Brazilian Journal of Medical and Biological Research (2003) 36: 807-813
ISSN 0100-879X

Factors associated with penicillin-nonsusceptible pneumococcal infections in Brazil

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Abstract

Resistance of *Streptococcus pneumoniae* is a worldwide, growing problem. Studies of factors associated with resistance to penicillin have not been conducted in Brazil. The objective of the present study was to evaluate factors associated with infection by *S. pneumoniae* not susceptible to penicillin. A prevalence study was conducted including all patients with a positive culture for *S. pneumoniae* in a hospital from July 1991 to December 1992 and the year 1994. Of 165 patients identified, 139 were considered to have clinically relevant infections and 88% of them had invasive infections. All infections were community acquired and consisted of pneumonia (44%) and of central nervous system (19%), pelvic or abdominal (12%), upper airway or ocular (12%), primary bloodstream (9%) and skin and soft tissue (5%) infections. Mortality was 25%. Susceptibility to penicillin was present in 77.6% of the isolates; 21.8% were relatively resistant, and one isolate was resistant (minimal inhibitory concentration = 4 μg/ml). Multivariate analysis showed that age below 4 years (odds ratio (OR): 3.53, 95% confidence interval (95%CI): 1.39-8.96) and renal failure (OR: 5.50, 95%CI: 1.07-28.36) were associated with lack of susceptibility to penicillin. Bacteremia occurred significantly less frequently in penicillin-nonsusceptible infections (OR: 0.34, 95%CI: 0.14-0.84), possibly suggesting that lack of penicillin susceptibility is associated with lower virulence in *S. pneumoniae*.

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Publication supported by FAPESP.

Received April 5, 2002
Accepted January 31, 2003

Key words
- *Streptococcus pneumoniae*
- Penicillin susceptibility
- Risk factors
- Prevalence study

Introduction

Pneumococcal infections are important worldwide because of their frequency and severity. *Streptococcus pneumoniae* is an important cause of pneumonia, meningitis and otitis media and is a frequent cause of bacteremia. Originally, susceptibility to penicillin was universal (1) but since the first report in 1967 (2), resistance has been a growing problem worldwide (3-11). Resistance to other antimicrobial drugs also occurs and multiresistance is progressively more frequent (12-18). Epidemiological studies of the risk factors for infection or colonization by penicillin-resistant pneumococci have been done but results are few and sometimes conflicting.

The objective of the present study was to evaluate factors associated with infection by *S. pneumoniae* not susceptible to penicillin.

Material and Methods

This was a prevalence study involving all patients under medical attention at the Hospital das Clínicas, University of São Paulo, São Paulo, SP, Brazil, with at least one positive culture for *S. pneumoniae* from any clinical specimen during the period from July 1991 to December 1992 and the year of 1994. Hospital das Clínicas is a tertiary care teaching hospital associated with the University of São Paulo. It has approximately 2000 beds divided among 5 buildings. The microbiology laboratory is central and serves all buildings. The hospital is a reference center for the city of São Paulo and outskirts (approximately 15 million inhabitants).

The cases in which the isolate did not remain viable for confirmation of penicillin susceptibility were excluded. Infections were diagnosed using the criteria described by Garner et al. (19). Colonization or carrier status was defined when the isolate was obtained from a usually nonsterile site. Only cases of infection were included in the analysis of factors associated with nonsusceptibility. The following infections were considered invasive pneumococcal disease: pneumonia, meningitis and other central nervous system (CNS) infections, pelvic and abdominal infections and any bacteremic infection documented by a positive blood culture.

Identification was performed at the Microbiology Laboratory, Hospital das Clínicas, and was based on morphology, optochin susceptibility and bile-solubility tests. Susceptibility testing for penicillin, tetracycline, chloramphenicol, erythromycin, sulfamethoxazole-trimethoprim, rifampin, ampicillin, vancomycin, cephalothin and 3rd generation cephalosporins, and serotyping were done at the Microbiology Laboratory of the Clinical Microbiology Department of the Federal University of Rio de Janeiro, Brazil. These results have been published elsewhere (8,10, 11). Susceptibility to penicillin was evaluated by obtaining the minimal inhibitory concentration (MIC) using the method of agar dilution. An isolate was considered susceptible if the MIC was below 0.1 μg/ml; an MIC from 0.1 to 1.0 μg/ml defined relative resistance, and an MIC above 1.0 μg/ml defined resistance. Relatively resistant and resistant isolates were considered penicillin nonsusceptible.

Patients were evaluated during hospitalization. When this was not possible data were obtained from their records. Autopsy data were available for 13 patients and were evaluated. The following data were collected: age, gender, diagnosis of infection, underlying conditions based on evaluation by the attending physician or on a clear indication in the patient's record (HIV infection, diabetes mellitus, chronic liver disease, chronic pneumopathy, cardiopathy, kidney failure, solid organ transplant, cancer, use of chemotherapy or steroids within the previous month), previous use of antimicrobial drugs, and present residence.

HIV infection was defined as a positive serologic test for HIV antibodies. Liver, kidney, heart and lung diseases were considered if there was a record of a previous medical follow-up for these conditions. The patients' present residences were divided into six zones: central (within a 5-km radius from the central point of the city), north, south, east, west, and outside the city perimeter. For the multivariate analysis the residence was considered central (within a 10-km radius from the central point) or peripheral. Information about current or previous use of antimicrobial agents was obtained from the patients or their relatives or from unmistakable evidence in the patients' chart. If not, antimicrobial use was considered unknown. Age was divided into three categories based on previously suggested high-risk age groups: younger than 4 years, from 4 to 64 years, and older than 64 years.

The data were organized using the EpiInfo software (version 6.02, Centers for Disease Control and Prevention, Atlanta, GA, USA).

The group of patients infected with penicillin-nonsusceptible *S. pneumoniae* was compared with the penicillin-susceptible infection group. For dichotomous variables the results were expressed as odds ratios and 95% confidence intervals and the chi-square test or Fisher exact test was applied. When there were more than two categories only the chi-square test was used. For continuous variables, the mean data were compared by the Kruskal-Wallis test. Significance was set at 0.05.

Variables with P≤0.20 in the univariate analysis were included in the multivariate analysis, which was performed by multiple logistic regression using the Stata program (version 7, StataCorp, College Station, TX, USA).

Results

The study involved 165 patients, 56% of whom were males. There were 107 adults (above 14 years of age) and 58 children. Mean (± SD) age was 43 ± 18 years for adults and 30 ± 32 months for children.

Most patients (76%) presented underlying conditions such as cancer (N = 20), liver disease (N = 18), HIV infection (N = 17), chronic lung disease (N = 13), steroid use within the previous month (N = 10), chronic heart disease (N = 8), renal failure (N = 8), and chemotherapy in the previous month (N = 8).

There were 128 (77.6%) isolates susceptible to penicillin, 21.8% relatively resistant, and one resistant isolate (MIC: 4 µg/ml). MIC50 for penicillin was 0.03 µg/ml and MIC90 was 0.25 µg/ml. Data concerning susceptibility to penicillin and other drugs and serotypes have been described elsewhere (8,10,11).

Twenty-six patients were considered to be noninfected carriers and 139 (84%) presented infection which was invasive in 122 (88%). The infections were: pneumonia (44%), CNS infections (19%), pelvic or abdominal infections (12%), upper airway or ocular infections (12%), primary bloodstream infections or endocarditis (9%), and skin or soft tissue infections (5%). Overall mortality was 25% for patients with pneumococcal infection, 33% in adults and 14% in children.

The results of univariate analysis can be seen in Table 1. Mean (± SD) age was 41.6 ± 17.4 years for patients with penicillin-susceptible infections and 44.5 ± 22.2 years for patients with nonsusceptible infection (P = 0.91). Among children, mean age was 18.1 ± 14.0 months for patients with penicillin-susceptible infections and 29.2 ± 28.9 months for patients with nonsusceptible infection (P = 0.15). Adequate information on previous antimicrobial use was only available for 37 cases of infection. The following variables were included in the multivariate analysis: age category, presence of bacteremia, cancer, renal failure, and present residence. Table 2 presents the final model of multiple logistic regression.

Discussion

There are relatively few studies involving factors associated with penicillin-nonsusceptible pneumococcal acquisition. Table 3 presents the most important studies in this area. Many involve a small number of patients or analyze only subsets of patients. In our study the factors associated with penicillin nonsusceptibility were: younger age, the absence of bacteremia and renal failure.

In many studies young age is considered to be a risk factor for penicillin-resistant infection (20-24) or colonization (25) for reasons that are not clear. This may reflect the high prevalence of antimicrobial use among young children, especially those in day care or in other situations of extended contact with other children (26). On the other hand, age has been considered to be an independent risk factor in multivariate analyses (20,27). The previous use of antimicrobial

Table 1. Factors associated with infection by penicillin-nonsusceptible *Streptococcus pneumoniae*: univariate analysis of categorical variables.

Variable		PNS	PS	Odds ratio	95% Confidence interval	P
Sex	Male	16	61	0.90	0.37-2.20	0.80
	Female	14	48			
Adult	Yes	15	73	0.49	0.20-1.22	0.09
	No	15	36			
Age category (years)	≤3	14	22			0.01
	4-64	13	77			
	≥65		10			
Invasive infection	Yes	23	99	0.33	0.10-1.10	0.06
	No	7	10			
Bacteremia	Yes	14	79	0.33	0.13-0.83	<0.01
	No	16	30			
Underlying disease	Yes	24	78	1.59	0.54-4.87	0.35
	No	6	31			
Cancer	Yes	1	14	0.23	0.01-1.86	0.19
	No	29	95			
HIV infection	Yes	5	12	1.62	0.44-5.65	0.53
	No	25	97			
Liver disease	Yes	3	13	0.82	0.17-3.46	1.00
	No	27	96			
Chronic lung disease	Yes	2	5	1.49	0.19-9.49	0.64
	No	28	104			
Kidney failure	Yes	4	4	4.04	0.77-21.25	0.07
	No	26	105			
Heart disease	Yes	2	4	1.88	0.22-13.03	0.61
	No	28	105			
Steroid use	Yes	2	6	1.23	0.16-7.41	0.68
	No	28	103			
Chemotherapy	Yes	1	7	0.50	0.02-4.44	1.00
	No	29	107			
Antimicrobial use	Yes	5	14	0.71	0.14-3.68	0.64
	No	6	12			
Zone of residence	Center	1	11			0.53
	North	5	10			
	South	3	17			
	East	15	46			
	West	5	16			
	Outside city perimeter	1	9			

PNS: infections caused by penicillin-nonsusceptible *S. pneumoniae*; PS: infections caused by penicillin-susceptible *S. pneumoniae*.

Table 2. Factors associated with infection by penicillin-nonsusceptible *Streptococcus pneumoniae*: multivariate analysis.

Variable	Odds ratio	95% Confidence interval	P
Age category (≤3 years)	3.53	1.39-8.96	0.008
Bacteremia	0.34	0.14-0.84	0.019
Renal failure	5.50	1.07-28.36	0.042
Residence in the center of the city	1.90	0.74-4.88	0.184
Cancer	0.34	0.04-2.99	0.329

Table 3. Studies of factors associated with penicillin-nonsusceptible *Streptococcus pneumoniae* infection or colonization.

Studies	Year	Type of study	Associated factors	Comments
Saah et al. (37)	1980	Case-control	None	21 cases (PNS)/18 controls
Pallares (18)	1987	Case-control	Pneumonia; previous hospitalization; ß-lactam use; nosocomial acquisition; critical condition	24 cases (PNS)/48 controls; only univariate analysis
Reichler et al. (23)	1992	Prevalence	Younger age	Only subset analysis of patients; mean age for PS: 43 months and for PNS: 32 months
Garcia-Leoni et al. (21)	1992	Prevalence	Age <10 years; fatal underlying disease; immune suppression; previous antimicrobial use	139 patients with infection
Tan et al. (32)	1993	Case-control	Previous antimicrobial use	Children: 43 cases (PNS)/66 controls
Nava et al. (22)	1994	Prevalence	ß-Lactam use; age <5 years; immune suppression	374 infections
Reichler et al. (24)	1995	Prevalence	Young age; previous hospitalization	Only subset analysis
Bedos et al. (20)	1996	Prevalence	Nosocomial acquisition; age <15 years; isolation from the upper respiratory tract, sinus or middle ear; HIV infection, use of ß-lactam in the previous 6 months	10,350 infections
Mannheimer et al. (38)	1996	Prevalence	White race; presence in a pediatric chronic facility	24 PNS/89 PS
Kronenberger et al. (26)	1996	Prevalence	Household members in day-care attendance	29 PNS/180 PS
Melander et al. (25)	1998	Prevalence	Previous antimicrobial use; younger age; male sex	1036 children in day care; only colonization
Einarsson et al. (30)	1998	Case-control	Previous antimicrobial use	Only pneumonia
Winston et al. (34)	1999	Prevalence	Previous hospitalization; absence of bacteremia	65 PNS/411 PS
Deeks et al. (27)	1999	Prevalence	Use of ampicillin or penicillin in the previous 3 months; private medical coverage; non-meningitis	274 children
Dejthevapor et al. (28)	2000	Prevalence	Previous antimicrobial use (OR: 18.4, 95%CI: 6.2-54.6)	73 PNS/51 PS
Diekema et al. (29)	2000	Ecologic	ß-Lactam prescriptions in the population	Compared resistance encountered at 23 medical centers in different cities with prescriptions per 100,000 inhabitants per month in the surrounding metropolitan area
Roberts et al. (39)	2001	Case-control	None	100 patients
Nasrin et al. (31)	2002	Cohort	ß-Lactam use	Only colonization and children <4 years

PNS: penicillin-nonsusceptible *S. pneumoniae*; S: penicillin-susceptible *S. pneumoniae*; OR: odds ratio; 95%CI: 95% confidence interval.

drugs has been the factor most frequently associated with nonsusceptibility (18,20-22,25,27-32). In our study reliable data on previous antimicrobial use were not available for most patients and could not be properly evaluated. However, one of the problems met when analyzing the use of antimicrobials as a factor associated with resistance is using a design such as the one used in the present study. If patients with susceptible and nonsusceptible pneumococcus are compared this may lead to a selection bias because patients with previous antimicrobial use probably will not have susceptible isolates or may not have pneumococci at all. When these groups are compared the non-use of antimicrobials may be associated with having a susceptible isolate and not an association of resistance with antimicrobial use, as suggested in the literature. It has been suggested that to evaluate the role of antimicrobial use in resistance, cases with resistant isolates should be compared with controls from the same population not chosen for presenting susceptible strains (33). We feel that the design used in our study may not be suited to evaluating the impact of antimicrobial use on resistance.

The finding that bacteremia was significantly more frequent among penicillin-susceptible *S. pneumoniae* infections is interesting and not easily explained. There have been studies with similar results in which invasive or severe infections have been associated with susceptibility to penicillin (20,25,34), suggesting that nonsusceptibility leads to less virulent strains. On the other hand, there has been an association between nonsusceptibility to penicillin and immune suppression (21,22), HIV infection (20), severe underlying disease or conditions (21), and, in our study, renal failure. The significance of this is unknown but may also be related to the possible inability of resistant strains to cause severe infections in immunocompetent patients. The mechanisms for this remain obscure and deserve further investigation.

Nosocomial infection and colonization by penicillin-resistant *S. pneumoniae* have been reported (35). Previous hospitalization is considered to be a risk factor in some studies (18,24,34). In our study the infections were community acquired. Renal failure was significantly associated with resistance even though it was a relatively rare event occurring in only 8 patients. We did not find evidence that these patients had previous hospitalizations which might explain resistance. Renal failure is a known risk factor for pneumococcal disease and is considered to be an independent prognostic factor for mortality (36) but, to our knowledge, an association of renal failure with resistance has not been described before. Further studies will be needed to confirm this finding.

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