New susceptibility breakpoints in antimicrobial resistance rates of invasive pneumococcal strains

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Abstract

Objective: To evaluate the impact of new penicillin susceptibility breakpoints on resistance rates of pneumococcal strains collected from children with pneumonia.

Methods: Pneumococcal strains collected from patients admitted with pneumonia were isolated at the clinical analysis lab of Hospital de Clínicas de Uberlândia, Uberlândia, Brazil, and sent to Instituto Adolfo Lutz, São Paulo, Brazil, for further identification, serotyping and determination of antimicrobial susceptibility.

Results: From April 1999 to December 2008, 330 strains of pneumococcus were sent to Instituto Adolfo Lutz; of these, 195 (59%) were collected from patients with pneumonia. One hundred strains collected from patients ≤ 12 years old were analyzed. The patients’ age ranged from 1 to 12.6 years old (with mean age of 2.4 and median of 1.7 years). Forty-seven patients were male. The strains were isolated from blood (42%) and pleural fluid (58%). There were 35 oxacillin-resistant strains: according to the criteria defined by the Clinical and Laboratory Standards Institute (CLSI) in 2007 [minimum inhibitory concentration (MIC) ≤ 0.06 µg/mL for susceptibility (S), 0.12 to 1 µg/mL for intermediate resistance (IR), and ≥ 2 µg/mL for full resistance (FR)], 22 strains had IR and 11 strains had FR. According to the current breakpoints defined by the CLSI in 2008 (≤ 2 µg/mL for S, 4 µg/mL for IR and ≥ 8 µg/mL for FR), only one strain had IR to penicillin. There was resistance to co-trimoxazole (80%), tetracycline (21%), erythromycin (13%), clindamycin (13%), and ceftriaxone (one strain simultaneously resistant to penicillin).

Conclusions: When the new breakpoints for in vitro susceptibility were applied, penicillin resistance rates dropped 97%, from 33 to 1%.


Introduction

Streptococcus pneumoniae, pneumococcus, is one of the most frequent agents of pneumonia, acute otitis media, meningitis, and sinusitis in children.1,2 It is responsible for high rates of morbidity and mortality among children younger than 5 years old and adults older than 65, especially in developing countries.2,3 Community pneumonia, one of the most common types of pneumococcal disease in children and adults, usually presents suggestive clinical and radiologic
susceptibility breakpoints for pneumococcal resistance - Wolkers PC et al.

The clinical evolution of the pneumococcal infection is influenced by several factors, such as age, underlying disease, topography, severity of infection, and treatment appropriateness.\textsuperscript{2} The treatment of pneumococcal infections is based on initial antibiotic therapy, which is usually empirical in terms of etiology and in vitro susceptibility to antimicrobial agents. After performing bacterial culture isolation and antibiogram test, the antibiotic therapy can be adequately adjusted. Penicillin is the drug of choice for many pneumococcal diseases\textsuperscript{1,2}; however, with the increasing description of penicillin-resistant strains since the 1980s, some alternative regimens have been suggested.\textsuperscript{6-9}

Based on the positive response to the treatment with β-lactam antibiotics (penicillin or ampicillin) of patients with invasive pneumococcal diseases (except for meningitis), even when caused by strains with minimum inhibitory concentration (MIC) up to 2.0 µg/mL, a redefinition of the susceptibility categories has been recommended.\textsuperscript{6}

With the purpose of monitoring the pattern of resistance to antimicrobial agents and the profile of pneumococcal serotypes in Latin America, the Pan American Health Organization (PAHO) created the Regional System for Vaccines (Sistema Regional de Vacinas, SIREVA) in 1993 with the participation of six countries, including Brazil. Since 1994, this program has been broadened to include other bacteria (\textit{Haemophilus influenzae} and \textit{Neisseria meningitidis} in addition to pneumococcus) and other countries (currently 20 countries take part in this project), being called SIREVA II.\textsuperscript{10-12} It is a laboratory surveillance program that analyzes (counts and describes) laboratory characteristics, such as serotype and in vitro resistance pattern of the strains collected. Since April 1999, Uberlândia, Brazil, has been participating in the national network of the SIREVA Project by sending pneumococcal strains isolated at the clinical analysis lab of Hospital de Clínicas de Universidade Federal de Uberlândia (HC-UFU), Uberlândia, to Instituto Adolfo Lutz (IAL), São Paulo, Brazil.

As the resistance pattern of antibiotics and the prevalence of pneumococcal serotypes vary according to different populations from different geographic regions and, probably, throughout time as well, it is extremely important to define the profile of the most prevalent serotypes in a given community and to establish the resistance rates to support the choice of the best initial empirical treatment.\textsuperscript{13}

**Objectives**

To assess resistance rates to penicillin and other antimicrobial agents in pneumococcal strains collected from children hospitalized at HC-UFU with diagnosis of pneumonia according to the traditional criteria,\textsuperscript{14} as well as penicillin resistance rates based on the current criteria,\textsuperscript{15} and the impact of the new criteria on penicillin resistance rates.

**Methods**

This is a prospective, case series, laboratory surveillance study, whose laboratory data on serotyping and in vitro susceptibility to antimicrobial agents of pneumococcal invasive strains were collected from patients hospitalized at HC-UFU with diagnosis of pneumonia. The index case is a pneumococcal strain isolated at the clinical analysis lab of HC-UFU from clinical specimens (blood or pleural fluid) of an inpatient with pneumonia. The patients’ individual medical record included identification data (name, birth date, age, sex, address, admission and discharge dates, and length of hospital stay) and clinical data such as medical history, diagnosis, antimicrobial treatment, survival or death, and bacterial source. The medical team providing care to each patient was responsible for the diagnosis and clinical management, without any interference from the laboratory surveillance. Aseptically collected specimens were appropriately processed and seeded in blood culture flasks (blood sample) or chocolate agar and blood agar plates (pleural fluid) within the shortest time possible after collection and immediately after being delivered at the laboratory. Only one pneumococcal isolate per patient was considered in the present study. Pneumococcal strains were isolated and identified at HC-UFU according to internationally described methods and sent to the Bacteriology Department of IAL for specimen confirmation, serotyping and definition of in vitro susceptibility to antimicrobial agents.\textsuperscript{16,17} Pneumococcal strains were freeze-dried in skim milk at 20% and adequately catalogued.

Antimicrobial susceptibility was assessed using the disc diffusion technique for oxacillin (1 µg), tetracycline, ofloxacin, chloramphenicol, erythromycin, sulfamethoxazole-trimethoprim (co-trimoxazole), vancomycin, and clindamycin in Mueller-Hinton agar plates supplemented with sheep blood at 5% according to the standardized technique.\textsuperscript{18,19} Oxacillin-resistant strains (inhibition halo \textleq{} 19 mm) were submitted to MIC definition for penicillin using broth microdilution and classified as susceptible (S) (MIC \textleq{} 0.06 µg/mL), intermediate resistant (IR) (MIC = 0.12 to 1 µg/mL), and full resistant (FR) (MIC \textgeq{} 2 µg/mL), according to the traditional criteria.\textsuperscript{14,19} According to the current criteria,\textsuperscript{15} values for interpretation of MIC results were \textleq{} 2 µg/mL for S, 4 µg/mL for IR, and \textgeq{} 8 µg/mL for FR. Oxacillin-resistant strains were submitted to ceftriaxone MIC definition and considered S when MIC was \textleq{} 1 µg/mL, IR with MIC = 2 µg/mL, and FR with MIC \textgeq{} 4 µg/mL.\textsuperscript{20} Oxacillin-susceptible strains (inhibition zone > 19 mm) were considered penicillin-susceptible and were not submitted to MIC definition according to the 2007 CLSI standard.
Results

During a period of 9 years and 8 months, from April 1999 to December 2008, 330 samples were sent to the IAL lab. Of these, 195 (59%) were collected from patients with diagnosis of pneumonia. Twenty of these samples were excluded because the specimens were dead when they were delivered to the lab (15) or did not have appropriate records (5). Of the remaining 175 samples, 100 were collected from patients aged ≤ 12 years and were analyzed; 47 (47%) were collected from male individuals. The patients’ age ranged from 1 and 12.6 years (mean age = 2.4 years, standard deviation = 2.3 years, and median = 1.7 years) with an interquartile range (IR25-75%) from 1 to 3 years. The number of isolates according to age group was 60 in children up to 2 years old, 32 in children from 2 to 5 years old, and eight among patients between 5 and 12 years old. The number of valid samples collected during each year from 1999 to 2008 was 14, 20, 8, 12, 19, 5, 6, 6, and 4, respectively. The sources of the strains were blood for 42 samples (42%) and pleural fluid for the remaining 58 (58%).

Thirty-five oxacillin-resistant strains (35%) were detected, and according to the traditional criteria,14,19 22 (22%) strains were IR to penicillin and 11 (11%) strains were FR (total of 33%). According to the current criteria,15 only one strain was IR to penicillin (Table 1).

The distribution of the penicillin-resistant strains (only one, collected from a child aged 53 months) did not show a significant difference according to age group (younger or older than 5 years old).

Reduced susceptibility to co-trimoxazole was detected in 80% of the strains tested (IR = 5 and FR = 75). Resistance to erythromycin and clindamycin was found in 13% of the strains, all of them simultaneously resistant to both antibiotics. Tetracycline resistance rate was 21%, and there was no resistance to chloramphenicol, ofloxacin, rifampicin, and vancomycin.

IR to ceftriaxone was detected in only one strain simultaneously resistant to penicillin.

Discussion

Penicillin resistance was detected in 33% of the strains analyzed – according to the traditional criterion of in vitro susceptibility,14,19 a higher rate than those of 25.9 and 25.6% found in both national10 and international3 studies that used the same criterion, respectively. The Brazilian study assessed 4,169 pneumococcal invasive strains, and 878 of these were collected from patients with pneumonia from 2000 to 2005.10 The surveillance system of the SIREVA project is based on volunteer participation and it has attracted the collaboration of several research centers from different Brazilian states and Latin American countries in the last 13 years.10-13,16,17,21-24 The distribution of results according to the geographical region has revealed heterogeneity regarding the findings in the Brazilian territory. For instance, among the 94 strains analyzed in the state of Minas Gerais from 1993 to 1998, 12.8% were IR and 2.1% were FR.17 When this study was enlarged, including samples collected up to 1999 from children up to 6 years old, the total resistance rate was 20.7%,13 a rate similar to the 20% value found in other local studies carried out in Brazil.25,26 In the state of Minas Gerais, local studies have revealed rates of 11.8% of oxacillin-resistant strains27 and 15.5% of penicillin-resistant pneumococcal invasive strains according to the traditional criteria.28

According to the traditional criteria, the classification as resistant is clinically important in cases of acute otitis media and meningitis, but it is not relevant in cases of pneumonia.6,8 In fact, studies involving children and adults with pneumonia caused by penicillin-resistant pneumococcal strains (MIC up to 2 µg/mL) or ceftriaxone-resistant pneumococcal strains (MIC up to 1 µg/mL) have demonstrated an effective response to the treatment with penicillin or third-generation cephalosporin.6,8 Pharmacokinetic and pharmacodynamic data have confirmed these findings. The presence of serum or tissue concentration of β-lactam antibiotics higher than the MIC for pneumococcus during at least 40 to 50% of the time interval between the doses is a predictive indicator of

Table 1 - Penicillin-resistance rates according to the 2007 CLSI and 2008 CLSI standards in pneumococcal strains collected from children hospitalized with pneumonia (1999 to 2008)

<table>
<thead>
<tr>
<th>Resistance</th>
<th>n#</th>
<th>%</th>
<th>n¹</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Intermediate</td>
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<tr>
<td>Full</td>
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<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total¹</td>
<td>33</td>
<td>33</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

CLSI = Clinical and Laboratory Standards Institute.
# According to the 2007 CLSI standard.
¹ According to the CLSI 2008 standard.

CLSI = Clinical and Laboratory Standards Institute.
Clinical effectiveness. Such objective is reached by using penicillin at doses of 8 to 15 million units per day (intervals from 4 to 6 hours) in adults and from 100,000 to 300,000 U/kg/day (4 to 6 doses) in children for the treatment of pneumonia caused by a pneumococcal strain with MIC ≤ 4 μg/mL.

New breakpoints for in vitro susceptibility to penicillin have been recently suggested. After adopting the new criteria, there was a decrease in the resistance rates of 97%, from 33 to 1%, in the present study. In Brazilian studies, it has been also possible to find a decrease in the penicillin-resistance rates when using different criteria. When analyzing 768 invasive strains collected during 2006, among the 168 strains from patients with pneumonia, the authors found a penicillin-resistance rate of 39.3% (IR = 21.4% and FR = 17.9%). When analyzing 874 pneumococcal invasive strains collected during 2007, among the 162 strains from patients with pneumonia, the authors found a penicillin resistance rate of 12.3% (IR) according to the current criterion. In spite of the fact that these results are related to different samples, one collection from 2006 and one from 2007, the decrease in the resistance rate was significant (68.7%) for such a short period of time; therefore, we may assume that the main variable was the change in the criterion used to define resistance.

Regarding to the susceptibility of strains to other antimicrobial agents, it is possible to conclude that the high resistance rate found for co-trimoxazole (80%) is in agreement with the rates reported in other Brazilian studies (78.5, 65.7, 54.5, and 59.1%), and such high value may compromise the indication of this chemotherapy drug for the treatment of pneumococcal infections. Resistance rates for erythromycin (13%) and clindamycin (13%) remained relatively low; however, these rates are higher than those reported in other Brazilian studies for: erythromycin (2.4, 4.8, 10.1, 5.7, 23 and 3.8%) and clindamycin (2.9 and 3.1).

The fact that all 13 erythromycin-resistant strains were also resistant to clindamycin suggests the manifestation of the MLSB phenotype, characterized by the resistance to macrolides, lincosamides and streptogramin B. There was not in vitro resistance to chloramphenicol, ofloxacin, rifampicin or vancomycin.

The fact that there was only one pneumococcal strain IR to cefotaxime results in an estimated resistance rate of 0.9%, which is lower than the rate found in population surveillance studies conducted in Brazil. Of the 412 invasive strains collected from patients without meningitis during 2006 and 2007, there was a cefotaxime resistance rate of 9% (37/412), which was divided into 8.7% of IR (36/412) and 0.3% of FR (1/412). In the USA, the Active Bacterial Core Surveillance (ABCs) Report detected, among 3,514 invasive strains collected during 2007, a cefotaxime resistance rate of 6.9% (IR = 5.5 and FR = 1.4%).

The initial treatment for most pneumococcal infections remains being empirical in terms of etiology and susceptibility to drugs. After the detection of the etiological agent, the choice of the antibiotic is influenced by the laboratory classification (as susceptible or resistant, according to the MIC), and the adoption of new breakpoints must increase the rates of strains reported as being susceptible and consolidate the importance of the use of penicillin for the treatment of pneumococcal pneumonia.

References


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