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Activity of HMR 3647 Compared to Those of Five Agents against *Haemophilus influenzae* and *Moraxella catarrhalis* by MIC Determination and Time-Kill Assay

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The microdilution MICs of HMR 3647, erythromycin A, azithromycin, clarithromycin, roxithromycin, and pristinamycin against 50/90% of 249 *Haemophilus influenzae* and 50 *Moraxella catarrhalis* isolates were 2/4, 0.06/0.125; 8/16, 0.25/0.25; 2/4, 0.06/0.125; 16/16, 0.25/0.25; 32/>32, 1/2; and 2/4, 0.5/0.5 μg/ml. Azithromycin was bactericidal against all 10 *H. influenzae* and 3 of 5 *M. catarrhalis* isolates and HMR 3647, erythromycin A, clarithromycin, roxithromycin, and pristinamycin were bacteriostatic, against all 15 strains after 24 h at the MIC.

Haemophilus influenzae and *Moraxella catarrhalis* remain important causes of respiratory tract infections (2, 10). The in vitro susceptibility of *H. influenzae* to macrolides is variable, with azithromycin showing the lowest MICs, followed by clarithromycin, erythromycin A, and roxithromycin. Pristinamycin and other streptogramins are also active in vitro against *H. influenzae* (5, 7, 8, 11, 12, 18). *M. catarrhalis* has been reported to be more susceptible to all of the above compounds than *H. influenzae* (2).

HMR 3647 is a new ketolide with expanded activity against many multiresistant (especially erythromycin A-resistant) gram-positive organisms (4, 7, 20); excellent activity against *M. catarrhalis* and activity against *H. influenzae* equivalent to that of azithromycin have recently been documented (1, 3, 4, 6, 7, 20). The problem of macrolide activity against *H. influenzae* is complicated by the difficulty of in vitro susceptibility testing (21). *Haemophilus* test medium (HTM), the medium recommended by the National Committee for Clinical Laboratory Standards (NCCLS), has a shelf life of only a few weeks and is therefore difficult to obtain commercially. Viability counts should also be performed on each suspension used for susceptibility testing (10, 21).

The current study attempted to shed light on the above by using freshly prepared HTM and Mueller-Hinton broth with added Fildes extract (MHF) to test the activity of HMR 3647, erythromycin A, azithromycin, clarithromycin, roxithromycin, and pristinamycin against 249 *H. influenzae* and 50 *M. catarrhalis* isolates by microdilution. The activity of each drug against 10 *H. influenzae* and 5 *M. catarrhalis* isolates was also tested by time-kill assay.

The organisms used were all recent clinical isolates. β-Lactamase testing was by the nitrocefin disk method (Cefinase; BBL Microbiology Systems, Cockeysville, Md.). Powders were obtained from the respective manufacturers. Microdilutions were performed on 249 *H. influenzae* and 50 *M. catarrhalis* strains by the NCCLS microdilution method (9). Inocula were prepared from chocolate agar plates (BBL) incubated for 24 h by the direct colony suspension method. Final organism suspensions in trays yielded colony counts of 3×10^5 to 8×10^5 CFU/ml. Inoculum checks were performed in every case; preparation of strains with inocula which did not conform to the standard was repeated.

Frozen microdilution trays (MicroMedia Systems Inc., Cleveland, Ohio) each contained all five antimicrobials prepared in freshly prepared HTM and MHF. HTM was prepared by adding 0.5% yeast extract, 15-μg/ml hematin, and 15-μg/ml NAD to cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.), and MHF was prepared by adding 1% yeast extract and 5% Fildes enrichment (Difco) to cation-supplemented Mueller-Hinton broth (Difco). Wells were inoculated with 100-μl suspensions and incubated in air at 35°C for 20 to 24 h (9). Standard quality control strains (9) were included in each run.

Time-kill experiments were carried out with HTM as previously described (13, 14). Dilutions required to obtain the correct inoculum were determined by prior viability studies with each strain. Only tubes containing an initial inoculum between 5×10^5 and 5×10^6 CFU/ml were acceptable. Viability counts of antibiotic-containing suspensions were performed at 0, 3, 6, 12, and 24 h, respectively (13, 14), on chocolate agar plates incubated for up to 48 h in CO_2. Colony counts were performed in duplicate, and means were taken.

Time-kill assays were analyzed by determining the number of strains which showed viable count decreases of 1, 2, and 3 $\log_{10}$ CFU/ml compared to the counts at 0 h. Drugs were considered bacteriostatic if they yielded a decrease in the count of <3 $\log_{10}$ CFU/ml compared to that at 0 h. With the sensitivity threshold (250 CFU/ml) and inocula used, bactericidal activity (99.9% killing and a decrease in the count of >3 $\log_{10}$ CFU/ml) could be determined when present. Bacterial carryover was minimized by dilution (13, 14).

Of 249 *H. influenzae* strains, 118 (47.4%) were β-lactamase positive. All 50 *M. catarrhalis* strains were β-lactamase producers. MICs did not differ for β-lactamase-positive and -negative *H. influenzae* strains (data not shown). Microdilution MICs are presented in Table 1. HMR 3647, azithromycin, and pristinamycin had the lowest MICs against *H. influenzae*, followed by erythromycin A, clarithromycin, and roxithromycin. All compounds were active against *M. catarrhalis*, with HMR 3647 and azithromycin having the lowest MICs. Although MICs were

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TABLE 1. MICs for *H. influenzae* and *M. catarrhalis* strains in HTM and MHF

Drug, organism, and medium	MIC (μg/ml)		
	Range	For 50% of isolates	For 90% of isolates
HMR 3647			
H. influenzae			
HTM	1.0–8.0	2.0	4.0
MHF	1.0–>8.0	4.0	8.0
M. catarrhalis			
HTM	0.06–0.25	0.06	0.125
MHF	0.06–0.25	0.125	0.125
Erythromycin A			
H. influenzae			
HTM	2.0–>16.0	8.0	16.0
MHF	2.0–>16.0	8.0	16.0
M. catarrhalis			
HTM	0.125–0.5	0.25	0.25
MHF	0.125–0.5	0.25	0.5
Azithromycin			
H. influenzae			
HTM	0.5–8.0	2.0	4.0
MHF	1.0–16.0	4.0	4.0
M. catarrhalis			
HTM	0.06–0.125	0.06	0.125
MHF	0.06–0.125	0.125	0.125
Clarithromycin			
H. influenzae			
HTM	4.0–>32.0	16.0	16.0
MHF	4.0–>32.0	16.0	32.0
M. catarrhalis			
HTM	0.25	0.25	0.25
MHF	0.25	0.25	0.25
Roxithromycin			
H. influenzae			
HTM	8.0–>32.0	32.0	>32.0
MHF	8.0–>32.0	>32.0	>32.0
M. catarrhalis			
HTM	0.25–4.0	1.0	2.0
MHF	0.5–4.0	1.0	2.0
Pristinamycin			
H. influenzae			
HTM	1.0–8.0	2.0	4.0
MHF	1.0–16.0	2.0	4.0
M. catarrhalis			
HTM	0.5–1.0	0.5	0.5
MHF	0.5–1.0	0.5	0.5

sometimes higher in MHF than in HTM, the values did not differ significantly (Table 1).

Azithromycin had the best kill kinetics against *H. influenzae*, with 99.9% killing of all strains after 24 h at the MIC. Pristinamycin was bactericidal against all strains after 24 h at twice the MIC. HMR 3647, erythromycin A, and clarithromycin had similar kill kinetics, with 99.9% killing of seven or eight strains at twice the MIC after 24 h and 90% killing of all strains after 24 h at two to four times the MIC. The kill kinetics of roxithromycin were slower. After 24 h, HMR 3647, erythromycin A, and clarithromycin had bacteriostatic activity at the MIC for all of the strains tested. All compounds were bactericidal at 24 h for three or four of the five *M. catarrhalis* strains at the MIC, and all were bacteriostatic at the MIC after 24 h.

Our results indicate that against both *H. influenzae* and *M. catarrhalis*, HMR 3647 and azithromycin had the greatest activity by MIC determination and azithromycin had the greatest activity by time-kill test followed by erythromycin A, clarithromycin, and roxithromycin. Pristinamycin gave MICs similar to those of HMR 3647 but showed more rapid kinetics of *H. influenzae* killing. Our HMR 3647 MICs were similar to those obtained by Felmingham et al. (4) and Wise and Andrews (20) but higher than those obtained by Agouridas and colleagues for HMR 3004, an older ketolide (1). However, ketolides consistently yield lower MICs than other macrolides, lincosamides, and streptogramins against these species (1, 3, 4, 6, 7, 20). Medium-related problems are probably responsible for published MIC differences.

Although HTM is recommended by the NCCLS as the method of choice for *Haemophilus* testing (9), the medium cannot be made reliably commercially and must be used within 2 to 3 weeks of in-house preparation for optimal growth. MICs in MHF did not differ significantly from those in HTM. Because most strains of *Haemophilus* and *Moraxella* have no specific macrolide-lincosamide-streptogramin mechanism such as *erm* or *mef* (16, 17), pharmacokinetic and pharmacodynamic factors (time above the MIC, area under the concentration-time curve/MIC, and concentration in specific body fluids) must also be taken into therapeutic consideration (10, 11, 19). There is an urgent need for a method of *Haemophilus* susceptibility testing which can readily be adapted for use in the clinical laboratory.

Despite the above technical problems, HMR 3647 and azithromycin had the lowest MICs of all of compounds tested. HMR 3647 was also bacteriostatic against all of the strains tested at the MIC after 24 h, with kill kinetics similar to those of erythromycin A and clarithromycin. HMR 3647 is very active against macrolide-susceptible and -resistant pneumococci (15), as well as other organisms responsible for community-acquired pneumonia (4, 7, 20). If results of pharmacokinetic, pharmacodynamic, and animal studies support its in vitro activity, clinical testing of HMR 3647 in respiratory infections is indicated.

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