Time-Kill Study of the Activity of Telithromycin against Macrolide-Resistant Streptococcus pneumoniae Isolates with 23S rRNA Mutations and Changes in Ribosomal Proteins L4 and L22

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By use of a time-kill methodology, the antipneumococcal activity of telithromycin was determined against macrolide-resistant S. pneumoniae isolates having mutations in the 23S rRNA gene and changes in the ribosomal proteins L4 and L22. Telithromycin had MICs ranging between 0.03 and 0.25 μg/ml and was bactericidal against four of seven strains after 24 h at two times the MIC.

Resistance to macrolides is being increasingly reported in clinical isolates of Streptococcus pneumoniae worldwide (2). In Germany, a sharp rise in resistance among pneumococcal strains isolated from patients with invasive and noninvasive disease was observed with the macrolides only recently (10, 11). The most prevalent mechanisms of macrolide resistance in S. pneumoniae are mediated by mef(A), a gene encoding an efflux pump, and erm(B), a 23S rRNA methylase that methylates adenine in position 2058, resulting in macrolide-lincosamide-streptogramin B resistance (13). In addition, mutations in the 23S rRNA gene and ribosomal proteins L4 and L22 have been shown to account for resistance in pneumococci (3, 4, 14). Such isolates have recently been described to be prevalent in Germany (12).

Ketolides are a new class of semisynthetic agents derived from erythromycin A which were designed to overcome erythromycin A resistance in S. pneumoniae. Ketolides are characterized by the replacement of the L-cladinose sugar at position 3 of the erythronolide A moiety by a keto group.

Telithromycin is the first ketolide available in Europe and the United States (since 2001 and 2004, respectively). Approved indications for its use in Europe are mild and moderate respiratory tract infections, including those caused by antibiotic-resistant S. pneumoniae (9). In this paper, the activity of telithromycin against macrolide-resistant S. pneumoniae isolates having mutations in the 23S rRNA gene and changes in the ribosomal proteins L4 and L22 was investigated by time-kill studies with seven pneumococcal strains.


MICs and characteristics of the seven strains are presented in Table 1. Only some of the 23S rRNA gene mutations, such as A2060G and A2061G (pneumococcal numbering), have been clearly shown to confer macrolide resistance in S. pneumoniae. The relevance to macrolide resistance of most of the other 23S rRNA gene mutations reported for the seven strains needs further investigation. Details of the resistance mechanism and the molecular epidemiology of these strains have been provided previously (12). Microbroth MICs were determined according to NCCLS recommendations (5) using cation-adjusted Mueller-Hinton broth with 5% lysed, defibrinated horse blood. Time-kill testing was performed as described previously (7, 8). Only initial inocula of 5 × 10^5 to 5 × 10^6 CFU/ml were acceptable. The lower limit of sensitivity of colony counts in viability testing was 300 CFU/ml. Time-kill results were analyzed by determining the change in log_{10} numbers of CFU/ml of −1, −2, and −3 at 0, 2, 4, 8, 12, and 24 h, compared to counts at 0 h. Antimicrobials were considered bactericidal at the lowest concentration that reduced the size of the original inoculum by ≥3 log_{10} CFU/ml (99.9%) over each of the time periods and were considered bacteriostatic if the inoculum’s size was reduced by 0 to <3 log_{10} CFU/ml. The problem of bacterial carryover was addressed by dilution (7, 8). Results of the time-kill studies for the seven strains are presented in Table 2.

Telithromycin was bactericidal for four of seven macrolide-resistant S. pneumoniae strains after 24 h at two times the MIC. S. pneumoniae PS 2938, a strain with a combination of an L4 alteration and a mutation in the 23S rRNA gene, showed a bacteriostatic effect as well as a very slow bactericidal effect after 12 h at eight times the MIC. Similar results were observed with strain PS 2909, which possesses an L22 mutation along with multiple alterations in the L4 protein. However, in contrast, the two pneumococcal strains with the S20N alteration in L4 (NRZ 288 and NRZ 769) showed a relatively rapid bactericidal effect.

Ortega and coworkers performed kill studies with 120 clinically significant S. pneumoniae isolates (60 susceptible and 60 highly resistant to erythromycin) and showed 99.9% killing of all erythromycin-resistant strains after 18 to 24 h of incubation (6). Even for strains with erythromycin MICs of ≥64 μg/ml, telithromycin was uniformly bactericidal at 0.25 μg/ml (6);
however, the collection of macrolide-resistant S. pneumoniae isolates of Ortega et al. did not include strains with the newly described macrolide resistance determinants included in the present investigation.

Abbanat and coworkers tested the in vitro activities of erythromycin A, telithromycin, and two investigational ketolides, JNJ-17155437 and JNJ-17155528, against the erm- or mef-containing pneumococci (1). Cell counts in telithromycin-treated broth cultures decreased by at least 3 log_{10} CFU over 24 h, indicating that the ketolides were bactericidal against these isolates over this time period (1). This effect was also observed with the erm- and mef-containing pneumococci included in the present investigation.

In summary, the characterization of ketolides with respect to their bacteriostatic or bactericidal activity may be important for dosing and ultimately for clinical utility. Macrolides and ketolides may be classified as bacteriostatic agents, with slow bacteriostatic activity observed at higher concentrations against selected clinical pathogens (9). However, as shown by the present study, the killing by telithromycin of S. pneumoniae isolates having the rare combination of mutations in the 23S rRNA gene and changes in the ribosomal proteins L4 and L22 is relatively low. Thus, the efficacy of telithromycin for such infections requires further investigation.

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### REFERENCES


