High-Level Telithromycin Resistance in a Clinical Isolate of *Streptococcus pneumoniae*

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A rare clinical isolate of *Streptococcus pneumoniae*, highly resistant to telithromycin, contained *erm*(B) with a truncated leader peptide and a mutant ribosomal protein L4. By transformation of susceptible strains, this study shows that high-level telithromycin resistance is conferred by *erm*(B), wild type or mutant, in combination with a _69GTG_71–to–TPS mutation in ribosomal protein L4.

Telithromycin (TEL), the first ketolide approved for clinical use, is a semisynthetic derivative of the macrolide erythromycin A. Modifications of the 14-membered macrolactone ring include the replacement of the L-cladinose sugar at position 3 with a ketone group and a carbamate extension at position C11-C12. Telithromycin and the macrolide antibiotics bind to the peptidyl transferase region of the large ribosomal subunit and inhibit protein synthesis by blocking the peptide exit tunnel (7, 33). Macrolides and ketolides also interfere with assembly of the 50S ribosomal subunit (3). The C11-C12 carbamate extension of telithromycin enables it to bind to A752 in domain II of 23S rRNA, in addition to the primary binding site of erythromycin, A2058, in domain V (7). As a result, telithromycin has a stronger binding affinity for the ribosome and therefore can overcome common macrolide resistance mechanisms including target modification and drug efflux (2, 13). The former is directed by a methylase, encoded by the *erm*(B) gene, which methylates a specific adenine residue (A2058) in domain V of the 23S rRNA to block macrolide binding. This process results in high-level resistance to macrolides, lincosamides (clindamycin), and streptogramin B, referred to as the MLSb phenotype (32). Mutations in the 23S rRNA and ribosomal proteins, involved in translation, that interrupt macrolide binding have also been described (10, 27, 28). The *mef* gene mediates active drug efflux that leads to low-level resistance to macrolides only (M phenotype) (26).

Pneumococcal resistance to telithromycin remains rare. In laboratory-generated telithromycin-resistant mutants, mutations were shown to occur in the *erm*(B) upstream region (31). Clinical isolates with reduced susceptibility to telithromycin have shown mutations to occur in *erm*(B) (29) and ribosomal proteins L4 and L22 (11, 17, 18, 28). Tait-Kamradt et al. (29) described a highly resistant clinical isolate of *Streptococcus pneumoniae*, BSF11524, isolated from the conjunctiva of a 1-year-old boy in Canada in 1996 and submitted to the Canadian Bacterial Surveillance Network as part of an ongoing pneumococcal resistance surveillance program. This occurred several years prior to the approval of telithromycin in Canada. No further telithromycin-resistant isolates have been detected by the same network (15, 20). The isolate was found to contain mutations in *erm*(B) and ribosomal protein L4. In this study we investigate BSF11524 further, to establish the mechanism of resistance in this rare isolate.

Pneumococci were routinely cultured at 37°C in 5% CO2 on Mueller-Hinton agar supplemented with 5% horse blood. MICs were determined by the agar dilution method according to CLSI guidelines (4) and the Etest (AB Biodisk, Solna, Sweden). CLSI breakpoints were used (5). For telithromycin breakpoints were ≤1 µg/ml for susceptibility, 2 µg/ml for intermediate, and ≥4 µg/ml for resistance. Serotyping was performed by the Quellung reaction with antisera from the Statens Serum Institut (Copenhagen, Denmark). Chromosomal DNA was extracted as previously described (23). PCR-based methods were used to screen for *erm*(B) and *mef*(A) (25). Genes encoding L4 and L22 and all four alleles encoding 23S rRNA were amplified according to previously described methods (10, 27). The *erm*(B) gene was amplified using forward primer ermBF (5’-CTTAGAAGCAACTTAAAGG-3’) and reverse primer ermBR (5’-ATCGTACAAATCTCCCCGTA G-3’). Amplified products were purified with the QIAquick gel extraction kit (QIAGEN Ltd., Surrey, United Kingdom). DNA sequencing was performed using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an Applied Biosystems Model 310 automated DNA sequencer.

BSF11524 is serotype 19A and is highly resistant to erythromycin (MIC, >256 µg/ml), clindamycin (MIC, >256 µg/ml), and telithromycin (MIC, >256 µg/ml). It is resistant to tetracycline (MIC, 12 µg/ml) and penicillin (MIC, 16 µg/ml) but susceptible to chloramphenicol (MIC, 2 µg/ml). It was confirmed, as described previously (29), to be *erm*(B) positive and *mef*(A) negative. The *erm*(B) gene contained an adenine base insertion in the control peptide creating a stop codon and resulting in the truncation of the control peptide to 10 amino acids. In addition, three mutations were found in *erm*(B): I75T, S100N, and H118R. Ribosomal protein L4 was found to contain the following mutations: E13Q, S20N, E30Q, _69GTG_71 to...
TPS, V88I, G98A, A128S, and S130E. Ribosomal protein L22 and all four alleles of 23S rRNA were wild type.

In order to confirm the role of the mutations in conferring telithromycin resistance, transformations were carried out. Two pneumococcal strains were used for transformation studies: an unencapsulated laboratory strain (R6), susceptible to all antibiotics, and a strain (PC13) representative of pneumococcal clone 13 (South Africa19A) (16). PC13 was used as a recipient strain for transformation studies due to the fact that R6 does not contain an \(erm(B)\) gene for homologous recombination. Attempts to introduce a wild-type \(erm(B)\) gene into R6 by means of electroporation and conjugation were unsuccessful. The PC13 strain was selected based on its containing a wild type for PC13. It is therefore highly likely to be the combination of additional interaction with A752 in domain II of 23S rRNA that confers high-level telithromycin resistance.

The \(e_{69GTG71}^{TPS}\) mutation occurred in all transformants. This mutation is in the S20N mutation. Transformants were not selected in the absence of an \(erm(B)\) gene; therefore, PC13 was transformed with a mutated L4 gene containing only the S20N mutation. Transformants were not selected in the presence of telithromycin. The role of the L4 mutations in the high-level resistance observed in the isolate. The reduced telithromycin susceptibility of the PC13ermB transformants in the absence of an \(erm(B)\) gene was investigated by transforming R6 (TEL MIC, 0.015 \(\mu g/mL\)) with the full-length L4 gene of BSF11524 and the L4Fr fragment containing only the \(e_{69GTG71}^{TPS}\) mutation. R6L4 and R6L4Fr transformants had telithromycin MICs of 0.12 \(\mu g/mL\) and erythromycin MICs of >256 \(\mu g/mL\) (Table 1).

The mutant \(erm(B)\) gene of BSF11524 reduces the susceptibility of PC13 to telithromycin; however, it does not confer the high-level resistance observed in the isolate. The reduced telithromycin susceptibility of the PC13ermB transformants in comparison with PC13 may be due to increased dimethylation of A2058 in 23S rRNA as a result of the truncated control peptide in \(erm(B)\) (8, 14). The full-length L4 gene and the fragment of L4 containing the \(e_{69GTG71}^{TPS}\) to-TPS mutation. R6L4 Fr and R6L4Fr transformants had telithromycin MICS of 0.12 \(\mu g/mL\) and erythromycin MICS of >256 \(\mu g/mL\) (Table 1).

The \(erm(B)\) gene of BSF11524 was associated with resistance to macrolides (21) and has been identified in telithromycin-nonsusceptible strains (1); therefore, PC13 was transformed with a mutant L4 gene containing only the S20N mutation. Transformants were not selected in the presence of telithromycin. The role of the L4 mutations in the high-level resistance observed in the isolate. The reduced telithromycin susceptibility of the PC13ermB transformants in the absence of an \(erm(B)\) gene was investigated by transforming R6 (TEL MIC, 0.015 \(\mu g/mL\)) with the full-length L4 gene of BSF11524 and the L4Fr fragment containing only the \(e_{69GTG71}^{TPS}\) mutation. R6L4 and R6L4Fr transformants had telithromycin MICS of 0.12 \(\mu g/mL\) and erythromycin MICS of >256 \(\mu g/mL\) (Table 1).

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### TABLE 1. Phenotypic and genotypic results of pneumococcal transformations

<table>
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<tr>
<th>Strain</th>
<th>(erm(B)^{a})</th>
<th>L4(^{b})</th>
<th>TEL</th>
<th>ERY</th>
<th>AZM</th>
<th>CLR</th>
<th>CLI</th>
<th>TET</th>
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\(a\) \(erm(B)\) gene; \(b\) L4 gene; \(c\) ERY, erythromycin; AZM, azithromycin; CLR, clarithromycin; CLI, clindamycin; TET, tetracycline; CHL, chloramphenicol.

The elevated telithromycin MIC of PC13 in comparison with PC13 was transformed with the \(erm(B)\) gene of BSF11524 and the L4Fr fragment containing only the \(e_{69GTG71}^{TPS}\) mutation of L4 from BSF11524. PC13ermB transformants had a telithromycin MIC of >256 \(\mu g/mL\). The S20N mutation in L4 of BSF11524 has been described (19, 28), and as shown here, when not combined with \(erm(B)\) it confers high-level erythromycin resistance but confers only reduced susceptibility to telithromycin. Erythromycin and telithromycin share a common binding site; however, telithromycin forms a tighter bond with the ribosome due to an additional interaction with A752 in domain II of 23S rRNA (7). The \(e_{69GTG71}^{TPS}\) to-TPS mutation in L4 may therefore destabilize the binding of telithromycin; however, it does not block it completely, as for erythromycin. Higher levels of resistance to telithromycin appear to be a result of a combination of mutations.
telithromycin MIC of 256 μg/ml with an A2058T mutation in 23S rRNA and a deletion in L22. A combination of an A2058G mutation in 23S rRNA and an RTAHIT insertion in L22 resulted in a telithromycin MIC of 16 μg/ml (17). In addition, a telithromycin-resistant isolate with a MIC of 8 μg/ml was found to contain an erm(B) gene, an S20N mutation in L4, and a number of mutations in 23S rRNA (22). A highly resistant BSF11524, with a K94Q mutation in riboprotein L22 (31), had a 210-bp deletion in the /H11006 site. J. Antimicrob. Chemother. 46:1295–1301.


