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In Vitro Susceptibilities of *Bordetella pertussis* and *Bordetella parapertussis* to Two Ketolides (HMR 3004 and HMR 3647), Four Macrolides (Azithromycin, Clarithromycin, Erythromycin A, and Roxithromycin), and Two Ansamycins (Rifampin and Rifapentine)

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When tested by agar dilution on Mueller-Hinton agar supplemented with 5% horse blood, the ketolides HMR 3004 and HMR 3647 were slightly more active (MIC at which 90% of the isolates were inhibited [MIC_{90}], 0.03 μg/ml) against *Bordetella pertussis* than azithromycin, clarithromycin, erythromycin A, and roxithromycin. Azithromycin (MIC_{90}, 0.06 μg/ml) was the most active compound against *B. parapertussis*. Rifampin and rifapentine were considerably less active.

Ketolides possess a mode of action that is similar to that of the structurally related macrolide-lincosamide-streptogramin compounds (7). The structure of these semisynthetic 14-membered-ring macrolides differs from that of erythromycin A by having a 3-keto group instead of an L-cladinose at position 3 on the erythronolide A ring (10). Evaluations of two new ketolides (HMR 3004 and HMR 3647; formerly RU 64004 and RU 66647, respectively) showed potent in vitro activity against many gram-positive and some gram-negative bacterial species (7, 8, 10). The susceptibilities of the fastidious gram-negative species *Bordetella pertussis* and *Bordetella parapertussis* to the new ketolides have not been studied before but are of considerable clinical interest, since the macrolide erythromycin is the drug of choice for treatment of pertussis patients. The ketolides were compared in the present evaluation with four macrolides (azithromycin, clarithromycin, erythromycin A, and roxithromycin).

Rifapentine is an antimicrobial agent of the ansamycin group of which rifampin and rifabutin are the best-known compounds. The in vitro activity of rifapentine against nonmycobacterial microorganisms is currently being studied (2).

The test procedure was similar to that in our previous studies (5, 6). Test substances of all antimicrobial agents were supplied by Hoechst Marion Roussel (Romainville, France) and were dissolved and diluted according to the manufacturer's instructions. Stock solutions were prepared at 2,000 μg/ml.

An agar dilution procedure in accordance with the recommendations of the National Committee for Clinical Laboratory Standards (9) was used. The test strains of *B. pertussis* ($n = 34$) and *B. parapertussis* ($n = 34$) were recent clinical isolates (1994 to 1997) from children in southern Germany. The strains had been identified by colony morphology, oxidase reaction, and agglutination with specific antisera. They had been stored at −70°C in glycerol soon after isolation. *B. pertussis* ATCC 9797 was also included in the study. For some antimicrobial agents, more than 34 isolates of *B. pertussis* were studied (see Table 1).

After being thawed, the strains were cultivated on antibiotic-free Mueller-Hinton agar (Difco, Augsburg, Germany) supplemented with 5% whole defibrinated horse blood. Several *Bordetella* colonies were inoculated into antibiotic-free Mueller-Hinton broth (Difco) with 5% horse blood and incubated at 36°C for 24 h. The broth then contained ca. 10^8 CFU/ml.

Serial twofold concentrations of antibiotics were incorporated into Mueller-Hinton agar supplemented with 5% horse blood. Inocula were applied with a multipoint inoculator, resulting in a final inoculum of 10^4 to 10^5 CFU per spot. Having been protected from desiccation, the plates were incubated at 36°C for 48 h (*B. parapertussis*) or 72 to 96 h (*B. pertussis*). MIC end point readings were done as recommended by the National Committee for Clinical Laboratory Standards (9). Antibiotic-free plates were inoculated before and after test plates to check for growth and purity. *Staphylococcus aureus* ATCC 29213 served as the control strain. All tests were performed in duplicate.

Table 1 shows the results of MIC determinations. Among the ketolides and macrolides, the novel ketolides HMR 3004 and HMR 3647 showed the highest activity against *B. pertussis* (MIC at which 90% of the isolates were inhibited [MIC_{90}], 0.03 μg/ml). The MIC_{90}s of azithromycin, clarithromycin, and erythromycin A were 1 dilution higher; the MIC_{90} of roxithromycin was 2 dilutions higher. The susceptibility of *B. pertussis* ATCC 9797 was not different from those of the German clinical isolates. Against *B. parapertussis*, azithromycin was the most active compound (MIC_{90}, 0.06 μg/ml), followed by the ketolides (MIC_{90}, 0.25 μg/ml) and the macrolides clarithromycin, erythromycin A, and roxithromycin (MIC_{90}, 0.5 μg/ml).

The ansamycins rifampin and rifapentine were less active against the two *Bordetella* species than the ketolides and macrolides, with rifapentine exhibiting less activity than rifampin against both species (Table 1).

This study demonstrated the excellent in vitro activity of two ketolides in comparison with four macrolides against the two *Bordetella* species as has been found in other investigations that studied different bacterial species (7, 8, 10).

The MICs of the four macrolides against the *Bordetella* spe-

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TABLE 1. Activities of two ketolides, four macrolides, and two ansamycins against *B. pertussis* and *B. parapertussis*

Organism	No. of strains	Antimicrobial agent	MIC (μg/ml)[a]		
			Range	50%	90%
B. pertussis	51	HMR 3004	0.008–0.03	0.015	0.03
	52	HMR 3647	0.004–0.06	0.015	0.03
	40	Azithromycin	0.008–0.06	0.03	0.06
	37	Clarithromycin	0.015–0.125	0.06	0.06
	34	Erythromycin A	0.03–0.06	0.03	0.06
	36	Roxithromycin	0.03–0.25	0.06	0.125
	34	Rifampin	0.25–2.0	0.5	1.0
	34	Rifapentine	1.0–4.0	1.0	2.0
B. parapertussis	34	HMR 3004	0.25–0.5	0.25	0.25
	31	HMR 3647	0.125–0.5	0.125	0.25
	34	Azithromycin	0.06–0.25	0.06	0.06
	32	Clarithromycin	0.25–2.0	0.5	0.5
	34	Erythromycin A	0.5	0.5	0.5
	34	Roxithromycin	0.5–1.0	0.5	0.5
	34	Rifampin	2.0–4.0	2.0	2.0
	34	Rifapentine	8.0	8.0	8.0

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

cies in the present investigation are in good agreement with those found in previous studies (3, 5). Two groups of authors have previously investigated by agar dilution the in vitro activity of rifampin against *B. pertussis* (1, 11) and found somewhat lower MICs than in the present study. This may be explained by the use of different media and different methodologies in various studies since susceptibility testing of *Bordetella* species lacks standardization (4). No comparative data from the literature are available for the activities of rifampin against *B. parapertussis* and for rifapentine against both species.

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