

## *Streptococcus pneumoniae*: susceptibility to penicillin and moxifloxacin<sup>\*, \*\*</sup>

*Streptococcus pneumoniae*: sensibilidade a penicilina e moxifloxacina

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### Abstract

**Objective:** To determine the minimum inhibitory concentrations (MICs) of parenteral penicillin and moxifloxacin against *Streptococcus pneumoniae* strains isolated at a hospital center. **Methods:** In-vitro, prospective study involving 100 *S. pneumoniae* isolates collected from patients who had been treated, between October of 2008 and July of 2010, at the *Hospital das Clínicas* complex of the University of São Paulo School of Medicine, located in the city of São Paulo, Brazil. The isolates were obtained from respiratory tract cultures or blood samples unrelated to meningitis, and they were tested for penicillin and moxifloxacin susceptibility by E-test. The MIC category interpretations were based on updated standards. **Results:** All isolates were fully susceptible to parenteral penicillin (MIC  $\leq 2$   $\mu\text{g/mL}$ ), and, consequently, they were also susceptible to amoxicillin, ampicillin, third/fourth generation cephalosporins, and ertapenem. Of the *S. pneumoniae* strains, 99% were also susceptible to moxifloxacin, and only one strain showed an MIC = 1.5  $\mu\text{g/mL}$  (intermediate). **Conclusions:** Our results showed high susceptibility rates to parenteral penicillin and moxifloxacin among *S. pneumoniae* isolates unrelated to meningitis, which differs from international reports. Reports on penicillin resistance should be based on updated breakpoints for non-meningitis isolates in order to guide the selection of an antimicrobial therapy and to improve the prediction of the clinical outcomes.

**Keywords:** Drug resistance; Penicillin G; Pneumococcal infections; *Streptococcus pneumoniae*; Respiratory tract infections.

### Resumo

**Objetivo:** Determinar a concentração inibitória mínima (CIM) de penicilina parenteral e moxifloxacina contra cepas de *Streptococcus pneumoniae* isoladas em um centro hospitalar. **Métodos:** Estudo in vitro prospectivo de 100 isolados de *S. pneumoniae* coletados de pacientes tratados entre outubro de 2008 e julho de 2010 no complexo do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, em São Paulo (SP). Os isolados foram obtidos de culturas do trato respiratório e de amostras de sangue não relacionadas a infecções meningéas e foram testados quanto à suscetibilidade a penicilina e moxifloxacina por *E-test*. As interpretações categóricas de CIM foram baseadas em padrões atualizados. **Resultados:** Todos os isolados foram suscetíveis a penicilina parenteral (CIM  $\leq 2$   $\mu\text{g/mL}$ ) e, conseqüentemente, eram também suscetíveis a amoxicilina, ampicilina, cefalosporinas de terceira e quarta geração e ertapenem. Quanto à moxifloxacina, 99% das cepas de *S. pneumoniae* também foram suscetíveis, e somente uma teve CIM = 1,5  $\mu\text{g/mL}$  (intermediário). **Conclusões:** Nossos resultados mostraram altas taxas de sensibilidade a penicilina parenteral e moxifloxacin nos isolados de *S. pneumoniae* não relacionados a meningite, o que difere de relatos internacionais. Relatos sobre resistência a penicilina devem ser baseados em pontos de corte atualizados para isolados não relacionados a meningite a fim de guiar a escolha terapêutica antimicrobiana e melhorar a predição dos desfechos clínicos.

**Descritores:** Resistência a medicamentos; Penicilina G; Infecções pneumocócicas; *Streptococcus pneumoniae*; Infecções respiratórias.

\* Study carried out in the Microbiology Laboratory, University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

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## Introduction

*Streptococcus pneumoniae* is the most common etiologic agent in pneumonia, meningitis, and middle ear infections. In the developed world, the annual incidence of invasive pneumococcal disease ranges from 11 to 17 per 100,000 population.<sup>(1)</sup> Among patients hospitalized with community-acquired pneumonia (CAP), the microbial agent most commonly isolated is *S. pneumoniae*, the prevalence of *S. pneumoniae*-related CAP being 9–55% among inpatients, compared with 5–9% among outpatients. In community-acquired infections, empirical treatment can be guided by local epidemiological surveillance studies.<sup>(2)</sup>

The penicillin resistance rates among pneumococcus strains have been increasing in various countries, especially in Asia; such rates can vary, and up-to-date local data are needed in order to improve the management of those infections. It is important to identify the source of the infection in order to improve the application of the current penicillin susceptibility breakpoints.<sup>(3)</sup> In a study conducted in three Latin American countries (Argentina, Brazil, and Mexico), an overall resistance to penicillin was detected in 15.3% of the isolates. The highest proportion of fully resistant isolates was observed in Mexico City (24.1%), and it was relatively low in most centers in Brazil (8.1%).<sup>(4)</sup> However, in 2008, the Clinical Laboratory Standards Institute (CLSI) updated the penicillin susceptibility breakpoints for *S. pneumoniae* with new category definitions according to the clinical source of the isolates (meningitis and non-meningitis), based on pharmacokinetic and pharmacodynamic evidence. In recent studies in Brazil,<sup>(5)</sup> none of the evaluated strains was resistant to penicillin—i.e., with a minimum inhibitory concentration (MIC)  $\geq 8$   $\mu\text{g/mL}$ —when the updated CLSI standards were applied.<sup>(6)</sup> The resistance rates of *S. pneumoniae* to respiratory fluoroquinolones, although very low in general (< 1%), have also emerged in some countries (Canada, for example), and, therefore, they should be submitted to close local monitoring.<sup>(5,7)</sup> The objective of the present study was to determine the MICs of penicillin and moxifloxacin against *S. pneumoniae* strains isolated at a tertiary care hospital.

## Methods

This was as an in-vitro, prospective study involving 100 *S. pneumoniae* isolates collected from patients (one sample per patient) who had been treated, between October of 2008 and July of 2010, at one of the ten hospitals that compose the *Hospital das Clínicas* complex of the University of São Paulo School of Medicine, located in the city of São Paulo, Brazil. The isolates were obtained from respiratory tract cultures or blood samples that were unrelated to meningeal infections (i.e., non-meningitis isolates), and they were tested for penicillin and moxifloxacin susceptibility by E test (AB Biodisk, Solna, Sweden). The MIC was determined in  $\mu\text{g/mL}$ , and the category interpretations were carried out based on updated standards.<sup>(6)</sup> The isolates were classified as susceptible, intermediate, and resistant to parenteral penicillin if the MIC was  $\leq 2.0$   $\mu\text{g/mL}$ , 4  $\mu\text{g/mL}$ , and  $\geq 8.0$   $\mu\text{g/mL}$ , respectively. Regarding moxifloxacin, the isolates were classified as susceptible, intermediate, and resistant if the MIC was  $\leq 1.0$   $\mu\text{g/mL}$ , 2  $\mu\text{g/mL}$ , and  $\geq 4.0$   $\mu\text{g/mL}$ , respectively. Quality control was carried out using *S. pneumoniae* ATCC 49619 in accordance with CLSI standards.<sup>(6)</sup>

All the tests and data analysis were carried out at the *Hospital das Clínicas* Microbiology Laboratory. This study was approved by the research ethics committee of the institution.

## Results

Of the 100 *S. pneumoniae* isolates, 49 were from respiratory tract cultures (BAL fluid samples, in 23; tracheal aspirates, in 19; pleural fluid, in 6; and oropharyngeal secretion, in 1), and 51 were from blood samples. The mean age of the patients was 38.4 years (range, 0.5–93.0 years), and the proportion of male patients was 65%.

Applying the susceptibility breakpoints for non-meningitis isolates, all isolates were fully susceptible to parenteral penicillin (MIC  $\leq 2$   $\mu\text{g/mL}$ ), with MIC at which 50% and 90% of the isolates are inhibited (MIC<sub>50</sub> and MIC<sub>90</sub>) of 0.012 and 0.500  $\mu\text{g/mL}$ , respectively (range, 0.002–1.500  $\mu\text{g/mL}$ ). Of the *S. pneumoniae* strains, 99% were also susceptible to moxifloxacin, and only one strain showed an MIC of 1.5  $\mu\text{g/mL}$  (intermediate), with both MIC<sub>50</sub> and MIC<sub>90</sub> of 0.012  $\mu\text{g/mL}$  (range, 0.064–1.500  $\mu\text{g/mL}$ ).

**Table 1** - Antimicrobial activity of moxifloxacin and penicillin to *Streptococcus pneumoniae* isolates (n = 100).

Antimicrobial	CLSI criteria, % of isolates			MIC, µg/mL		
	Resistant	Intermediate	Susceptible	MIC50	MIC90	Range
Moxifloxacin <sup>a</sup>	0	1	99	0.125	0.125	0.064-1.500
Penicillin <sup>b</sup>	0	0	100	0.012	0.500	0.002-1.500

CLSI: Clinical and Laboratory Standards Institute; and MIC: minimum inhibitory concentration. Moxifloxacin breakpoint limits: MIC ≤ 1 µg/mL (susceptible); MIC = 2 µg/mL (intermediate); and MIC ≥ 4 µg/mL (resistant). <sup>a</sup>Penicillin breakpoint limits: MIC ≤ 2 µg/mL (susceptible); MIC = 4 µg/mL (intermediate); and MIC ≥ 8 µg/mL (resistant).

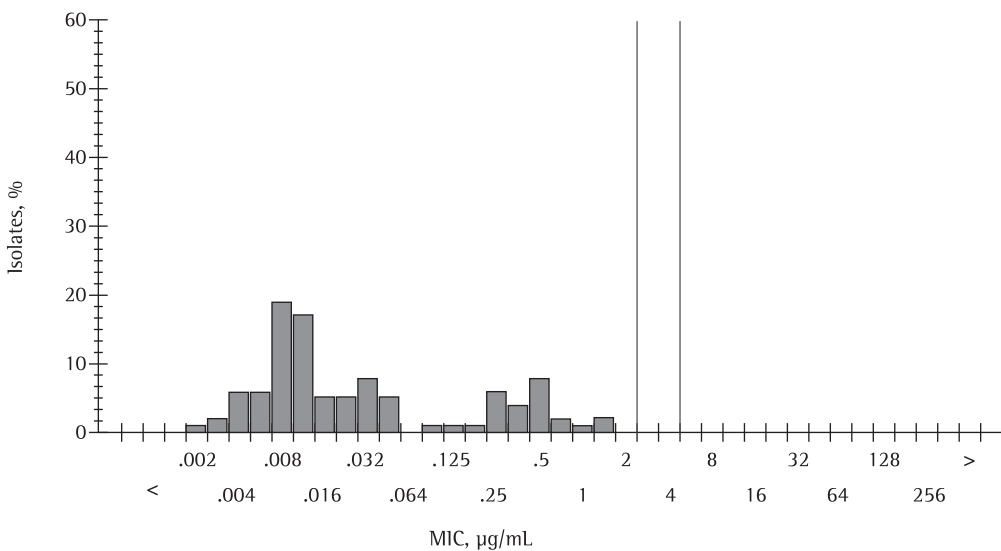
mL; Table 1). Figures 1 and 2 summarize the distribution of the isolates regarding their MIC values for both drugs.

**Discussion**

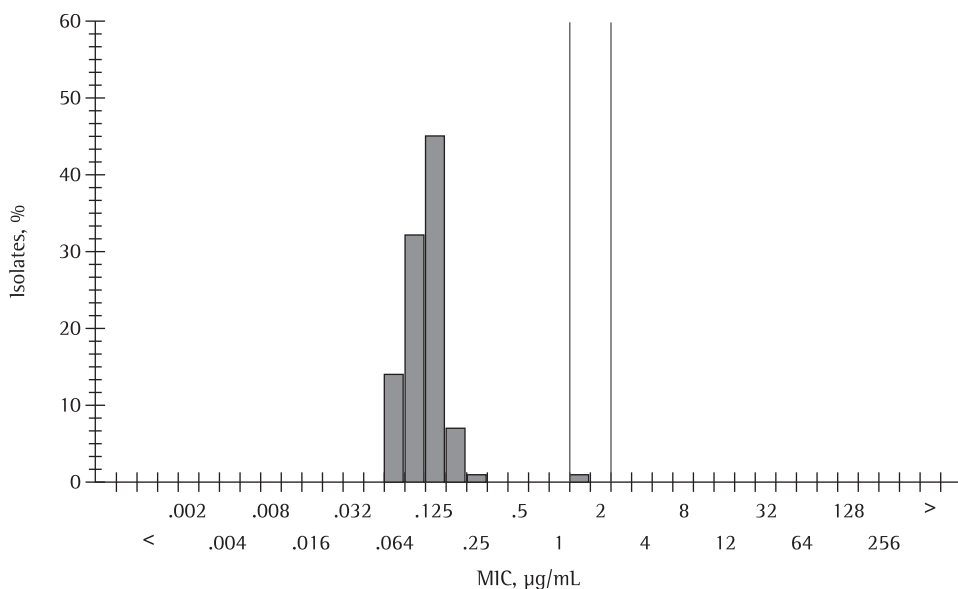
Penicillin-resistant *S. pneumoniae* strains can structurally modify penicillin-binding proteins, allowing the synthesis of peptidoglycans despite the presence of penicillin. In the present study, parenteral penicillin resistance was not observed, and moxifloxacin resistance was very low (only 1 isolate was classified as intermediate). It is of note that those isolates were related to respiratory infections and that the blood isolates were unrelated to meningitis. In January of 2008, the CLSI published new susceptibility breakpoints for *S. pneumoniae* (µg/mL) from isolates unrelated to meningitis (i.e., from respiratory sites or other sites).

All of the isolates analyzed had a penicillin MIC ≤ 2 µg/mL, and the resistance breakpoint for isolates unrelated to meningitis is now

defined as MIC ≥ 8 µg/mL. The previous penicillin MIC breakpoints for susceptible, intermediate, and resistant *S. pneumoniae* strains were ≤ 0.06 µg/mL; 0.12-1.00 µg/mL, and ≥ 2 µg/mL, respectively, but only from meningitis-related isolates.<sup>(8)</sup> Before 2008, even a patient infected with a penicillin resistant *S. pneumoniae* strain could have a good clinical outcome, due to the differences in the parenteral penicillin pharmacokinetics, and that was one of the major reasons why the CLSI adopted new breakpoints in their standards, which led to improved clinical and laboratory correlations regarding respiratory infections. More importantly, it has been reported that the use of the new breakpoints, in order to decide which patients would benefit from treatment with penicillin, have not caused an increase in case-fatality rates. The results obtained with parenteral penicillin can be extrapolated for amoxicillin, ampicillin, cephalosporins (third and fourth generations), and ertapenem. However, the new criteria do not apply to penicillin V



**Figure 1** - Distribution of the isolates regarding minimum inhibitory concentration (MIC) of penicillin against *Streptococcus pneumoniae*. Solid lines are breakpoint limits (susceptible: MIC = 2 µg/mL; intermediate: MIC = 4 µg/mL; and resistant: MIC = 8 µg/mL).



**Figure 2** - Distribution of the isolates regarding minimum inhibitory concentration (MIC) of moxifloxacin against *Streptococcus pneumoniae*. Solid lines are breakpoint limits (susceptible: MIC = 1 µg/mL; intermediate: MIC = 2 µg/mL; resistant: MIC = 4 µg/mL).

(oral), since it has different pharmacokinetic parameters, and isolates are considered resistant if they show an MIC  $\geq 0.06$  µg/mL. In Brazil, during the 1990s, the prevalence of resistance in the southeastern region of the country was under 5%, and, by 2003, resistance among invasive pneumococcal isolates ranged from 7.2% and 8.1%; these rates, however, are related to the old breakpoints.<sup>(9)</sup> One recent surveillance study in Brazil showed that, with the new breakpoints, penicillin resistance dropped from 33% to 1%, and only 1 isolate showed intermediate resistance to this medication.<sup>(10)</sup> In that study, however, despite the very low penicillin resistance rates, those isolates showed co-trimoxazole, tetracycline, erythromycin, and clindamycin resistance rates of 80%, 21%, 13%, and 13%, respectively, whereas only 1 isolate showed ceftriaxone resistance.<sup>(10)</sup>

In a review carried out in the United States, the effects of the new penicillin susceptibility breakpoints were reported, and an overall decrease in the rates of intermediate and resistant strains was found, as expected.<sup>(11)</sup> In our study, resistance among other classes was not evaluated, and this represents a limitation.

In our study, 99% of the isolates were fully susceptible to moxifloxacin, and only 1 isolate showed intermediate resistance. Similar results

were obtained in another study, in which 426 *S. pneumoniae* isolates showed 99.3% susceptibility to moxifloxacin.<sup>(12)</sup> Moxifloxacin resistance rates have been reported to be 0.1-0.3% in the United States and 0.3-1.4% in Canada, compared with approximately 0.9% and 0.3% in Europe and Asia, respectively.<sup>(3,13-15)</sup> Mendes et al. also found a very low overall prevalence of resistance to fluoroquinolones in Latin America (0.8%), complete resistance to levofloxacin (MIC  $\geq 8$  µg/mL) being observed in 1 isolate (0.4%) in Brazil and 3 (1.5%) in Mexico.<sup>(4)</sup>

Moxifloxacin has greater activity against pneumococci because it has a higher affinity for DNA gyrase than do older generations of fluoroquinolones. It has been demonstrated that moxifloxacin less commonly selects for resistant strains, mainly because it is bulkier and less hydrophilic, exhibiting a decreased affinity for the efflux mechanism.<sup>(16)</sup> Although some reports have described the development of resistance in *S. pneumoniae* strains during treatment with fluoroquinolones, resistance to moxifloxacin appears to be less common, due to a favorable profile, which is related to a low mutant prevention concentration in comparison with other fluoroquinolones.<sup>(7,17)</sup> In recent studies, the efficacy of moxifloxacin in the treatment of patients with CAP has been evaluated, and the

use of fluoroquinolone therapy has been found to promote earlier patient discharge, as well as to improve treatment compliance and quality of life, potentially resulting in cost savings. Although the overall use of fluoroquinolones has been increasing, investigations have revealed a decrease in fluoroquinolone-resistant pneumococci.<sup>(18)</sup>

The in-vitro antimicrobial activity of medications using locally isolated strains is a key issue in the selection of appropriate therapies for respiratory infection. However, factors, such as the immune status, age, and co-morbidities (regarding patients), as well as doses, side effects, pharmacokinetics, pharmacodynamics, and costs (regarding medications) should be taken into consideration as well. In accordance with various guidelines for CAP, monotherapy with a fluoroquinolone should be considered only in outpatients with CAP and co-morbidities or in those who received recent antibiotic treatment, as well as in inpatients with mild to moderate disease as an alternative to a combination of a beta-lactam and a macrolide.<sup>(5)</sup> Another group of authors suggested the use of amoxicillin plus a  $\beta$ -lactamase inhibitor as the primary treatment option for CAP, and the use of fluoroquinolone should be limited to adults for whom the initial treatment failed, patients who are allergic to alternative agents, such as penicillin, or patients with *S. pneumoniae* infection that was proven to be highly resistant to penicillin.<sup>(19)</sup>

In conclusion, *S. pneumoniae* resistance to parenteral penicillin and moxifloxacin was very low in the isolates investigated. Reports on penicillin resistance should be based on the updated CLSI breakpoints for non-meningitis isolates in order to improve the prediction of the clinical outcomes. Ongoing surveillance should be encouraged in order to track possible changes in this trend.

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