Diminished Killing of Pneumococci by Pristinamycin Demonstrated by Time-Kill Studies

Pristinamycin is a synergistic combination of streptogramin A (pristinamycin IIA) and streptogramin B (pristinamycin IB) compounds, licensed in France and some other European countries. This oral antibiotic has been considered as an alternative treatment for infections due to penicillin- and macrolide-resistant *Streptococcus pneumoniae* because streptogramins remain active against streptococci and pneumococci irrespective of their macrolide susceptibility status (10, 11). However, some treatment failures have been reported and may not be explained by pristinamycin resistance in vitro (2, 3). During its evaluation in our laboratory, the Vitek-II system (bioMérieux, Balmes-les-Grottes, France) classified a few strains among a selected collection of 100 multiresistant pneumococci as pristinamycin resistant (8a). Because this system uses a kinetic turbidimetric measurement of bacterial growth in the presence or absence of a known antibiotic concentration (9), we decided to perform time-kill studies to investigate the killing effect of pristinamycin against three strains classified as pristinamycin resistant by the Vitek II system and other selected isolates.

Eight clinical isolates and two reference pristinamycin-resistant *S. pneumoniae* strains (SP5500 [CIP104.448] and SP8906 [CIP104.486]) (6) were used in this study. Powders of known potency were obtained from Abbott, Rungis, France (erythromycin), Pharmacia & Upjohn, Paris La Défense, France (clindamycin), and Rhone-Poulenc Rorer, Paris, France (spiramycin and pristinamycin). Disk diffusion susceptibility and MICs were determined by the agar dilution method as previously described (13). To differentiate between the susceptible and intermediate-resistant categories or between the intermediate-resistant and resistant categories, the breakpoints recommended by the Comité de l’Antibiogramme de la Société Française de Microbiologie (5) were, respectively, 1 and 4 μg/ml for erythromycin and spiramycin, 1 and 2 μg/ml for pristinamycin, and 2 μg/ml for clindamycin (no intermediate-resistant category). Time-kill effects were studied by growing bacteria (original inoculum, 10^6 CFU/ml) in brain heart infusion containing increasing dilutions of pristinamycin and spreading 0.05 ml of 10-fold serial dilutions of the culture onto blood agar plates at fixed times (13). The limit of detection of the technique was 20 CFU/ml. A bactericidal effect was defined as ≥99.9% killing of the original inoculum (reduction of viable bacteria ≥3 log₂). Significant antibiotic carryover effect was excluded as initial bacterial counts of susceptible and control strains (inocula, 10^2 and 10^6 CFU/ml) were not changed in the presence or absence of a large amount of pristinamycin (1X to 8X the MIC). *S. pneumoniae* ATCC 49619 was used as a quality control strain for MIC determination and time-kill assays.

All clinical isolates were resistant to erythromycin (MIC > 128 μg/ml) and exhibited cross-resistance to spiramycin and clindamycin (MICs > 128 μg/ml), but they appeared to be susceptible to pristinamycin (MICs, 0.25 to 2 μg/ml). Reference strains SP5500 and SP8906 were susceptible to erythromycin (MIC, 0.125 μg/ml) and clindamycin (MIC, 0.125 μg/ml) but were resistant to spiramycin (MICs > 128 μg/ml); the MIC of pristinamycin was 8 μg/ml. Time-kill results (Table 1) were similar to those previously reported for pristinamycin, quinupristin-dalfopristin, and RPR106972 (1, 12): pristinamycin demonstrated a marked bactericidal activity against five of the eight clinical isolates tested, which were then classified as time-kill assay-susceptible strains. On the other hand, a more limited bactericidal effect was observed with pristinamycin during the first 6 h against the two pristinamycin-resistant pneumococci SP5500 and SP8906 and against the three remaining clinical strains (time-kill assay-resistant strains). The latter three strains had been classified as pristinamycin resistant by the Vitek-II system; they were, however, classified as susceptible to this antibiotic by disk diffusion and MIC determination assays. Against these five strains, pristinamycin was bactericidal only after 24 h at the higher concentrations (2X to 8X the MIC, i.e., 2 to 64 μg/ml).

In this study, we have identified and studied five pristinamycin-time-kill assay-resistant strains of *S. pneumoniae*. Time-kill curves of these resistant strains exhibit an unusual diminution of the bactericidal effect of the streptogramin combination compared to regular susceptible strains. Because none of the previous studies investigating the efficacy of streptogramins against streptococci and pneumococci had reported such diminished bactericidal effect of pristinamycin, the cross-resistance to macrolides-lincosamides-streptogramin B (MLS) was always considered to preserve synergism between streptogramin components A and B (1, 10–12). However, reduced bactericidal activity of pristinamycin was demonstrated against some erythromycin-resistant *Enterococcus faecium* (4, 7) and *Staphylococcus aureus* (8) strains and was related to expression of a ribosomal *erm* methylase according to the MLS phenotype seen in disk diffusion susceptibility testing (4, 7, 8).

The incidence and the relevance of the diminished bactericidal effect of pristinamycin in *S. pneumoniae* remain unknown because this effect was not predicted by disk diffusion susceptibility testing or MIC determination using the agar dilution method. One should keep in mind that the strains we have tested had been selected because they were determined to be multiresistant; thus, no epidemiological information can be drawn from our observation.

In conclusion, our report demonstrates the absence of a reliable correlation between killing kinetics and usual routine laboratory tests for pristinamycin susceptibility testing of some pneumococci and suggests that changes in laboratory practices may be needed to efficiently detect this form of resistance. Because the new Vitek-II system is not yet widely used, we suggest that time-kill assays may improve detection of such pristinamycin killing-resistant pneumococci.

Continued on following page
<table>
<thead>
<tr>
<th>Pristinamycin concentration</th>
<th>Time</th>
<th>MIC (μg/ml)</th>
<th>Mean log CFU/ml</th>
<th>Time-kill assay-susceptible strains (100% killing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2 h</td>
<td>0.25</td>
<td>+1.18 ± 0.54</td>
<td>+1.18 ± 0.54</td>
</tr>
<tr>
<td>0.5× MIC</td>
<td>4 h</td>
<td>0.125</td>
<td>+0.95 ± 0.44</td>
<td>+0.95 ± 0.44</td>
</tr>
<tr>
<td>1× MIC</td>
<td>6 h</td>
<td>0.25</td>
<td>+0.72 ± 0.38</td>
<td>+0.72 ± 0.38</td>
</tr>
<tr>
<td>2× MIC</td>
<td>24 h</td>
<td>0.5</td>
<td>+0.38 ± 0.25</td>
<td>+0.38 ± 0.25</td>
</tr>
<tr>
<td>8× MIC</td>
<td>2 h</td>
<td>1.0</td>
<td>+0.25 ± 0.14</td>
<td>+0.25 ± 0.14</td>
</tr>
</tbody>
</table>

*Strains and pristinamycin MICs are as follows: SP40409 and SP40314, 0.25 mg/ml; SP43512, SP40305, and SP43643, 0.5 mg/ml.

**REFERENCES**

ERRATUM

Letter to the Editor: Diminished Killing of Pneumococci by Pristinamycin Demonstrated by Time-Kill Studies

LAURENT SCHLEGEL, GENEVIÈVE SISSIA, ANNICK FRÉMAUX, AND PIERRE GESLIN

Centre National de Référence des Pneumocoques, Service de microbiologie, Centre Hospitalier Intercommunal,
40 Avenue de Verdun, 94010 Créteil Cedex, France