Antimicrobial resistance of *Streptococcus pneumoniae* isolates in 1999 and 2000 in Madrid, Spain: a multicentre surveillance study

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Resistance to first-line antimicrobial agents in *Streptococcus pneumoniae* is increasing worldwide and the new fluoroquinolones may provide a good alternative. The antimicrobial susceptibility to levofloxacin and 13 other antibiotics of 300 isolates of *S. pneumoniae*, isolated in the Madrid community in 1999 and 2000, was determined. A total of 65.6% of isolates were penicillin intermediate or resistant strains. A high percentage of resistance to macrolides, clindamycin, tetracycline and chloramphenicol was also observed, mainly in penicillin-resistant strains. All but one strain was susceptible to levofloxacin.

**Introduction**

*Streptococcus pneumoniae* is the most common cause of respiratory tract infections, including otitis, sinusitis, pneumonia and infectious exacerbation of chronic bronchitis, as well as potentially life-threatening systemic infections such as meningitis and bacteraemias. Until a few years ago, penicillin was the treatment of choice for infections caused by this microorganism. However, since the middle of the 1980s, pneumococci with structural changes in the penicillin binding protein (PBP) have been detected in many parts of the world, which has resulted in a decrease in susceptibility not only to penicillin but also to other β-lactam antibiotics.1–4 Some strains have MICs between 0.1 and 1 mg/L for penicillin, enabling treatment of extrameningeal infections with high dosages. However, the existence of strains with MICs ≥ 2 mg/L and the requirement to treat central nervous system (CNS) infections caused by pneumococci with MICs ≥ 0.1 mg/L make it necessary to search for alternative treatments.

The association of decreased susceptibility to β-lactams with resistance to other antibiotics, mainly macrolides,1,2 and the appearance of strains with tolerance but not resistance to vancomycin3 generate a genuine therapeutic problem.

The new generation fluoroquinolones, with increased activity for *S. pneumoniae*, have been shown to be a good therapeutic option for treatment of infections caused by multi-resistant pneumococci.

This study aimed to describe the susceptibility of *S. pneumoniae* isolated in the Madrid community in 1999 and 2000 for levofloxacin and 13 other antibiotics.

**Materials and methods**

In 1999 and 2000, 300 non-duplicated strains of *S. pneumoniae* isolated in 12 participating microbiology laboratories, which represent 10 out of the 11 Health Authority Areas of Madrid, were collected. The sample size was proportionally stratified according to the number of inhabitants in each area. Susceptibility testing was centralized in our laboratory.

The antibiotics tested were: penicillin G (Sigma Chemical Co., St Louis, MO, USA), amoxycillin (SmithKline Beecham, Toledo, Spain), cefuroxime (Glaxo Wellcome, Madrid, Spain), cefaclor (Sigma Chemical Co.), cefixime (Merck, Barcelona, Spain), cefotaxime (Sigma Chemical Co.), erythromycin (Sigma Chemical Co.), azithromycin (Pfizer Inc., New York, NY, USA), miocamycin (Menarini, Barcelona, Spain), clindamycin (Sigma Chemical Co.), tetracycline (Sigma Chemical Co.), chloramphenicol (Sigma Chemical Co.), ciprofloxacin (Bayer Q.F., Barcelona, Spain) and levofloxacin (Hoechst-Marion-Roussel, Romainville, France).

Antimicrobial susceptibility testing was performed by the agar dilution method in Mueller–Hinton agar with 5% sheep blood according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines.5 *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC...
29212 and *S. pneumoniae* ATCC 49619 strains were used as controls.

**Results and discussion**

The results of the susceptibility study are summarized in Table I and the susceptibility to macrolides–lincosamides according to penicillin susceptibility is summarized in Table II.

A high prevalence of isolates with decreased susceptibility to β-lactams was observed in this study, and 65.6% of the strains tested had penicillin MICs $\geq 0.12$ mg/L, which is greater than any previously described in Europe.1,2 In Asiatic countries, higher values have been described, with up to 73% of strains being non-susceptible in Taiwan.3 Of all the strains in our study, 40.6% are considered penicillin resistant (MIC $\geq 2$ mg/L) and only 25% show intermediate susceptibility. This is the inverse of the trend existing until now, in which all the strains with decreased susceptibility to penicillin were found to be so at the expense of the intermediate ones. Baquero et al.1 have already found more strains with MICs $\geq 2$ mg/L (36.5%) than those with MICs between 0.12 and 1 mg/L (23.6%).

Strains with decreased susceptibility to penicillin show a significant increase in the MICs for all the β-lactams. This increase is directly proportional to the increase in the penicillin MICs, so that amoxycillin has an MIC50 and an MIC90 that are one dilution lower than penicillin, cefotaxime two dilutions lower, cefaclor and cefixime three dilutions higher as a minimum, and cefuroxime an equal MIC50 and an MIC90 one dilution higher. These ratios obtained in the context of global values are the same as those observed in almost all strains when considered individually.

Amoxycillin and cefotaxime are the most active β-lactam antibiotics, with MIC50s of 2 and 1 mg/L, respectively. Although cefotaxime MICs are lower than those of amoxycillin, the percentage susceptibility is higher with amoxycillin (90.6%) than with cefotaxime (71.9%). This apparent paradox is explained by the new cut-off points established for amoxycillin by the NCCLS, which consider that all strains with MICs $\leq 2$ mg/L are susceptible to amoxycillin.6 Cefuroxime is the most active oral cephalosporin of the three tested in this study, with 53.5% of the strains being susceptible. The other two, cefaclor and cefixime, show the worst activity against the *S. pneumoniae* strains studied, with MIC50s $> 32$ and $> 16$ mg/L, respectively.

The resistance to penicillin in *S. pneumoniae* is directly related to the type of sample from which it originates.1,2 In our experience, the greatest percentage of decreased susceptibility to penicillin has been found in pneumococci from otic exudates (90.9%) and from the conjunctiva (80.9%), while those isolated from respiratory and blood culture samples have 62.4 and 53.8% resistance, respectively. Of the six strains isolated from CSF, five were susceptible and only one showed resistance (16.6%).

A high prevalence of global resistance to macrolides was observed: 36.1% to erythromycin, with a clear association between this and the decreased susceptibility to penicillin;

**Table I.** Susceptibility pattern of 300 isolates of *S. pneumoniae* isolated in 1999 and 2000

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
<th>Percentage of isolates in categorya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>MIC50</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>$\leq 0.03$–8</td>
<td>1</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>$\leq 0.03$–8</td>
<td>0.5</td>
</tr>
<tr>
<td>Cefuroxime p.b</td>
<td>$\leq 0.03$–&gt;8</td>
<td>1</td>
</tr>
<tr>
<td>Cefuroxime o.c</td>
<td>$\leq 0.03$–8</td>
<td>1</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>$\leq 0.25$–&gt;32</td>
<td>8</td>
</tr>
<tr>
<td>Cefixime</td>
<td>$\leq 0.12$–&gt;16</td>
<td>8</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>$\leq 0.03$–8</td>
<td>0.25</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>$\leq 0.12$–&gt;16</td>
<td>$\leq 0.12$–&gt;16</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>$\leq 0.12$–&gt;16</td>
<td>$\leq 0.12$–&gt;16</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>$\leq 0.12$–&gt;16</td>
<td>$\leq 0.12$–&gt;16</td>
</tr>
<tr>
<td>Miconycin</td>
<td>$\leq 0.25$–&gt;16</td>
<td>$\leq 0.25$–&gt;16</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>$\leq 1$–&gt;8</td>
<td>$\leq 1$–&gt;8</td>
</tr>
<tr>
<td>Cloramphenicol</td>
<td>$\leq 1$–&gt;8</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>$\leq 0.25$–&gt;8</td>
<td>1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>$\leq 0.12$–8</td>
<td>1</td>
</tr>
</tbody>
</table>

NA, no NCCLS breakpoint criteria.

aS, susceptible; I, intermediate susceptibility; R, resistant.

bAccording to the criteria established for parenteral administration.

cAccording to the criteria established for oral administration.
16% resistance to erythromycin in the penicillin-susceptible strains. 47.3% in the intermediate-resistant strains and 48.3% in the penicillin-resistant strains. This association, well known from an epidemiological perspective but unexplainable with respect to its molecular basis, clearly limits the use of macrolides in infections produced by pneumococci with reduced susceptibility to β-lactams. It is also remarkable that there are no differences in macrolide resistance between penicillin-intermediate and -resistant strains. The most frequent molecular mechanism for macrolide resistance in pneumococci is ribosomal methylation mediated by the presence of the erm gene, which bestows resistance to all the macrolides and lincosamides and is present in 29.9% of our isolates. The active efflux of antibiotic mediated by the presence of the mef gene represents a small percentage of macrolide resistance in Europe, and has a characteristic resistance pattern (M phenotype). The difference between the resistance to erythromycin (36.1%) and clindamycin (29.9%) is produced at the cost of the strains that display the M phenotype, i.e. 6.2% of the strains studied.

As described previously, levofloxacin shows good activity against tested pneumococci. All strains except one (MIC 8 mg/L) were susceptible, with an MIC₉₀ of 1 mg/L. Fluoroquinolone resistance has been described in some studies where 2% of strains have ciprofloxacin MICs > 4 mg/L, with an association with penicillin resistance.

Resistance of *S. pneumoniae* to frequently used antimicrobial agents, together with significant local differences in resistance patterns even within the same community, necessitate local surveillance studies to formulate protocols for empirical treatment. New fluoroquinolones, owing to their activity and favourable pharmacokinetic properties, will play an important role in this empirical treatment. The indiscriminate use of fluoroquinolones may render them useless in the short term, but the progressive increase of resistance to first-line antimicrobial agents seems to justify their choice. Ciprofloxacin was introduced at the start in the 1980s, and although there has been a significant increase in resistance in *Escherichia coli*, resistance in pneumococci has, practically, been low. Perhaps this is because fluoroquinolones have not been administered widely in children, the principal reservoir of *S. pneumoniae*.

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References


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