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In Vitro Activities of the New Ketolide Antibiotics HMR 3004 and HMR 3647 against *Streptococcus pneumoniae* in Germany

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The comparative in vitro activity of HMR 3004 and HMR 3647, new ketolide antibiotics, was tested by a standard agar dilution technique against 221 pneumococcal strains, including isolates with intermediate levels of resistance to penicillin and erythromycin-resistant isolates. The ketolides were more active than other macrolides and showed excellent activity against erythromycin-resistant strains. All the strains were inhibited by ≤2 μg of HMR 3004/ml or by ≤0.5 μg of HMR 3647/ml.

The worldwide incidence of infections caused by pneumococci resistant to penicillin G and other antimicrobials has increased at an alarming rate during the past two decades (1, 12, 17). In Europe, Spain, France, and Hungary have recorded the highest incidences of resistance to penicillin G as well as to other antibiotics among clinical isolates of *Streptococcus pneumoniae* (6, 13). In contrast, studies in Germany carried out between 1979 and 1995 revealed only a low rate of intermediate resistance to penicillin G (10, 14, 20, 21). Nevertheless, *S. pneumoniae* isolates with reduced susceptibility to penicillin G, including strains for which the MICs were ≥2 μg/ml and which caused therapeutic problems in a few cases, have been reported (19). Pneumococci with reduced sensitivity to penicillin G are often found to also be resistant to erythromycin A and other 14-membered-ring macrolides, such as clarithromycin and roxithromycin, and to azithromycin.

The increased erythromycin A resistance has become a worldwide problem. A recent 30-center national surveillance study by Doern and coworkers has documented erythromycin A resistance rates of 19 to 20% with isolates with an intermediate level of resistance to penicillin and of 49% with penicillin-resistant isolates recovered from outpatients in the United States (4). In France resistance to erythromycin A, which was first detected in 1976, increased to 20% in 1984 and reached 29% in 1990 (7). In Germany the rate of pneumococcal resistance to erythromycin A is low (3.2% [21]) and more often seen in children (18).

HMR 3004 and HMR 3647 are new ketolides. The ketolides are semisynthetic 14-membered-ring macrolides, characterized by a 3-keto group instead of the L-cladinose sugar of the erythronolide A ring. Previous studies have documented the antipneumococcal activity of this group of antibiotics (2, 3, 5, 8, 9, 16, 22).

In the present investigation 221 representative isolates, including 86 consecutive pneumococcal strains isolated between October 1996 and March 1997, were randomly selected from a collection of strains obtained from blood cultures or cerebrospinal fluid or other normally sterile body sites as part of the 1992-to-1997 national surveillance study of the antimicrobial resistance of *S. pneumoniae* in Germany (17).

The MICs of HMR 3004 (Hoechst Marion Roussel, Romainville, France), HMR 3647 (Hoechst Marion Roussel), penicillin G (Grünenthal, Stolberg, Germany), amoxicillin (SmithKline Beecham Pharmaceuticals, Munich, Germany), erythromycin A (Hoechst Marion Roussel), azithromycin (Pfizer, Karlsruhe, Germany), roxithromycin (Hoechst Marion Roussel), clarithromycin (Abbott, Wiesbaden, Germany), josamycin (Hoechst Marion Roussel), ofloxacin (Hoechst Marion Roussel), and tetracycline (Sigma Chemicals, Munich, Germany) were determined by a standard agar dilution technique with Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, United Kingdom) supplemented with 5% sheep blood (Oxoid), following the recommendation of the National Committee for Clinical Laboratory Standards (15).

Suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by growth from blood agar plates in 2 ml of Mueller-Hinton broth (Difco, Detroit, Mich.). The suspensions were diluted 1:10 to obtain a final inoculum of 10^4 CFU per spot. Plates were inoculated with a 19-prong replicating device and incubated overnight in ambient air at 37°C. The effects of the incubation atmosphere on HMR 3004 and HMR 3647 agar dilution MICs for 19 pneumococcal strains were determined by incubation of agar plates in both ambient air and in a 5 to 7% CO_2 atmosphere. The standard quality control strain *S. pneumoniae* ATCC 49619 was included in each run. The activity of clindamycin (Upjohn, Heppenheim, Germany) was determined for the erythromycin-resistant strains. The erythromycin-resistant strains were further screened for the type of macrolide-lincosamide-streptogramin B (MLS) resistance (constitutive versus inducible) by disk diffusion on Mueller-Hinton agar (BBL, Heidelberg, Germany) supplemented with 5% sheep blood with disks (Oxoid, Wesel, Germany) containing erythromycin A (15 μg) and clindamycin (2 μg). Inducible resistance was characterized by blunting of the clindamycin inhibition zone when the clindamycin disk was placed at a distance of 12 mm from the erythromycin A disk.

The MICs at which 50% of the isolates are inhibited (MIC_{50}s), MIC_{90}s, and MIC ranges for the four groups of pneumococcal strains investigated are shown in Table 1. HMR 3004 and HMR 3647 were highly active, with MIC_{90}s of ≤0.03 to 2 μg/ml. There was no significant difference between the activities of the two ketolides. Ofloxacin activity was independent of penicillin

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TABLE 1. In vitro activity of the new ketolides HMR 3004 and HMR 3647 compared with those of other macrolides, ofloxacin, tetracycline, amoxicillin, penicillin G, and tetracycline in Germany

S. pneumoniae resistance phenotype[a]	No. of isolates tested	Antimicrobial agent	MIC (μg/ml)		
			MIC_{50}	MIC_{90}	Range
Pen^s Ery^s	169	HMR 3004	≤0.03	0.125	≤0.03–0.125
		HMR 3647	≤0.03	0.06	≤0.03–0.125
		Erythromycin A	0.125	0.5	0.06–0.5
		Azithromycin	0.06	0.125	≤0.03–0.25
		Roxithromycin	0.25	1	0.125–2
		Clarithromycin	0.125	0.25	≤0.03–0.5
		Josamycin	0.5	1	0.125–2
		Ofloxacin	2	4	0.5–8
		Tetracycline	0.25	8	0.06–≥32
		Amoxicillin	0.016	0.016	≤0.008–0.06
		Penicillin G	0.016	0.016	≤0.008–0.03
Pen^s Ery^i/Ery^r	20	HMR 3004	0.06	0.25	≤0.03–0.5
		HMR 3647	0.06	0.25	≤0.03–0.5
		Erythromycin A	≥32	≥32	1–≥32
		Azithromycin	16	≥32	0.06–≥32
		Roxithromycin	≥32	≥32	0.125–≥32
		Clarithromycin	8	≥32	0.06–≥32
		Josamycin	1	≥32	0.25–≥32
		Clindamycin	2	≥32	0.06–≥32
		Ofloxacin	2	4	0.5–4
		Tetracycline	8	≥32	0.125–≥32
		Amoxicillin	≤0.008	0.016	≤0.008–0.06
		Penicillin G	≤0.008	0.016	≤0.008–0.03
Pen^i Ery^s	24	HMR 3004	≤0.03	≤0.03	≤0.03
		HMR 3647	≤0.03	≤0.03	≤0.03–0.06
		Erythromycin A	0.125	0.125	0.06–0.25
		Azithromycin	0.125	0.25	0.03–0.25
		Roxithromycin	0.25	0.5	0.25–0.5
		Clarithromycin	0.125	0.25	0.06–0.25
		Josamycin	0.5	0.5	0.125–0.5
		Ofloxacin	2	4	2–≥16
		Tetracycline	4	≥32	0.5–≥32
		Amoxicillin	0.25	1	0.03–1
		Penicillin G	0.25	1	0.125–1
Pen^i Ery^i/Ery^r	8	HMR 3004	0.06	2	≤0.03–2
		HMR 3647	≤0.03	0.25	≤0.03–0.25
		Erythromycin A	16	≥32	8–≥32
		Azithromycin	16	≥32	2–≥32
		Roxithromycin	16	≥32	2–≥32
		Clarithromycin	8	≥32	1–≥32
		Josamycin	1	≥32	0.125–≥32
		Clindamycin	0.25	≥32	0.125–≥32
		Ofloxacin	2	4	1–4
		Tetracycline	≥32	≥32	0.25–≥32
		Amoxicillin	0.125	0.5	0.06–1
		Penicillin G	0.25	1	0.125–1

[a] Pen^s, penicillin susceptible (MIC ≤ 0.06 μg/ml); Ery^s, erythromycin susceptible (MIC ≤ 0.25 μg/ml); Pen^i, intermediate level of resistance to penicillin (MIC 0.125 to 1 μg/ml); Ery^i/Ery^r, intermediate level of resistance to erythromycin or erythromycin resistant (MIC ≥0.5 μg/ml).

G susceptibility (MIC_{90}, 4 μg/ml). Thirteen of the 28 erythromycin-resistant strains were found to be clindamycin susceptible (MIC ≤ 0.25 μg/ml [presumptive M phenotypes]). The MICs of the ketolides (HMR 3004 MIC_{90}, 0.06 μg/ml; HMR 3647 MIC_{90}, 0.125 μg/ml) and all other macrolides (erythromycin A MIC_{90}, 16 μg/ml; roxithromycin MIC_{90}, ≥32 μg/ml; azithromycin MIC_{90}, 16 μg/ml; clarithromycin MIC_{90}, 8 μg/ml) for these strains were lower than those for the 15 strains belonging to the constitutive MLS phenotype of erythromycin A resistance, for all of which the macrolide MICs were ≥32 μg/ml. The MICs of the ketolides for these strains were slightly elevated (HMR 3004 MIC_{90}, 0.5 μg/ml; HMR 3647 MIC_{90}, 0.25 μg/ml). Inducible MLS resistance was not detected. Josamycin was active against clindamycin-susceptible strains (MIC_{90}, 0.5 μg/ml). Among 86 consecutive pneumococcal strains isolated between October 1996 and March 1997, resistance to penicillin G or amoxicillin was not detected. Only 95.4% of the strains were found to be erythromycin susceptible. The highest rate of resistance was recorded for tetracycline, with 4.7% of the strains resistant. The effect of the incubation atmosphere on ketolide MICs versus *S. pneumoniae* was investigated by simultaneously determining MICs in CO_2 and ambient air. These experiments showed that the MICs of both ketolides were higher when strains were incubated in a CO_2 atmosphere. For HMR 3647, a twofold increase of MIC_{90} was observed; the MIC_{90} of HMR 3004 was four dilution steps higher when plates were incubated in a CO_2 atmosphere.

The results of this study reflect the excellent activity of both ketolides (HMR 3004 and HMR 3647) against German *S. pneumoniae* isolates and confirm previous findings of American study groups (2, 3, 5, 8, 9, 16, 22).

As expected, erythromycin-resistant strains showed cross-resistance to the other macrolides and to azithromycin. The absence of erythromycin A cross-resistance may prove to be the major advantage of HMR 3004 and HMR 3647, particularly since erythromycin resistance has gradually increased in many parts of the world, including Germany. Ketolide MICs in our study were found to be a few dilutions higher for erythromycin-resistant strains than for erythromycin-susceptible strains, but the values were still lower than those for the other macrolides.

The results of the current study are also notable for the influence of a CO_2 incubation atmosphere on ketolide MICs. The MICs of HMR 3004 and HMR 3647 were consistently two to four times higher when plates were incubated in a CO_2 atmosphere. The CO_2 incubation may lead to a marked decrease of pH in test media or perhaps alter the growth characteristics of test strains, in turn leading to a decrease of ketolide activity. As far as we know, the prevalence of pneumococcal isolates from systemic infections showing reduced susceptibility to penicillin G in Germany is one of the lowest published to date worldwide (12). In contrast to the situation in Germany, studies from Spain (resistance rate, 44.3%), Hungary (57.8%), and France (>40%) report very high incidences of strains exhibiting reduced susceptibility to penicillin G (6, 11, 13). In the present study no penicillin G resistance was detected in the group of consecutive strains isolated between October 1996 and March 1997, which confirms previous findings by our working group of a very low level of penicillin G resistance in Germany (1.8% of strains with an intermediate level of resistance to penicillin in 1992 to 1994) (21).

In summary, HMR 3004 and HMR 3647 show great potential for the treatment of pneumococcal infections. Clinical studies are warranted to test this hypothesis.

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