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In Vitro Activities of Ketolide HMR3647, Macrolides, and Other Antibiotics against *Lactobacillus*, *Leuconostoc*, and *Pediococcus* Isolates

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Testing of susceptibility to 13 antibiotics was performed with 90 isolates of *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. MICs at which 90% of the isolates tested were inhibited by HMR3647, erythromycin, and ciprofloxacin were 0.015, 0.125 and 32 μg/ml, respectively. The penicillin MIC was ≥16 μg/ml against 26.2% of the studied *Lactobacillus* sp. isolates and 50% of *Lactobacillus plantarum*. HMR3647 showed excellent activity against these genera.

Lactobacillus, *Leuconostoc*, and *Pediococcus* spp. are commonly found as natural microflora in the mucous membranes of humans and animals, in dairy products, and on some plant surfaces (2, 21). Nevertheless, they are increasingly recognized as opportunistic pathogens involved in human infections (1, 3, 5, 8, 10, 11, 14, 17, 22). Intrinsic resistance to glycopeptides is well documented in these genera of lactic acid bacteria (LAB) (13, 26, 31); however, there are few reports on susceptibilities of LAB to other antibiotics. The purpose of this work was to determine the susceptibility pattern to 13 antibiotics in 90 LAB isolates: 60 *Lactobacillus* isolates (*L. plantarum*, 28; *L. paracasei*, 17; *L. brevis*, 8; *L. rhamnosus*, 3; *L. casei*, 1; *L. fermentum*, 1; *L. curvatus*, 1; *L. pentosus*, 1); 18 *Leuconostoc* isolates (*L. mesenteroides*, 10; *L. pseudomesenteroides*, 1; *L. oenos*, 1; *Leuconostoc* spp., 6); and 12 *Pediococcus* isolates (*P. pentosaceus*, 10; *P. acidilactici*, 2). They were identified as previously recommended (6, 15, 15a) and by using the API50 CH system (Biomérieux). MICs were determined by the National Committee for Clinical Laboratory Standards agar dilution method (19) using brain heart infusion agar (BHIA) and an atmosphere of 5% CO_2. Results in BHIA were compared with those obtained in Mueller-Hinton agar supplemented with 5% sheep blood (MHA-B). Strains grew better in BHIA than in MHA-B, and therefore, the former agar medium was used for MIC determinations. Control strains used were *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. Tested antibiotics were the following: ketolide HMR3647, erythromycin A, clarithromycin, roxithromycin, azithromycin, levofloxacin, and teicoplanin (all supplied by Hoechst Marion Roussel); spiramycin and penicillin (Sigma); pristinamycin I (Rhône-Poulenc Rorer); vancomycin (Elli Lilly); and ciprofloxacin (Bayer).

The susceptibility testing results are shown in Table 1 and 2. The MICs at which 90% of isolates were inhibited (MIC_{90}s) of the macrolides tested for 60 *Lactobacillus* isolates were in the range of 0.03 to 0.5 μg/ml (Table 1). Similar data have been obtained by others (23, 26). The MIC_{90} of HMR3647 for these isolates was 0.015 μg/ml, 3 dilutions lower than that of erythromycin. One of the studied *Lactobacillus* isolates, *L. paracasei* J19, was resistant to all macrolides (MICs in the range of 16 to 256 μg/ml). The MIC of HMR3647 for this isolate was 0.5 μg/ml, lower than those of macrolides and higher than those found for susceptible isolates. Erythromycin resistance mediated by plasmids has been previously reported for *Lactobacillus* (7, 9, 20, 28), and different genes encoding methylases, such as *ermGT* (28), *ermBC* (7), and *ermAM* (20), have been reported. Only one *Lactobacillus* isolate was susceptible to vancomycin, and five were susceptible to teicoplanin. MICs of penicillin for 16 of 60 *Lactobacillus* isolates (26.6%) were ≥16 μg/ml, but no β-lactamase activity was detected. MICs of penicillin or ampicillin for most *Lactobacillus* isolates referred to in the literature were ≤2 μg/ml (12, 26). Ciprofloxacin had a poor activity against our *Lactobacillus* isolates (MIC ≥ 4 μg/ml in 60% of them). *L. plantarum* showed the highest level of resistance to all tested antibiotics except macrolides (Table 2). The respective MIC_{90}s of penicillin and ciprofloxacin were ≥128 and 32 μg/ml for *L. plantarum* and were 1 and 2 μg/ml for *L. paracasei*. The modal MICs of penicillin and ciprofloxacin for *L. brevis* were 4 μg/ml and 16 to 32 μg/ml, respectively (Fig. 1).

All studied *Leuconostoc* isolates were susceptible to macrolides, with MIC_{90}s in the range of 0.03 to 0.5 μg/ml (Table 1). The MIC_{90} of HMR3647 was 0.015 μg/ml, 3 dilutions lower than that of erythromycin. Similar results have previously been reported (18, 26). Penicillin MICs were always ≤2 μg/ml. Ciprofloxacin and levofloxacin were poorly active against *Leuconostoc*, showing MICs of ≥4 μg/ml for 72.2 and 61.1% of the isolates, respectively.

For all but one of the studied *Pediococcus* isolates erythromycin MICs were ≤0.25 μg/ml. The MIC_{90} of HMR3647 for these isolates was 0.015 μg/ml. Marie-Bigot et al. (18) have reported similar results. *P. acidilactici* AR63 was the only macrolide-resistant *Pediococcus* isolate found in our study (MICs of ≥64 μg/ml). The MIC of HMR3647 for this isolate was 4 μg/ml. The mechanism of resistance involved was studied by PCR, and a positive result was obtained when degenerate *erm* primers were used (4). Nevertheless, negative results were obtained when specific primers for amplification of *ermA*, *ermB*, *ermC* (25), or *ermTR* (16), as well as *msrA* (30) and *mefA*/*E* (24), were assayed. A high-molecular-weight plasmid was observed in *P. acidilactici* AR63, and its putative relationship with macrolide resistance was studied by filter conjugation using erythromycin-sensitive *E. faecalis* JH2-2 and *E. faecium* GE-1 as

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TABLE 1. In vitro activities of different antibiotics against LAB isolates from various genera[a]

Microorganisms (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[b]		
		Range	50%	90%
Lactobacillus spp. (60)	Erythromycin A	0.03–256	0.06	0.125
	Clarithromycin	0.0015–128	0.015	0.03
	Roxithromycin	0.007–128	0.06	0.125
	Azithromycin	0.0035–128	0.06	0.125
	HMR3647	≤0.0007–0.5	0.007	0.015
	Spiramycin	0.06–16	0.25	0.5
	Pristinamycin I	≤0.25–16	2	8
	Vancomycin	4–≥4,096	≥4,096	≥4,096
	Teicoplanin	2–≥4,096	2,048	≥4,096
	Penicillin	0.25–≥128	2	≥128
	Ciprofloxacin	0.5–64	8	32
	Levofloxacin	0.125–16	4	8
Leuconostoc spp. (18)	Erythromycin A	0.03–0.125	0.06	0.125
	Clarithromycin	0.007–0.03	0.03	0.03
	Roxithromycin	0.03–0.5	0.125	0.5
	Azithromycin	0.03–0.125	0.125	0.125
	HMR3647	≤0.0007–0.015	0.007	0.015
	Spiramycin	0.25–0.5	0.25	0.5
	Pristinamycin I	≤0.25–8	2	8
	Vancomycin	512–≥4,096	1,024	≥4,096
	Teicoplanin	512–≥4,096	1,024	≥4,096
	Penicillin	0.25–2	0.5	2
	Ciprofloxacin	1–32	4	16
	Levofloxacin	1–8	4	8
Pediococcus spp. (12)	Erythromycin A	0.06–≥1,024	0.125	0.25
	Clarithromycin	0.007–128	0.06	0.06
	Roxithromycin	0.007–1,024	0.06	0.5
	Azithromycin	0.06–128	0.125	0.25
	HMR3647	≤0.0007–4	0.007	0.015
	Spiramycin	0.25–≥64	0.5	1
	Pristinamycin I	1–128	4	8
	Vancomycin	512–2,048	1,024	1,024
	Teicoplanin	512–≥4,096	2,048	≥4,096
	Penicillin	0.5–2	1	2
	Ciprofloxacin	32–64	32	64
	Levofloxacin	4–16	8	16
Total LAB (90)	Erythromycin A	0.03–≥1,024	0.06	0.125
	Clarithromycin	0.0015–128	0.015	0.06
	Roxithromycin	0.007–≥1,024	0.06	0.25
	Azithromycin	0.0035–128	0.06	0.125
	HMR3647	≤0.0007–4	0.007	0.015
	Spiramycin	0.06–≥64	0.5	0.5
	Pristinamycin I	≤0.25–128	4	8
	Vancomycin	4–≥4,096	2,048	≥4,096
	Teicoplanin	2–≥4,096	2,048	≥4,096
	Penicillin	0.25–≥128	1	64
	Ciprofloxacin	0.5–64	8	32
	Levofloxacin	0.125–16	4	8

[a] Agar dilution in BHIA and 5% CO_2.
[b] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

recipients. Results were negative in all cases. Erythromycin resistance has previously been reported for *Pediococcus* strains (26, 31). One *P. acidilactici* strain with macrolide resistance related to a determinant homologous to the *ermAM* gene located on a nontransferable 46 kb plasmid has previously been reported (27).

For all *Leuconostoc* and *Pediococcus* isolates penicillin MICs were ≤2 μg/ml. MIC ranges of ciprofloxacin for *Pediococcus* and *Leuconostoc* were 32 to 64 and 1 to 32 μg/ml, respectively. All isolates of both genera showed high-level glycopeptide resistance (MIC ≥ 512 μg/ml). This group of organisms is sometimes mistaken for enterococci, but the latter genus is usually susceptible to vancomycin.

MICs were determined in MHA-B medium and were compared with those obtained in BHIA for 78 LAB isolates in this study. MICs were similar in both media, although they tended to be 1 dilution higher in BHIA (data not shown).

In summary, with few exceptions, the LAB tested in this work were susceptible to all macrolides and the ketolide HMR3647. For all tested *Pediococcus* and *Leuconostoc* isolates penicillin MICs were ≤2 μg/ml, whereas for 26% of *Lactobacillus* isolates MICs were ≥16 μg/ml. Ciprofloxacin MICs for all *Pediococcus* isolates, 60% of *Lactobacillus* isolates, and 72% of *Leuconostoc* isolates were ≥4 μg/ml. Ecological balance modifications of the natural species in the human gut flora by antibiotic action are a matter of growing concern. This paper shows that differences in susceptibility to widely used groups of antibiotics occur among different genera, as well as among species within a given genus. Therefore, population replacement derived from antibiotic pressure would not come unexpectedly. Thus, for instance, *L. plantarum* (resistant to penicillin and ciprofloxacin) might replace *L. para-*

TABLE 2. In vitro activities of different antibiotics against *Lactobacillus* spp.[a]

Microorganisms (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[b]		
		Range	50%	90%
L. plantarum (28)	Erythromycin A	0.03–0.125	0.06	0.125
	Clarithromycin	0.0007–0.06	0.007	0.06
	Roxithromycin	0.03–0.5	0.06	0.25
	Azithromycin	0.0035–0.125	0.06	0.125
	HMR3647	≤0.0007–0.015	0.007	0.015
	Spiramycin	0.25–1	0.25	0.5
	Pristinamycin I	2–16	4	16
	Vancomycin	1,024–≥4,096	≥4,096	≥4,096
	Teicoplanin	1,024–≥4,096	≥4,096	≥4,096
	Penicillin	2–≥128	8	≥128
	Ciprofloxacin	4–64	16	32
	Levofloxacin	0.125–16	4	8
L. paracasei (17)	Erythromycin A	0.03–256	0.06	0.125
	Clarithromycin	0.0035–128	0.03	0.03
	Roxithromycin	0.015–128	0.125	0.125
	Azithromycin	0.03–128	0.06	0.06
	HMR3647	≤0.0007–0.5	0.007	0.015
	Spiramycin	0.25–16	0.5	0.5
	Pristinamycin I	0.25–2	0.5	2
	Vancomycin	4–≥4,096	1,024	≥4,096
	Teicoplanin	4–≥4,096	512	1,024
	Penicillin	0.25–2	0.5	1
	Ciprofloxacin	0.5–2	1	2
	Levofloxacin	0.5–2	2	2
L. brevis (8)	Erythromycin A	0.03–0.125	—[c]	—
	Clarithromycin	≤0.0007–0.06	—	—
	Roxithromycin	0.007–0.25	—	—
	Azithromycin	0.0035–0.125	—	—
	HMR3647	≤0.0007–0.015	—	—
	Spiramycin	0.125–0.5	—	—
	Pristinamycin I	1–16	—	—
	Vancomycin	128–≥4,096	—	—
	Teicoplanin	8–≥4,096	—	—
	Penicillin	0.25–128	—	—
	Ciprofloxacin	2–32	—	—
	Levofloxacin	2–8	—	—
Lactobacillus spp. (7)	Erythromycin A	0.03–0.06	—	—
	Clarithromycin	0.015–0.06	—	—
	Roxithromycin	0.06–0.125	—	—
	Azithromycin	0.03–0.25	—	—
	HMR3647	≤0.0007–0.007	—	—
	Spiramycin	0.06–0.5	—	—
	Pristinamycin I	0.25–8	—	—
	Vancomycin	128–≥4,096	—	—
	Teicoplanin	2–512	—	—
	Penicillin	0.25–≥128	—	—
	Ciprofloxacin	0.5–4	—	—
	Levofloxacin	0.25–4	—	—

[a] Agar dilution in BHIA and 5% CO_2.
[b] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[c] —, too few isolates for MIC determinations.

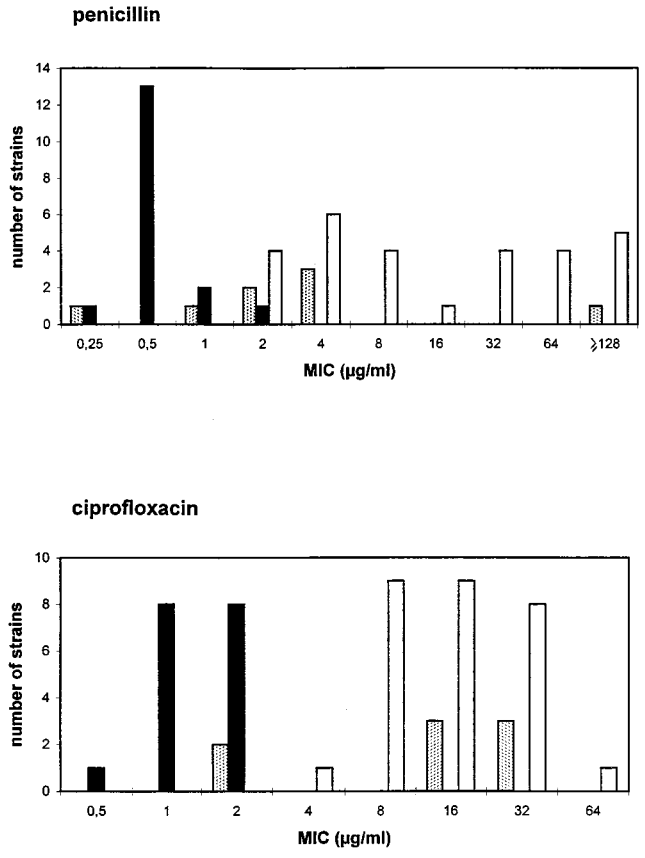

FIG. 1. Graph of penicillin and ciprofloxacin MICs for 53 *Lactobacillus* isolates. White bars, *L. plantarum*; black bars, *L. paracasei*; stippled bars, *L. brevis*.

casei (susceptible to both types of drugs). The clinical or evolutionary implications of these changes are currently unknown. More attention should be paid to the selection of naturally (or acquired) resistant microorganisms that are members of the normal human microbiota.

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