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# In Vitro Activities of the Novel Ketolide Telithromycin (HMR 3647) against Erythromycin-Resistant *Streptococcus* Species

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**The in vitro susceptibilities of 184 erythromycin-resistant streptococci to a novel ketolide, telithromycin (HMR 3647), were tested. These clinical isolates included 111 *Streptococcus pyogenes*, 18 group C streptococcus, 18 group G streptococcus, and 37 *Streptococcus pneumoniae* strains. The MICs for all but eight *S. pyogenes* strains were ≤0.5 μg/ml, indicating that telithromycin is active in vitro against erythromycin-resistant *Streptococcus* strains. All strains for which MICs were ≥1 μg/ml had an *erm*(B) resistance gene and six strains for which MICs were ≥4 μg/ml had a constitutive *erm*(B) gene (MIC range, 4 to 64 μg/ml). Interestingly, for *S. pneumoniae* strains with a constitutive *erm*(B) gene, MICs were ≤0.25 μg/ml (MIC range, ≤0.008 to 0.25 μg/ml). Our in vitro data show that for *S. pyogenes* strains which constitutively express the *erm*(B) methylase gene, MICs are so high that the strains might be clinically resistant to telithromycin.**

Ketolides represent a new generation of macrolides, in which a 3-keto group replaces L-cladinose in the lactone ring. Ketolides have shown to be more active in vitro than other macrolides against various gram-positive bacteria such as *Enterococcus* species (6, 24), *Staphylococcus aureus* (6, 13), and *Streptococcus* species, including erythromycin-resistant *Streptococcus pneumoniae* and *Streptococcus pyogenes* strains (6, 16, 18). On the other hand, methicillin-resistant and some erythromycin-resistant *S. aureus* strains, as well as some *Staphylococcus epidermidis* strains (1, 18), seem to be resistant to ketolides (1, 6, 13).

In streptococci, there are two well-characterized macrolide resistance mechanisms: target site modification and active drug efflux. Target site modification is mediated by methylases encoded by the *erm* (erythromycin ribosome methylation) genes (21, 26). Methylation of A2058 of the peptidyl transferase loop of 23S rRNA causes resistance to macrolides as well as to lincosamides and streptogramin B antibiotics (the $MLS_B$ resistance phenotype) (26). The expression of the *erm* genes can be either constitutive or inducible (27). In streptococci, *erm* genes are carried on both the chromosome and plasmids (2, 21) and are associated with conjugative transposons (25). The active-efflux mechanism, encoded by the *mef* (macrolide efflux) genes, is more specific and causes resistance only to 14- and 15-member-ring macrolides (the M resistance phenotype) (3, 22). Expression of *mef* genes is constitutive (C. Arpin, M. H. Canron, P. Noury, and C. Quentin, Letter, J. Antimicrob. Chemother. **44:**133–134, 1999). The *mef* genes are chromosomal (11, 17) and, at least in the case of *S. pyogenes*, can be transferred by conjugation (11).

The present work was carried out to study the activity of a novel ketolide, telithromycin (HMR 3647), against *Streptococcus* species with known macrolide resistance determinants. In addition, its activity against nine *S. pneumoniae* strains with an unknown macrolide resistance mechanism was tested.

## MATERIALS AND METHODS

**Bacterial strains.** Altogether 184 erythromycin-resistant streptococcal strains (MIC, ≥1 μg/ml) selected by the erythromycin resistance mechanism and 51 erythromycin-susceptible streptococcal strains (MIC, ≤0.25 μg/ml) were analyzed. These strains included 131 *S. pyogenes*, 28 group C streptococcus (GCS), 29 group G streptococcus (GGS), and 47 *S. pneumoniae* strains (Table 1). Erythromycin-resistant *S. pyogenes* strains were collected from the United States, Argentina, and various European countries between 1986 and 1997. Erythromycin-susceptible *S. pyogenes* strains were collected from Finland as described previously (9, 11). The GCS and GGS strains were collected in the North Karelian region in Finland between 1992 and 1995 (12). *S. pneumoniae* strains were obtained from microbiological laboratories situated all over Finland between 1994 and 1998. Each laboratory identified the strains using their own standard microbiology techniques and sent the strains to the Antimicrobial Research Laboratory of the National Public Health Institute, Turku, Finland, where the identification was further confirmed based on colony morphology and hemolysis on Blood Agar Base (Oxoid Ltd., Basingstoke, Hampshire, England) plates supplemented with 7.5% sheep blood. For *S. pyogenes*, GCS, and GGS strains the Streptex test (Murex Biotech Ltd., Kent, England) was also used, and for *S. pneumoniae*, the Optochin Disc (Oxoid Ltd.) or Slidex Pneumo-Kit (bioMérieux SA, Marcy l'Etoile, France) was also used. Two control strains, *S. pyogenes* ATCC 10389 and *S. pneumoniae* ATCC 49619, were tested together with the studied strains.

**MIC testing.** MIC testing was done using the agar dilution technique. The bacteria were cultured for 20 h in air at 35°C on Mueller-Hinton II (Becton Dickinson Microbiology Systems, Cockeysville, Md.) agar plates supplemented with 5% sheep blood. The antibiotics used were telithromycin (HMR3647), erythromycin, azithromycin, clarithromycin (Hoechst Marion Roussel [Aventis Pharma], Romainville Cedex, France), quinupristin-dalfopristin (30/70) (RP59500), spiramycin (Rhône-Poulenc Rorer, Vitrysur-Seine, France), and clindamycin (Sigma-Aldrich Chemie, Gmbh, Steinheim, Germany). If available, NCCLS MIC breakpoints were used (15); otherwise, interpretation of MIC results was done based on the distribution of MICs.

**Phenotyping.** The double-disk method with erythromycin (diffusible content, 78 μg) and clindamycin (diffusible content, 25 μg) (Neo-sensitabs; A/S Rosco, Taastrup, Denmark) disks was used for classification of macrolide resistance phenotypes. The disks were placed 15 to 20 mm apart on Mueller-Hinton II agar plates supplemented with 5% sheep blood. Bacteria were cultured for 20 h in 5% $CO_2$ at 35°C. After incubation, blunting of the clindamycin zone of inhibition

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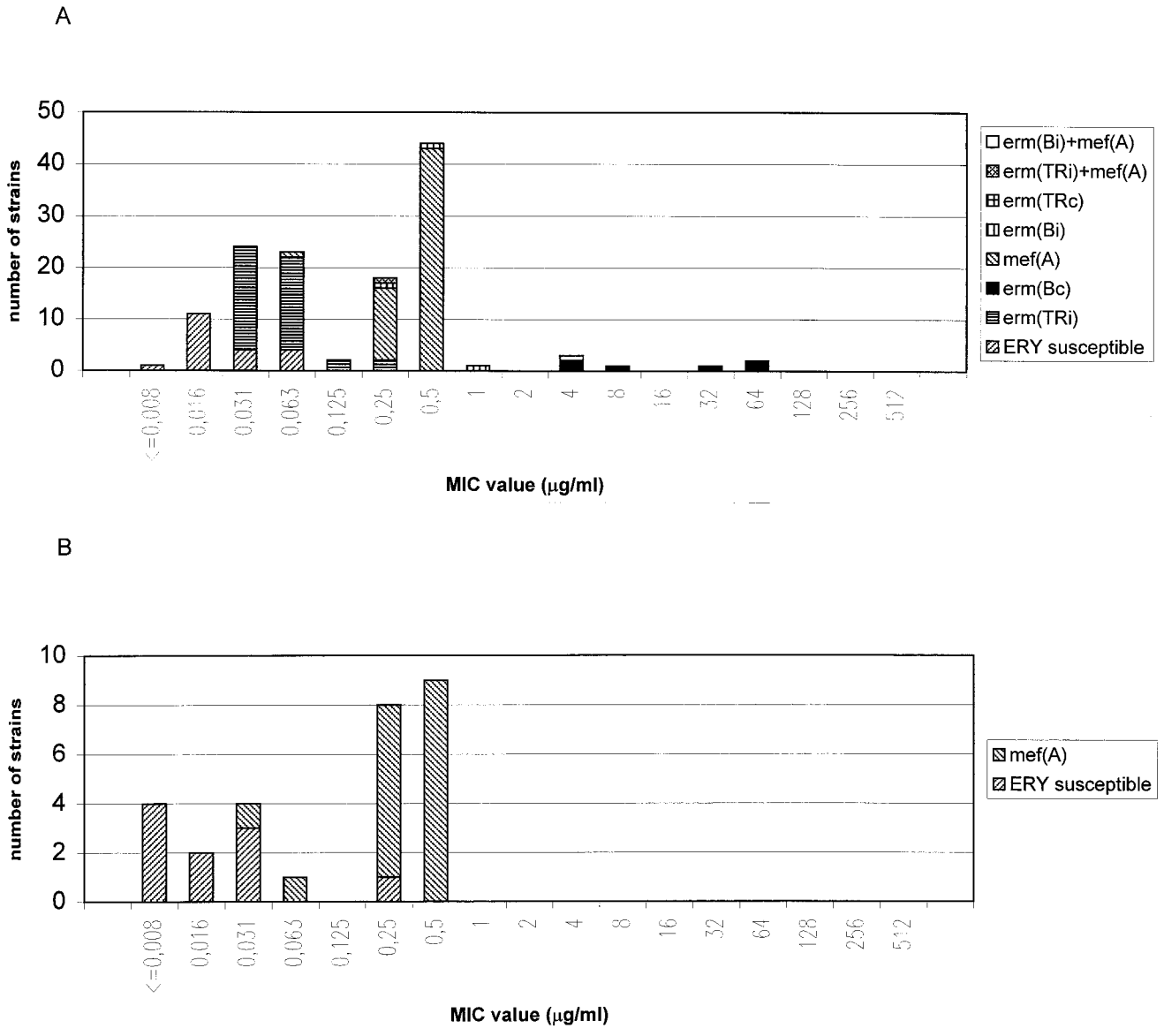


FIG. 1. Distribution of macrolide resistance genes of *S. pyogenes* (A), GCS (B), GGS (C), and *S. pneumoniae* (D) strains according to telithromycin MICs Abbreviations: ERY, erythromycin; c, constitutive; i, inducible; erm, erythromycin ribosome methylation genes; mef, macrolide efflux genes. See text for details of the novel resistance phenotype.

proximal to the erythromycin disk was considered to indicate inducible $MLS_B$ resistance (20). In addition to strains with well-characterized macrolide resistance types (the $MLS_B$ and M phenotypes), nine *S. pneumoniae* strains with a novel resistance phenotype were included in the analyses. In these nine strains no known macrolide resistance gene has been found by PCR. These strains are resistant to erythromycin, azithromycin, and clarithromycin, and the spiramycin and clindamycin MICs for these strains are elevated compared to those for strains with the M resistance phenotype, although MICs of clindamycin are not as high as for strains with a constitutive *erm*(B) resistance gene. Resistance to clindamycin is not inducible (unpublished observations). A similar phenotype for *S. pneumoniae* has been previously described for clinical isolates (8) and recently also for laboratory strains carrying mutations in 23S rRNA or ribosomal protein L4 (23).

**Resistance gene determinations and clonality studies.** Macrolide resistance genes were determined using PCR as described previously (11). The clonality of six *S. pyogenes* strains was studied using serotyping (14), Vir typing (5), and random amplified polymorphic DNA analysis (19).

## RESULTS AND DISCUSSION

In this study, the in vitro activities of telithromycin and six other antibiotics against 184 erythromycin-resistant and 51 erythromycin-susceptible *Streptococcus* strains were studied (Table 1 and Fig. 1). In general, telithromycin was more active than 14- and 15-member-ring macrolides (erythromycin, azithromycin, and clarithromycin) against erythromycin-resistant *Streptococcus* strains. MICs of telithromycin were ≤0.5 μg/ml for all but eight *S. pyogenes* strains (see below). Quinupristin-dalfopristin, a mixture of streptogramin A and B antibiotics, showed activity similar to that of telithromycin, although MICs were somewhat higher (MIC at which 90% of the isolates are inhibited [$MIC_{90}$], 0.5 to 4 μg/ml). Clindamycin

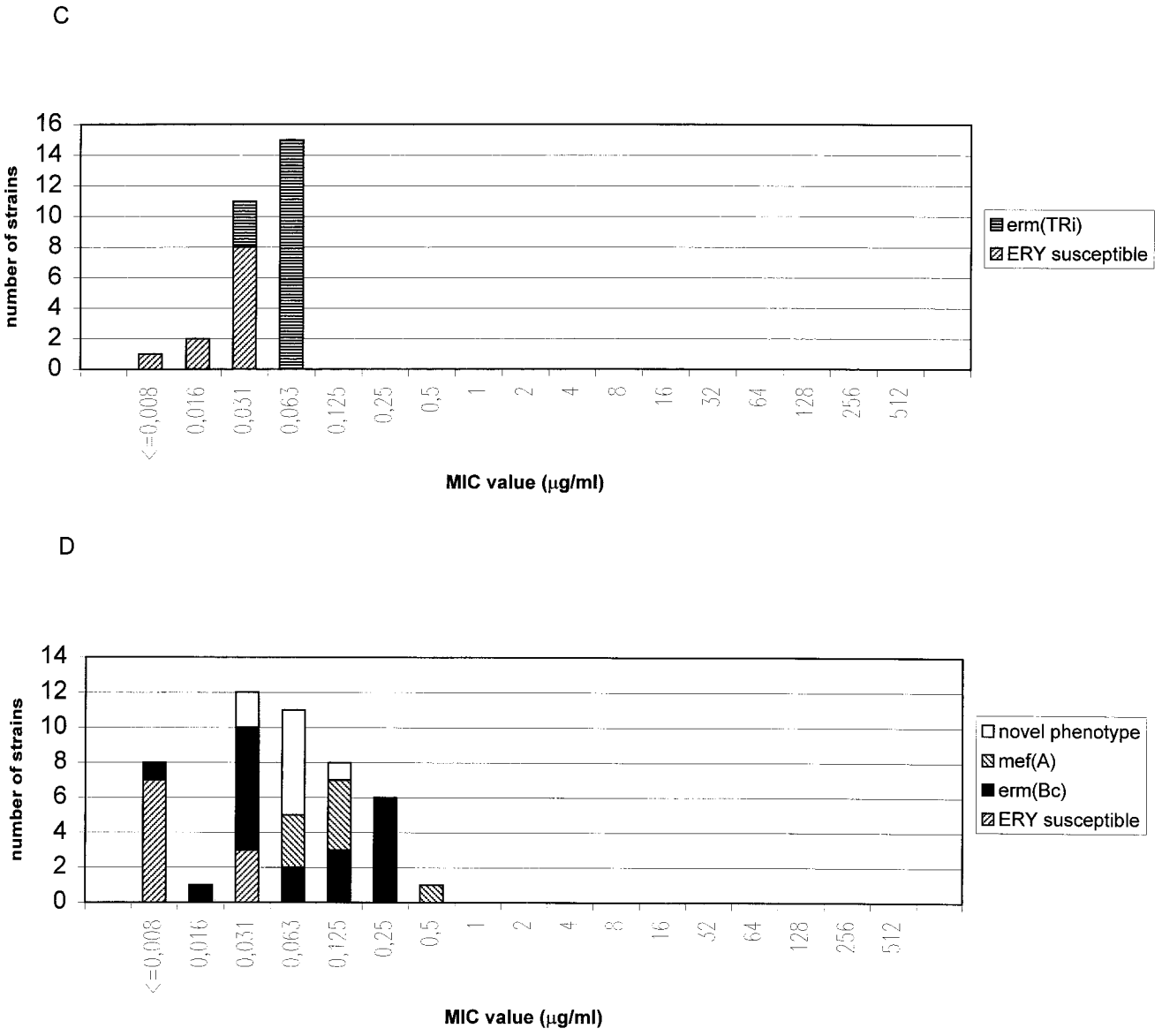


FIG. 1—*Continued.*

(a lincosamide) was active against strains with an inducible *erm* methylase gene ($MIC_{90}$, 0.063 to 0,5 μg/ml) and was even more active than telithromycin against strains with a *mef* gene ($MIC_{90}$, 0.063 to 0.125 μg/ml). The clindamycin MICs for strains with a constitutive *erm* methylase gene were high ($MIC_{90}$, >64 μg/ml). Also, *S. pneumoniae* strains with the novel resistance phenotype were intermediate or resistant to clindamycin ($MIC_{90}$, 4 μg/ml). Spiramycin (a 16-member-ring macrolide) showed activity similar to that of telithromycin against strains with a *mef* resistance gene. However, the spiramycin MICs for all strains with an *erm* methylase gene (inducible or constitutive) and for *S. pneumoniae* strains with the novel resistance phenotype were elevated ($MIC_{90}$, 4 to >64 μg/ml).

The eight *S. pyogenes* strains for which the telithromycin MICs were elevated included one strain with an inducible *erm*(B) gene (MIC, 1 μg/ml), one strain with two resistance genes [an inducible *erm*(B) gene and a *mef*(A) gene; MIC, 4 μg/ml], and, most importantly, six strains with a constitutive *erm*(B) gene ($MIC_{90}$, 64 μg/ml). These six *S. pyogenes* strains belonged to six different serotypes (T12M22 opacity factor positive, T12M12 opacity factor negative, T1M1 opacity factor negative, T12M66 opacity factor positive, T6M6 opacity factor negative, and T28R28 opacity factor positive), six different Vir types, and six different random amplified polymorphic DNA types, indicating that the strains were not of the same clonal origin. In contrast to the case for *S. pyogenes* strains, telithromycin MICs for gene *S. pneumoniae* strains with the constitutive *erm*(B) were low ($MIC_{90}$, 0.25 μg/ml). This finding is concordant with a previous study where telithromycin MICs for *S. pneumoniae* strains with the constitutive $MLS_B$ phenotype of erythromycin resistance were low ($MIC_{90}$, 0.25 μg/ml) (16). Similar to the case for *S. pyogenes*, telithromycin MICs for constitutively erythromycin-resistant *S. aureus* strains have

TABLE 1. *Streptococcus* strains characterized according to their macrolide resistance determinants and MICs

| Organism | Gene[a] | *n* | MIC (μg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Telithromycin (HMR 3647) | | | Erythromycin | | | Clindamycin | | |
| | | | Range | 50% | 90% | Range | 50% | 90% | Range | 50% | 90% |
| *S. pyogenes* | S | 20 | 0.008–0.063 | 0.016 | 0.063 | ≤0.031–0.25 | 0.063 | 0.125 | ≤0.031–0.125 | 0.063 | 0.063 |
| | *erm*(TR$^i$) | 42 | 0.031–0.25 | 0.063 | 0.063 | 0.5–>64 | 4 | 8 | 0.063–>64 | 0.125 | 0.25 |
| | *erm*(TR$^c$) | 1 | 0.25 | | | >64 | | | >64 | | |
| | *erm*(B$^i$) | 2 | 0.5–1 | | | >64 | | | 0.25 | | |
| | *erm*(B$^c$) | 6 | 4–64 | 8 | 64 | >64 | >64 | >64 | >64 | >64 | >64 |
| | *mef*(A) | 58 | 0.063–0.5 | 0.5 | 0.5 | 4–16 | 8 | 8 | ≤0.031–0.125 | 0.063 | 0.063 |
| | *erm*(TR$^i$) + *mef*(A) | 1 | 0.25 | | | 8 | | | 0.063 | | |
| | *erm*(B$^i$) + *mef*(A) | 1 | 4 | | | >64 | | | 0.25 | | |
| bsc | S | 10 | ≤0.008–0.25 | 0.016 | 0.031 | ≤0.031–0.125 | 0.063 | 0.063 | ≤0.031–0.125 | 0.125 | 0.125 |
| | *mef*(A) | 18 | 0.031–0.5 | 0.25 | 0.5 | 1–8 | 4 | 8 | 0.063–0.125 | 0.125 | 0.125 |
| bsg | S | 11 | ≤0.008–0.031 | 0.031 | 0.031 | 0.063–0.25 | 0.063 | 0.063 | ≤0.031–0.25 | 0.063 | 0.125 |
| | *erm*(TR$^i$) | 18 | 0.031–0.063 | 0.063 | 0.063 | 1–>64 | 4 | 64 | 0.063–0.5 | 0.125 | 0.5 |
| *S. pneumoniae* | S | 10 | ≤0.008–0.031 | ≤0.008 | 0.031 | ≤0.031–0.063 | ≤0.03 | ≤0.031 | ≤0.031–0.063 | 0.063 | 0.063 |
| | *erm*(B$^c$) | 20 | ≤0.008–0.25 | 0.063 | 0.25 | 64–>64 | >64 | >64 | 0.25–>64 | >64 | >64 |
| | *mef*(A) | 8 | 0.063–0.5 | 0.125 | 0.5 | 1–4 | 2 | 4 | 0.063–0.125 | 0.063 | 0.125 |
| | Novel phenotype[b] | 9 | 0.031–0.125 | 0.063 | 0.125 | 64–>64 | >64 | >64 | 0.5–4 | 1 | 4 |

[a] i, inducible; c, constitutive; S, erythromycin-susceptible strain; *erm* = erythromycin ribosome methylation genes; *mef*, macrolide eflux genes.
[b] See text for details.

been shown to be elevated (6). Telithromycin MICs for *S. pyogenes* and GGS strains which had an inducible *erm*(TR) gene were low ($MIC_{90}$, 0.063 μg/ml). In contrast to the case for the constitutive *erm*(B) gene, constitutive expression of the *erm* (TR) gene in one *S. pyogenes* strain did not increase the telithromycin MIC.

An interesting question is why the Erm(B) methylase does not protect *S. pneumoniae* as well as it protects *S. pyogenes* against telithromycin. The Erm(B) methylases of *S. pneumoniae* and *S. pyogenes* are nearly identical (99% similarity at the amino acid level [21]), so it is unlikely that the differences in the *erm*(B) genes can explain differences in susceptibility to telithromycin. Structural differences in the 23S rRNA molecules or other ribosomal components are more likely explanations. Ketolides as well as other macrolides interact with the peptidyl transferase loop in the V domain of the 23S rRNA. For example, both telithromycin and erythromycin protect positions A2058, A2059, and G2505 of the 23S rRNA against chemical modification (7, 28). It has also been shown that hairpin 35 in domain II of the 23S rRNA constitutes a part of the binding site of macrolides and ketolides (7, 28). Telithromycin protects against chemical modification, and erythromycin enhances chemical modification, of residue A752 in hairpin 35 (7). Actually, using different ketolide derivatives it has been shown that interaction with hairpin 35 is essential for the antimicrobial activity of ketolides (4). Methylation of A2058 of the peptidyl transferase loop in the V domain confers resistance to $MLS_B$ antibiotics by inhibiting the binding of the antibiotics to the ribosomes. However, as we have shown here with *S. pneumoniae* and as has been shown with several other bacteria (6, 18), this methylation does not protect against telithromycin. It has been proposed that although methylation of A2058 weakens the binding of macrolides and ketolides to the ribosomes by interfering with the interaction between the antibiotic and the residues on the peptidyl transferase loop, it does not prevent the strong interaction of ketolides with hairpin 35. This interaction therefore is probably enough to bind the antibiotic to the ribosome and to prevent protein synthesis (28). The same is true with mutations in the peptidyl transferase loop, which can inhibit binding of macrolides but not ketolides to the ribosomes if ketolides have alkyl-aryl 11/12 lactone ring extensions, which can make contact with hairpin 35 and the drug (4). It might be that in *S. pyogenes* the interaction of telithromycin with hairpin 35 is weaker than that in *S. pneumoniae* and because of that, telithromycin can not bind to the ribosomes of *S. pyogenes* if A2058 is methylated. Structural differences in the ribosomes of the two bacteria may thus explain the differences in the interactions between telithromycin and hairpin 35.

Quite recently, Tait-Kamradt et al. (23) demonstrated that in *S. pneumoniae* mutations in the 23S rRNA or ribosomal protein L4 can cause resistance to macrolides. Mutations in the pepditYl transferase loop of the 23S rRNA caused phenotypes that were similar but not identical to those described in this work for *S. pneumoniae* strains without any known macrolide resistance genes. The type, number, and positions of mutations in the peptidyl transferase loop affect the phenotype, so it is possible that the strains we describe here also carry resistance-causing mutations in 23S rRNA molecules.

Telithromycin is a novel ketolide that belongs to the macrolide family of antibiotics. Our work in addition to several other studies, indicates that the new ketolides are active in vitro against various gram-positive bacteria, including strains that are resistant to other macrolides (6, 16). Telithromycin MICs were high only for *S. pyogenes* strains with the constitutive *erm*(B) resistance gene. In recent years these strains have comprised about 10% of all erythromycin-resistant *S. pyogenes* strains in Finland (11) and several other countries (10). Although the presence of a constitutive *erm*(B) gene in *S. pyogenes* varies in different countries (10), it seems that telithromycin has good in vitro activity against most *S. pyogenes* strains.

TABLE 1—*Continued*

| MIC (μg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Azithromycin | | | Clarithromycin | | | Spiramycin | | | Quinupristin-dalfopristin (RP59500) | | |
| Range | 50% | 90% | Range | 50% | 90% | Range | 50% | 90% | Range | 50% | 90% |
| 0.063–0.5 | 0.125 | 0.25 | ≤0.031–0.125 | 0.031 | 0.063 | 0.25–1 | 0.25 | 0.5 | 0.25–1 | 0.5 | 1 |
| 0.5–>64 | 16 | 32 | 0.25–>64 | 2 | 2 | 0.5–64 | 1 | 8 | 0.25–1 | 0.5 | 0.5 |
| >64 | | | >64 | | | 64 | | | 0.25 | | |
| >64 | | | >64 | | | >64 | | | 0.5 | | |
| >64 | >64 | >64 | >64 | >64 | >64 | >64 | >64 | >64 | 0.5–1 | 0.5 | 1 |
| 1–16 | 4 | 8 | 1–8 | 4 | 8 | 0.125–2 | 0.25 | 0.5 | 0.25–1 | 0.5 | 0.5 |
| 8 | | | 8 | | | 0.5 | | | 0.25 | | |
| >64 | | | >64 | | | >64 | | | 0.5 | | |
| 0.063–0.25 | 0.125 | 0.25 | ≤0.031–0.063 | 0.031 | 0.063 | 0.125–0.5 | 0.25 | 0.5 | 0.5–2 | 1 | 2 |
| 1–4 | 4 | 4 | 0.25–4 | 4 | 4 | 0.125–1 | 0.5 | 1 | 0.5–4 | 1 | 2 |
| 0.125–1 | 0.125 | 0.125 | ≤0.031–0.25 | ≤0.031 | 0.063 | 0.125–1 | 0.5 | 0.5 | 0.5–1 | 1 | 1 |
| 4–>64 | 16 | >64 | 0.5–>64 | 1 | 8 | 0.5–4 | 2 | 4 | 1–2 | 1 | 1 |
| ≤0.031–0.125 | 0.125 | 0.125 | ≤0.031 | ≤0.031 | ≤0.031 | 0.125–0.25 | 0.125 | 0.25 | 0.5–2 | 1 | 2 |
| >64 | >64 | >64 | 8–>64 | >64 | >64 | 16–>64 | >64 | >64 | 0.5–4 | 2 | 4 |
| 1–4 | 2 | 4 | 1–4 | 2 | 4 | 0.125–0.25 | 0.125 | 0.25 | 0.5–1 | 1 | 1 |
| 32–>64 | 64 | >64 | 16–>64 | >64 | >64 | >64 | >64 | >64 | 2–4 | 4 | 4 |


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