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Activity of HMR 3647 Compared to Those of Six Compounds against 235 Strains of *Enterococcus faecalis*

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Agar dilution was used to test the activities of HMR 3647, erythromycin A, azithromycin, clarithromycin, roxithromycin, clindamycin, and quinupristin-dalfopristin against 235 strains of *Enterococcus faecalis*. HMR 3647 was the most active compound (MICs at which 50 and 90% of the isolates are inhibited [MIC_{50} and MIC_{90}, respectively] of 0.06 and 4.0 μg/ml, respectively). The MIC_{50} and MIC_{90} (with the MIC_{50} given first and the MIC_{90} given second; both in micrograms per milliliter) for other compounds were as follows: 4.0 and >32.0 for erythromycin A, 16.0 and >32.0 for azithromycin, 2.0 and >32 for clarithromycin, 32.0 and >32.0 for roxithromycin, 32.0 and >32.0 for clindamycin, and 8.0 and 16.0 for quinupristin-dalfopristin. All compounds were only bacteriostatic.

Enterococci are increasing causes of serious systemic infections, especially in debilitated hosts (6, 9, 20). The problem is complicated by the inherent drug resistance of these species as well as recently developed resistance to previously active drugs. *Enterococcus faecalis* has developed resistance to ampicillin (chromosomal and plasmid mediated), high-level resistance to aminoglycoside, and (rarely) resistance to glycopeptide. *Enterococcus faecium* is inherently more resistant than *E. faecalis*, with higher rates of glycopeptide resistance (1, 4–6, 8, 9, 12, 14, 16, 18–20).

The ketolides are a new group of compounds characterized by a 3-keto function replacing the cladinose moiety of other erythromycin A derivatives (3). Previous studies have reported improved activities of HMR 3004 and HMR 3647 against *E. faecalis* (especially vancomycin-susceptible strains) over those of erythromycin A, azithromycin, clarithromycin, roxithromycin, clindamycin, and quinupristin-dalfopristin (2, 3, 7, 10, 11, 13, 21–23). This study sheds further light on these findings by (i) determination of the MICs of HMR 3647, erythromycin A, azithromycin, clarithromycin, roxithromycin, clindamycin, and quinupristin-dalfopristin against 235 *E. faecalis* strains and (ii) examination of the activities of these compounds against 10 strains by microdilution and time-kill studies.

All organisms were isolated from individual patients. β-Lactamase-negative strains were recent clinical isolates from Hershey Medical Center and University Hospital of Cleveland, Ohio, and were not selected for specific antimicrobial resistance. β-Lactamase-producing and vancomycin-resistant strains were obtained from sources cited in Acknowledgments.

Agar dilution by standard methodology (15) was performed on Mueller-Hinton agar. β-Lactamase testing was done with nitrocefin disks (Cefinase; BBL Microbiology Systems, Cockeysville, Md). For β-lactamase-producing strains, β-lactam agar dilution MICs were repeated with inocula of 10^6 CFU/spot. Erythromycin A breakpoints were ≤0.5 μg/ml (susceptible), 1.0 to 4.0 μg/ml (intermediate resistant), and ≥8.0 μg/ml (resistant). High-level gentamicin resistance was defined as MICs of >500 μg/ml. For the purposes of this study, moderate susceptibility to clindamycin was defined as MICs between 8.0 and 32.0 μg/ml (22). Vancomycin resistance was defined as MICs of ≥16.0 μg/ml. For 10 selected strains examined by time-kill analysis, microbroth dilution MICs were performed by using National Committee for Clinical Laboratory Standards methodology and cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (15).

Time-kill studies were performed as described previously (17), using cation-adjusted Mueller-Hinton broth (Difco). Viability counts were performed at 0, 3, 6, 12, and 24 h. Data were analyzed by determining the number of strains which yielded −1, −2, and −3 $\Delta\log_{10}$ CFU/ml compared to the counts at time 0 h. Antimicrobial agents were considered bactericidal at the lowest concentration which reduced the original inoculum by >3 $\log_{10}$ CFU/ml (99.9%) and bacteriostatic if the inoculum was reduced by <3 $\log_{10}$ CFU/ml. Antibiotic carryover was minimized by dilution (17). All strains were tested with final inocula of 5×10^5 to 5×10^6 CFU/ml. For strains with macrolide MICs of >64.0 μg/ml, time-kill studies were performed with HMR 3647 and quinupristin-dalfopristin only.

Results of agar dilution MICs for the 235 strains tested are presented in Table 1. HMR 3647 was the most active, with MICs at which 50 and 90% of the isolates are inhibited (MIC_{50} and MIC_{90}, respectively) of 0.06 and 4.0 μg/ml, respectively. The MIC_{50} and MIC_{90} (with the MIC_{50} given first and the MIC_{90} given second; both in micrograms per milliliter) for other compounds were as follows: 4.0 and >32.0 for erythromycin A, 16.0 and >32.0 for azithromycin, 2.0 and >32.0 for clarithromycin, 32.0 and >32.0 for roxithromycin, 32.0 and >32.0 for clindamycin, and 8.0 and 16.0 for quinupristin-dalfopristin. When enterococci were divided into six groups (namely, (i) gentamicin susceptible, (ii) high-level gentamicin resistant, (iii) β-lactamase producing, (iv) erythromycin A susceptible and moderately clindamycin susceptible, (v) erythromycin A and clindamycin resistant, and (vi) vancomycin resistant), HMR 3647 yielded the lowest MICs against strains susceptible to gentamicin (MIC_{50}, 0.03 μg/ml; MIC_{90}, 4.0 μg/ml) and erythromycin A (MIC_{50}, 0.03 μg/ml; MIC_{90}, 0.06 μg/ml). HMR 3647 MICs for the 102 strains with erythromycin A MICs in the intermediate range (1.0 to 4.0 μg/ml) were ≤0.008 to 0.125 μg/ml; clindamycin MICs for these strains ranged from 8.0 to 32.0 μg/ml. The highest MICs for all compounds were in β-lactamase-producing strains. The clindamycin MICs of all eryth-

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TABLE 1. MICs of drug substances against 235 *E. faecalis* strains

E. faecalis strains (no. of strains)	Drug	MIC (μg/ml)		
		Range	50%	90%
Gentamicin susceptible (164)	HMR 3647	≤0.008–16	0.03	4.0
	Erythromycin A	0.25–>32.0	2.0	>32.0
	Azithromycin	0.5–>32.0	8.0	>32.0
	Clarithromycin	0.125–>32.0	2.0	>32.0
	Roxithromycin	0.5–>32.0	16.0	>32.0
	Clindamycin	4.0–>32.0	32.0	>32.0
	Quinupristin-dalfopristin	≤1.0–32.0	8.0	8.0
High-level gentamicin resistant[a] (71)	HMR 3647	≤0.008–16.0	1.0	8.0
	Erythromycin A	0.5–>32.0	>32.0	>32.0
	Azithromycin	2.0–>32.0	>32.0	>32.0
	Clarithromycin	0.25–>32.0	>32.0	>32.0
	Roxithromycin	4.0–>32.0	>32.0	>32.0
	Clindamycin	16.0–>32.0	>32.0	>32.0
	Quinupristin-dalfopristin	2.0–32.0	8.0	16.0
β-Lactamase producing (10)	HMR 3647	0.016–16.0	8.0	16.0
	Erythromycin A	0.5–>32.0	>32.0	>32.0
	Azithromycin	2.0–>32.0	>32.0	>32.0
	Clarithromycin	0.25–>32.0	>32.0	>32.0
	Roxithromycin	4.0–>32.0	>32.0	>32.0
	Clindamycin	16.0–>32.0	>32.0	>32.0
	Quinupristin-dalfopristin	4.0–32.0	16.0	16.0
Erythromycin A susceptible[b] and moderately clindamycin susceptible[d] (26)	HMR 3647	≤0.008–0.06	0.03	0.06
	Erythromycin A	0.25–0.5	0.5	0.5
	Azithromycin	0.5–16.0	2.0	2.0
	Clarithromycin	0.125–0.5	0.25	0.5
	Roxithromycin	0.5–8.0	2.0	4.0
	Clindamycin	8.0–32.0	32.0	32.0
	Quinupristin-dalfopristin	2.0–8.0	8.0	8.0
Erythromycin A resistant[c] and clindamycin resistant[e] (107)	HMR 3647	0.03–16.0	2.0	8.0
	Erythromycin A	8.0–>32.0	>32.0	>32.0
	Azithromycin	8.0–>32.0	>32.0	>32.0
	Clarithromycin	2.0–>32.0	>32.0	>32.0
	Roxithromycin	16.0–>32.0	>32.0	>32.0
	Clindamycin	>32.0	>32.0	>32.0
	Quinupristin-dalfopristin	1.0–32.0	8.0	16.0
Vancomycin resistant[f] (13)	HMR 3647	0.06–16.0	2.0	8.0
	Erythromycin A	2.0–>32.0	>32.0	>32.0
	Azithromycin	8.0–>32.0	>32.0	>32.0
	Clarithromycin	1.0–>32.0	>32.0	>32.0
	Roxithromycin	8.0–>32.0	>32.0	>32.0
	Clindamycin	32.0–>32.0	>32.0	>32.0
	Quinupristin-dalfopristin	4.0–16.0	8.0	16.0
All strains (235)	HMR 3647	≤0.008–16.0	0.06	4.0
	Erythromycin A	0.25–>32.0	4.0	>32.0
	Azithromycin	0.5–>32.0	16.0	>32.0
	Clarithromycin	0.125–>32.0	2.0	>32.0
	Roxithromycin	0.5–>32.0	32.0	>32.0
	Clindamycin	8.0–>32.0	32.0	>32.0
	Quinupristin-dalfopristin	1.0–32.0	8.0	16.0

[a] >500 μg/ml.
[b] ≤0.5 μg/ml.
[c] ≥8.0 μg/ml.
[d] 8.0 to 32.0 μg/ml.
[e] ≥64.0 μg/ml.
[f] ≥16.0 μg/ml. Nine strains had the VanA phenotype, and four strains had the VanB phenotype.

romycin A-susceptible strains (erythromycin A MICs of ≤0.5 μg/ml) were 8.0 to 32.0 μg/ml, while the clindamycin MICs of erythromycin A-resistant strains (erythromycin A MICs of ≥8.0 μg/ml) were ≥64.0 μg/ml. Vancomycin MICs for vancomycin-resistant strains were 16.0 μg/ml for one strain and ≥256.0 μg/ml for the remaining 12 strains. Teicoplanin testing (5, 20) showed that nine strains had phenotypes consistent with the VanA phenotype and four strains had phenotypes consis-

tent with the VanB phenotype. HMR 3647 MICs of VanA strains were 1.0 to 16.0 μg/ml, and those of VanB strains were 0.06 to 8.0 μg/ml.

The 10 strains tested by time-kill analysis had various susceptibilities to erythromycin A, gentamicin, and vancomycin, with one β-lactamase-producing organism. All compounds were bacteriostatic (0.1- to 1.9-log decrease compared to the counts at 0 h) only. No 99 or 99.9% killing was observed with any compound.

Our results indicate that, while overall activity of HMR 3647 was greater than other agents, in vitro activity varied considerably, with the MICs varying with susceptibilities to other macrolides, lincosamides, and nonmacrolides. Similar findings were reported for HMR 3004 (21), and HMR 3647 (22). However, lower ketolide MICs were reported for β-lactamase-producing strains (MIC_{50} and MIC_{90} of 0.03 and 0.04 μg/ml, respectively, compared to our values of 8.0 and 16.0 μg/ml, respectively) (21, 22). Different strains and different techniques may be responsible for this discrepancy. Time-kill studies showed that compounds were uniformly bacteriostatic, with HMR 3647 yielding the lowest MICs. While strains of *E. faecalis* susceptible or intermediately resistant to erythromycin A were highly susceptible to HMR 3647 (MICs of ≤0.125 μg/ml), strains with high-level resistance to erythromycin A (≥8.0 μg/ml) and clindamycin (≥64.0 μg/ml) were often but not always inhibited by ≤4.0 μg of HMR 3647 per ml. Because of the intrinsic resistance of most enterococci to lincosamides, it is possible that macrolide resistance in these strains was inducible, rather than constitutive, despite a phenotype which might suggest the latter pattern (21, 22). Studies with *E. faecalis* strains with known mechanisms of resistance are necessary to resolve this issue.

Because approximately 50% of strains resistant to both erythromycin A and clindamycin at ≥64.0 μg/ml were inhibited by 1.0 to 4.0 μg of HMR 3647 per ml, concentrations of HMR 3647 which fall into the intermediate category for erythromycin A, in vivo studies would be useful to determine whether such isolates are truly susceptible to the ketolide. This question is of special importance, considering the current high rates of macrolide resistance among *E. faecalis* strains and the limited therapeutic options available for treatment of infections caused by these organisms (21, 22).

Our results indicate that HMR 3647 yielded the lowest MICs against all groups of *E. faecalis* strains tested, with bacteriostatic activity comparable to those of other compounds. Quinupristin-dalfopristin MIC_{50} and MIC_{90} were ≥8.0 μg/ml. Our results indicate a possible use for HMR 3647 in treatment of *E. faecalis* infections, especially those caused by erythromycin-susceptible strains. In vitro studies require confirmation by pharmacokinetic studies and animal and human clinical studies.

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