillin, Amoxicillin, Cefotaxime, Erythromycin, Levofloxacin, Tetracycline and Rifampin. Minimal inhibitory concentrations (MICs) of the antibiotics tested were determined using the microdilution method and interpreted according to the breakpoints suggested by the National Committee for Clinical and Laboratory Standard (NCCLS, 2004) [13]. S. pneumoniae ATCC 49619 was included in all runs as a control.

3. Results

3.1. Epidemiological data

During the 14 months of survey 4576 children were recruited (69% of the 6651 eligible subjects) for blood culture testing. Table 1 reports the total amount of children in whom blood cultures were performed, compared to the total amount
of eligible children and the number of cultures positive for Sp in each centre. Non-enrolment was due to variable attitude in performing blood cultures in different centres. Different blood cultures systems were not significantly associated with differing rates of Sp bacteremia.

Data on the presence of an antibiotic treatment started in the week before the time of blood sampling were available in 3493 blood cultures: pre-treatment occurred in 1119 (32%).

Blood cultures were positive for Sp in 55 cases, with a rate of 1.2/100 blood cultures. (CI 95%: 0.9–1.6).

The mean age of the positive cases was 19 months (median age: 16 months; range: 1–59 months); 70% were under 2 years of age and 41% under 1 year (Table 2). Males were 40 (73% of cases). No clear season distribution was observed, but no case occurred in the hottest summer months (July and August).

### 3.2. Clinical data

Clinical data were available only in 44 positive cases. All of them had been recruited for fever: 58% of them for two febrile peaks (rectal temperature ≥ 38.0 °C), and 42% for one febrile peak (rectal temperature ≥ 38.5 °C). Two cases also had neutropenia conditions. Organ localisation was present in 21 (48%) cases, and nine of them had pneumonia.

### Table 2

**Age distribution of patients with blood cultures positive for Sp**

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–6 months</td>
<td>13</td>
</tr>
<tr>
<td>6–12 months</td>
<td>9</td>
</tr>
<tr>
<td><strong>0–1 year</strong></td>
<td><strong>22 (40.9)</strong></td>
</tr>
<tr>
<td>12–18 months</td>
<td>10</td>
</tr>
<tr>
<td>18–24 months</td>
<td>6</td>
</tr>
<tr>
<td><strong>1–2 years</strong></td>
<td><strong>16 (29.5)</strong></td>
</tr>
<tr>
<td>2–3 years</td>
<td>9 (15.9)</td>
</tr>
<tr>
<td>3–4 years</td>
<td>4 (6.8)</td>
</tr>
<tr>
<td>4–5 years</td>
<td>4 (6.8)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>55 (100)</strong></td>
</tr>
</tbody>
</table>

Table 3 summarizes the diagnosis at discharge. Median length of hospitalization was 8 days (range: 1–56). The mean (±S.D.) peak of fever was 39.4 (±0.6) °C (range: 38.0–40.3 °C). No fatality was reported.

Blood cultures were performed after a mean duration of fever of 3.1 days (S.D. = 3.5 days; range: 0.5–19 days). Blood culture was performed in 67.4% of cases at admission, in other cases during hospitalization, but always during the first day, including 27% of cases in which Sp positivity was detected in a second blood culture, after the first one was negative for Sp.

Only 5 of the 44 Sp positive cultures for which clinical data are available had underlying chronic diseases (Sickle cell anaemia; Congenital malformation; Allergy-Asthma-Atopic Dermatitis; Malignancy; Ulcerative colitis). None of the Sp positive cases had been vaccinated for Sp.

Antimicrobial therapy had been started before hospitalization in 38.5% of cases (mean duration: 3 days; range: 1–6 days) and the most frequently used antibiotics were Amoxicillin (30%), Clarithromycin (20%), Azithromycin (10%), Ceftriaxone (10%), Cefitiben (10%), Cefaclor (20%).

In 48%, antimicrobial therapy had been given during hospitalization, before blood testing (Amoxicillin in 7.7%; Ampicillin + Sulbactam in 7.7%; Ceftriaxone in 30.8%; Cef-
tazidime in 23.1%; Ceftriaxone in 15.4%; Vancomycin in 7.7%, Clarithromycin in 7.7% of cases).

Antibiogram was performed in 67% of cases and was considered in the choice of therapy in half the cases.

3.3. Microbiological data

Of the 55 cases found positive for Sp, 42 were available for serotyping and definition of resistance profile at the central laboratory of the Microbiological Institute in Genoa.

Serotype distribution of the isolates is reported in Table 4. As the serotypes included in the heptavalent conjugate vaccine (4, 6B, 9V, 14, 18C, 19F, 23F) were more commonly encountered in children younger than 2 years of age, taking the potential cross-reactivity of three serotypes (23A, 6A and 9N) into account, the expected vaccine “coverage” and the total potential vaccine “coverage” can be respectively calculated as 70% and 83% under 2 years of age, and 58% and 67% in the interval between 2 and 5 years of age.

High resistance to Penicillin was observed in 7.1%, of isolates; intermediate resistance in 14.3%, with a cumulative total resistance profile of 21.4% of the isolated strains. Resistance to Erythromycin and Tetracycline was observed in 42.9% and 35.7% of isolated, respectively. No resistance was detected to Amoxicillin, Cefotaxime, Levofloxacin, Rifampin.

All isolates with reduced sensitivity to Penicillin belonged to serotypes included in the heptavalent conjugate vaccine (19F = 5 strains; 23F = 2 strains; 6B = 2 strains). Sixtyone percent of strains resistant to Macrolides belonged to serotypes included in the heptavalent conjugate vaccine; taking the cross-reactivity of 23A, 6A and 9N into account, the vaccine “coverage” can be calculated as 88%.

4. Discussion

Our results, and the interpretation of them, might be affected by some important study limitations:

- total enrolment rate was low; the differences in the number of recruited children, and especially in the proportion of blood culture performed and rate of blood culture positive for Sp among the ten centers suggest different risks for Sp bacteremia in different settings.
- a possible differing enrolment criteria by center cannot be excluded. The study was conducted in Children’s Hospitals, recovering cases of General Paediatrics, with no theoretical risk for selection of patients due to the specialization of the hospitals, but it was hardly possible to monitor attitudes of single centers, and of different M.D.s in the same center. Centers with over 90% enrolment had very low rates of bacteremia (more similar to the published rates of occult bacteremia in the literature); sites with lower enrolment levels had higher rates of bacteremia: this may just indicate that they were only performing blood cultures on children in frank sepsis or with known meningitis, and therefore had higher rates of bacteremia associated with those clinical entities.
- furthermore, as we did not collect clinical data of children whose blood cultures were negative, we cannot tell if cultures were performed more often in sicker children than in children with mild diseases at centers with higher rates of bacteremia.
- it is also possible that different Sp serotypes contribute differently to disease severity. Therefore, if some centers only obtained blood cultures in critically ill patients such as those with meningitis, serotypes with a propensity to cause severe disease might be over represented in this study.
- even if different blood cultures systems were not significantly associated with differing rates of Sp bacteremia, a possible effect of blood culture system on rate of detected bacteremia cannot be definitely excluded.

However, the results of this survey add important information to the epidemiology of IPD in Italian children. In the population studied we detected a rate of Sp bacteremia of 1.2/100 children, similar to the figures reported by studies performed in the U.S.A.: 1.6/100 children with pneumonia treated as outpatients [14] and 1.9/100 febrile outpatients with no focal infection or immunosuppression [15].

Sp is the most common causative organism of occult bacteremia [16,17] and might be difficult to diagnose [18]: neither an elevated total white blood cell count, an elevated absolute neutrophil count, nor an increased percentage of bands are highly predictive [19]. Serious adverse outcome is an uncommon result of bacteremia in the absence of sepsis or meningitis [20].

The incidence of IPD in Spain has been reported as 76 per 100,000 for children aged 0–24 months, 45 per 100,000 for children aged 0–48 months and 16.6 per 100,000 for children

<table>
<thead>
<tr>
<th>Serotype/serogroup</th>
<th>Number of isolated (aged &lt;2 years)</th>
<th>Number of isolated (aged 3–5 years)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>8</td>
<td>1</td>
<td>9(21)</td>
</tr>
<tr>
<td>23F</td>
<td>5</td>
<td>1</td>
<td>6(14)</td>
</tr>
<tr>
<td>19F</td>
<td>4</td>
<td>1</td>
<td>5(12)</td>
</tr>
<tr>
<td>9V</td>
<td>1</td>
<td>2</td>
<td>3(7)</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3(7)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2(4)</td>
</tr>
<tr>
<td>6B</td>
<td>1</td>
<td>1</td>
<td>2(4)</td>
</tr>
<tr>
<td>7F</td>
<td>1</td>
<td>1</td>
<td>2(4)</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2(4)</td>
</tr>
<tr>
<td>33F</td>
<td>1</td>
<td>1</td>
<td>2(4)</td>
</tr>
<tr>
<td>6A</td>
<td>1</td>
<td>1</td>
<td>2(4)</td>
</tr>
<tr>
<td>9N</td>
<td>2</td>
<td>–</td>
<td>2(4)</td>
</tr>
<tr>
<td>18C</td>
<td>1</td>
<td>–</td>
<td>1(3)</td>
</tr>
<tr>
<td>23A</td>
<td>1</td>
<td>–</td>
<td>1(3)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>12</td>
<td>42</td>
</tr>
</tbody>
</table>

Serotypes included in the heptavalent conjugate vaccine are: 4, 6B, 9V, 14, 18C, 19F, 23F.

a The remaining 13 isolates of the total 55 were unavailable.
aged 0–14 years [21], and has been increasing during the last decade [22]. The global incidence of hospital-based IPD in Catalonia is reported as 10.5 per 100,000 persons/year; in children <2 years it is six times higher (59.6 per 100,000 persons-year), with pneumonias (rate of 26.2 per 100,000 persons-year) and non-focal bacteremias (rate of 22.1 per 100,000 persons-year) being especially frequent [23].

In Greece, the overall annual incidence rate of IPD was reported as 44 per 100,000 children <14 years of age, whereas the incidence rate for children <5 year of age was 100 per 100,000 [24]; the mean annual incidence rates of pneumococcal meningitis among children aged <1, <2, and <7 years were respectively reported as 17.4, 12.4, and 4.3 per 100,000, and the mean annual incidence rates of bacteremia in the same groups of age respectively as 30.1, 32.5, and 14.0 per 100,000 children [25].

The higher prevalence of Sp bacteremia in children admitted to hospital aged less than 2 years found by our survey is also confirmed in other areas [16,17,26].

Pretreatment with antibiotics may lead to an underestimation, reducing the cases of invasive disease, but in our experience it was as common as reported by other authors [27]; in our results antibiotic pre-treatment in Sp positive cases was slightly more common than in the whole recruited population.

Mortality due to bacteraemia in children without underlying conditions is reported as rare [28]. In our experience underlying medical conditions were present in a low percentage of children with blood cultures positive for Sp, much lower than the figures (27–47%) reported by other studies [10,18,27,29–31]. This should induce health authorities to consider the importance of a vaccination strategy extended to all newborn babies, not restricted to “high risk” cases.

In our study, with the exception of summer months, Sp bacteremias were distributed all around the year, while in other experiences about 50% of the cases of IPD in childhood occurred between February and May [32].

The distribution of the most frequently isolated Sp serotypes in our results mostly overlaps with existing Italian data [7,12]. Seventy percent of serotypes isolated in the first 2 years of life and 58% of the isolated between 2 and 5 years of age in our survey are represented in the newly licensed heptavalent pneumococcal conjugate vaccine. Coverage might be considerably higher, considering potentially cross-reacting serotypes. This confirms published data from different countries [10,16,22], and previous Italian data reporting 72–79% of the isolates in children related to serotypes included in the heptavalent conjugate vaccine [7,9].

Over the past few decades, the emergence of antibiotic-resistant pneumococcal strains has been reported worldwide, especially in children [26,33], even if recent studies on community-acquired pneumonia failed to provide incontrovertible evidence for a direct link between in vitro resistance and treatment failure [34], and current practice suggests that intermediate resistance to penicillin is of little clinical significance in non-meningitic systemic pneumococcal infections [35]. Our data show that penicillin-resistance is an ever-increasing phenomenon also in Italy: 7.1% of strains showed a high resistance and 14.3% an intermediate resistance, which accounts for a total resistance profile that has exceeded 20% of the isolates. This confirms the recently described Italian trends [36,37], with less resistance than reported elsewhere [20,26,38]. Careful monitoring of antibiotic susceptibility and outcome of therapy is necessary to continually reassess current recommendations for treatment, but, considering that serotypes most commonly associated with penicillin resistance (9V, 23F, 6B, 19F, 14) are contained in the heptavalent vaccine, a preventive strategy with an effective vaccine is the best options to such a public health problem.

The results of this study show that the heptavalent pneumococcal conjugate vaccine may prevent the majority of pneumococcal bacteremia episodes in Italian children less than 5 years of age; this finding should lead to less pressure to use broad-spectrum antibiotics, less antibiotic resistance and reduced need for laboratory testing and hospitalization [39,40]. A better assessment of the epidemiology of Sp infection in Italian paediatric population is fundamental to the decision regarding the offering of the new effective heptavalent conjugate vaccine to all the Italian paediatric population [41]. Our study indicates that inclusion of a pneumococcal conjugate vaccine in the primary immunisation programme in Italy would have a considerable effect on the morbidity associated with IPD.

Appendix A


References


