Community Acquired Pneumonia Among Pediatric Outpatients in Salvador, Northeast Brazil, with Emphasis on the Role of Pneumococcus

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Pneumonia is one of the leading causes of hospitalization and death among children in developing countries, and mortality due to pneumonia has been associated with *S. pneumoniae* infection. This investigation was designed to describe the antimicrobial susceptibility and serotype patterns of pneumococcal strains recovered from the blood of children with community-acquired pneumonia (CAP) and to assess the clinical findings of pneumococcal bacteremic patients with pneumonia. In a 26 month prospective study, blood cultures were obtained as often as possible from children (<16 years of age) diagnosed with CAP in two emergency rooms. Antimicrobial drug susceptibility tests and serotyping were performed when pneumococcus was identified. We studied 3,431 cases and cultured blood samples from 65.5% of those. Pneumococcus was recovered from 0.8% of the blood samples. The differences in age, somnolence, wheezing and hospitalization among children with and without pneumococcal bacteremia were statistically significant. Pneumococcal bacteremia was age-related (mean 1.63 ± 1.55; median 0.92) and associated with somnolence and hospitalization among children with CAP. One strain was recovered from pleural fluid. Penicillin resistance was detected in 21.0% (4/19) of the strains at an intermediate level, whereas 63.0% of the strains were resistant to trimethoprim-sulfamethoxazole. The most common serotypes were 14 and 6B, and these serotypes included the resistant strains. Eight of our 18 isolates from blood were of types included in the heptavalent conjugate pneumococcal vaccine, recently licensed in the USA.
Key Words: *Streptococcus pneumoniae*, antimicrobial resistance, serotypes, blood culture, community-acquired pneumonia, children.

Acute respiratory infection (ARI), particularly pneumonia, continues to be one of the leading causes of hospitalization and death among children in most developing countries [1-3]. Since mortality from preventable diseases has diminished markedly as the result of worldwide systematic application of oral rehydration therapy and vaccination, pneumonia is one of the challenges to face at the present time [4, 5]. According to several studies, mortality due to ARI is associated with bacterial infections, caused especially

by *S. pneumoniae* and *H. influenzae* [6-9]. Since the early reports from Boston in 1965, Australia in 1967, and South Africa in 1977, penicillin-nonsusceptible pneumococci have been reported with increasing frequency of infection around the world [10-16], including Latin America [17-21]. It has become increasingly important, therefore, to consider the use of the conjugate pneumococcal vaccine which is immunogenic in children over 2 months of age [22]. The efficacy of an adequate pneumococcal vaccine will depend on the existence of detailed, reliable and representative data on prevalence and serotype distribution of *S. pneumoniae* in the region where the vaccine is to be used [23]. Since 1993, the Pan American Health Organization has been conducting an Epidemiological Surveillance Study to determine the relative prevalence of capsular types of *S. pneumoniae* which cause invasive disease, particularly pneumonia, and their patterns of antimicrobial resistance in Latin America (SIREVA-VIGIA) [23]. In Brazil, reports of the bacteriologic characteristics of *S. pneumoniae* are limited.[18,24,25] In Salvador, a large city in Northeast Brazil, there is no information on the antimicrobial susceptibility and distribution of serotypes of pneumococcal strains isolated from children diagnosed with pneumonia. The purposes of the present investigation are to provide such information and to assess the clinical findings of pneumococcal bacteremic patients with pneumonia.

Materials and Methods

Study design and population

We conducted a prospective study as part of the SIREVA-VIGIA in Salvador, in Northeast Brazil [23], from September, 1997, to October, 1999. We attempted to enroll all identified cases of pneumonia among children seen in the Emergency Room (ER) of the Professor Hosannah de Oliveira Pediatric Center (PHOPC) and the Pediatric ER of the Aliança Hospital (AH) in Salvador. Data collection was based on a questionnaire that asked for demographic and clinical findings. A large proportion (64.3%) of the questionnaires were filled out immediately after each case identification by the primary physician. The diagnosis of pneumonia was based on either the WHO case definition criteria (ie., cough and tachypnea) [26], and/or on crackles on auscultation and/or on radiological findings. Chest x-ray was read by the duty pediatrician during the consultation. All collected data and all emergency room records were reviewed by the research group members once a week beginning in March, 1998, at the PHOPC, and in May, 1998, at the AH. Retrospectively, the same group also completed the questionnaires for those patients characterized as having pneumonia whose questionnaires were not completed prospectively. Each case was cross-referenced with the Bacteriology Laboratory log of the respective hospital, in order to verify the collection of blood and/or pleural fluid for culture.

Bacteriologic data

One blood culture and/or pleural fluid culture was obtained in as many cases as possible, before the start of the antimicrobial therapy. Data concerning the use of immediately previous antibiotic therapy was collected, but only intravenous antibiotic treatment was used as a criterion for exclusion.

At the PHOPC laboratory, the blood and pleural fluid specimens (1.0 ml to 3.0 ml of blood or pleural fluid) were immediately inoculated into 30 ml of Brain Heart Infusion broth with SPS (0.025%) and incubated at 35°C. All broths were sub-cultured after 24 hrs, 48 hrs and at 7 days of incubation onto Brain Heart Infusion agar with 5% sheep blood and on chocolate agar at 35°C. At the AH laboratory, 0.5 ml to 4.0 ml of the collected specimens were immediately inoculated into 20 ml of supplemented Brain Heart Infusion broth and incubated in Bact/Alert Organon at 35°C for 7 days. Whenever the Bact/Alert Organon displayed a positive result, the broth was sub-cultured onto Columbia agar with 5% sheep blood and onto chocolate agar at 35°C in a 5% CO_2 incubator for 18 hrs to 24 hrs. *Streptococcus pneumoniae* was distinguished from other α-haemolytic streptococci by

using the bile solubility test and the optochin disc test. The research group members were informed about all isolated bacteria from children attending both ERs in order to cross-reference them with the clinical records. The strains isolated during the study were sent to the Central Laboratory of Bahia (LACEN) and then by airplane to the Adolfo Lutz Institute, in São Paulo, where the bacteriologic identification was rechecked and the antimicrobial drug susceptibility tests and serotyping were performed. Pneumococcal resistance to penicillin was screened initially by using a 1µg oxacillin disc. Penicillin resistance was considered probable with an oxacillin zone size less than 20 mm. Disc-diffusion was used for surveillance of resistance to other antibiotics including chloramphenicol, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, ofloxacin, vancomycin, and tetracycline. The non-susceptible strains by oxacillin disc were submitted for the determination of minimal inhibitory concentration (MIC) testing against penicillin by using the broth microdilution method (with Mueller-Hinton broth supplemented with 2% to 5% lysed horse blood) (NCCLS, 2000) [27]. Seroptyping of pneumococcus was done by the Neufeld-Quellung reaction using sera produced by the Statens Seruminstitut, Copenhagen, Denmark.

Study setting

The PHOPC is a public university hospital located in a central area of Salvador, the capital of Bahia, in Northeast Brazil. The population of Salvador is approximately 2,800,000 inhabitants. The PHOPC serves children of predominantly lower socio-economic status who live in Salvador and adjacent cities. The AH is a general private hospital located in a middle class area of Salvador. Its Pediatric ER attends children from middle to middle-upper and high socio-economic status. The physicians in both ERs were all pediatricians trained to use the WHO case definition of pneumonia, and they were all asked to collect blood and/or pleural fluid for culture whenever a patient was eligible for this study after receiving the consent of the child's guardian.

Data analysis

Statistical analyses were performed by using the Statistical Package for the Social Sciences (SPSS 9.0). Differences in proportions were assessed by the Pearson Chi Square test or Fisher's exact test, as appropriate. Means of continuous variables were compared by Mann-Whitney U. Confidence Interval (95%) was reported for mean difference. The statistical tests were two tailed, with a significance level of 0.05. The study was approved by the institutional review board of each hospital and by the Ethics Committee of the Faculty of Medicine of the Federal University of Bahia.

Results

By active surveillance, a total of 3,431 cases of pneumonia among 3,221 children were identified during the study period. These totals were comprised of 2,476 cases from the PHOPC and 955 cases from the AH. The intervals between the separate episodes among the patients with more than 1 infection were, in all instances, greater than 1 month. One hundred seventy of the patients had 2 or more separate episodes of pneumonia. Of all patients, 54.3% were male and 45.7% were female. The median age was 1.92 years (range 2 days to 15.5 years, mean 2.87 ± 2.78 years). Tachypnea and crackles were reported in 57.7% and 68.3% of the cases, respectively. Chest x-ray was performed in 93.2%, and radiologically confirmed infiltrate was reported in 96.0%. Considering the radiological pattern, the infiltrates were classified as interstitial (24.7%), alveolar (51.0%) or mixed (interstitial-alveolar) (20.3%). All children received antimicrobial therapy. Blood cultures were done in 73.7% (704/955) at the AH, and in 62.3% (1,542/2,476) at the PHOPC (overall 65.5%). Contamination was detected in 4.1% of the blood culture sets. The frequency of isolating *S. pneumoniae* from blood was 0.8% (18/2,246) [0.9% (14/1,542) at the PHOPC, 0.6% (4/704) at the AH; p=0.4]. The

Table 1. Significant differences among children with community acquired pneumonia when blood culture was performed

Characteristic	Pneumococcus		Total	p value
	Yes	No		
Age (years)				
Mean±SD	1.63 ± 1.55	2.91 ± 2.79	2.90 ± 2.78	0.044
Median	0.92	2.00	2.00	
range	0.17 - 5.92	0.02 - 15.50	0.02 - 15.50	
Wheezing†	11.8 (2/17)	47.5 (935/1967)	47.2 (937/1984)	0.003
Somnolence†	50.0 (8/16)	19.1 (357/1870)	19.4 (365/1886)	0.005
Hospitalization†	66.7 (12/18)	29.3 (653/2228)	29.7 (666/2246)	0.0005

† Results are reported in % (n/N).

Table 2. Frequency of serotypes and antimicrobial resistance to penicillin of *S. pneumoniae* isolates recovered in sterile fluids from children with pneumonia

Serotype	Hospital		Total (%)
	PCPHO	AH	
1	1	0	1 (5.3)
5	1	0	1 (5.3)
6A	1	1	2 (10.5)
6B	1	2(1*)	3 (15.7)
9N	1	0	1 (5.3)
10A	1	0	1 (5.3)
14	4(3*)(1†)	0	4 (21.0)
15¶	1	1	2 (10.5)
19A	2	0	2 (10.5)
20	1	0	1 (5.3)
23F	1	0	1 (5.3)
Total	**15**	**4**	**19 (100.0)**

*Penicillin non-susceptible strains.
†Strain isolated from pleural fluid, penicillin susceptible.
¶Serotypes in serogroup 15 were not distinguished.

significant differences in demographic and clinical findings among the groups of children with and without pneumococcus as identified from blood cultures are shown in Table 1. The mean difference of age between those groups was 1.28 (95% CI 0.5 - 2.06). No significant differences were noticed in the frequency of tachypnea, crackles, cough, ability to drink, chest retraction, fever (axillary temp > 37.5 °C during the consultation), chronic underlying illness, day care center attendance, chest x-ray performance, radiological infiltrate patterns, or gender. Antimicrobial use during the 72 hours before the blood was obtained for culture was reported in 17.6% of the cases and such information was not reported on 22.8% of the questionnaires. *Streptococcus pneumoniae* was isolated from 1 patient who reported the use of trimethoprim-sulfamethoxazole (1/305, 0.3%). The isolation rate among patients who reported no use of antimicrobials during 72 hours before the collection of blood for culture was 0.9% (13/1,428) (p=0.49). We also observed that 1.8% (12/666) of the hospitalized patients had pneumococcus isolated from blood culture in comparison to 0.4% (6/1,580) of the patients who were not hospitalized (p=0.001).

Nineteen strains of pneumococcus were isolated during the study period; 18 were isolated from blood and 1 was isolated from pleural fluid (blood culture was not drawn from this child). Penicillin-resistance was detected in 21.0% (4/19) of the strains isolated from different children living in different quarters of Salvador, all of them at the intermediate level; 3 of the resistant strains were isolated from children visiting the PHOPC, and 1 was isolated from a child visiting the AH. The 3 resistant strains isolated at the PHOPC were serotype 14, and the 1 resistant strain isolated at the AH was serotype 6B (Table 2). The most prevalent serotype was 14, the same serotype most frequently resistant in this study. All isolates were sensitive to chloramphenicol, clindamycin, ofloxacin, tetracycline, erythromycin and vancomycin, but only 37.0% of the strains were sensitive to trimethoprim-sulfamethoxazole.

Discussion

We found a 0.8% yield of pneumococcus in blood cultures. This rate was similar to those reported in several studies of the etiology of pediatric community acquired pneumonia (CAP). [28-32] The overwhelming majority of cases of CAP during childhood are caused by respiratory viruses [33]. *Streptococcus pneumoniae* has been estimated, by serological methods, to be responsible for one third of all cases of acute pneumonias [9, 31, 32]. However, the proportion of children with pneumonia who are bacteremic at the time blood is obtained for culture is uncertain [34]. Some data are available from studies of concurrent cultures of blood and lung aspirate. Blood cultures were positive in only 13.0% of patients with bacterial pneumonia, documented by lung punctures, as reported by Silverman, et al. [35]. Although the bacteriologic procedures were not the same at both laboratories, the difference of isolates was not statistically significant.

The present study provided evidence that bacteremia among children with CAP was age-related (Table 1). This fact may be related to the immaturity of childrens' immune systems during the first years of life. Somnolence and hospitalization were associated with pneumococcal bacteremia, whereas wheezing was not. This finding is reasonable since hospitalized patients usually appear more severely ill than do those who are managed as outpatients, and somnolence is regarded as a severity sign. It was expected, therefore, that hospitalized and somnolent patients would have a greater chance of being bacteremic. On the contrary, wheezing was associated with nonbacteremic pneumococcal cases. Perhaps this can be explained by the association of wheezing with infection by respiratory viruses [36].

We found that 17.6% of the patients were using antimicrobial drugs before the collection of blood. It is widely known that the previous use of antimicrobial drugs can decrease the isolation rate of pathogenic bacteria. The difference between the proportions of pneumococci isolated as related to previous antimicrobial use was not statistically significant, probably because of the small number of strains studied.

The use of trimethoprim-sulfamethoxazole did not interfere with the isolation of *S. pneumoniae* from 1 child; this strain was susceptible to that drug but compliance could not be certified.

Until now, the results of pneumococcal susceptibility tests and serotyping in this study are in accord with data from other Brazilian states (Table 2) [18, 24, 25]. These results suggest that penicillin G can still be used as initial therapy to treat children in our region diagnosed with pneumococcal pneumonia. Penicillin remains adequate to treat pneumonia caused by *S. pneumoniae* with an intermediate level of resistance to penicillin [37]. The 19 strains studied were distributed among 11 serotypes; the most frequent serotypes were 14, 6B, 6A, 19A and serogroup 15. The penicillin-nonsusceptible strains (14, 6B) were among the most frequent serotypes. These data agree with results reported by Ko, et al. [38], who studied 221 pneumococcal isolates recovered in cerebrospinal fluid from patients with meningitis in Salvador, when infections with serotypes 14 and 6B were responsible for 83.0% of the 29 penicillin non-susceptible cases (all intermediate level). By comparing the serotype distribution of our pneumococcal strains with those represented in the heptavalent pneumococcal conjugate vaccine recently licensed in the USA [39], we found that 3 of our 11 serotypes (6B, 14, 23F) were contained in that vaccine, 2 of the 5 (14, 6B) most frequent, the same penicillin-nonsusceptible ones. Although there are 90 capsular types of pneumococcus, relatively few types are responsible for most disease in children [33], with types 6A, 6B, 14 and 19A commonly found in pediatric disease worldwide. The predominance of penicillin-resistant serotypes 6B and 14 have been described worldwide [17-21, 40-42].

It is important to emphasize that caution must be taken in interpreting the features of *S. pneumoniae* reported here because of the small number of strains studied. Culturing blood was not feasible for children seen in the ER of the PHOPC before this investigation, when blood was drawn only from hospitalized patients. Blood culturing was achieved due to the support provided for this study.

The increasing prevalence of penicillin-nonsusceptible *S. pneumoniae* has influenced the decision to order blood cultures for patients with pneumonia [43]. In spite of the low rate of isolation in this study (0.8%), blood culture remains the gold standard for the diagnosis of pneumococcal pneumonia [43, 44]; it is also the most practical way to identify the causative organism, mainly when data derived from susceptibility testing and serotype patterns are the goals.

Acknowledgements

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