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ACUTE BACTERIAL MENINGITIS CAUSED BY *Streptococcus pneumoniae* RESISTANT TO THE ANTIMICROBIAN AGENTS AND THEIR SEROTYPES

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Abstract – The main objectives of this study are to evaluate the resistance rates of *Streptococcus pneumonia* to penicillin G, ceftriaxone and vancomycin in patients with meningitis; to analyze possible risk factors to the antimicrobian resistance; to describe the serotypes detected and to suggest an initial empirical treatment for meningitis. The sensitiveness and serotypes of all isolated *S. pneumoniae of* patients with acute bacterial meningitis received by the Paraná State Central Laboratory from April 2001 to august 2002 have been evaluated. One hundred *S. pneumoniae* have been isolated, of which 15% were resistant to penicillin, 1% to cephalosporin and 0% to vancomycin. The serotypes most found were 14 (19%), 3 and 23F (10% each). When only the resistant serotypes were analyzed, the most prevalent was the 14 with 44%. The risk factors found in relation to the *S. pneumoniae* resistance were: age under one year old (p=0.01) and previous use of antibiotic (p=0.046). The resistance rates found, which were moderate to penicillin, low to cephalosporin and neutral to vancomycin, suggest the isolated use of a 3rd generation cephalosporin as an initial empirical therapy for the treatment of acute bacterial meningitis with a communitarian background.

KEY WORDS: *Streptococcus pneumoniae*, pneumococcus, meningitis, antimicrobial resistance, cerebrospinal fluid.

Meningite bacteriana aguda por *Streptococcus pneumoniae* resistente aos antimicrobianos e seus sorotipos

Resumo – Este estudo teve como objetivo avaliar as taxas de resistência de *Streptococcus pneumoniae*, isolados de pacientes com meningite, à penicilina G, ceftriaxona e vancomicina; avaliar possíveis fatores de risco para resistência antimicrobiana; descrever os sorotipos encontrados e sugerir a terapêutica empírica inicial para meningite. Foram isoladas 100 amostras de *S. pneumoniae*, encontrando-se 15% de resistência à penicilina, 1% à cefalosporina e 0% à vancomicina. Os sorotipos mais encontrados foram 14 (19%), 3 e 23F (10% cada). Analisando-se os resistentes, o sorotipo 14 (44%) também foi o mais freqüente. Os fatores de risco para resistência de *S. pneumoniae* encontrados foram: idade menor que um ano (p=0,01) e o uso prévio de antibiótico (p=0,046). As taxas de resistência encontradas, moderada a penicilina, baixa para cefalosporina e nula para vancomicina, sugerem como terapêutica empírica inicial para tratamento da meningite bacteriana aguda de origem comunitária, a cefalosporina de terceira geração isoladamente.

PALAVRAS-CHAVE: *Streptococcus pneumoniae*, pneumococo, meningite, resistência antimicrobiana, líquor.

Streptococcus pneumoniae is one of the main causing agents for the respiratory tract infections, meningitis and sepsis, with high rates of lethality and morbidity. The evaluation of Paraná State's data since 2002 shows that the main etiological agent of the specified acute bacterian meningitis was the *Neisseria meningitidis*, with 948 cases (occurrence ratio of 2.3%) followed by the *S. pneumoniae, with* 358 cases (occurrence ratio of 0.9%). The pneumococcal meningitis recorded, in this period, the highest lethality level of 32%[1]. The efforts to decrease the pneumococcal infection's mortality are based, mainly, in the quick diagnosis and on an adequate antimicrobial therapy. In the last four decades, with the development of the *S. pneumoniae* resistant to the majority of drugs, commonly uti-

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lized for the treatment of their infections, including the penicillin, the matter has become of great importance. As a result, it has been considered a problem of public health, with a growing number of studies being conducted in this regard[2]. From 1980 onwards, pneumococcal resistance to penicillin rapidly increased and currently resistant strains are found in practically all continents, sometimes with rates above 70% and often associated with the resistance to other antibiotics[3]. In Brazil, according to data from Regional Vaccination System Project (SIREVA), the intermediate resistance rates to penicillin isolated from the invasive illnesses in children under six years of age varied from 24.6% in 2000 to 25.4% in 2005. High-level resistance varied from 5.7% to 16.5% in that same period[4].

The pneumococcal resistance to penicillin is due to the decrease of the antibiotic's affinity to the pneumococcal's cellular wall proteins (PBP - penicilin binding proteins). There is a progressive reduction of sensitiveness to the penicillin and, in varied degrees, to other β-lactamic antibiotics, causing the need of higher drug concentration to inhibit the bacterial growth[5]. This is a gradual chromosomically mediated process, probably by successive mutations in the genetic code and/or by the replacement of chromosomal segments for fragments derivated from other streptococci species[6]. In parallel to penicillin resistance, the pneumococcus can develop resistance to other β-lactamic drugs and to other antimicrobials, which is called multiple resistance or multiresistance, when present in three or more groups of antibiotics, being it more commonly detected amongst the isolated already penicillin resistant. In Brazil, there are reports of 4% multiresistance, with variations in the rates among the many antibiotics (cefotaxime 2.6%; sulfamethoxazole 65%; tetracycline 14.6%; erythromycin 6.2%; chloramphenicol 1.3%; and rifampicin 0.7%)[7].

There are no reports of *S. pneumoniae* strains resistant to vancomycin up to the moment. The epidemiological indicators are fundamental to evaluate, plan and implement control measures and adequate treatment for the reduction of morbidity and mortality due to pneumococcal infections. In Parana State, the profile of the pneumococcal resistance is unknown. And there is also a lack of data about the circulating serotypes, making difficult a consistent prophylaxis with the new heptavalent conjugated pneumococcal vaccine.

This study was made to evaluate the resistance rates of the *Streptococcus pneumoniae* to the main antibiotics utilized in the treatment, the penicillin G, ceftriaxone and vancomycin, in patients with acute bacterial meningitis; and also to evaluate the possible risk factors to the *S. pneumoniae* resistance and to describe the *S. pneumoniae* serotypes found in the region.

METHOD

The study was conducted on prospective basis, analyzing all positive cultures for *S. pneumoniae* isolated from samples of the cerebrospinal fluid (CSF) or serum from acute bacterial meningitis patients, which were received from the Paraná State Central Laboratory (Laboratório Central do Paraná - LACEN) in the period from April 2001 to August 2002.

A total of 3545 samples of patients with a clinical condition similar to acute bacterial meningitis were analyzed. Etiological agent was identified in 436 samples (12%) (Table 1).

The *S. pneumoniae* was isolated by culture in 100 cases which formed the study group. Only one isolated was from blood culture, with the data of CSF of that patient being also evaluated. All other strains were isolated from the CSF.

The identification of the bacteria was made through biochemical tests according to the ASM (American Society of Microbiology) Manual[8]. The sensitiveness to the antimicrobial agents – penicillin, ceftriaxone and vancomycin – was done using the E-test strips (AB BIODISK, Piscataway, New Jersey, USA), following the CLSI 2007 guidelines[9]. The minimum inhibitory concentration levels (MIC) in μg/mL for the bacteria to be considered sensitive, intermediate resistant or high-level resistant in relation to a drug were, respectively: ≤0.06; from 0.12 to 1.0 and

Table 1. Etiology of acute bacterial meningitis by age bracket in the State of Paraná: April 2001 – August 2002.

Identified agent	Age bracket (years)											
	<2		6–19		6–19		20–50		>50		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
S. pneumoniae	53	34.9	8	14.5	33	34.0	33	35.5	6	15.3	133	30.5
N. meningitidis B type	49	32.2	29	52.7	32	33.0	18	19.3	3	7.7	131	30.0
N. meningitidis C type	7	4.6	3	5.5	5	5.2	2	2.2	0	0	17	3.9
N. meningitidis Y/W135	1	0.7	3	5.5	3	3.1	3	3.2	0	0	10	2.3
H. influenzae B type	11	7.2	7	12.7	3	3.1	1	1.1	0	0	22	5.1
Others	31	20.4	5	9.1	21	21.6	36	38.7	30	77	123	28.2
Total	152	100	55	100	97	100	93	100	39	100	436	100

≥2.0 for penicillin; ≤0.5; 1.0; and ≥2.0 for ceftriaxone; and with only one sensitiveness cut when ≤1.0 for vancomycin[10].

The isolated strains were forwarded to the Adolf Lutz Institute (São Paulo SP, Brazil) for the purpose of confirming the species and serotypes base on the Neufeld-Quellung technique[10].

The risk factors were collected from epidemiological records of compulsory notification, patient's medical records, information obtained from the attending physician for each case, the responsible person for the patient, or the patients themselves.

In order to analyze the data, the samples were divided in two groups according to the penicillin susceptibility. The Pearson Correlation Coefficient and the Mann-Whitney non-parametric tests (through the "primer of biostatistics" software), Chi-square and Fisher's Exact (through the Epi-Info software) were utilized for the statistical analysis. The level of significance adopted was lower than 5% ($p<0.05$).

This study was duly evaluated and approved by the Independent Ethical Committee on Research in Human Beings of the Hospital das Clinicas of the Paraná State Federal University (UFPR - Universidade Federal do Paraná).

RESULTS

Epidemiology

The sample group sensitive to penicillin (N=85), presented 59 (69%) isolated male patients, with ages ranging from one day to 73 years old, with a median of 16 years old.

The second group of samples resistant to penicillin (N=15), were eight (53%) isolated from male patients, with a median age of a year old, varying from three months to 50 years old.

Resistances and serotypes

From the 15 penicillin resistant samples which were isolated, 14 (93%) have shown an intermediate resistance and only one (7%) a high-level resistance (MIC 2 μg/mL). This same sample (1%) was the only one that showed resistance to cephalosporin (MIC 1 μg/mL). All the samples were sensible to vancomycin.

Evaluating the penicillin resistance rate in relation to the age, patients younger than five years old had a 26,5% resistance rate and patients under two years old had a 30% rate.

Sixty-eight (68) samples were serotyped (Table 2). In patients under two years old, the most common serotype found was the 14 present in 12 cases (52%). The serotypes 6B, 7F, 18C and 19F appeared in two cases (8,7%) and the remaining 8, 9V, 19F, 23B appeared in just one case (4,4%).

Risk factors

The group of patients with resistant samples not only presented a lower age median (one year old) compared to the other group (16 year old) ($p=0.01$) but also a higher number of previous use of antibiotic (45.5% whilst the other group had a 16.4% rate) ($p=0.046$). The previous use of antibiotic was mainly secondary to upper airway tract infection (acute otitis media and tonsillitis), within a two months period before the meningitis.

In the sensitive group, most cases occurred from July and August. However, in the resistant group, cases of meningitis were observed during all period studied (Figure).

The other possible risk factors characteristics investigated are described in Table 3.

Antibiotic therapy used

Sixty-six percent have made use of third generation cephalosporin (ceftriaxone of cefotaxime) as sole drug (61%) or associated to vancomycin (5%), 27% penicillin or ampicillin, and 12% vancomycin. In seven of these patients that use vancomycin (70%), the drug was introduced during the evolution of the case. Thirteen patients (16%) needed to have an antibiotic change due to bad clinical evolution, but only three of these patients presented the penicillin resistant *S. pneumoniae*.

Clinical outcome

The total lethality was 29%, of which 21% in the group of patients with resistant samples and 31% in the group of patients with sensitive samples ($p=0.366$).

Table 2. Isolated S. pneumoniae serotypes in meningitis in the State of Paraná in the period from April 2001 to August 2002 – 68 serotyped samples.

Serotype	Nº samples	%
14*	13	19
3 and 23F*	7	10
19F	5	7.4
18C and 7F	4	5.9
10A, 6A and 6B*	3	4.4
4, 8, 22F, 9V and 24F	2	2.9
5*, 7C, , 12F, 15B, 17, 18A, 18B, 23B and 35A	1	1.5

*Resistant samples – serotype 14: 4 (44%); 23f: 3 (33%); 6b: 1 (11%); 5: 1 (11%).

Table 3. Risk factors for S. pneumoniae resistance in the studied population.

Characteristics	Sensitive group		Resistant group		p value
	N	%	N	%	
Patient's origin					
Capital	41	48	5	33	0.431
Countryside	44	52	10	67	
Source					
Hospital strain	4	5	1	7	0.572
Community strain	79	95	14	93	
Race					
Caucasians	42	76	8	73	0.532
Blacks	2	4	0	0	
Indian	1	2	0	0	
Mestizo	10	18	3	27	
Age median (years)	16		1		0.010
Prior use of antibiotic					
Yes	9	16.4	5	45.5	0.046
No	46	83.6	6	54.5	
Baseline disease					
Yes*	17	30	5	46	0.262
No	39	70	6	55	
Other infectious focus					
Yes**	14	26	0	0	0.223
No	41	74	11	100	
Institutionalized patient					
Yes	3	4	1	8	0.458
No	77	96	12	92	
Secondary case					
Yes	2	3	1	7	0.446
No	65	97	14	93	
Pneumococcal vaccination					
Yes	0	0	1	24	0.183
No	67	100	3	76	

Main causes: *History of encephalic cranial traumatism; **Upper respiratory infections.

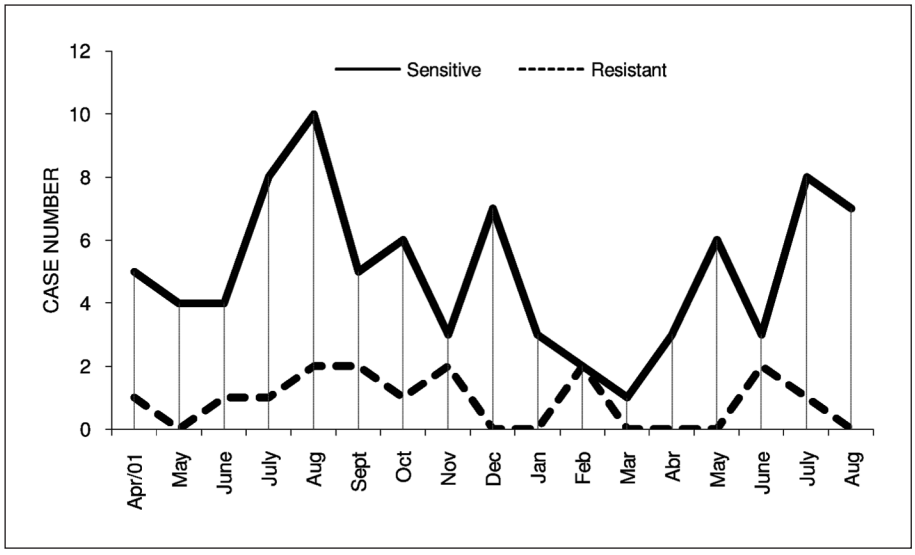

Figure. Seasonal distribution of the meningitis cases in the studied groups

Table 4. Evaluation of the cytological and biochemical characteristics of the CSF of patients of the studied groups.

Characteristics	N	Mean	Standard deviation	Median	p value
Sensitive group					
Number of cells (mm^3)	78	3 431.5	9531.4	400.5	0.975
PM (%)	72	83.1	19.8	–	0.848
LM (%)	72	14.6	18.0	10.0	0.608
Proteins (mg/dL)	67	630.8	1325.0	227.2	0.077
Glucose (mg/dL)	76	19.5	26.8	6.8	0.518
Resistant group					
Number of cells (mm^3)	15	1 531.7	2 010.3	251.0	
PM (%)	15	80.9	19.9	–	
LM (%)	15	19.8	20.9	18.0	
Proteins (mg/dL)	15	207.5	171.0	116.0	
Glucose (mg/dL)	14	20.3	27.9	4.4	

Amongst the 68 patients who survived, some neurological complications such as convulsions, abscesses, motor deficit, dyslalia, hydrocephaly, and coma were observed in 27 patients (47%) of the group of sensitive samples and in seven patients (63%) of the resistant sample group (63%). One patient (2%) of the sensitive sample group presented other clinical complications such as sepsis and pneumothorax, while two (18%) of the resistant sample group presented shock and renal failure (p=0.489).

Nine patients (16%) of the sensible sample group presented sequels. The most common sequel presented in this group was the cranial nerve III involvement. Only one patient of the resistant sample group (9%) presented a sequel, dyslalia (p=0.066).

Death occurred later in patients who presented the resistant *S. pneumoniae* than in patients of the sensitive group (median of 6.0 and 3.0 days) (p=0.339). The non neurological clinical complications were also predominant in the patients of the resistant group in comparison to the patients of the sensitivity group (18% versus 2%) (p=0.066).

CSF characteristics

The cytological and biochemical characteristics of the CSF in both groups are indicated in Table 4. The total protein concentration in the CSF samples of the patients with resistant *S. pneumoniae* was lower than in the patients with sensible *S. pneumoniae* (median 227.2 mg/dL and 116.0 mg/dL) (p=0.077). No significant difference was observed in the variables studied.

DISCUSSION

This is the first study of local rates describing a resistance rate to the penicillin of 15%, with the predominance of intermediate resistance. However, this rate increases to 30% when analyzed a population of greater risk for pneumococcal invasive diseases, two years old and younger. These results are similar to other studies conducted in the same period in other Brazilian regions[11-13].

Pneumococcal resistance to the cephalosporin group has increased gradually. Studies conducted in North Carolina show a decrease in the susceptibility to cefotaxime from 85% in 1996 to 77%, in 2000, leading to failure in the treatment of meningitis with cephalosporin[14,15]. In these studies, only one child presented elevated MIC (1.0 μg/mL for cephalosporin and 2.0 μg/mL for penicillin). It was a strain serotype 14, isolated in an eight-month old child, without co-morbidities and who had taken adequately antibiotic therapy for acute otitis media four months before the meningitis. This patient did not attend regularly to a day care center and had good evolution without sequels with the use of ceftriaxone (only therapy).

In this study, no strains were resistant to vancomycin, being this drug indicated as a therapeutic option in the case of multiresistant strains, together with the cephalosporin, never sole, due to little penetration in the central nervous system. Even when there is a pneumococcal resistance to cephalosporin, such an association can be indicated, combined or not to rifampicin[15,16].

The pneumococcal resistance presents a large amount of risk factors: the extremes of age, seasons of the year, guest's health conditions, history of recurring infections, previous use of antibiotics, frequent attendance to day-cares centers or to closed environments, recent hospitalization, type of infection and the nature of the material analyzed. Resistant samples are more easily isolated in nasopharyngeal smears, expectorations and middle ear secretions than in relation to invasive strains obtained from blood or CSF[17].

The antimicrobial resistance rates vary according to the characteristics of the population studied. It has been demonstrated a higher prevalence of resistance in lower age children, as observed in this study. This is justified by the higher incidence of respiratory infections in this age bracket and, consequently, to the greater use of antibiotics. The greater use of antibiotics, often inadequate, in the infant population, not only makes it more susceptible to pneumococcal infections, but mainly the major reservoir and source of transmission of resistant strains[18].

From the 68 serotyped strains in this study, 47 (69%) are reported as the most frequent in Brazil the serotypes 3, 4, 5, 6A, 6B, 9V, 14, 18C, 19F, 23F[13]. The casuistic of the present study is small for a definitive conclusion about the distribution of the pneumococcal serotypes in our region. Nevertheless, it was possible to observe that in two year old children or younger the serotype 14 continued to be the most frequent and that 83% of the isolated serotypes are present in the heptavalent conjugated vaccine. In this study, it was observed a larger coverage than what have been published in Brazil, around 70%[5]. This reinforces the importance of knowing the region's most prevalent serotypes, considering that there is already available an effective vaccine against pneumococcus, which can be applied in two year old children or younger. One of the greatest problems of the vaccination with the heptavalent pneumococcal vaccine, besides the lack of knowledge of the circulating serotypes, is its high costs. However there are already studies proving that the cost-effectiveness relation of the vaccine is favorable, since there has been an important decrease in the invasive pneumococcal illness, in all age brackets, in the countries that have introduced the pneumococcal vaccine as a routine in the vaccine national calendar[18-21].

Some serotypes, 6A, 6B, 9V, 14, 19A, 19F, and 23F[22-24], are associated to resistance, colonization and infection in pediatric patients, making this population of greater risk, as well as it was perceived in this study. Several authors described the relation between a resistant *S. pneumoniae* infection and the previous use of antibiotics during the three months before the clinical disease[24]. In this casuistic, a statistical difference, between the two studied groups, was found. In the group of patients resistant to penicillin there was a greater use of antibiotics, mainly in the last two months before the clinical disease and due to respiratory tract infections.

There are not studies on cytological and biochemical characteristics in the CSF related to bacterial resistance. In this study, the protein concentration in the CSF in the samples of the group of sensitive patients was five times greater than the standard concentration value, whilst in the group of resistant patients it was only two and a half times greater than the standard value, indicating trend. Possibly, if the number of samples is increased, a statistical difference can be confirmed.

Since the concentration of antimicrobials in the CSF is limited, due to the blood-brain barrier, the need to know the local susceptibility of the pneumococcal invader to the antimicrobials is essential for the initial empirical treatment to be safe and adequate. In meningitis, differently from other infection sites, even when occurs intermediate resistance, it is mandatory to change the therapy.

The importance of the *S. pneumoniae* resistance to penicillin is a world health issue, and due to its variation throughout the regions in the world, each local must know its incidence to better adjust an efficient therapy.

The high levels of resistance found in this study confirm the need of a constant evaluation of the resistance profile, considering that with the data found in Paraná State, the initial empirical therapy for the treatment of meningitis of communitarian background, in the age brackets of patients, where the pneumococcus is one of the main etiology, must be done on a routine basis with a third generation cephalosporin and, at the moment, without the need of initial complementary therapy with vancomycin. The empiric association with vancomycin is only recommended when the pneumococcal resistance rate to cephalosporin is equal or higher than 5%[25].

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