High Prevalence of the \textit{ermB} Gene among Erythromycin-Resistant \textit{Streptococcus pneumoniae} Isolates in Germany during the Winter of 2000-2001 and In Vitro Activity of Telithromycin

Michael Kresken,1* Beate Henrichfreise,1,2 Simone Bagel,1 Johannes Brauers,1 and Bernd Wiedemann1,2


Received 20 November 2003/Returned for modification 1 March 2004/Accepted 3 April 2004

Of 595 isolates of \textit{Streptococcus pneumoniae} from outpatients with respiratory tract infections, collected from 17 microbiology laboratories, 14.1% were resistant to erythromycin. Eighty-three erythromycin-resistant isolates were genetically analyzed, 83.1% of which harbored the \textit{ermB} gene. Only four isolates (4.8%) harbored the \textit{mefA} gene. Telithromycin exhibited potent activity against all isolates.

The two most common mechanisms of macrolide resistance among \textit{Streptococcus pneumoniae} isolates are efflux of the drug mediated by a transport protein encoded by the \textit{mefA} (subclass \textit{mefE}) gene and target-site modification due to a methylase encoded by the \textit{ermB} gene (subclass \textit{ermAM}) (15, 20). Isolates harboring the \textit{mefA} gene display low-level resistance to 14- and 15-membered-ring macrolides, whereas isolates with the \textit{ermB} genotype display high-level resistance to all macrolides, lincosamides, and group B streptogramins (2, 6, 15).

Telithromycin, the first compound of the ketolide class, is highly active against pneumococcal isolates, including both \textit{ermB}- and \textit{mefA}-mediated macrolide-resistant isolates (4, 7, 13, 17).

(This study was presented in part at the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, 27 to 30 September 2002, San Diego, Calif.)

A total of 595 \textit{S. pneumoniae} isolates from outpatients with respiratory tract infections were collected from 17 medical microbiology laboratories throughout Germany during two surveillance studies conducted between October 2000 and April 2001. The inclusion of only one isolate per patient was permitted. Susceptibility testing was performed by broth microdilution with reference to the guidelines of the National Committee of Clinical Laboratory Standards (9). Broth microdilution plates were purchased from Merlin GmbH, Bornheim, Germany. The antibacterial agents tested were erythromycin, clindamycin, telithromycin, penicillin, tetracycline, and trimethoprim-sulfamethoxazole (1:19 ratio). NCCLS breakpoints were applied to categorize susceptibility patterns (10).

Erythromycin-resistant isolates were tested for the presence of the \textit{ermB} or \textit{mefA} gene using a duplex PCR (18). Pulsed-field gel electrophoresis (PFGE) was used to determine clonal relationships between erythromycin-resistant isolates (16).

Of the 595 isolates, 84 (14.1%) and 76 (12.8%) were resistant to erythromycin and clindamycin, respectively. Seventy-six (90.5%) and 34 (40.5%) out of the 84 erythromycin-resistant isolates were resistant to tetracycline and intermediate (\(n = 3\)) or resistant (\(n = 31\)) to trimethoprim-sulfamethoxazole, respectively. In addition, 18 (21.4%) of the 84 erythromycin-resistant isolates were nonsusceptible to penicillin, though none were penicillin resistant (MIC, \(\geq 2\) mg/liter). All erythromycin-resistant isolates were highly susceptible to telithromycin (MICs of \(\leq 0.06\) [\(n = 83\)] and 0.125 [\(n = 1\)] mg/liter).

Eighty-three out of the 84 erythromycin-resistant isolates were available for duplex PCR and PFGE. Sixty-nine (83.1%) and four (4.8%) isolates were positive for the \textit{ermB} and \textit{mefA} genes, respectively. None of the isolates harbored both resistance genes. Ten (12.0%) isolates hybridized neither with the \textit{ermB} probe nor with the \textit{mefA} probe. PFGE analysis revealed 17 different clones. Three clones (I to III) comprised a total of 66 (79.5%) isolates were predominant. Isolates of all three clones showed the \textit{ermB} genotype and were resistant to both erythromycin and clindamycin. Clone I isolates were additionally resistant to tetracycline only, whereas clone II isolates showed also resistance to tetracycline and trimethoprim-sulfamethoxazole (one isolate tested intermediate to trimethoprim-sulfamethoxazole). Isolates of clone III were additionally resistant to tetracycline and trimethoprim-sulfamethoxazole and intermediate to penicillin (Table 1).

Erythromycin resistance varied markedly between the centers; the local resistance rate ranged from 0% to 45%. Isolates of clone I were detected in two centers located in western (Essen) and southern (Stuttgart) Germany, whereas those of clone II were detected in one center of southern Germany (Rosenheim) and in two centers of eastern Germany (Rostock and Görlitz), and those of clone III were found in three centers of eastern Germany (Greifswald, Görlitz, Rostock) (Fig. 1).

The rate of erythromycin resistance (14.1%) found in the present study confirms the findings of two other recently conducted studies in Germany. Reinert et al. reported erythromycin resistance to be present in 17.4% of 333 \textit{S. pneumoniae} isolates collected from pediatric outpatients with respiratory tract infections (13). Of the 325 pneumococci isolated in the 1999-2000 PROTEKT surveillance study in Germany, 15.7% were erythromycin resistant (5). However, in the present study,
12.8% of all isolates were resistant to clindamycin, compared to 8.7% reported by Reinert et al. (13). In our study, 80% of the erythromycin-resistant isolates harbored the \( \text{ermB} \) gene, and 5% harbored the \( \text{mefA} \) gene. In previous studies from Germany, rates for \( \text{ermB} \) were in the range of 40.8 to 74.0%, and those for \( \text{mefA} \) were in the range of 20.5 to 51.0% (3, 11–13). \( \text{ermB/mefA} \) ratios of 80% have been reported from various other European countries, such as Belgium, France, Italy, and Spain, whereas \( \text{mefA} \)-mediated efflux was found to be the predominant mechanism in isolates in the United States and Canada (3).

Ten isolates of our series with a macrolide resistance phenotype were negative for the \( \text{ermB} \) and \( \text{mefA} \) genes. Other mechanisms found to confer macrolide resistance in pneumococci are mutations in the 23S rRNA or alterations in the ribosomal proteins L4 and L22 and a methylase encoded by the \( \text{ermA} \) (subclass \( \text{ermTR} \)) gene (3, 4, 14, 19).

The high rate of occurrence of \( \text{ermB} \) in our study was related to the dissemination of three clones. Resistance to other classes of antibacterial agents was widespread among these isolates. This was especially true for resistance to tetracycline. We did not look for the underlying mechanism of tetracycline resistance, but the association of erythromycin resistance and tetracycline resistance may be due to the conjugative transposon \( \text{Tn1545} \), which encodes erythromycin resistance via the \( \text{ermB} \) gene and tetracycline resistance via the \( \text{tetM} \) gene (1). In our study, 23.2% of \( \text{ermB} \)-positive isolates were intermediate to penicillin. This figure corresponds to the rate of 19.5% reported by Reinert et al. in a previous study in Germany (12).

In vitro macrolide resistance in \( S. \) pneumoniae infections due to both the efflux and the methylase mechanisms has been demonstrated to result in clinical failure (8). Telithromycin appears to be an alternative to macrolides for the treatment of community-acquired respiratory tract infections, especially in areas with a high prevalence of macrolide-resistant pneumococci.

This work was supported in part by Aventis Pharma Germany. We are grateful to Gabriele Bierbaum and Hans-Georg Sahl for giving us the opportunity to perform PFGE in their laboratory. We also thank Monika Pinkwart and Andrea Reipert for their support with PFGE.

### TABLE 1. Erythromycin-resistant clones of \( S. \) pneumoniae identified by PFGE

<table>
<thead>
<tr>
<th>Clone</th>
<th>No. of isolates</th>
<th>Genotype</th>
<th>MIC phenotype</th>
<th>MIC phenotype</th>
<th>MIC of telithromycin (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>33(^a)</td>
<td>+</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>II</td>
<td>17</td>
<td>+</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>+</td>
<td>–</td>
<td>R</td>
<td>I/R(^c)</td>
</tr>
<tr>
<td>IV</td>
<td>3(^b)</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>VI</td>
<td>1</td>
<td>–</td>
<td>+</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>VII</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>VIII</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>IX</td>
<td>1</td>
<td>+</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>X</td>
<td>1</td>
<td>–</td>
<td>+</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>XI</td>
<td>1</td>
<td>–</td>
<td>+</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>XII</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>XIII</td>
<td>1</td>
<td>+</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>XIV</td>
<td>1</td>
<td>+</td>
<td>–</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>XV</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>XVI</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>XVII</td>
<td>1</td>
<td>–</td>
<td>+</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

\(^a\) Thirty-one isolates had identical PFGE patterns, and two isolates showed slightly different patterns (difference, less than four bands).

\(^b\) Two isolates had identical PFGE patterns, and one isolate showed a slightly different pattern (difference, less than four bands).

\(^c\) The MIC was 2/38 mg/liter for one isolate and 4/76 mg/liter for 16 isolates.

\(^d\) For MIC phenotypes, R, S, and I indicate that the bacteria are resistant, susceptible, or intermediate to the indicated drug, respectively.

FIG. 1. Spread of three erythromycin-resistant \( S. \) pneumoniae clones with the \( \text{ermB} \) genotype in Germany.
REFERENCES


