

Susceptibility of *Streptococcus pneumoniae* to Penicillin in the State of Minas Gerais, Brazil from 1997-2004

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We monitored the susceptibility to penicillin of invasive strains of *Streptococcus pneumoniae* in two reference laboratories; 502 positive cultures from patients with an active invasive infectious process were analyzed. *Streptococcus pneumoniae* was identified through conventional procedures, and the oxacillin disc diffusion method was used to check for penicillin susceptibility. Statistical analysis included calculations of the frequency distribution, with 95% confidence intervals (CI), as well as chi-square tests and chi-square for linear trend for temporal analysis of susceptibility. The bacterium was isolated from patients less than a year old (40.7% of the isolates), from infants (55.9%), and from individuals less than 15 years old (64.4%). The majority (88.2%, 95% CI = 85.5%-91.1%) of the 502 isolates were susceptible to penicillin. There was no significant temporal trend of elevation of resistance rate during the study period ($p=0.56$). We conclude that resistance of *S. pneumoniae* to penicillin is not yet an important clinical-epidemiological concern in the State of Minas Gerais. To provide necessary support for the adoption of therapeutic and prophylactic measures, epidemiological surveillance should be implemented at a national level to monitor the profile of susceptibility/resistance of *S. pneumoniae* to penicillin and other antimicrobials.

Key Words: *Streptococcus pneumoniae*, antimicrobial resistance, invasive disease

Infections by *Streptococcus pneumoniae* significantly increase morbidity and mortality in children and the elderly, based on studies conducted internationally, and especially in developing countries, where the problem is considerably greater. This bacterium is one of the most frequent etiological pathogens in otitis, pneumonias and meningitis. [1,2] According to a WHO estimate, pneumonias are responsible for approximately 5 million child deaths per year in developing countries, 30% of which are caused by pneumococcus [1].

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Nowadays, there is an increasing incidence of resistance of *S. pneumoniae* to penicillin, which disrupts the treatment of infant and elderly patients with previous history of hospitalization and treatment with b-lactams [2]. This phenomenon has also been occurring with other antimicrobials, such as tetracycline, chloramphenicol, erythromycin, trimethoprim-sulfamethoxazole, rifampin, and during the last decade, cephalosporins [3]. Continuous epidemiological surveillance of the susceptibility profile in infections by pneumococcus should be implemented, with periodical local and regional assessments spanning various years, allowing the establishment of strategies for control and treatment.

During the last two decades, several studies have been performed in Brazil on the susceptibility pattern of the pneumococci to penicillin and other antimicrobials. The prevalence of intermediately susceptible and resistant strains ranged from zero to 26.7% from 1981 to 2004 [4-13]. However, due to the continental dimensions of Brazil, this tremendous

effort is still relatively limited, and it does not offer a trustworthy epidemiological view.

We examined the susceptibility pattern of *S. pneumoniae* strains to penicillin isolates between 1997 and 2004 in the state of Minas Gerais.

Material and Methods

Design, local and time period of study

We made a descriptive study, in which the susceptibility of invasive isolates of *S. pneumoniae* to penicillin was analyzed from January 1997 to December 2004. This study was conducted in the Bacteriology Section of the Medical Biology Department, Ezequiel Dias Foundation (FUNED), State Health Authority and in the Federal University of Minas Gerais (UFMG) General Hospital.

The Medical Biology Department of FUNED takes part in the Brazilian public health laboratories network, coordinated by the Ministry of Health.

Inclusion and exclusion criteria

Cultures positive for *S. pneumoniae* were collected from patients with invasive infectious illnesses in progress; one isolate was obtained per patient. Blood samples and/or cerebrospinal or pleural fluid were collected from patients, regardless of the usage of antimicrobials. Positive cultures from biological materials from other non-sterile sources were excluded from the analysis.

Laboratory methods

Streptococcus pneumoniae was identified through its typical morphology on blood agar, and chocolate agar, as indicated by Gram staining, and by susceptibility to optochin, bile solubility and alpha hemolysis [14]. The Kirby-Bauer *in vitro* disk diffusion method was used to determine antibiotic resistance throughout the study, using 1 µg oxacillin discs, as recommended in the *National Committee for Clinical Laboratory*

Standards (NCCLS) [15]. Growth inhibition zones were measured after 20-24 hours of incubation. Intermediate or elevated resistance to penicillin was considered probable when the oxacillin inhibition zone size was less than 20 mm in diameter [16].

Protocol and data collection

The following variables were recorded in a standardized protocol, especially devised for this investigation: sex, age at diagnosis, place of residence, origin of material, institution, culture date, and antimicrobial susceptibility tests.

Statistical aspects

All of the 502 isolates of *S. pneumoniae* available from the records of the two laboratories were included. Data was organized and analyzed using the software EpiInfo, version 6.04 [17]. Frequency distribution calculations were made (expressed as 95% confidence intervals) to characterize the susceptibility/resistance of the pneumococcus to penicillin. Additionally, we used chi-square tests to compare proportions and chi-square for linear trends to determine the significance of the temporal distribution of the susceptibility/resistance. A *p* value < 0.05 was considered statistically significant.

Ethical aspects

The experimental protocol was approved by the Ethics in Research Committee of the Federal University of Minas Gerais.

Results

During the period from January 1997 to December 2004, 502 invasive isolates were collected (Table 1).

The samples came from cerebral spinal fluid (69.5%) and blood (28.8%); most isolates were from cases of meningitis and were processed at FUNED (71.2%). Among these samples, most (62.7%) came from male patients.

Age was available for 64.1% of the patients. Infants (less than 2 years old) accounted for 28.7% of the cases (95% CI, 38.6%-49.4%). The age group with the highest number of isolates (23.5%, 95% CI, 30.9%-41.3%) was children younger than one year. Among the age groups, patients below the age of 15 accounted for 40.6% of the isolates positive for pneumococcus, whereas patients older than 60 years of age accounted for only 6.0%. Approximately half of the isolates were patients living in Belo Horizonte, the capital of the state of Minas Gerais; there was no record of residence for 27.5% of the isolates.

The susceptibility and resistance rates of the pneumococcus isolates to penicillin during the study period were 88.2% (95% CI, 85.5%-91.1%) and 11.8% (95% CI, 9.0%-14.6%), respectively. The evolution of resistance rates of pneumococcus to penicillin was recorded during seven years (Table 2).

Fifty-nine isolates were resistant to penicillin. In 1997, all of the 56 strains were sensitive to this antibiotic. Between 1998 and 2004, the resistance rate ranged from 9.1 to 17.1%. There was no temporal trend of increase in the resistance rate ($p=0.56$). Despite the lack of statistical significance, excluding the year 1997, during which no resistant strain was identified, the *odds ratio* estimates for each year in the period 1998-2004 were 1.00, 0.68, 0.89, 1.01, 0.78, 1.41 and 0.96, respectively, when compared to 1997.

Susceptibility patterns of pneumococcus to penicillin for each type of biological material were recorded (Table 3).

Approximately 70% of the susceptible strains were collected from cerebrospinal fluid. A single isolated strain in pleural liquid was classified as resistant; 11.1% and 12.2% of the isolates from cerebral spinal fluid and blood, respectively, were considered resistant.

Table 4 describes the characteristics of the 54 invasive isolates with growth inhibition zones smaller than 20mm around the oxacillin disc and therefore defined as strains of elevated and intermediate resistance to penicillin.

Among the 54 resistant isolates, 63% were from males. Among the 43 strains resistant to penicillin from patients whose age was known, 33 of them (56%) were

collected in patients younger than two years of age (95% CI, 51%-79%), indicating the importance of this type of infection for pediatric patients.

Resistant isolates from cerebrospinal fluid accounted for 69.5% of the samples. Most of the isolates (71.2%) were processed at FUNED, due to its importance as a central laboratory in the Public Health Care System. About 56% of the resistant isolates came from the metropolitan region of Belo Horizonte.

Finally, there were no significant differences ($p>0.25$) in the distribution of susceptible versus resistant isolates by age group, sex, origin of biological material, institution, and patient place of residence.

Discussion

The susceptibility profile of *S. pneumoniae* to antibiotics can be determined through a number of methods. Determination of a minimum inhibitory concentration (MIC) constitutes the *gold-standard*, by which strains inhibited by concentrations inferior or equal to 0.06 mg/mL are considered susceptible to penicillin G; strains are considered to have intermediate resistance when MIC is 0.1-1.0 $\mu\text{g/mL}$, and they have elevated or complete resistance when these values are greater than 2 $\mu\text{g/mL}$ [16].

Assessment of the susceptibility pattern of pneumococcus can also be determined by the disc diffusion method, which is widely available and is low in cost. It is particularly useful as a screening method and as an indicator of susceptibility to penicillin. Discs containing 1 μg of oxacillin are stable and there is a high correlation between results with these discs and MIC values [17]; MIC calculation is a more complex method, which is available in very few institutions in Brazil. In the assessment of susceptibility/resistance to penicillin, the oxacillin discs gave a sensitivity greater than 99% and a specificity of 80% for MIC determination. [18]

The disc diffusion method is more sensitive (rarely are resistant strains classified as susceptible) than specific (6% to 40% of susceptible strains can be classified as resistant to penicillin) [17]. According to

Table 1. Descriptive characteristics of invasive isolates of *Streptococcus pneumoniae* studied from 1997-2004

Variable	N	%
Biological material		
Cerebrospinal fluid	361	71.9
Blood	139	30.0
Others	1	0.3
Institution		
FUNED	368	73.3
University Hospital	134	26.7
Sex		
Male	331	65.9
Female	171	34.1
Age group (years)		
< 1	118	23.5
1-2	26	5.2
3-15	60	11.9
16-60	94	18.7
> 60	30	6.0
Unknown	174	34.7
Patient location of residence		
Metropolitan region of Belo Horizonte	248	49.4
Countryside	116	23.1
Unknown	138	27.5
Susceptibility to penicillin G		
Susceptible	443	88.2
Resistant	59	11.8

Table 2. Pattern of susceptibility/resistance of *Streptococcus pneumoniae* to penicillin G through the disc diffusion method in 502 invasive isolates

Year	Susceptible N (%)	Resistant* N (%)	Total N (100%)
1997	56 (100.0)	0 (0.0)	56
1998	71 (87.7)	10 (12.3)	81
1999	58 (82.9)	12 (17.1)	70
2000	63 (86.3)	10 (13.7)	73
2001	72 (87.8)	10 (12.2)	82
2002	39 (84.8)	7 (15.2)	46
2003	50 (90.9)	5 (9.1)	55
2004	34 (87.2)	5 (12.8)	39
Total	443 (88.2)	59 (11.8)	502

*indicates intermediate and elevated resistance

Table 3. Origin and susceptibility of *Streptococcus pneumoniae* to penicillin through the disc diffusion method from 1997-2004

Source	Susceptible		Resistant	
	N	%	N	%
Cerebrospinal fluid	310	71.5	41	69.5
Blood	123	28.5	17	28.8
Pleural fluid	0	0	1	1.7
Total	433	100.0	59	100.0

Table 4. Descriptive characteristics of the 54 invasive isolates of *Streptococcus pneumoniae* resistant to penicillin studied from 1997-2004

Variable	N	%
Sex		
Male	37	62.7
Female	22	37.3
Age group (years)		
< 1	24	40.7
1-2	9	15.2
3-15	5	8.5
16-60	12	20.3
> 60	zero	Zero
Unknown	9	15.3
Material		
Cerebrospinal fluid	41	69.5
Blood	17	28.8
Pleural fluid	1	1.7
Institution		
FUNED	42	71.2
University Hospital	17	28.8
Local of residence		
City of Belo Horizonte	33	55.9
Countryside	17	28.9
Unknown	9	15.2

the American Academy of Pediatrics [19], disc diffusion reliably identifies every susceptible pneumococcus (growth inhibition zones equal or greater than 20mm). However, it does not allow distinction between strains with complete resistance from those with intermediate resistance to penicillin.

In Brazil, a continental-scale country, there are relatively few studies assessing susceptibility patterns of pneumococcus. During the last 23 years, in an examination of studies with invasive samples, resistance rates have ranged from 0 to 25% [4-13]. The state of Minas Gerais has an area almost the size of France, and though it is only one state, our sample is larger than in the previous studies. In a comparison with several other Brazilian states, the state of Minas Gerais is nowadays in an intermediate epidemiological situation (11.7% resistance), especially after 1998, the year in which the resistance was 12.3% [11].

Kertesz et al. conducted a prospective multicentric study (named SIREVA Project) in six Latin American countries, including Brazil; 1,649 invasive samples from children younger than five years of age were studied. The proportion of isolates resistant to penicillin ranged from 12% in Colombia to 47.3% in Mexico, where the highest levels of complete penicillin resistance were reported (21.3%). The mean rate of resistance in the six countries was 24.9%, including 8.2% elevated resistance [20]. In a comparison with North American countries, the rates found in our study were lower than those found in Canada (30.2%) [21], the United States (43.8%) [21] and Mexico (48%) [20].

The rates of resistance observed in some European countries, such as Spain (58%) [22], France (32%) [23], and Hungary (58%) [24] were much higher than those we found in the state of Minas Gerais. The tendency was reversed when our results were compared with levels from Germany (0.8%) [25], Sweden (2.0%) [26], and Italy (5.5%) [27].

Among Asian countries, Korea has the world's highest rate of resistance, involving more than 70% of the isolates, among which 33% presented complete resistance to penicillin [28]. In a

retrospective study conducted in that country from 1985-1996, resistance rates in isolates from children younger than 12 years of age with invasive infections ranged from zero in 1989 to 89% in 1995, while the rate of resistance in 1996 was 69% [28].

The resistance rate to penicillin in our study was highest among infants (59.2%), similar to what is known from other studies [6,9,20], probably due to a greater utilization of broad-spectrum antimicrobials and a higher prevalence of pneumococcal infections in patients of that age group. Similar results were reported by Ko et al. [11], who have found that 71.0% of isolates from infants were resistant to penicillin. These findings suggest that more attention should be given to this age group.

A map of the profile of resistance/susceptibility of *S. pneumoniae* should be made through studies similar to ours, which ideally should include determination of MIC as well as implementation of an effective epidemiologic surveillance system with continuous monitoring of resistance levels. This would require a network of laboratories in charge of performing the available microbiological tests for all invasive isolates. The diffusion method in oxacillin discs should be used as a screening method, and in the following phase the resistant isolates should be submitted to conventional MIC determination, or the E-test should be applied, which is a more practical and rapid method, with virtually 100% agreement with MIC [29].

The systematic use of blood cultures in the routine care of children with pneumonia and meningitis, especially among those younger than five years of age, is of paramount importance and would contribute effectively towards the recognition of local/regional/national patterns of resistance, allowing for the development of strategies of pneumococcal infection control.

In order to reduce the risk of colonization and dissemination, and the development of disease due to resistant strains in communities, preventive measures should include anti-pneumococcal vaccination of all children and the judicious usage of antimicrobials, especially β -lactams [30,31].

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