Susceptibility of \textit{Mycoplasma pneumoniae} to Several New Quinolones, Tetracycline, and Erythromycin

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\textit{Mycoplasma pneumoniae} (39 strains) was most susceptible to two quinolones, WIN 57273 and sparfloxacin, with MICs for 90\% of the strains (MIC\textsubscript{90}) of 0.125 and 0.25 \mu g/ml, respectively. It was susceptible to ofloxacin and ciprofloxacin at 2 \mu g/ml and to lomefloxacin and fleroxacin at 4 \mu g/ml. The MIC\textsubscript{90} of erythromycin was 0.062 \mu g/ml, and that of tetracycline was 1 \mu g/ml.

\textit{Mycoplasma pneumoniae} is a major cause of pneumonia and accounts for as much as 20\% of total pneumonia (2). The recommended therapies are tetracycline or erythromycin, both of which are efficacious in reducing the duration of symptoms (21). Because of the increasing resistance of conventional bacteria to penicillin and tetracycline (18), the quinolones (26) may be used more frequently. Accordingly, it will be of increasing importance to know the comparative susceptibilities of clinical strains of \textit{M. pneumoniae} to various quinolones. In this study, we report the susceptibilities of \textit{M. pneumoniae} to quinolones, tetracycline, and erythromycin as determined by the agar dilution method.

The reference strains of \textit{M. pneumoniae} used were AP-164 (8) and the type strain FH (16). Thirty-seven clinical isolates from Seattle were tested, which included the 12 strains from 1964 to 1974 which have been compared antigenically (24). The clinical isolates were tested within five passages from the patient. Strains were stored frozen at \(-70\textdegree\)C. Inocula for susceptibility testing were grown in a broth medium consisting of dialysate broth (6) supplemented with 20\% horse serum, 5 mM glucose, 0.001\% phenol red, and penicillin at 200 U/ml. Broth cultures were used for inocula no more than 2 days after the pH began to drop as judged by a change in the pH indicator (usually 3 to 5 days when a culture was diluted 1:100 into fresh medium). The agar dilution susceptibility testing procedure closely followed that described for \textit{Mycoplasma hominis} (9). Briefly, portions (25 ml) of H agar (7) containing various quantities of antimicrobial agent were poured into plastic petri dishes (100 mm square), and plates were held for 48 h in the dark at room temperature to eliminate excess surface moisture. The soy peptone used for both H agar and dialysate broth was obtained from Sheffield Chemical (Norwich, N.Y.), and the media were prepared as described previously (6, 7). Plates were inoculated with 25-\mu l samples of cultures diluted 1:10, 1:100, and 1:1,000 by using a Steers replicator (23). Plates were incubated in the dark in a moist atmosphere of 5\% CO\textsubscript{2} in air and observed at 8, 16, and 25 days with a dissecting microscope at \times 40 magnification. The pH of the agar was 6.85, and the agar remained at that pH throughout incubation. The MIC was the least amount of antimicrobial agent which completely prevented the formation of 30 to 300 colonies on a spot as determined by colony count of spots on control plates (9). The MIC\textsubscript{50} and MIC\textsubscript{90} were the least amounts of antimicrobial agent which prevented the growth of 50 and 90\% of the strains, respectively.

The following quinolones were used: ciprofloxacin (Miles Inc. Pharmaceutical Division, West Haven, Conn.), fleroxacin (AM-833; Hoffmann-La Roche, Nutley, N.J.), lomefloxacin (NY 198; G. D. Searle, Mt. Prospect, Ill.), ofloxacin (Ortho Pharmaceutical, Raritan, N.J.), sparfloxacin (CI-978, AT 4140; Parke-Davis, Ann Arbor, Mich.), and WIN 57273 (Sterling Research Group, Rensselaer, N.Y.). Tetracycline and erythromycin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Quinolones (10 mg) were suspended in 10 ml of H\textsubscript{2}O, and 0.025 ml of 10 N NaOH was added to aid solubilization. Erythromycin was dissolved in 10\% ethanol in water, and tetracycline was dissolved in water. Fresh preparations were made up on the day of the test and sterilized by filtration through an 0.22-\mu m-pore-size filter (Millipore Corp., Bedford, Mass.).

The susceptibilities of 39 strains were determined by the agar dilution method, with the endpoint observed at day 16 (Table 1). \textit{M. pneumoniae} was highly susceptible to WIN 57273 and sparfloxacin, with MIC\textsubscript{90} of 0.125 and 0.25 \mu g/ml, respectively, values which approach the MIC\textsubscript{90} of 0.062 \mu g/ml found for erythromycin (Table 1). Nakamura et al. found a value of 0.1 \mu g/ml for sparfloxacin by the agar dilution method (12). The MIC\textsubscript{90} of 2 \mu g/ml for ciprofloxacin and ofloxacin and 4 \mu g/ml for fleroxacin and lomefloxacin are close to the levels attainable in tissue (26). In other studies, the MIC\textsubscript{90} of ciprofloxacin was 0.25 to 1.0 \mu g/ml by broth dilution (1) and 1 \mu g/ml by agar dilution (12, 17). MIC\textsubscript{90} of ofloxacin were 0.8 to 1.6 \mu g/ml (15) by broth dilution and 1 \mu g/ml by agar dilution (12, 17). The range in susceptibility for each quinolone was narrow (Table 1); thus, there was no evidence of resistance of any of these strains or a change in susceptibility over time.

The susceptibilities of \textit{M. pneumoniae} to ofloxacin, fleroxacin, and lomefloxacin were about twofold less than those for \textit{Mycoplasma hominis} and about the same as those for \textit{Ureaplasma urealyticum} (9). In the case of ciprofloxacin, \textit{M. pneumoniae} was twofold less susceptible than \textit{M. hominis} and fourfold more susceptible than \textit{U. urealyticum}. The susceptibilities of \textit{M. pneumoniae} to quinolones roughly parallel the susceptibilities of gram-positive bacteria to the same quinolones (5, 12, 26), a result similar to our previous data for \textit{M. hominis} and \textit{U. urealyticum} (9). The two most active compounds against \textit{M. pneumoniae}, WIN 57273 and sparfloxacin, also had the highest activities against gram-positive bacteria among the quinolones tested (5, 12).
TABLE 1. Comparison of the activities of quinolones and erythromycin and tetracycline for 39 *M. pneumoniae* strains

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>50%</th>
<th>90%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinolones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WIN 57273</td>
<td>0.125</td>
<td>0.125</td>
<td>0.031-0.125</td>
<td></td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25-0.5</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0-2.0</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>2.0</td>
<td>4.0</td>
<td>2.0-4.0</td>
<td></td>
</tr>
<tr>
<td>Fleroxacin</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0-8.0</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5-1.0</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.031</td>
<td>0.062</td>
<td>0.031-0.062</td>
<td></td>
</tr>
</tbody>
</table>

* Quinolones are listed in order of descending activity. Quinolones with the same activity are listed alphabetically.
* MICs determined at day 16 of incubation.

Colony formation by *M. pneumoniae* is slow, and colonies on control plates first appear at 2 to 3 days and reach maximal size at 2 weeks. When plates were observed at various times of incubation, the MICs of WIN 57273, sparfloxacin, and floxacin were the same at 8 and 16 days. The MICs of ofloxacin, ciprofloxacin, and lomefloxacin increased twofold from day 8 to day 16. Determining an endpoint for tetracycline at day 8 was not possible because the colonies were barely visible even at 0.25 µg/ml (1/4 the eventual MIC). No other antimicrobial agent tested showed such an effect. Erythromycin showed a shifting endpoint: the MIC at day 8 was 0.015 µg/ml on day 8, 0.062 µg/ml on day 16, and 0.125 at day 25. This shifting endpoint may explain the wide variations in the reported susceptibilities of *M. pneumoniae* to erythromycin. By using the broth dilution method, two ranges of MICs have been reported: 0.001 to 0.01 µg/ml (4, 14, 15, 22, 25) and 0.1 to 1.0 µg/ml (20). By agar dilution, MICs of 0.008 to 0.063 µg/ml (3, 11, 19) and 0.8 to 1.6 µg/ml (10) have been reported. These differences are probably not the result of low-level resistance to erythromycin since the strains reported as resistant have been highly resistant (400 µg/ml [13]).

Standardization of susceptibility testing of *M. pneumoniae* is difficult because of the slow growth of the organism even though the agar medium contains 20% serum. For quinolones, changes in the incubation period made only a twofold difference in MIC, whereas four- to eightfold differences were found with erythromycin and tetracycline. Incubation periods of 7 days could be used for determination of quinolone endpoints, but an incubation period of as much as 14 days appears necessary for determination of susceptibilities to tetracycline and erythromycin. Although these results do not predict how effective quinolones might be in treatment of *M. pneumoniae* pneumonia, they provide a ranking of the relative activities of a number of newer quinolones.

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