Emergence of a *Streptococcus pneumoniae* isolate resistant to streptogramins by mutation in ribosomal protein L22 during pristinamycin therapy of pneumococcal pneumonia

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**Objectives:** The aim of this study was to characterize the mechanism of resistance to macrolides and streptogramins of a *Streptococcus pneumoniae* strain isolated from blood cultures in an 80-year-old patient suffering from severe pneumonia unsuccessfully treated with pristinamycin.

**Methods:** Resistance genes *erm*(B) and *mef*(A) were searched for by PCR. Portions of genes for domains V and II of the 23S rRNA (*rrl*) and genes for ribosomal proteins L4 (*rplD*) and L22 (*rplV*) were amplified by PCR from total genomic DNA and sequenced.

**Results:** Resistance genes *erm*(B) and *mef*(A) were not detected. Only mutation in the *rplV* gene encoding ribosomal protein L22 was detected. The strain contained a six amino acid insertion (107KRTAHI108) in the C-terminus of the ribosomal protein L22.

**Conclusions:** This is the first report of emergence of a pneumococcus resistant to streptogramins by mutation in ribosomal protein L22 during treatment with pristinamycin.

Keywords: macrolide resistance, pneumococcus, riboprotein, *rplV*

**Introduction**

*Streptococcus pneumoniae* is an important cause of community-acquired pneumonia. Penicillins are the treatment of choice of these infections and macrolides constitute an alternative in the case of intolerance to the drugs. However, increasing resistance to macrolides and related antibiotics [macrolides–lincosamides–streptogramins (MLS)] among *S. pneumoniae* clinical isolates constitutes a worldwide problem.1 In pneumococci, MLS resistance is mediated by two major mechanisms: target site modification and drug efflux.1 Ribosomal alteration is mediated by a methyltransferase, encoded by the *erm*(B) gene [or rarely the *erm*(A) gene], that methylates the A2058 residue (*Escherichia coli* numbering) in domain V of the 23S rRNA and confers the so-called MLSB phenotype of cross-resistance among macrolides, lincosamides and streptogramins B. Active efflux is mediated by the *mef*(A) gene [formerly *mef*(E)] that codes for an efflux pump and is responsible for the so-called M phenotype characterized by unique resistance to 14- and 15-membered-ring macrolides (azithromycin, erythromycin, clarithromycin and roxithromycin).1 In addition, mutations in 23S rRNA and the ribosomal proteins L4 and L22 (encoded by the *rplD* and *rplV* genes, respectively) have been infrequently described in pneumococci.2 Resistance to macrolides can reach percentages exceeding 50% in certain countries and reduced susceptibility to erythromycin has become more frequent than reduced susceptibility to penicillin in Europe overall.3 Telithromycin, a ketolide, and pristinamycin, an oral streptogramin, are molecules related to macrolides, which have demonstrated activity against pneumococci resistant to macrolides.4 Pristinamycin is a combination of streptogramin A-type (pristinamycin IIA) and streptogramin B-type (pristinamycin IA) antibiotics. Although Erm methylases confer cross-resistance to macrolides, lincosamides and streptogramins B, synergism between the two streptogramin components is maintained, explaining the activity of the streptogramin combination.2 Here, we report an *S. pneumoniae* isolate resistant to pristinamycin, an oral streptogramin, and mutated in ribosomal protein L22 isolated from blood cultures of a patient with lower respiratory tract infection unsuccessfully treated with pristinamycin.
**S. pneumoniae L22 ribosomal mutant**

### Materials and methods

**Bacterial strains and antimicrobial susceptibility testing**

The strain of *S. pneumoniae* HM8989 was isolated from two blood cultures taken from an 80-year-old male patient suffering from severe pneumonia. He had been treated empirically with pristinamycin (3 g day) for 1 week for bronchial infection and was hospitalized at Henri Mondor hospital ( Créteil, France) for persistent fever and increasing dyspnoea. The *S. pneumoniae* isolate was phenotypically identified and antimicrobial susceptibility was tested by the disc diffusion method. MICs of MLS antibiotics were determined by the agar dilution method on Mueller–Hinton agar medium, according to the recommendations of Comité de l’Antibiogramme de la Société Française de Microbiologie. The reference strain *S. pneumoniae* ATCC 49619 was included as a control.

**PCR and DNA sequence analysis**

Bacterial genomic DNA was extracted using the QIAmp DNA Mini Kit (Qiagen, Courtaboeuf, France). Resistance genes *erm* (B) and *mef* (A) and portions of genes for domains V and II of the 23S rRNA (*rrl*) and genes for ribosomal proteins L4 (*rplD*) and L22 (*rrl*) were amplified using PCR with specific primers, as previously described. PCR products were then sequenced in both directions using the ABI PRISM 3100 automatic sequencer (Applied Biosystems, Courtaboeuf, France). For identification of ribosomal mutations, sequences were compared with the genomic sequences of *S. pneumoniae* TIGR4 and R6 (GenBank accession numbers NC003098 and NC003028, respectively). Comparisons were done using the software available over the Internet at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/).

### Results

**Susceptibility to antimicrobial agents**

By the disc diffusion method, the pneumococcal strain HM8989 was fully susceptible to all β-lactams tested with MICs of penicillin G, ampicillin and cefotaxime equal to 0.016, 0.016 and 0.008 mg/L, respectively. It also showed an intrinsic low-level resistance to aminoglycosides (streptomycin, kanamycin and gentamicin) and was susceptible to tetracyclines, chloramphenicol, rifampicin, co-trimoxazole, levofloxacin and vancomycin. In contrast, it exhibited a very uncommon profile of resistance to macrolides and related antibiotics with an intermediate susceptibility to erythromycin and pristinamycin. The strain remained susceptible to lincomycin. No D-shaped zone was visible when the disc of erythromycin was placed close to the disc of clindamycin.

MICs of MLS antibiotics are shown in Table 1. MICs of erythromycin, clarithromycin, azithromycin, telithromycin and spiramycin for the strain HM8989 were 32–64-fold greater than those for the reference strain *S. pneumoniae* ATCC 49619. Despite the 32-fold increased MIC of telithromycin, the isolate remained categorized as susceptible to this antibiotic. MIC determination confirmed that the isolate was fully susceptible to lincosamides with MICs 2–4-fold lower than those for the reference strain. In addition, the isolate was resistant to the streptogramin combination quinupristin/dalfopristin with an 8-fold increase in the MIC of quinupristin, whereas the MIC of dalfopristin was the same. As expected, this strain was classified as intermediate to pristinamycin (MIC = 2 mg/L), with MICs of pristinamycin IA and pristinamycin IIA equal to 16 and 4 mg/L, respectively.

### Discussion

Macrolide resistance in *S. pneumoniae* is mostly mediated by *erm* (B), a 23S rRNA methylase, and by *mef* (A), a gene encoding an efflux pump. Since pristinamycin is still active against pneumococcal isolates displaying either mechanism, 2 this oral antibiotic represents an attractive option for the empirical treatment of respiratory tract infections. This report shows that resistance to streptogramins may emerge in clinical isolates through mutation in ribosomal protein L22. This insertion corresponded to a six amino acid insertion (107KRTAHI108) tandemly duplicated (Figure 1).

### Table 1. MICs of MLS antibiotics for *S. pneumoniae* HM8989 and for control strain, *S. pneumoniae* ATCC 49619

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S. pneumoniae HM8989</th>
<th>S. pneumoniae ATCC 49619</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>1</td>
<td>0.016</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>8</td>
<td>0.12</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0.12</td>
<td>0.5</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Quinupristin</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Dalfopristin</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Quinupristin/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dalfopristin</td>
<td>16</td>
<td>0.25</td>
</tr>
<tr>
<td>Pristinamycin IA</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Pristinamycin IIA</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Pristinamycin IIA</td>
<td>2</td>
<td>0.12</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>0.25</td>
<td>0.008</td>
</tr>
</tbody>
</table>

No *erm* (B) methylase gene or *mef* (A) efflux gene could be detected by PCR with specific primers. No mutations were found in the *rrl* gene (domains II and V) or in the *rplD* gene for ribosomal protein L4. However, the strain displayed an 18 nucleotide tandem duplication at the 3' end of the *rrl* gene encoding ribosomal protein L22. This insertion corresponded to a six amino acid insertion (107KRTAHI108) tandemly duplicated (Figure 1).

Mutation in the *rplV* gene

No *erm* (B) methylase gene or *mef* (A) efflux gene could be detected by PCR with specific primers. No mutations were found in the *rrl* gene (domains II and V) or in the *rplD* gene for ribosomal protein L4. However, the strain displayed an 18 nucleotide tandem duplication at the 3' end of the *rrl* gene encoding ribosomal protein L22. This insertion corresponded to a six amino acid insertion (107KRTAHI108) tandemly duplicated (Figure 1).

Discussion

Macrolide resistance in *S. pneumoniae* is mostly mediated by *erm* (B), a 23S rRNA methylase, and by *mef* (A), a gene encoding an efflux pump. Since pristinamycin is still active against pneumococcal isolates displaying either mechanism, 2 this oral antibiotic represents an attractive option for the empirical treatment of respiratory tract infections. This report shows that resistance to streptogramins may emerge in clinical isolates through mutation in ribosomal protein L22. This insertion corresponded to a six amino acid insertion (107KRTAHI108) tandemly duplicated (Figure 1).
then, resistance to pristinamycin in clinical isolates of pneumococci from France has not been reported.\textsuperscript{10} Mutations in protein L22 seem to be uncommon in clinical isolates. In epidemiological surveys, mutations occurring in the 3' end of the rplV gene have recently been shown to confer unusual MLS-resistance patterns in clinical isolates [R22C, G95D, insertion \textsuperscript{93}VRPR\textsuperscript{53}, A101P, tandem duplications (\textsuperscript{108}RTAHI\textsuperscript{109} or \textsuperscript{108}RTAHIT\textsuperscript{109}), C117T].\textsuperscript{2} Emergence of an L22 mutant during treatment with azithromycin of a fatal pneumococcal pneumonia in France in 2002 was linked to mutations at the C-terminus of L22. This isolate contained a tandem duplication \textsuperscript{108}RTAHI\textsuperscript{109} with resistance to erythromycin, azithromycin and quinupristin/dalfopristin (MICs 2–4 mg/L). The mechanism of resistance by mutation in L22 seems to be uncommon in clinical isolates. In epidemiological surveys, mutations occurring in the 3' end of rplV are described (\textsuperscript{92}VRPR\textsuperscript{93}, A101P, tandem duplications \textsuperscript{108}RTAHI\textsuperscript{109} or \textsuperscript{108}RTAHIT\textsuperscript{109}, C117T).\textsuperscript{9} The tandem duplication that we found in the C-terminus of L22 in \textit{S. pneumoniae} is very similar. The tandem duplication that we found in the C-terminus of L22 in \textit{S. pneumoniae} by L22 mutations. The mechanism of resistance by mutation in L22 in \textit{S. pneumoniae} has not been studied, but is probably similar. The tandem duplication that we found in the C-terminus of the L22 protein \textsuperscript{107}KRTAHI\textsuperscript{108} was not exactly the same as those that were previously described \textsuperscript{108}RTAHIT\textsuperscript{109} among clinical isolates of macrolide-resistant \textit{S. pneumoniae}.\textsuperscript{2} However, these two close variations could be responsible for similar conformational changes in the riboprotein. In the literature, there is only one report of a macrolide-resistant pneumococcal mutant emerging during empirical treatment. Finally, L22 mutations did not affect the susceptibility to lincosamides but compromised the potency of the ketolides, with an increase in MIC of telithromycin from 0.008 to 0.25 mg/L.

This report confirms the role of tandem duplications of ribosomal target protein L22 in MLS resistance in \textit{S. pneumoniae}. This is the first report of emergence of a pneumococcal isolate resistant to streptogamins by mutation in ribosomal protein L22 during treatment with pristinamycin. Finally, it also highlights the risk of emergence of these mutants that present increased MICs of telithromycin.

### Transparency declarations

None to declare.

### References


12. Tu D, Blaha G, Moore PB \textit{et al.} Structures of MLS\textsubscript{B}K antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. \textit{Cell} 2005; \textbf{121}: 257–70.