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RESISTANCE OF *STREPTOCOCCUS PNEUMONIAE* TO ANTIMICROBIALS IN SÃO PAULO, BRAZIL: CLINICAL FEATURES AND SEROTYPES

Anna Sara S. LEVIN (1), Lucia Martins TEIXEIRA (2), Juliana Furian SESSEGOLO (2) & Antonio Alci BARONE (1)

SUMMARY

To study resistance to antimicrobials, serotypes and clinical features of *S. pneumoniae* in S. Paulo, Brazil, 50 patients with a positive culture were evaluated: 7 were considered carriers and 43 had pneumococcal infections. Pneumonia and meningitis were the most commom infections. Mortality was 34% and underlying diseases were present in 70%. Relative resistance to penicillin occurred in 24% and complete resistance was not detected. Resistance to tetracycline was 32% and to sulfamethoxazole/trimethoprim 32%; one strain had intermediate susceptibility to erythromycin; no resistance was present for chloramphenicol, rifampin or vancomycin. Resistance to at least one of the drugs tested occurred in 62%. Results by the E-test for penicillin were similar to those by the agar dilution method. There were 24 different serotypes and 74% of the strains belonged to the 23-valent vaccine including all the penicillin-resistant strains. In this study *S. pneumoniae* caused severe infections and presented a high resistance rate to commonly used antimicrobials. Routine surveillance of resistance and the use of vaccination, as well as the restriction of inappropriate use of antimicrobials, are recommended in São Paulo, Brazil.

KEYWORDS: *Streptococcus pneumoniae;* Pneumococcal infections; Resistance; Serotypes.

INTRODUCTION

Invasive pneumococcal infections are an important group among the bacterial infectious diseases especially because of their severity, complications and mortality. Another important aspect of these infections is the emergence of resistance to various commonly used antimicrobial agents that has occurred in many parts of the world. Few studies have been made in Latin America[14, 15], and it is important to know the situation in our environment as an orientation for empirical treatment of pneumococcal infections.

The objective of this study is to describe the clinical features of infections by *Streptococcus pneumoniae*, test susceptibility to various antimicrobial drugs (including penicillin) and determine the serotypes present in São Paulo, Brazil.

METHODS

From July to December 1991 50 patients of Hospital das Clínicas of the University of São Paulo with a positive culture for *S. pneumoniae* were studied.

Infections were defined based on criteria from the Centers for Disease Control-1988, and 7 patients were considered to be colonized but not infected by the microorganism.

Clinical information was obtained by following-up the patients and from their medical charts.

Microbiological identification of *S. pneumoniae* was made based on morphology, optochin susceptibility and bile-solubility tests.

(1) Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil.
(2) Laboratório de Apoio Microbiológico do Departamento de Microbiologia Médica do Instituto de Microbiologia da Universidade Federal do Rio de Janeiro, R.J., Brasil.
Correspondence to: Anna Sara Shafferman Levin. Rua Harmonia, 564/52, 05435-000 São Paulo, SP, Brazil. E-mail: nivel@usp.br. Phone: 55-11-815-0709, FAX: 55-11-881-1140

Susceptibility tests to the following antimicrobial agents were performed by the agar dilution method using recommendations by the NCCLS: penicillin, chloramphenicol, cotrimoxazol, erythromycin, rifampin, tetracycline and vancomycin.

The E-test (AB Biodisk) was used for testing susceptibility to penicillin.

All susceptibility testing was performed in the Microbiology Laboratory of the Department of Microbiology of the Federal University of Rio de Janeiro.

Serotyping was performed using the capsular precipitin reaction with anti-serum supplied by the Centers for Disease Control, Atlanta, USA. The Danish nomenclature was used.

RESULTS

Clinical features: Among the 50 patients studied pneumonia and meningitis were the most common infections. Seven patients were carriers and 43 had pneumococcal infection. There were 12 children (ages: 15 days to 3 years; mean: 1 year and 8 months) and 38 adults (ages: 24 to 83 years; mean: 41 years). Twenty-two patients were female (44%).

The distribution of infections and the mortality rate can be seen on Table 1.

Overall mortality was 34%. Among the carriers 2 deaths occurred unrelated to *S. pneumoniae*. Among the cases of infection total mortality was 34.9%; among the children 16.7% and among adults 41.9%.

Bacteremia occurred in 69.8% of the infections and mortality was 50%. No deaths occurred among non-bacteremic cases.

Underlying diseases occurred in 70% of the patients: cancer (7 patients); HIV infection (5); severe hepatopathy (4); chronic pneumopathy (3); diabetes mellitus (3); alcoholism (3); neurologic disease (3); CSF leak (2); and chronic otitis media; severe burn; severe coronary disease; rheumatic disease; bone-marrow transplant; renal failure; nephrotic syndrome; severe leukopenia (one patient each). Three patients had more than one disease.

Mortality was 40% among patients with underlying diseases and 20% among the others.

Only 2 patients had nosocomial pneumococcal infections.

Resistance to penicillin: Minimal inhibitory concentrations (MIC) of the 50 samples tested varied from 0.015 to µ1 g/ml (Table 2), and 12 (24%) presented relative resistance to penicillin (MIC from 0.12 to 1 µg/ml). Among these 12 relatively resistant strains 5 were susceptible to all the other drugs tested, 6 were resistant to cotrimoxazol (MIC of at least 4/76 for trimethoprim and sulfamethoxazole respectively), and 1 was resistant to tetracycline.

There were 4 bacteremic infections among the cases of resistance to penicillin and death occurred in 3 of these patients. Two relatively resistant samples were isolated from carriers.

One 72 year-old patient presented meningitis and evolved with a complete cure receiving IV penicillin G (24 million units daily) although the MIC for the infecting strain was 0.25 µg/ml.

E-test: The MIC values obtained using the E-test varied from 0.016 to µ1 g/ml, and MIC_{50} and MIC_{90} were 0.023 and 0.125 µg/ml respectively. Compared to the agar dilution

TABLE 1
Infectious diagnosis of the 50 patients studied.

Infectious Diagnosis	Number of Cases (%)	Adults (%)	Children (%)	Mortality
Pneumonia	17 (34%)	11 (28.9%)	6 (50%)	35.3%
Meningitis	10 (20%)	9 (23.7%)	1 (8.3%)	40%
Peritonitis	3 (6%)	2 (5.3%)	1 (8.3%)	66.7%
Primary Bacteremia	3 (6%)	3 (7.9%)	–	66.7%
Cellulitis	2 (4%)	1 (2.6%)	1 (8.3%)	–
Conjunctivitis	2 (4%)	–	2 (16.7%)	–
Sinusitis	2 (4%)	2 (5.3%)	–	–
Others*	4 (8%)	3 (7.9%)	1 (8.3%)	25%
Carriers	7 (14%)	7 (18.5%)	–	28.6%
Total	50 (100%)	38 (100%)	12 (100%)	15 (34%)

* one case of each: skin infection, appendicitis, amigdalitis, otitis media.

TABLE 2
Susceptibility of *S. pneumoniae* to 8 antimicrobial agents tested (50 samples).

Antimicrobial	Variation*	MIC_{50}*	MIC_{90}*
Chloramphenicol	2-8	4	4
Erythromycin	0.015-1	0.03	0.12
Penicillin	0.015-1	0.03	0.25
Rifampin	0.015-0.25	0.06	0.12
Tetracycline	0.12-64	1	64
Trimethoprim/Sulfamethoxazole	0.06/1.2-16/304	0.5/9.5	8/152
Vancomycin	0.12-0.5	0.25	0.5

* Values expressed in μg/ml.

test MICs obtained were the same or varied 1 titer in all the samples except for 2 in which the interpretation was not affected.

Considering the agar dilution test as the gold-standard relative resistance to penicillin was detected by the E-test with a sensitivity of 75%, specificity of 97.4%, a positive predictive value of 90%, and a negative predictive value of 92.5%.

Resistance to other antimicrobial agents: Among the 50 samples 32% presented resistance to tetracycline (MIC above 8 μg/ml) and 4% showed intermediate susceptibility (MIC of 8 μg/ml); 32% were resistant to cotrimoxazol (MIC above 2/38 μg/ml for trimethoprim and sulfamethoxazole respectively). One sample presented intermediate susceptibility to erythromycin (MIC of 1 μg/ml).

Resistance to at least one of the drugs tested occurred in 62% of the samples. 26% of the samples were resistant to 2 drugs simultaneously. There were no samples resistant to 3 drugs.

MICs for all the drugs tested can be seen in Table 2. There were 6 patterns of resistance:

Penicillin only: 5 samples (10%);

Penicillin and cotrimoxazol: 6 samples (12%);

Penicillin and tetracycline: 1 sample (2%);

Tetracycline only: 9 samples (18%);

Cotrimoxazol only: 4 samples (8%);

Tetracycline and cotrimoxazol: 6 samples (12%).

Serotyping: The 50 samples tested belonged to 24 serotypes as can be seen in Figure 1. Thirty-seven samples (74%) belonged to serotypes included in the 23-valent anti-pneumococcal vaccine. Considering that the vaccine can provide protection against serotypes 6A and 18B the proportion can be considered to be 80%.

The 12 samples relatively resistant to penicillin belonged to 5 serotypes included in the vaccine: 14, 23F, 3, 6B and 19A.

Among the samples susceptible to penicillin but resistant to other drugs 68.4% were of serotypes included in the vaccine (78.9% if included serotype 6A).

DISCUSSION

The most common pneumococcal infections in this study were pneumonia and meningitis accounting for 54% of the patients. Bacteremia was present in 30 patients (60%) or 69.8% of the infections, and death only occurred among the bacteremic infections. Mortality among bacteremic infections was 50%. The high mortality rate among *S. pneumoniae* infections in this study might be a reflection of the strict criteria used to define pneumococcal infections, leading to a large proportion of severe cases (70% of the patients had underlying diseases). It must however be noted that pneumococcal disease occurs more frequently among patients with chronic pulmonary or cardiac disease, diabetes mellitus, renal failure, nephrotic syndrome, organ transplants, HIV infection and other immunosupressive conditions.

Mortality among patients with underlying diseases was 40% compared to 20% among the others but this difference was not considered statistically significant.

Only 2 cases were considered nosocomial infections probably endogenous infections from a community-acquired carrier state as opposed to what has been shown in South Africa where nosocomial transmission of resistant strains occurred[6].

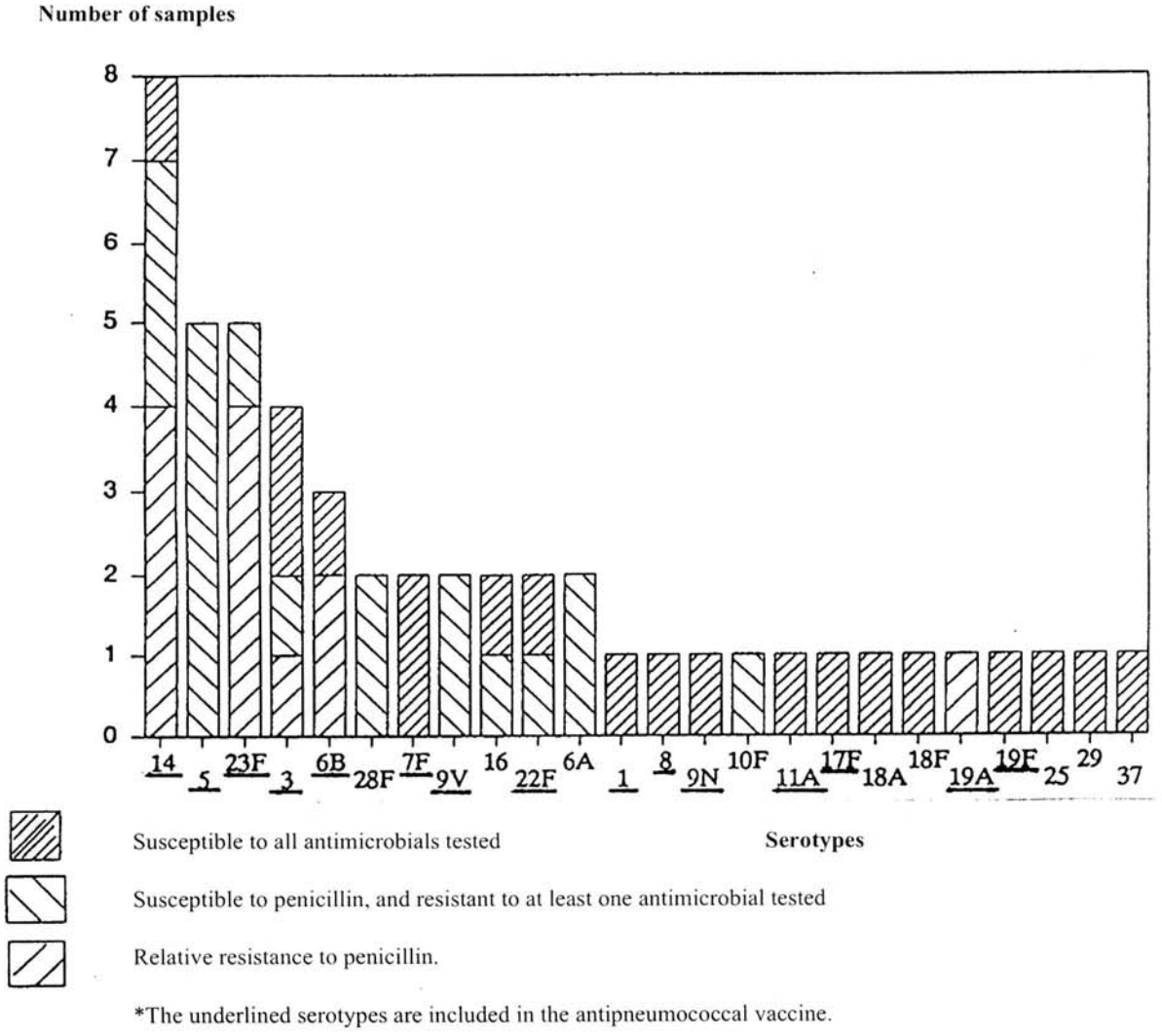

Fig. 1: Serotype distribution of the 50 samples of *S. pneumoniae*.

There were 7 asymptomatic carriers of *S. pneumoniae* in this study, 2 of which were relatively resistant to penicillin. Considering that approximately 1 in each 6 carriers will develop a pneumococcal infection within a month, the high proportion of resistance among asymptomatic carriers might be an important therapeutic problem. As there are no well established methods of prevention of transmission of these resistant strains, immunization of the high risk population should be considered. The flaw is that there is a poor response to the vaccine among children under 2 years and some studies have demonstrated the importance of the transmission in day-care settings within this age group[4, 13].

The anti-pneumococcal vaccine available in Brazil is the American 23-valent. Among the 50 samples studied 74% belonged to serotypes included in the vaccine, and if we consider cross-protection for serotypes 6A and 18B this figure goes up to 80% which indicates that the use of the vaccine in our country is valid.

Resistance of *S. pneumoniae* occurred in 24% of the samples studied; however all were relatively resistant (MIC up to 1 μg/ml) and no full resistance was encountered.

In various countries resistance to penicillin has been an emerging therapeutic problem, i.e. South Africa, Spain, Papua-New Guinea, Hungary, Rumania, Bangladesh, France, Israel, and some regions of the USA with the presence of full resistance (MIC above 1 μg/ml) as well as relative resistance[1]. In Brazil there probably has

been a gradual increase in penicillin resistance since 1981 as can be seen in 2 previous studies: the first study[15], in Rio de Janeiro, from 1981 to 1982 showing the absence of resistance to penicillin. However in this study there were samples with MICs of 0.03 and 0.06 g/ml. The second study by the same group[14] demonstrated 14.7% of relative resistance to penicillin during the period of 1988 to 1990. Our study confirms these findings.

Our study showed a high incidence of resistance to other drugs commonly used to treat pneumococcal infections – 32% to tetracycline and 32% to cotrimoxazol. Considering that strains with intermediate susceptibility should be considered resistant for practical means, the incidence of resistance to tetracycline was 36%, comparable to that of countries such as Spain[2, 7, 9], South Africa[12], Hungary[10], Rumania[11] and France[3]. Many antimicrobials are sold over the counter in Brazil without any need of a medical prescription and are usually administered inappropriately. Tetracycline and cotrimoxazol are among the most popular drugs used in this way and this might explain the high resistance encountered in this study. Antibiotics are drugs that have been considered unique because of their ability to treat populations as well as individuals[8] and the high usage of antimicrobials can affect the susceptibility of the whole pool of *Streptococcus pneumoniae* in the general population, mainly carriers. Two measures can be taken in order to control resistance of *S. pneumoniae* in Brazil: restrictions on the sale and prescription of antimicrobials; and an increase in hygiene that goes with the improvement of sanitary conditions leading to a decreased need for these drugs.

With regard to erythromycin one sample presented intermediate susceptibility. Resistance to erythromycin should be monitored because of the importance of the macrolides in the treatment of community-acquired pneumonia.

Sixty-two percent of the samples presented resistance to either one or two of the drugs tested. No samples was resistant to 3 drugs. No resistance to chloramphenicol, rifampin or vancomycin was encountered.

Chloramphenicol might be considered an alternative in the treatment of pneumococcal disease; however there have been reports of failures in the treatment of meningitis caused by penicillin-resistant *S. pneumoniae*, although these cases showed susceptibility to chloramphenicol "in vitro". The mechanisms involved in these failures are not yet well understood[5].

Rifampin could be an alternative drug; however there have been reports of the emergence of resistance during treatment[7], limiting its use.

Vancomycin demonstrated good activity "in vitro" and can be considered a useful drug to treat infections by *S. pneumoniae* resistant to other drugs.

Cephalothin may be useful in the treatment of infections by resistant pneumococci; however in the presence of high levels of resistance to penicillin the alterations in PBPs will probably also limit its effectiveness.

Possible good alternative treatments of community-acquired meningitis in our environment are the 3rd generation cephalosporins, as the presence of relative resistance to penicillin is prevalent and adequate levels of penicillin in the central nervous system might not be achieved. Until results of CSF Gram stain and culture are ready, the initial treatment with these cephalosporins should be considered because of their broad spectrum that includes *S. pneumoniae* (resistant to penicillin or not), *Neisseria meningitidis* and *Haemophilus influenzae* (important in pediatric patients).

The E-test proved to be a simple and reproducible quantitative method for the detection of resistance to penicillin by *S. pneumoniae*. The results obtained by the E-test compared well to those by the agar dilution method, although it led to erroneous interpretations in 3 cases.

The presence of resistance of *S. pneumoniae* to penicillin and other commonly used drugs in our environment makes it important to maintain constant surveillance for drug resistance especially in material obtained from clinically significant sites such as blood, CSF, pleural fluid and other usually sterile sites.

RESUMO

Resistência antimicrobiana de *Streptococcus pneumoniae* em São Paulo, Brasil: quadro clínico e sorotipos.

Com a finalidade de estudar resistência a antimicrobianos, sorotipos e quadro clínico de *Streptococcus pneumoniae* em São Paulo, Brasil, foram avaliados 50 pacientes com culturas positivas: 7 foram considerados portadores e 43 infectados. Pneumonia e meningite foram as infecções mais freqüentes. A letalidade foi de 34% e doenças de base estiveram presentes em 70%. Resistência relativa a penicilina ocorreu em 24% e a resistência completa não foi detectada. Resistência a tetraciclina ocorreu em 32% e a sulfametoxazol/trimetoprim em 32% e houve uma cepa com sensibilidade intermediária a eritromicina. Não houve resistência a cloranfenicol, rifampicina ou vancomicina. Em 62% dos casos houve resistência a pelo menos uma das drogas testadas. Resultados

de sensibilidade utilizando o E-teste resultaram semelhantes aos pelo método de diluição em ágar. Houve 24 sorotipos diferentes e 74% das cepas pertenciam a sorotipos vacinais incluindo todas as cepas resistentes a penicilina. Neste estudo *Streptococcus pneumoniae* causou infecções graves e houve uma alta incidência de resistência a drogas comumente utilizadas. Os autores recomendam a vigilância rotineira para resistência e o uso adequado de vacinação, além da restrição do uso inadequado de antimicrobianos.

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