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In Vitro Activities of a New Lipopeptide, HMR 1043, against Susceptible and Resistant Gram-Positive Isolates

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The purpose of this study was to compare the activity of HMR 1043 with those of daptomycin and teicoplanin against gram-positive isolates. Susceptibility tests were performed for 52 strains, 26 parental strains, including staphylococcal, streptococcal, enterococcal, and listerial strains, and 26 HMR 1043-resistant mutants obtained from parental strains by using the Szybalski method. Agar dilution and disk diffusion susceptibility tests were performed by the procedures outlined by the NCCLS. HMR 1043 demonstrated good activity against susceptible and resistant gram-positive bacteria. The activity of HMR 1043 in vitro was less influenced by the presence of calcium ions than that of daptomycin. Susceptibility test breakpoints were not defined because of the poor correlation coefficients obtained with the different disks tested.

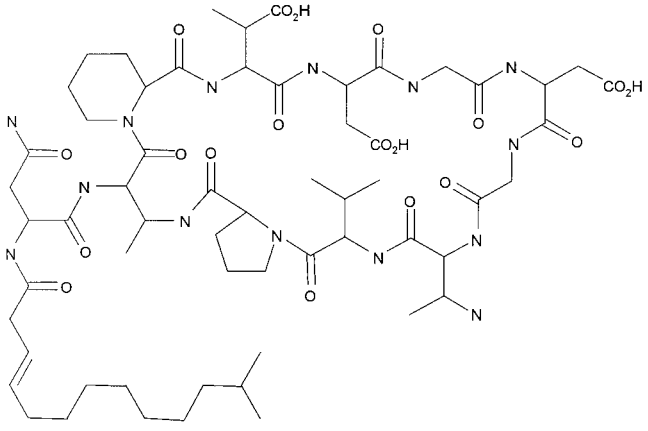
HMR 1043 is a new antibiotic belonging to the class of cyclic lipopeptides that includes daptomycin (6). The structure of HMR 1043 is shown in Fig. 1. These drugs are being developed for the treatment of severe infections due to antibiotic-resistant gram-positive cocci. Daptomycin is active against susceptible and resistant gram-positive isolates, such as vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, glycopeptide-intermediate staphylococci, and penicillin-resistant pneumococci (2, 4, 8, 11, 12). The antibacterial activity of daptomycin requires the presence of calcium cations. Previous studies have recommended a calcium supplementation of 50 mg/liter for broth microdilution susceptibility tests (1, 5, 7) and of 9 mM (200 mg/liter) for agar dilution susceptibility tests (3).

The objective of this study was to compare the activity of HMR 1043 with those of daptomycin and teicoplanin against susceptible and resistant gram-positive isolates, to investigate the effect of calcium supplementation, and to interpret the zone size obtained with different HMR 1043 disks. The Szybalski method (13) was used for the preliminary selection of mutants, because of the rarity of truly resistant gram-positive strains.

The 26 parental strains included 18 clinical and 8 reference isolates: 5 *Staphylococcus aureus* strains (including 1 with intermediate resistance to teicoplanin and 1 vancomycin-intermediate *Staphylococcus aureus* strain), 4 *Staphylococcus epidermidis* strains (including 1 resistant to teicoplanin), 2 *Staphylococcus haemolyticus* strains (including 1 resistant to teicoplanin), 3 *Enterococcus faecalis* strains (1 possessing the *vanA* gene and 1 possessing the *vanB* gene), 3 *Enterococcus faecium* strains (1 with the *vanA* gene and 1 with the *vanB* gene), 2 *Listeria monocytogenes* strains, 3 *Streptococcus pneumoniae* strains (including 1 penicillin resistant), 2 *Streptococcus pyogenes* strains, and 2 *Streptococcus agalactiae* strains.

HMR 1043-resistant mutants were selected by plating 10^9 CFU of overnight cultures of the 26 parental strains grown in Mueller-Hinton (MH) medium on MH agar containing an HMR 1043 gradient up to 32 times the MIC (13). Spontaneously resistant colonies appeared after incubation at 37°C for 24 to 48 h. The colony grown in the highest drug concentration was taken as the mutant strain. Susceptibility tests were performed for the 52 strains, the 26 parental strains and 26 mutants.

HMR 1043 and teicoplanin were kindly provided by Aventis (Romainville, France), and daptomycin was provided by Lilly Research Laboratories (Indianapolis, Ind.). HMR 1043, daptomycin, and teicoplanin concentrations ranged from 0.0038 to 16 μg/ml. Disks with 7.5, 15, 30, 60, and 120 μg of HMR 1043 were prepared. Agar dilution and disk diffusion susceptibility tests were performed by the procedures outlined by the National Committee for Clinical Laboratory Standards (9, 10), with MH agar supplemented with 5% whole sheep blood when



HMR 1043

FIG. 1. Structure of HMR 1043.

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TABLE 1. In vitro activity of HMR 1043 and other agents on parental and mutant strains[a]

Organism	Strain	MIC (μg/ml)				
		HMR 1043		Daptomycin		Teicoplanin in MH
		MH	CSMH	MH	CSMH	
Staphylococcus aureus						
Methicillin susceptible ATCC 25923	1	1	0.5	0.5	0.25	1
Methicillin susceptible	2	0.5	0.5	0.25	0.125	1
Methicillin susceptible	2M*	2	1	0.25	0.125	1
Methicillin resistant	3	1	0.5	0.25	0.25	1
Teicoplanin intermediate	4	1	1	0.5	0.25	16
Teicoplanin intermediate	4M	4	2	1	0.25	16
VISA	5	4	2	1	0.25	16
Staphylococcus epidermidis						
Methicillin susceptible ATCC 12228	6	0.5	0.5	0.25	0.125	1
Methicillin susceptible	6M	4	1	0.25	0.125	2
Methicillin susceptible	7	1	0.5	0.25	0.125	1
Methicillin susceptible	7M	4	2	2	0.5	1
Methicillin resistant	8	1	0.5	0.25	0.125	2
Methicillin resistant	8M	4	2	1	0.25	2
Teicoplanin resistant	9	1	1	0.25	0.125	8
Teicoplanin resistant	9M	8	4	2	0.5	8
Staphylococcus haemolyticus						
Teicoplanin susceptible	10	0.5	0.5	0.125	0.125	4
Teicoplanin resistant	11	0.5	0.5	0.25	0.125	16
Teicoplanin resistant	11M	8	4	0.5	0.25	16
Enterococcus faecalis ATCC 29212	12	4	2	1	0.125	0.25
	VanA 13	1	0.5	2	0.25	16
	VanB 14	4	2	1	0.25	0.25
Enterococcus faecium ATCC 35667	15	2	1	2	0.25	0.5
	VanA 16	2	1	2	0.5	>16
	VanB 17	4	1	0.5	0.125	0.5
Listeria monocytogenes ATCC 19115	18	8	4	4	0.5	0.5
	18M	>16	4	4	0.5	0.5
	19	8	2	2	0.25	0.5
Streptococcus pneumoniae						
Penicillin susceptible ATCC 49619	20	0.125	0.125	0.125	0.03	0.125
Penicillin susceptible	20M	1	1	0.125	0.06	0.125
Penicillin susceptible	21	1	0.5	0.125	0.03	0.125
Penicillin susceptible	21M	4	0.5	ND	ND	ND
Penicillin resistant	22	0.5	0.25	0.125	0.03	0.125
Streptococcus pyogenes ATCC 19615	23	1	0.5	1	0.125	0.125
	24	1	0.5	0.06	0.03	0.125
Streptococcus agalactiae ATCC 13813	25	1	1	0.25	0.06	0.25
	26	1	0.5	0.25	0.06	0.125

[a] M*, mutant strain with decreased susceptibility; VISA, vancomycin-intermediate *Staphylococcus aureus*; ND, not determined.

streptococcal and listerial strains were tested. The following media were used: base MH agar containing 100 mg of Ca^{2+} per liter and MH agar supplemented with up to 207 mg (9 mM) of Ca^{2+} per liter (CSMH) from a 20-g/liter $CaCl_2$ solution. MICs and inhibition zone diameters were read after 18 h of incubation at 37°C.

Comparative antimicrobial activity. The comparative MICs of HMR 1043, daptomycin, and teicoplanin are shown in Table 1. The activity of HMR 1043 was not influenced by the resistance of staphylococci to methicillin or teicoplanin, the resistance of pneumococci to penicillin, or the resistance of enterococci to vancomycin (*vanA* or *vanB* type). HMR 1043 was two- to eightfold less active than daptomycin against listerial isolates. Mutants selected from staphylococci intermediate or resistant to teicoplanin were 4- to 16-fold less susceptible than the parental strains (HMR 1043 MICs, 4 to 8 μg/ml). MICs of daptomycin for parental and mutant streptococcal and listerial strains were identical. Mutants selected from the *Staphylococcus epidermidis* clinical strains were twofold to eightfold more resistant than the parental strains, with daptomycin MICs of ≤2 μg/ml; MICs of teicoplanin for parental and mutant strains were comparable. Streptococci were more susceptible to teicoplanin than staphylococci.

Comparisons of HMR 1043, daptomycin, and teicoplanin MICs by regression statistics yielded poor correlation coeffi-

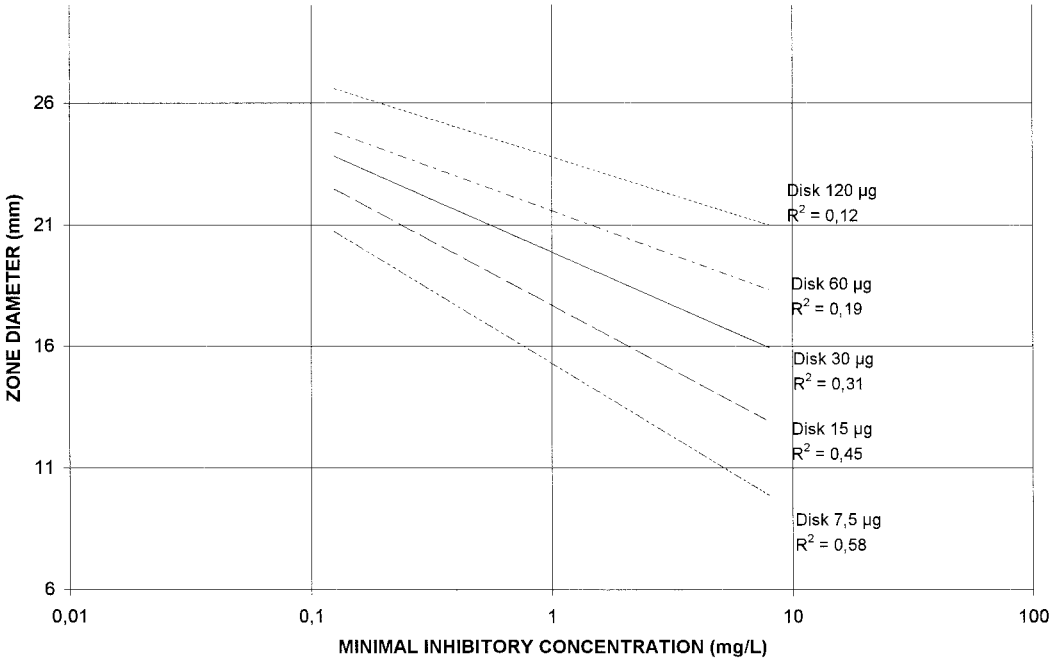

FIG. 2. Correlations between HMR 1043 MICs and zone diameters around 7.5-, 15-, 30-, 60-, and 120-μg HMR 1043 disks on MH agar.

cients as follows: HMR 1043 versus daptomycin, $r = 0.51$; HMR 1043 versus teicoplanin, $r = 0.02$. These data imply that HMR 1043 is more related to daptomycin than to teicoplanin.

Effect of calcium on activity. The comparative MICs of HMR 1043 and daptomycin tested on MH agar and on CSMH are shown in Table 1. The addition of calcium had little effect on HMR 1043 activity: MICs generally varied no more than twofold. Daptomycin MICs determined in CSMH were fourfold lower than those obtained in unsupplemented MH agar: HMR 1043 versus daptomycin, $r = 0.36$.

Disk diffusion tests. Correlations among HMR 1043 MICs and zone diameters around 7.5-,15-, 30-, 60-, and 120-μg HMR 1043 disks on MH agar and CSMH are shown in Fig. 2 and 3, respectively. The correlation coefficients for the four different

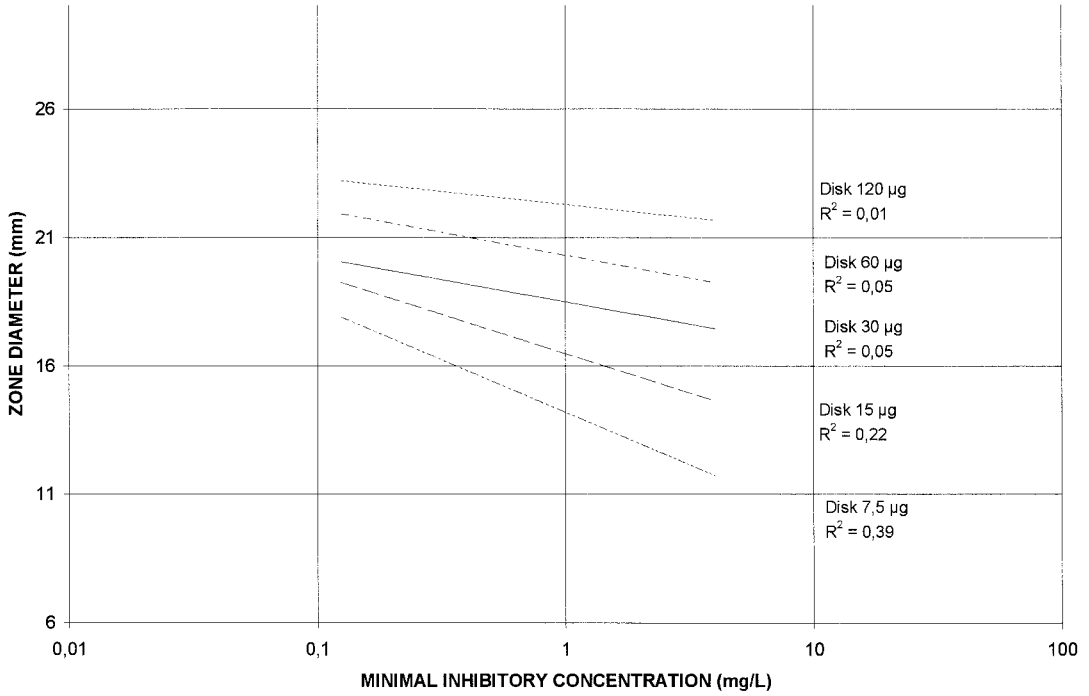

FIG. 3. Correlations between HMR 1043 MICs and zone diameters around 7.5-, 15-, 30-, 60-, and 120-μg HMR 1043 disks on CSMH.

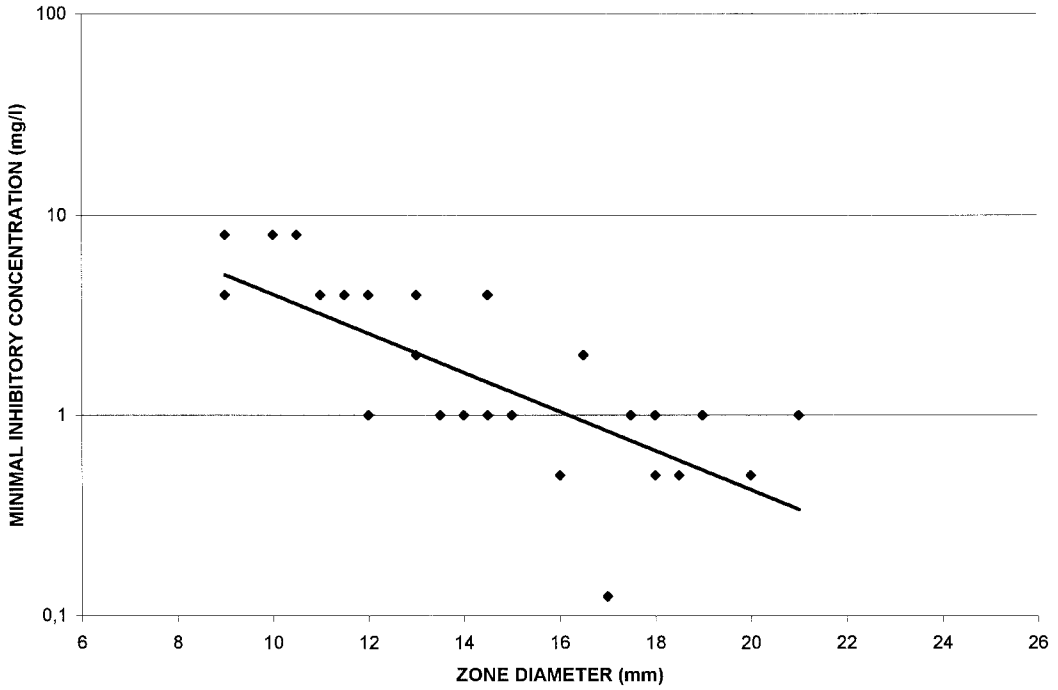

FIG. 4. Scattergram showing correlations between HMR 1043 MICs and zone diameters around 7.5-μg HMR 1043 disks on MH agar.

disks were poor (r range, 0.12 to 0.58) and lower when disk diffusion tests were performed in CSMH (r range, 0.01 to 0.39). The best correlation was obtained with the 7.5-μg disk on MH agar ($r = 0.58$) versus 0.39 on CSMH. Scattergrams of the disk zone diameters and HMR 1043 MICs determined in MH agar and in CSMH are shown in Fig. 4 and 5, respectively. Therefore, zone diameters remained small: a MIC of 1 μg/ml was correlated with a zone diameter of 16 mm, and a MIC of 4 μg/ml was correlated with a zone diameter of 11 mm (Fig. 4). Disks with 7.5 μg of HMR 1043 performed better, with zone diameters very similar to those determined in MH agar.

HMR 1043 demonstrated good activity against susceptible

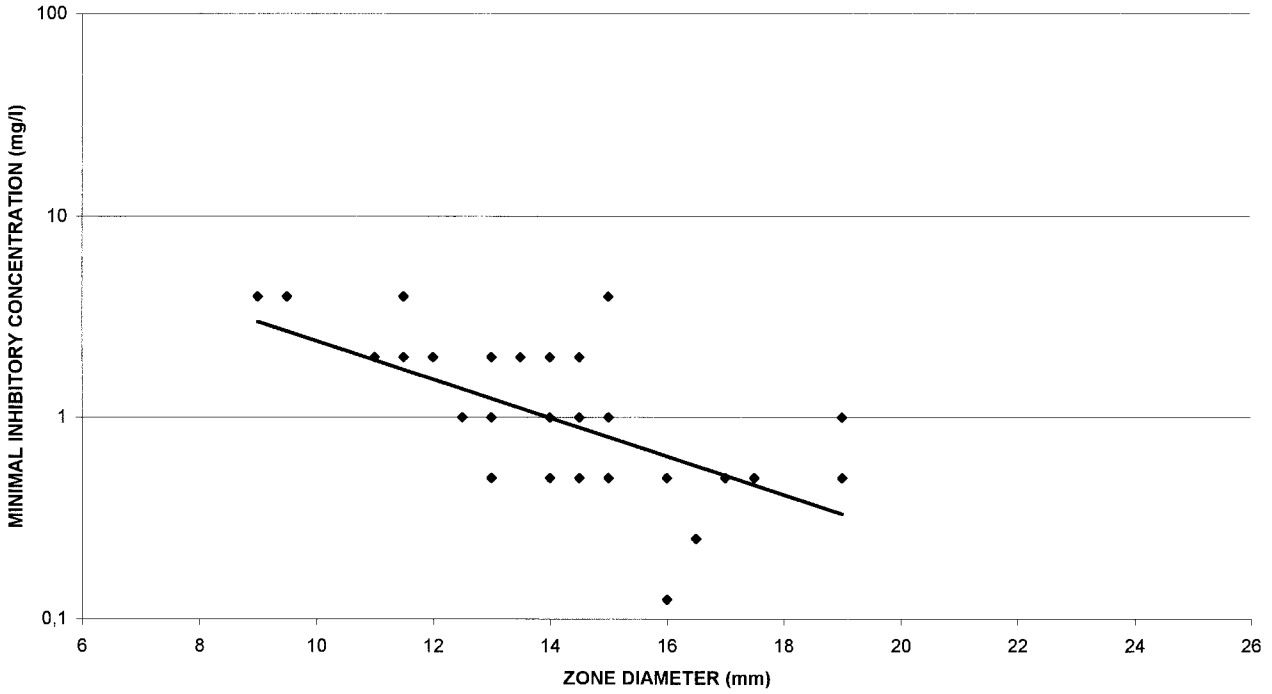

FIG. 5. Scattergram showing correlations between HMR 1043 MICs and zone diameters around 7.5-μg HMR 1043 disks on CSMH.

and resistant gram-positive bacteria. The activity of HMR 1043 in vitro is less influenced by the presence of calcium ions than that of daptomycin. The activities of HMR 1043 and daptomycin against staphylococcal and streptococcal parental strains were similar, but daptomycin remains more active than HMR 1043 against enterococcal and listerial strains. Mutants selected from teicoplanin-intermediate or -resistant coagulase-negative staphylococci were 8-fold to 16-fold more resistant to HMR 1043 than their parental strains (MICs $\geq$ 4 μg/ml) and remained susceptible to daptomycin (MICs $\leq$ 2 μg/ml). Selection of in vitro susceptibility test breakpoints for HMR 1043 was difficult because of the poor correlation coefficients obtained with the different disks tested.

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