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NOTES

In Vitro Activity of Telithromycin against Macrolide-Susceptible and Macrolide-Resistant Pharyngeal Isolates of Group A Streptococci in the United States

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In vitro susceptibility testing of 2,797 group A streptococcus (GAS) isolates demonstrated that telithromycin was fully active against all macrolide-susceptible strains and among 80 of 115 macrolide-resistant GAS expressing the M phenotype. Telithromycin resistance was identified in 2 of 45 strains expressing the inducible macrolide-lincosamide-streptogramin B phenotype and four of nine isolates expressing the constitutive macrolide-lincosamide-streptogramin B resistance phenotype.

Macrolides, particularly azithromycin, are prescribed increasingly for treatment of pharyngitis due to group A streptococcus (GAS). A high prevalence of macrolide resistance among GAS has been recognized in Europe and Southeast Asia for many years (2, 4, 7), although the overall rates of macrolide resistance among GAS in the United States have remained relatively low (1, 3, 6, 9, 12, 21, 23). Telithromycin, a member of a new class of antimicrobials known as ketolides, has been reported to be active against GAS expressing the macrolide (M) and inducible macrolide, lincosamide, and streptogramin (MLS) resistance phenotypes while being less active against isolates expressing the constitutive MLS phenotype (8, 19). This report summarizes results of susceptibility testing of GAS isolates collected as part of a multicenter prospective surveillance study during the 2002–2003 respiratory season.

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Pharyngeal isolates of GAS were collected from clinical microbiology laboratories of nine academic centers across the United States, with susceptibility testing performed at a central laboratory (Pittsburgh, Pa.). Participating centers were located in Ann Arbor, MI; Columbus, OH; Durham, NC; Houston, TX; Little Rock, AR; Nashville, TN; Newark, NJ; Pittsburgh, PA; and San Diego, CA. Fifty isolates of GAS per month were requested from each site from 1 September 1 2002 to 31 May 31 2003. This study was approved by the Institutional Review Boards of each participating center.

Susceptibility to erythromycin and clindamycin was screened using Kirby-Bauer disks (BBL Becton Dickinson, Sparks, MD) on Mueller-Hinton agar (16). The MIC was determined using the E-test (AB Biodisk, Piscataway, NJ) for isolates identified as nonsusceptible (intermediate or resistant susceptibility) to erythromycin or clindamycin by Kirby-Bauer disk screening. Susceptibility testing was also performed against azithromycin, clarithromycin, levofloxacin, gatifloxacin, and telithromycin using broth microdilution methods by a commercial reference laboratory (Clinical Microbiology Institute, Williamsville, Oregon). Breakpoints used for this study were those approved by the National Committee for Clinical Laboratory Standards (NCCLS) for GAS (17). Since NCCLS telithromycin MIC breakpoints for GAS are not available, those defined in Europe were used: susceptible, $\leq$0.5 μg/ml; intermediate, 1.0 to 2.0 μg/ml; and resistant, $\geq$4.0 μg/ml (13).

Macrolide-resistant isolates were designated as expressing the M phenotype, the inducible MLS phenotype (MLS_i), or the constitutive MLS phenotype (MLS_c) using the double disk diffusion test (20). The presence of *mef*(A), *erm*(B), and *erm*(A) resistance genes was detected by PCR amplification for isolates expressing telithromycin resistance (14). The genetic relatedness of telithromycin-nonsusceptible isolates (MIC $\geq$ 1.0) was investigated by field inversion gel electrophoresis (15).

Results of susceptibility testing performed for erythromycin, quinolones, and telithromycin on 2,797 isolates of GAS are

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TABLE 1. Overview of in vitro susceptibility testing for pharyngeal isolates of group A streptococci from nine sites across the United States

Drug or GAS isolate group	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	No. (%) of nonsusceptible isolates[a]	No. (%) of resistant isolates[b]
Erythromycin					
All isolates (n = 2,797)	0.06–>128.0	0.06	0.06	169 (6.0)	169 (6.0)
M phenotype (n = 115)	1.00–32.0	16.00	32.00	115 (4.1)	115 (4.1)
MLS_i (n = 45)	2.00–>128	4.00	>128.00	45 (1.6)	45 (1.6)
MLS_c (n = 9)	>128.0	>128.0	>128.00	9 (0.3)	9 (0.3)
Quinolone					
All isolates (n = 2,797)					
Levofloxacin	0.25–16.0	0.50	1.00	12 (0.43)	10 (0.36)
Gatifloxacin	0.06–4.0	0.25	0.50	12 (0.43)	4 (0.14)
Telithromycin					
All isolates (n = 2,797)	0.015–32.0	0.03	0.06	46 (1.6)	6 (0.2)
Erythromycin-resistant isolates (n = 169)	0.03–32.0	0.50	1.00	46 (27.2)	6 (3.6)
M phenotype (n = 115)	0.25–1.0	0.50	1.00	35 (30.4)	0 (0.0)
MLS_i (n = 45)	0.03–8.0	0.03	0.06	2 (4.4)	2 (4.4)
MLS_c (n = 9)	1.0–32.0	3.00	8.00	9 (100)	4 (44.4)

[a] Nonsusceptible, MIC of ≥0.5, 4.0, 2.0, and 1.0 for erythromycin, levofloxacin, gatifloxacin and telithromycin, respectively.
[b] Resistance, MIC of ≥1.0, 8.0, 4.0, and 4.0 for erythromycin, levofloxacin, gatifloxacin, and telithromycin, respectively.

shown in Table 1; 169 (6.0%) isolates were resistant to erythromycin, with 115 (4.1%), 45 (1.6%), and 9 (0.3%) of the total isolates expressing the M, MLS_i, and MLS_c resistance phenotypes, respectively. All erythromycin-resistant isolates were also resistant to clarithromycin and azithromycin. Levofloxacin and gatifloxacin were highly active against the GAS isolates; 12 isolates were nonsusceptible to both agents; 10 of these were resistant to levofloxacin, while only 4 were resistant to gatifloxacin. Susceptibilities to either quinolone agent and the macrolides were unrelated.

Six (0.2%) isolates were resistant to telithromycin, and 40 isolates had an intermediate MIC of telithromycin. All nonsusceptible isolates were also macrolide resistant. None of the macrolide-resistant GAS expressing the M phenotype was resistant to telithromycin. However, all 115 of the M-phenotype isolates (including 35 nonsusceptible isolates) had higher MICs than those observed for macrolide-susceptible GAS. Two of 45 macrolide-resistant GAS (erythromycin MIC, >128 μg/ml) expressing the MLS_i phenotype were resistant to telithromycin (MICs of 4 and 8 μg/ml). In contrast, all nine GAS isolates expressing the MLS_c phenotype were nonsusceptible to telithromycin; four were resistant.

PCR was carried out to determine the genetic mechanism of macrolide resistance for isolates expressing in vitro resistance to telithromycin. Amplifiable product consistent with the presence of *erm*(*B*) was identified for the two MLS_i isolates and one of four of the MLS_c isolates that were resistant to telithromycin. No PCR product [*erm*(A), *erm*(*B*), or *mef*(A)] was identified from the remaining three telithromycin-resistant isolates or four telithromycin-intermediate isolates of GAS expressing the MLS_c phenotype.

The genetic relationships among 46 isolates of macrolide-resistant GAS with a telithromycin MIC of ≥1 μg/ml were determined using field inversion gel electrophoresis. Five distinct clones were identified among 35 M-phenotype isolates with a telithromycin MIC of 1 μg/ml. A single unrelated clone accounted for two MLS_i isolates that were resistant to telithromycin. Both of these isolates were recovered during the same month from the same participating site. Finally, four clones recovered from three participating sites accounted for nine MLS_c isolates.

Telithromycin is the first of the new class of ketolide antibiotics to achieve approval from regulatory agencies in Europe and the United States. Results of this study demonstrate the overall high level of in vitro activity of telithromycin against pharyngeal isolates of GAS even in the presence of macrolide resistance. Only 6 of 169 macrolide-resistant GAS were resistant to telithromycin. The M phenotype accounted for two-thirds of the macrolide-resistant GAS isolates, none of which were resistant to telithromycin. However, these strains were noted to have approximately a 10-fold increase in MICs of telithromycin compared to macrolide-susceptible isolates. The observation of a relative decrease in activity of telithromycin and ABT-773 (a second ketolide agent) against strains of GAS and *Streptococcus pneumoniae* expressing the M phenotype has been previously reported (5, 18, 19, 22).

Previous studies have demonstrated that telithromycin resistance in GAS is found exclusively in macrolide-resistant isolates expressing *erm*(B) (8, 10, 19). Consistent with this, we demonstrated the presence of *erm*(B) in two strains of GAS expressing the MLS_i phenotype that were resistant to telithromycin. However, only one of four telithromycin-resistant isolates that expressed the MLS_c resistance phenotype and none of four isolates of MLS_c GAS with an intermediate MIC of telithromycin demonstrated the presence of *erm*(B). There was no evidence of the presence of *erm*(A) or *mef*(A) within these seven GAS isolates. A recent report identified the presence of an adenine-to-guanine mutation at position 2058 of the 23S rRNA in a telithromycin-resistant GAS isolate expressing the MLS_c phenotype (11). This target site for methylation by *erm*(B) may explain why a mutation at this site results in the MLS_c phenotype. The possibility that our isolates contain this or other mutations is under investigation.

Results of this study provide additional evidence regarding

the activity of telithromycin against both macrolide-susceptible and -resistant GAS. The recent licensure of telithromycin in Europe and the United States will surely lead to its increased use. Accordingly, future surveillance will be necessary to track the development of altered patterns of susceptibility to telithromycin in GAS.

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