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In Vitro Activities of the Ketolides Telithromycin (HMR 3647) and HMR 3004 Compared to Those of Clarithromycin against Slowly Growing Mycobacteria at pHs 6.8 and 7.4

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The in vitro activities of HMR 3647 (telithromycin) and HMR 3004, two novel semisynthetic ketolides, were investigated and compared with that of the reference macrolide drug, clarithromycin, against 34 strains of slowly growing mycobacteria at pHs 6.8 and 7.4, as determined radiometrically. The MICs at pH 7.4 were about 1 to 2 dilutions lower than those observed at pH 6.8. In terms of the highest to the lowest activity, the three antibiotics could be classified as follows: clarithromycin > HMR 3004 > HMR 3647. Among the species tested, *Mycobacterium bovis* BCG, *M. ulcerans*, *M. avium*, and *M. paratuberculosis* were moderately susceptible to HMR 3004 and HMR 3647 (MICs at pH 7.4, ≤5.0 and ≤20.0 μg/ml, respectively, versus ≤1.25 μg/ml for clarithromycin), whereas *M. tuberculosis*, *M. africanum*, *M. bovis*, and *M. simiae* were resistant (MICs, ≥10.0 and ≥40.0 μg/ml, respectively, at pH 7.4). Although not more active than clarithromycin in vitro, the high level of intracellular accumulation of the two ketolides inside phagocytes warrants further screening in experimental animal models.

HMR 3647 (telithromycin) and HMR 3004 are two novel semisynthetic ketolide antibiotics that have been found to be highly active against a variety of microorganisms (1, 3, 4, 5, 10, 14, 15, 16). However, only two studies have yet dealt with the evaluation of the antimycobacterial activities of ketolide drugs; one investigation (C. Truffot-Pernot, N. Lounis, J. F. Chantot, and J. Grosset, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F167, p. 142, 1995) described the MICs of HMR 3004 for three species (*Mycobacterium marinum*, *M. xenopi*, and *M. avium*), whereas another (7) reported the in vitro MICs of HMR 3647 for five rapidly growing species (*M. fortuitum*, *M. chelonae*, *M. peregrinum*, *M. abscessus*, and *M. mucogenicum*). Consequently, the present investigation was aimed at the evaluation of the in vitro activities of HMR 3647 and HMR 3004 compared with that of a reference compound, clarithromycin, against a panel of slowly growing mycobacterial species both at pH 6.8 and at pH 7.4.

All the strains used in this study (Table 1) were from our own culture collection and were grown as fresh Löwenstein-Jensen (LJ) slants at 37°C; *M. ulcerans*, however, was grown at 30°C. For drug activity studies, the bacteria were scraped from the LJ slants, homogenized with 2-mm glass beads, and resuspended in sterile distilled water at an optical density of 650 nm of 0.15. MICs were determined radiometrically with the BACTEC 460-TB apparatus (Becton Dickinson, Towson, Md.) in a confined atmosphere with 7H12 broth as a function of the ability of the microorganisms to catabolize ^{14}C-palmitic acid and by automatically measuring the amount of $^{14}CO_2$ released, as reported previously for *M. tuberculosis* and nontuberculous mycobacteria (11, 12, 13). Due to differences in the growth kinetics of the species studied, the initial bacterial inoculum added to the BACTEC vials was adjusted as follows (11, 12, 13): after preculture of a strain in a 7H12 vial to a growth index (GI) of 500, the drug-containing vials were inoculated with 0.1 ml of the preculture directly in the case of the *M. tuberculosis* complex, *M. ulcerans*, and *M. paratuberculosis*, whereas they were inoculated with 0.1 ml of a 1:100 diluted preculture in the case of *M. avium* and *M. simiae*. In all experiments with *M. paratuberculosis*, the vials with 7H12 medium were supplemented with 2 μg of mycobactin J (Rhône-Mérieux, Lyon, France) per ml to promote bacterial growth, as reported previously (12). The MICs were interpreted by meauring the change in the daily GI (ΔGI) for the drug-containing vials compared with the GI for the control vials, as reported previously (11, 12, 13). As macrolides are significantly more active at a slightly alkaline pH than the pH 6.8 routinely used to screen drugs for antimycobacterial activity, the MICs were determined in parallel both at pH 6.8 and at pH 7.4, as previously reported for determination of the in vitro activity of clarithromycin against mycobacteria (11). The activities of both drugs against all organisms tested were compared with the in vitro activity of the reference macrolide drug clarithromycin, assessed in parallel with duplicate vials inoculated with the same bacterial inoculum that was used to inoculate vials containing HMR 3647 and HMR 3004.

For selected strains, the bactericidal actions of the three drugs were compared at both pHs by determining bacterial viable counts in parallel, as reported previously (13). For this purpose, the numbers of CFU of each strain per milliliter were determined both at the time of inoculation of BACTEC vials (time zero) and at the end of the experiment, as follows: 0.1 ml of culture from the BACTEC vials was removed and was successively diluted 10-fold in sterile double-distilled water to give 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. A total of 0.1 ml of each of these dilutions was plated onto 7H11 agar medium, and the resulting bacterial counts were enumerated after 21 days of incubation. For *M. paratuberculosis* growth, the 7H11 agar plates were supplemented with 2 μg of mycobactin

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TABLE 1. BACTEC MICs of the ketolides HMR 3647 (telithromycin) and HMR 3004 compared to that of clarithromycin at pHs 6.8 and 7.4 for slowly growing mycobacteria

Strain	MIC (μg/ml)					
	HMR 3647		HMR 3004		Clarithromycin	
	pH 6.8	pH 7.4	pH 6.8	pH 7.4	pH 6.8	pH 7.4
M. tuberculosis						
Type strain H37Rv	>40.0	>40.0	20.0	10.0	20.0	5.0
Clinical isolate 900145	>40.0	>40.0	40.0	10.0	20.0	5.0
Clinical isolate 900216	>40.0	>40.0	40.0	10.0	20.0	5.0
M. africanum						
Type strain ATCC 25420	>40.0	>40.0	40.0	20.0	20.0	5.0
Clinical isolate 901376	>40.0	>40.0	>40.0	40.0	40.0	20.0
Clinical isolate 920640	>40.0	>40.0	20.0	10.0	10.0	2.5
M. bovis						
Type strain ATCC 19210	>40.0	>40.0	20.0	10.0	10.0	2.5
Clinical isolate 910882	>40.0	>40.0	>40.0	40.0	>40.0	40.0
Clinical isolate 7576	>40.0	>40.0	20.0	10.0	10.0	2.5
M. bovis BCG						
BCG-Pasteur	40.0	20.0	10.0	2.5	0.6	0.3
BCG-Denmark	20.0	10.0	2.5	1.25	0.15	0.07
BCG-Glaxo	20.0	10.0	5.0	1.25	0.3	0.15
M. ulcerans[a]						
Type strain ATCC 19423	>40.0	20.0	20.0	5.0	1.25	0.6
Clinical isolate 94118	>40.0	20.0	20.0	5.0	1.25	0.6
M. simiae						
Type strain ATCC 25275	40.0	20.0	20.0	5.0	20.0	10.0
AIDS isolate 91-098	>40.0	40.0	40.0	10.0	20.0	10.0
AIDS isolate 92-039	>40.0	>40.0	>40.0	10.0	10.0	5.0
AIDS isolate 94-120	>40.0	>40.0	>40.0	20.0	40.0	10.0
AIDS isolate 96-005	>40.0	40.0	40.0	10.0	20.0	5.0
AIDS isolate 96-012	>40.0	40.0	40.0	10.0	20.0	5.0
M. avium						
Type strain ATCC 25291	5.0	1.25	2.5	0.3	0.6	0.15
Clinical isolate 711	10.0	2.5	2.5	0.6	1.25	0.3
Clinical isolate 969	10.0	2.5	2.5	0.6	1.25	0.3
Clinical isolate 1110	40.0	20.0	10.0	2.5	2.5	0.6
Clinical isolate 1257	≥40.0	10.0	10.0	1.25	2.5	0.6
Clinical isolate 1295	≥40.0	20.0	10.0	2.5	2.5	1.25
AIDS isolate HIV733	5.0	1.25	2.5	0.6	1.25	0.3
AIDS isolate HIV804	>40.0	20.0	10.0	2.5	2.5	1.25
AIDS isolate HIV827	40.0	10.0	10.0	1.25	2.5	0.6
AIDS isolate HIV1423	>40.0	20.0	10.0	2.5	2.5	0.6
M. paratuberculosis[b]						
Type strain ATCC 19698	>40.0	5.0	>40.0	1.25	5.0	0.6
Animal isolate 1077	40.0	2.5	10.0	1.25	2.5	0.3
Animal isolate 7912	20.0	2.5	5.0	1.25	0.6	0.15
Human isolate 2569	10.0	2.5	5.0	0.6	0.6	0.15

[a] Incubated at 30°C.
[b] The growth medium was supplemented with 2 μg of mycobactin J per ml.

J per ml. These experimental conditions avoided the possibility of an accidental loss of bacterial viability due to carryover of drugs to the solid medium. All the drugs were tested at fixed concentrations of 1.25, 2.5, and 5.0 μg/ml, as it covered the range of the expected peak concentrations of clarithromycin in human serum. HMR 3647, HMR 3004, and clarithromycin were provided by Hoechst-Marion-Roussel, Romainville, France. All the stock solutions were initially prepared in dimethyl sulfoxide (DMSO) and serially diluted in sterile distilled water prior to use. Equivalent amounts of DMSO had no growth inhibitory effects on control bacteria treated likewise.

The MICs obtained for a panel of 34 mycobacterial strains (Table 1) showed that the activities of all three drugs were significantly higher at pH 7.4 than at the pH routinely used to screen drugs for antimycobacterial activity (pH 6.8). As shown previously for clarithromycin (11), the MICs at pH 7.4 were about 1 to 2 dilutions lower than those observed at pH 6.8. For the members of the *M. tuberculosis* complex, the MICs of all

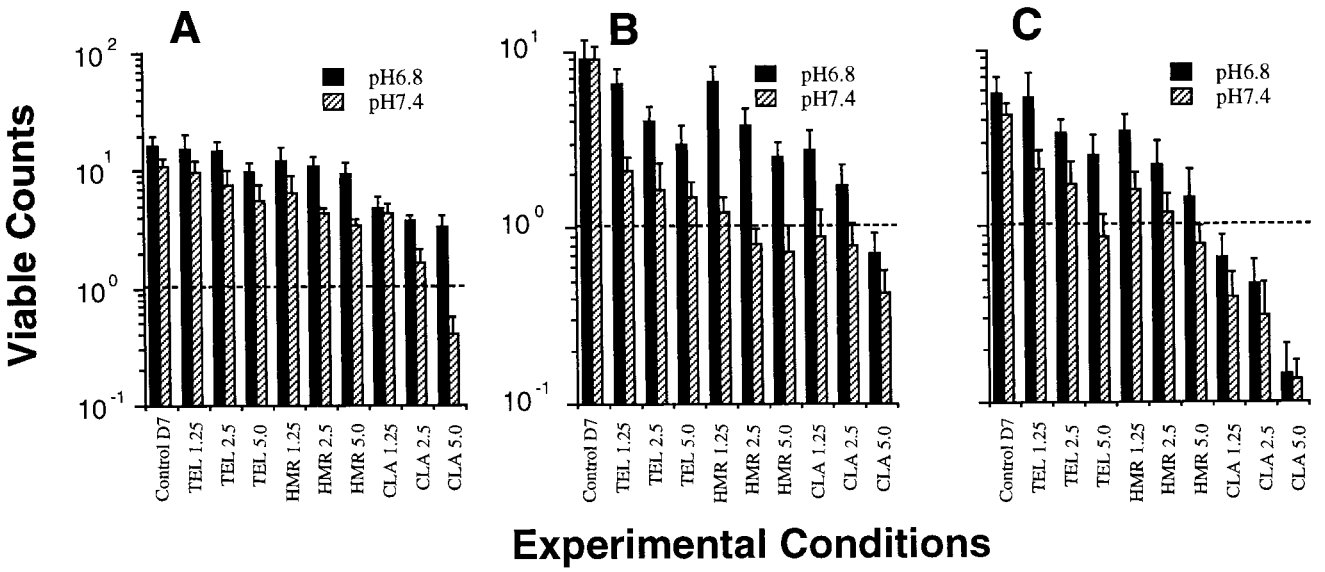

FIG. 1. Comparative bactericidal effects of telithromycin (HMR 3647), HMR 3004, and clarithromycin in BACTEC 7H12 vials against *M. africanum* ATCC 25420 (A), *M. bovis* ATCC 19210 (B), and *M. bovis* BCG-Pasteur (C) at fixed concentrations of 1.25, 2.5, and 5.0 μg/ml. Results illustrate the mean ± standard error viable counts in the presence of selected concentrations of various drugs after 7 days of incubation at 37°C compared to the growth in the untreated control vials. The initial inoculum at time zero was taken as 1 and is represented by the dotted lines. Values after the drug abbreviations indicate the concentration (in micrograms per milliliter). Abbreviations: Control D7, control at day 7; TEL, telithromycin or HMR 3647; HMR, HMR 3004; CLA, clarithromycin.

three drugs were lowest for *M. bovis* BCG (MIC ranges at pH 7.4: clarithromycin, 0.07 to 0.3 μg/ml; HMR 3004, 1.25 to 2.5 μg/ml; HMR 3647, 10 to 20 μg/ml), whereas the MICs were ≥2.5, ≥10, and >40 μg/ml for the remaining three species, i.e., *M. tuberculosis*, *M. africanum*, and *M. bovis*, respectively, (Table 1). For the other 22 strains of slowly growing nontuberculous mycobacteria that still remain difficult to treat, our results showed that except for *M. simiae*, which was highly resistant to all three drugs, the two isolates of *M. ulcerans* tested as well as a majority of the *M. avium* and *M. paratuberculosis* isolates tested were susceptible to the drugs when they were used at pH 7.4 (Table 1). Once again, clarithromycin was the most active drug (MIC range, 0.15 to 1.25 μg/ml), followed by HMR 3004 (MIC range, 0.6 to 5 μg/ml) and HMR 3647 (MIC range, 1.25 to 20.0 μg/ml). Thus, in terms of the highest to the lowest activity, the three antibiotics could be classified as follows: clarithromycin > HMR 3004 > HMR 3647 (the MICs of the corresponding drugs were 1 to 2 dilutions higher for each successive drug in the same order). Regarding the two ketolide drugs, *M. bovis* BCG, *M. avium*, *M. paratuberculosis*, and *M. ulcerans* were moderately susceptible to HMR 3004 and HMR 3647 (MICs at pH 7.4, ≤5.0 and ≤20.0 μg/ml, respectively, versus ≤1.25 μg/ml for clarithromycin), whereas *M. tuberculosis*, *M. africanum*, *M. bovis*, and *M. simiae* were resistant (MICs, ≥10.0 and ≥40.0 μg/ml, respectively, at pH 7.4).

When the spectra of activity of the three drugs described above were compared by determining the bacterial viable counts obtained with fixed concentrations of 1.25, 2.5, and 5.0 μg of each of the three drugs per ml for *M. africanum*, *M. bovis*, and *M. bovis* BCG, none of the drugs was found to be bactericidal (Fig. 1). Although *M. bovis* BCG was the most susceptible organism among the members of the *M. tuberculosis* complex, even the most bactericidal drug, clarithromycin, used at 5.0 μg/ml killed ≥90% of the initial bacterial inoculum (the corresponding values for HMR 3004 and HMR 3647 at pH 7.4 were about 70 and 60%). These bactericidal activities are significantly below the 99% killing of the initial bacterial inoculum that is required to consider a drug bactericidal for *M. tuberculosis* (13). However, the killing effects of all three drugs were significantly higher at pH 7.4 than at pH 6.8 (Fig. 1).

Because of the natural resistance of *M. avium* and *M. paratuberculosis* to most of the antituberculous drugs, the bactericidal activities of the three compounds were further compared at pH 6.8 and 7.4 against seven *M. avium* isolates and two *M. paratuberculosis* isolates at fixed concentrations of 1.25, 2.5, and 5.0 μg/ml. As can be seen from the results obtained (Fig. 2), clarithromycin was the most bactericidal drug, followed by HMR 3004 and HMR 3647, with the highest activity being observed at pH 7.4. If bacterial killing was considered the ability to effectively reduce viable counts below the initial bacterial inoculum, irrespective of the level of killing itself, clarithromycin used at 5.0 μg/ml reduced the numbers of CFU per milliliter in the test vials below the initial inoculum for all seven *M. avium* and two *M. paratuberculosis* isolates at pH 7.4, whereas HMR 3004 did so for five and one isolates, respectively, and HMR 3647 did so for three and one isolates, respectively (Fig. 2). If a bactericidal effect was considered a reduction of the initial bacterial inoculum by ≥2 logs, as previously defined for *M. tuberculosis*, then clarithromycin alone was bactericidal for two *M. avium* isolates and one *M. paratuberculosis* isolate at 5 μg/ml and for one *M. avium* isolate and one *M. paratuberculosis* isolate at 2.5 μg/ml (Fig. 2). On the contrary, none of the ketolides was really bactericidal. However, for difficult-to-treat nontuberculous mycobacteria such as *M. avium* that are resistant to multiple drugs, a reduction of the initial inoculum by ≥1 log may be considered an indication (provided that a drug has excellent pharmacokinetic parameters) that it is a potential candidate drug that may be used with other drugs in combination therapy. Thus, if a reduction of the initial bacterial inoculum by ≥1 log was considered indirect evidence for an eventual killing effect of the drug, then clarithromycin (5.0 μg/ml [pH 7.4]) showed a bactericidal effect against six *M. avium* isolates and one *M. paratuberculosis* isolate, whereas HMR 3004 had a bactericidal effect against two

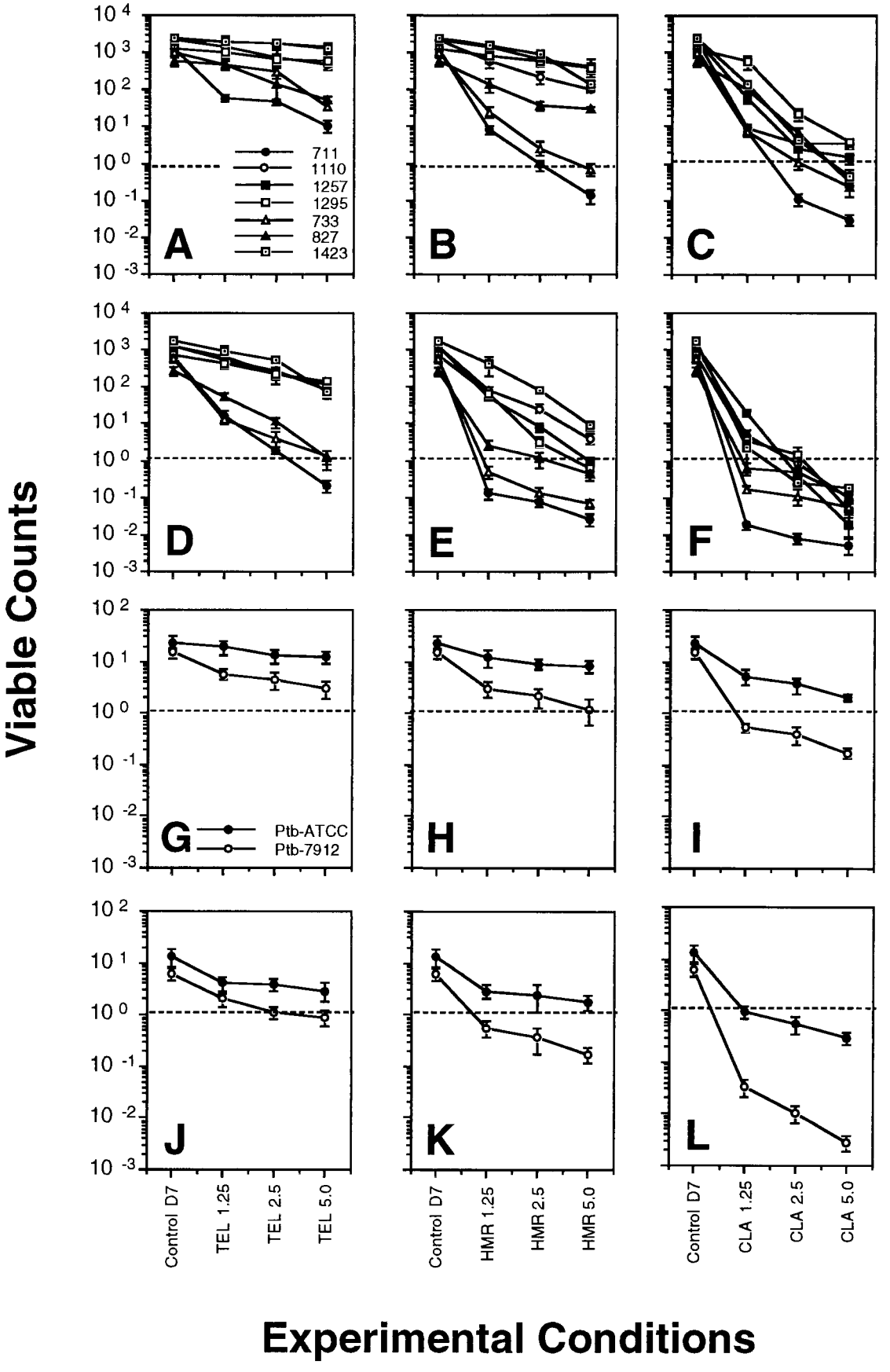

FIG. 2. Comparative bactericidal effects of telithromycin (HMR 3647), HMR 3004, and clarithromycin in BACTEC 7H12 vials against seven *M. avium* isolates (A to F) and two *M. paratuberculosis* isolates (G to L). The drugs were screened both at pH 6.8 (panels A to C for *M. avium* and panels G to I for *M. paratuberculosis* [Ptb]) and at pH 7.4 (panels D to F for *M. avium* and panels J to L for *M. paratuberculosis*). See the legend to Fig. 1 for the definitions of the abbreviations used.

M. avium isolates and one *M. paratuberculosis* isolate and HMR 3647 had a bactericidal effect against only one *M. avium* isolate (Fig. 2).

Derived from the parental compound erythromycin A, the semisynthetic 14-membered macrolides HMR 3647 (telithromycin) and HMR 3004 belong to a new chemical family, the ketolides, that possess in the macrolactone ring a 3-keto group instead of the L-cladinose moiety of erythromycin A; HMR 3647 contains a carbamate group linked to an imidazolium and pyridinium nucleus at C-11-12, whereas HMR 3004 contains a quinoline side chain linked to the 11-12 position of the 3-keto-6-methoxy erythromycin A skeleton (1, 4; C. Agouridas, Y. Benedetti, A. Denis, C. Fromentin, S. Gouin D'Ambrieres, O. Le Martret, and J. F. Chantot, Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F164, p. 227, 1994). Like erythromycin A, the ketolides act by apparently inhibiting bacterial protein synthesis through their binding to the 50S ribosomal subunit (10) and are highly potent against a variety of organisms that include common pathogens involved in respiratory tract infections such as *Streptococcus pneumoniae* (14), *Haemophilus influenzae*, and *Moraxella catarrhalis* (3); atypical pathogens involved in lower respiratory tract infections such as *Chlamydia pneumoniae* (15), *Legionella pneumophila*, and *Mycoplasma pneumoniae* (1, 5, 16); and gram-positive organisms such as *Staphylococcus*, *Streptococcus*, and *Enterococcus* spp. (10).

The ketolides HMR 3004 and HMR 3647 were found to be more active than other macrolide drugs against a variety of organisms including *Bordetella pertussis* (9), non-*Bacteroides fragilis* group anaerobes (6), and unusual aerobic and anaerobic pathogens from human and animal bites (8). However, the results of the present investigation show that despite an interesting spectrum of activity against mycobacteria in general (Table 1; Fig. 1 and 2), the HMR 3004 MICs were nearly always 1 to 2 dilutions higher than those of clarithromycin, whereas the MICs of HMR 3647 were a further 1 to 2 dilutions higher than those of HMR 3004. Thus, in terms of the highest to the lowest activity, the three antibiotics may be classified in the order clarithromycin > HMR 3004 > HMR 3647, which is in agreement with previous findings of Truffot-Pernot et al. (35th ICAAC) for HMR 3004 against *M. marinum*, *M. xenopi*, and *M. avium* and those of Fernandez-Roblas et al. (7) for HMR 3647 against the rapidly growing mycobacterial species *M. fortuitum*, *M. chelonae*, *M. peregrinum*, *M. abscessus*, and *M. mucogenicum*.

Although our in vitro results do not support the fact that HMR 3647 and HMR 3004 may serve as better alternatives to the macrolide drug clarithromycin, their high levels of intracellular accumulation inside phagocytes warrants that further screening of these novel ketolide drugs should be performed with experimental animal models. Indeed, equally active in experimental murine or guinea pig models of infection (2, 5), the two ketolides described above actively concentrate intracellularly, with cellular concentration to extracellular concentration ratios as high as 348 ± 27.1 for HMR 3647 and 461 ± 14.8 for HMR 3004 within 3 h of incubation (17, 18). Thus, it is possible that significantly higher intracellular concentrations of HMR 3647 and HMR 3004 may result in high levels of drug activity in intracellular models of infection, which is the case with difficult-to-treat opportunistic mycobacterial infections such as those caused by *M. avium* and *M. paratuberculosis*.

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