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In Vitro Activity of YM133, a New Semisynthesized Macrolide

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YM133, the 4″-*O*-(4-methoxyphenyl)acetyltylosin, is a new macrolide. The in vitro activity of YM133 was compared with those of erythromycin, josamycin, and rokitamycin by an agar dilution method. YM133 inhibited 90% of the tested isolates of *Streptococcus pneumoniae*, *Legionella* spp., and anaerobic bacteria at ≤1.56 μg/ml. The drug inhibited 90% of erythromycin-resistant staphylococci and *Streptococcus pyogenes* at ≤50 μg/ml. YM133 showed activity against erythromycin-, josamycin-, and rokitamycin-resistant (MIC ≥ 100 μg/ml) strains of staphylococci, streptococci, *Bacteroides* spp., and *Clostridium* spp. Enterococci were less susceptible to other YM133-like macrolides. Unlike other macrolides, YM133 showed killing activity, and the MBC/MIC ratios of YM133 for several strains were 1:32, whereas those of erythromycin were 4:1,024. In a time-kill curve study, the reduction of viable cells started within 2 h after the addition of YM133.

Recently, several new macrolides have been developed by chemical modification of existing macrolides to improve their pharmacokinetics or to widen their antimicrobial spectra (1, 2, 4, 6, 7). In staphylococci and streptococci, there are many strains which are resistant to 14-membered macrolides and susceptible to 16-membered macrolides, but a large number of strains are resistant to both 14- and 16-membered macrolides. One way of developing a new macrolide is to give an efficacy to strains which are resistant to usual macrolides (11, 18). But at this time, there are no new macrolides which have sufficient efficacy to resistant strains (4).

A newly developed tylosin derivative, YM133 [4″-*O*-(4-methoxyphenyl)acetyltylosin] (formerly code name IMC-XV), was effective against erythromycin-resistant strains of *Staphylococcus aureus* (3, 8, 13, 14, 20), so we tested the activity of YM133 against clinical isolates.

In this article, we describe the in vitro activities of YM133 compared with those of rokitamycin, josamycin, erythromycin, clindamycin, and oleandomycin.

(A part of this work was presented at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy [17].)

MATERIALS AND METHODS

Antibiotics. Antibiotics used in this study were YM133, josamycin (Mercian Corp., Tokyo, Japan), rokitamycin (Toyo Jozo Co., Shizuoka, Japan), and oleandomycin, clindamycin, and erythromycin (Japan Upjohn, Tokyo, Japan).

Bacterial strains. Clinical isolates of various species were obtained from several hospitals in Japan and maintained in our laboratory.

Of the set of macrolide-resistant *S. aureus* strains, three strains (MS15026, MS15009/pMS97, and MS15027) were derived from strain MS15009 by transduction of macrolide resistance with S2 phage, and the rest were clinical isolates. The donors in transduction were resistant isolates which belonged to the macrolide-resistant group A, B, or C, and the transductants were designated MS15026, MS15009/pMS97, and MS15027, respectively. The grouping of macrolide-resistant strains was according to the classification of Mitsuhashi and Inoue (10).

Determination of MIC. MICs were determined by the twofold agar dilution method as previously described (19), with sensitivity disk agar (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) for staphylococci. Heart infusion agar (Nissui) was used for *Streptococcus pyogenes*, enterococci, and other species unless otherwise noted. Heart infusion agar supplemented with 5% horse blood was used for *Streptococcus pneumoniae*. Heart infusion agar supplemented with 5% Fildes enrichment (Difco Laboratories, Detroit, Mich.) was used for *Haemophilus influenzae*. GC agar (Difco Laboratories) supplemented with 1% hemoglobin (Difco Laboratories) and 1% IsoVitalX (Becton Dickinson and Co., Paramus, N.J.) was used for *Neisseria gonorrhoeae*. GAM agar (Nissui) was used for obligate anaerobes. For *Legionella* spp., *Legionella* CYE agar base and *Legionella* growth supplement (Oxoid Ltd., Basingstoke, Hampshire, England) were used (9). An overnight culture of each strain in suitable broth was diluted in buffered saline with gelatin (8.5 g of NaCl, 0.6 g of KH_2PO_4, 0.3 g of K_2HPO_4, 0.1 g of gelatin per liter of distilled water). About 10^4 CFU of bacteria per spot was inoculated onto agar media, each containing an amount of one of the drugs. Inoculated plates were incubated at 37°C for 18 h unless otherwise noted. *S. aureus* strains were incubated at 30°C. *N. gonorrhoeae* strains were incubated by a candle extinction jar method (15). Anaerobic bacteria were incubated in an anaerobic jar for 48 h.

Determination of bactericidal activity. MBCs were determined by the methods of Pearson et al. (12) and Taylor et al. (16), but several points were modified. Medium used was heart infusion broth (Nissui). Glass tubes containing 1 ml each of serial twofold dilutions of the drugs in heart infusion broth were inoculated with precultured bacteria to a final inoculum of about 10^6 CFU/ml. Tubes were incubated at 37°C for 18 h. After the MICs were determined, each broth was centrifuged in a polypropylene tube, and the cells were washed once with physiological saline. The organisms were resuspended to 1 ml of buffered saline with gelatin, and a 0.1-ml sample of suspension was spread onto the sensitivity disk agar plate. Plates were incubated at 37°C for 48 h. The concentration of drug when the number of colonies was less than 0.1% of initial bacterial concentration was defined as

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TABLE 1. Comparative in vitro activity of YM133

Strain (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Erythromycin-resistant[b] *S. aureus* (42)	YM133	0.78–12.5	3.13	6.25
	Rokitamycin	0.39–>100	>100	>100
	Josamycin	0.78–>100	>100	>100
	Erythromycin	6.25–>100	>100	>100
Erythromycin-susceptible[b] *S. aureus* (58)	YM133	0.39–1.56	0.78	1.56
	Rokitamycin	0.20–0.78	0.39	0.39
	Josamycin	0.20–3.13	0.78	0.78
	Erythromycin	0.05–3.13	0.20	1.56
Erythromycin-resistant[b] *S. epidermidis* (30)	YM133	0.39–>100	6.25	50
	Rokitamycin	0.39–>100	>100	
	Josamycin	0.39–>100	>100	>100
	Erythromycin	6.25–>100	>100	>100
Erythromycin-susceptible[b] *S. epidermidis* (62)	YM133	0.39–3.13	0.78	1.56
	Rokitamycin	0.20–1.56	0.39	0.39
	Josamycin	0.39–3.13	0.78	0.78
	Erythromycin	0.10–0.39	0.20	0.39
Erythromycin-resistant[b] *S. pyogenes* (56)	YM133	0.78–25	12.5	12.5
	Rokitamycin	0.78–>100	>100	>100
	Josamycin	1.56–>100	>100	>100
	Erythromycin	6.25–>100	>100	>100
Erythromycin-susceptible[b] *S. pyogenes* (97)	YM133	0.39–3.13	0.39	0.78
	Rokitamycin	0.05–0.78	0.10	0.20
	Josamycin	0.05–0.78	0.20	0.20
	Erythromycin	0.025–12.5	0.025	0.05
S. pneumoniae (42)	YM133	0.05–>100	0.20	0.78
	Rokitamycin	0.025–100	0.05	0.78
	Josamycin	0.025–>100	0.10	6.25
	Erythromycin	≤0.013–>100	0.05	3.13
E. faecalis (100)	YM133	0.20–>100	0.78	>100
	Rokitamycin	0.20–>100	0.78	>100
	Josamycin	0.39–>100	1.56	>100
	Erythromycin	0.05–>100	1.56	>100
E. faecium (33)	YM133	0.78–>100	3.13	>100
	Rokitamycin	0.39–>100	1.56	>100
	Josamycin	0.78–>100	6.25	>100
	Erythromycin	0.10–>100	6.25	>100
B. catarrhalis (42)	YM133	0.39–>100	1.56	3.13
	Rokitamycin	0.05–>100	0.20	0.78
	Josamycin	0.20–>100	0.78	1.56
	Erythromycin	0.10–>100	0.20	0.78
N. gonorrhoeae (51)	YM133	0.39–12.5	6.25	6.25
	Rokitamycin	0.10–6.25	1.56	3.13
	Josamycin	0.20–6.25	3.13	6.25
	Erythromycin	0.05–3.13	0.78	1.56
Legionella spp. (15)	YM133	0.39–3.13	1.56	1.56
	Rokitamycin	≤0.025–0.20	0.10	0.10
	Josamycin	0.10–0.78	0.39	0.78
	Erythromycin	0.39–3.13	0.78	1.56
H. influenzae (94)	YM133	3.13–100	25	50
	Rokitamycin	0.39–25	12.5	12.5
	Josamycin	1.56–50	12.5	50
	Erythromycin	0.78–6.25	3.13	6.25
C. difficile (21)	YM133	≤0.05–0.39	0.39	0.39
	Rokitamycin	≤0.05–6.25	3.13	6.25
	Josamycin	≤0.05–100	50	100
	Erythromycin	≤0.05–50	12.5	50
	Clindamycin	0.10–100	>100	>100
C. perfringens (16)	YM133	≤0.05–0.78	0.10	0.20
	Rokitamycin	≤0.05–6.25	0.10	0.20
	Josamycin	0.10–100	0.39	0.39
	Erythromycin	0.39–>100	0.78	1.56
	Clindamycin	≤0.025–100	0.39	1.56
B. fragilis (35)	YM133	0.10–6.25	0.39	0.78
	Rokitamycin	≤0.05–100	0.20	3.13
	Josamycin	0.10–>100	0.39	100
	Erythromycin	0.78–>100	3.13	>100
	Clindamycin	≤0.025–>100	0.10	>100

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] The MICs of erythromycin for erythromycin-resistant *S. aureus*, *S. epidermidis*, and *S. pyogenes* were ≥6.25 μg/ml, and MICs for erythromycin-susceptible strains were <6.25 μg/ml.

TABLE 2. Antibacterial activity of YM133 against macrolide-resistant *S. aureus*

Group[a] and strain	MIC (μg/ml) of drug				
	YM133	Rokitamycin	Josamycin	Oleandomycin	Erythromycin
S					
209P JC-1	0.39	0.20	0.20	0.78	0.10
Terajima	0.78	0.39	0.78	1.56	0.20
MS353	0.78	0.39	0.78	1.56	0.20
MS15009	0.39	0.20	0.39	1.56	0.10
A					
MS13134	6.25	>100	>100	>100	>100
MS16005	12.5	>100	>100	>100	>100
MS16020	6.25	>100	>100	>100	>100
MS16016	3.13	>100	>100	>100	>100
MS15026	1.56	>100	>100	>100	>100
B					
MS12714	1.56	0.39	1.56	>100	>100
MS12786	0.78	0.39	0.78	>100	>100
MS16004	1.56	0.78	1.56	>100	>100
MS16007	1.56	0.78	3.13	>100	>100
MS15009/pMS97	0.39	0.39	0.78	>100	>100
C					
MS12711	0.39	0.20	0.39	3.13	1.56
MS12731	0.39	0.20	0.39	3.13	1.56
MS16018	0.78	0.39	0.78	3.13	12.5
MS16061	0.78	0.39	0.78	6.25	12.5
MS15027	0.39	0.39	0.78	3.13	3.13

[a] Grouping of macrolide-resistant strains was as follows: S, macrolide susceptible; A, group A (14- and 16-membered-macrolide resistant); B, group B (14-membered-macrolide resistant); C, group C (erythromycin resistant).

MBC. Time-kill kinetics were determined by the method of Kojima et al. (9), by using heart infusion broth as testing medium and heart infusion agar as colony-forming medium.

RESULTS

Antibacterial activity against clinical isolates. The ranges of MICs, the MICs for 50% of the isolates (MIC_{50}s), and the MICs for 90% of the isolates (MIC_{90}s) of YM133, rokitamycin, and other reference drugs for 794 clinical isolates are shown in Table 1. Against erythromycin-resistant staphylococci, YM133 was most active and inhibited 90% of the isolates at ≤12.5 μg/ml, but YM133 was two- to fourfold less active than other macrolides against erythromycin-susceptible strains. Against erythromycin-resistant *S. pyogenes*, YM133 was most active and inhibited 90% of the strains at ≤12.5 μg/ml. YM133 was 2- to 16-fold less active against erythromycin-susceptible *S. pyogenes* than were other macrolides. Against *S. pneumoniae*, YM133 was most active and inhibited 90% of the isolates at ≤0.78 μg/ml. Against enterococci, the activity of YM133 was equal to those of other macrolides. Against *H. influenzae*, *Branhamella catarrhalis*, and *N. gonorrhoeae*, YM133 was two- to eightfold less active than erythromycin. For *Legionella* spp., the MIC_{50} and MIC_{90} of rokitamycin were 0.10 μg/ml, whereas those of YM133 were 1.56 μg/ml.

YM133 inhibited anaerobic bacteria at 0.05 to 6.25 μg/ml, and the MIC_{90}s (0.20 to 0.78 μg/ml) were equal to or two times higher than the MIC_{50}s (0.10 to 0.39 μg/ml) of this drug, whereas both the MIC_{50}s and MIC_{90}s of erythromycin were higher than those of YM133. Rokitamycin inhibited *Clostridium difficile* and *Clostridium perfringens* strains at

TABLE 3. Bactericidal activity of YM133 and reference agents

Strain	Antibiotic	MIC (μg/ml)[a]			MBC (μg/ml)[b]			MBC/MIC
		Range	50%	90%	Range	50%	90%	Range
S. aureus	YM133	0.78–1.56	1.56	1.56	1.56–6.25	3.13	3.13	2–4
	Rokitamycin	0.39–0.78	0.78	0.78	1.56–100	3.13	25	2–128
	Josamycin	0.78–1.56	1.56	1.56	6.25–50	25	50	4–32
	Erythromycin	0.20–3.13	0.39	0.78	1.56–100	12.5	25	4–128
S. epidermidis	YM133	0.39–3.13	0.78	3.13	1.56–100	6.25	25	2–32
	Rokitamycin	0.20–0.78	0.39	0.78	0.78–>100	6.25	100	2–>128
	Josamycin	0.39–1.56	0.78	1.56	6.25–>100	12.5	>100	8–>128
	Erythromycin	0.20–12.5	0.39	0.39	1.56–>100	>100	>100	4–>512
S. pyogenes	YM133	0.20–0.78	0.39	0.39	0.20–1.56	0.78	1.56	1–4
	Rokitamycin	0.05–0.39	0.10	0.20	0.10–0.78	0.39	0.78	1–8
	Josamycin	0.10–0.39	0.20	0.39	0.39–>100	6.25	100	2–>256
	Erythromycin	0.05–0.20	0.05	0.20	0.78–100	12.5	100	16–1024

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] 50% and 90%, MBCs for 50 and 90% of isolates tested, respectively.

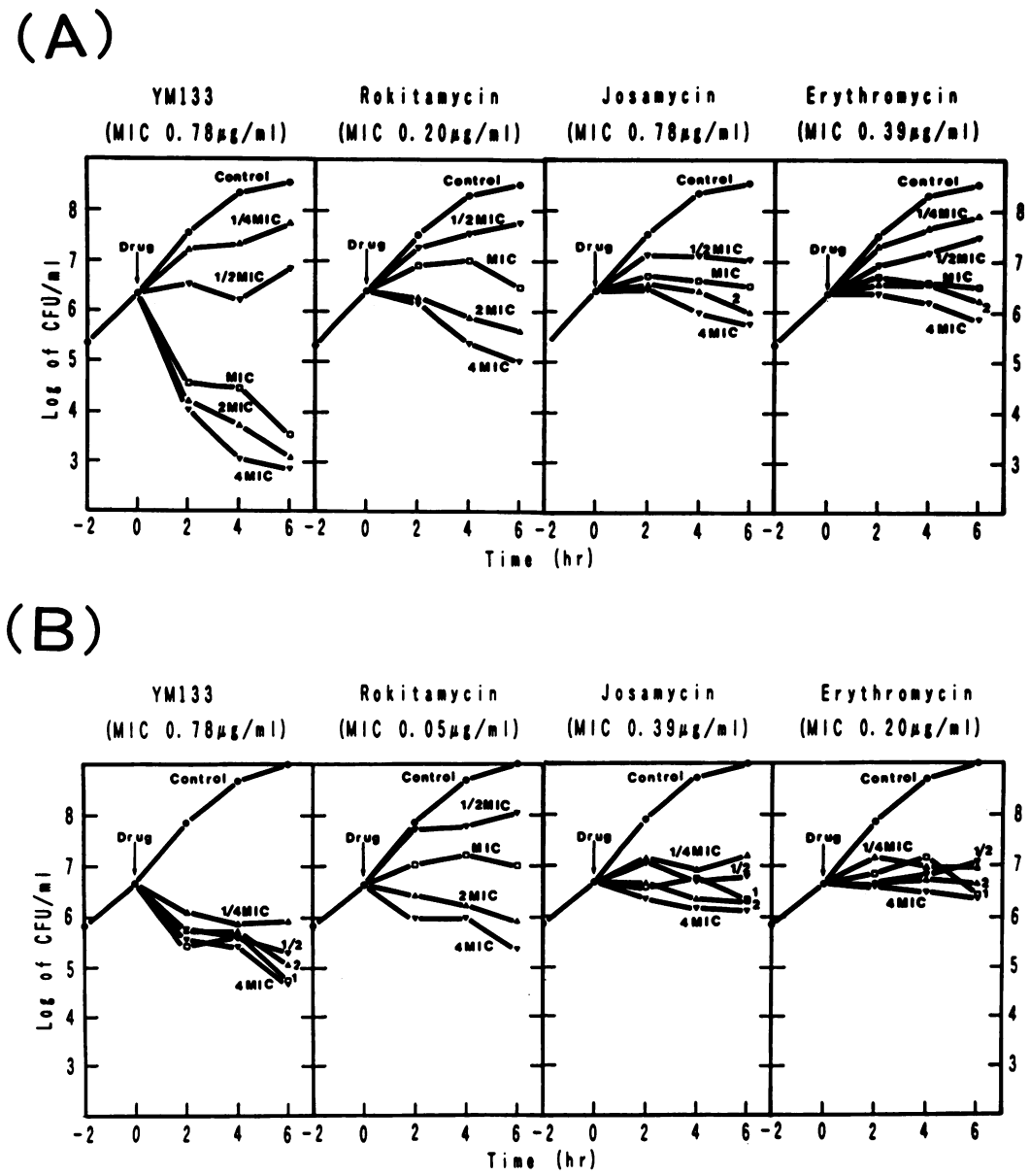

FIG. 1. Time-kill curves of YM133 and reference agents against (A) *S. aureus* MS353 and (B) *S. pyogenes* Cook.

less than 6.25 μg/ml, but some strains of *Bacteroides fragilis* were resistant to the drug. The MIC_{90}s of clindamycin for *C. difficile* and *B. fragilis* were >100 μg/ml and higher than those of YM133 and rokitamycin.

Antibacterial activity against macrolide-resistant strains. The MICs of YM133 for the set of *S. aureus* strains are shown in Table 2.

For the group C strains (erythromycin resistant), the MICs of erythromycin (1.56 to 12.5 μg/ml) and oleandomycin (3.13 to 6.25 μg/ml) were higher than those for susceptible strains (0.10 to 0.20 and 0.78 to 1.56 μg/ml, respectively), whereas the MICs of YM133, rokitamycin, and josamycin were equal to those for susceptible strains. For the group B strains (erythromycin and oleandomycin resistant), the MICs of erythromycin and oleandomycin were higher than 100 μg/ml. In contrast, those of YM133, rokitamycin, and josamycin were one- to twofold higher than those for groups B and C strains. The MICs of all drugs except YM133 were higher than 100 μg/ml for the group A strains, whereas those of YM133 were 1.56 to 25 μg/ml.

Bactericidal activity. The MICs, MBCs, and MBC/MIC ratios for ten susceptible strains each of clinical isolates of *S. aureus*, *Staphylococcus epidermidis*, and *S. pyogenes* are shown in Table 3. For *S. aureus*, the MBC/MIC ratios of YM133 were 2:4, whereas the ratios of erythromycin were 4:128 and those of rokitamycin were 2:128. For *S. epidermidis*, though the MBCs for 90% of the strains tested (MBC_{90}s) of the drugs were higher than those for *S. aureus*, the MBC_{90} of YM133 was lower than those of reference drugs. The MICs and MBCs of YM133 and rokitamycin for *S. pyogenes* strains were lower than those for *S. aureus* and *S. epidermidis* strains, and the MBC/MIC ratios of these drugs for *S. pyogenes* strains were lower than those for staphylococci.

Time-kill curves of YM133 and reference drugs against *S.*

aureus MS353 and *S. pyogenes* Cook are shown in Fig. 1A and B, respectively. Erythromycin and josamycin did not reduce the CFU even at four times the MIC. Rokitamycin reduced the CFU to about 1/10 of the initial number at four times the MIC after 6 h. YM133 reduced the CFU rapidly after the drug was added, and the numbers of cells remaining with the MIC of YM133 after 2 h were 1.4% (*S. aureus* MS353) and 12.5% (*S. pyogenes* Cook) of initial bacterial numbers.

DISCUSSION

Recently, various macrolide antibiotics have been developed (1, 2, 4, 6, 7). However, no macrolide which has activity against the 16-membered macrolide-resistant strains has been developed. YM133 showed activity against these strains (3, 13, 14), and we compared the activity with those of other macrolides.

YM133 was most potent against erythromycin-resistant staphylococci and *S. pyogenes*. The MICs of YM133 for these strains were lower than those of other drugs. Against erythromycin-susceptible strains of staphylococci and *S. pyogenes*, YM133 was less potent than other macrolides. Erythromycin was most potent of the drugs tested against *B. catarrhalis*, *N. gonorrhoeae*, and *H. influenzae*, and YM133 was less active than other drugs against these strains. Rokitamycin showed potent activity against *Legionella* spp., whereas YM133 showed weak activity. Against anaerobes, YM133 was most potent, and the reference drugs were not sufficiently active. It was reported that rokitamycin was active against anaerobes (6) and that clindamycin was most active against anaerobes among the macrolide-lincosamide-streptogramin B antibiotics (1). In this test, the MIC_{50}s and MIC_{90}s of YM133 for anaerobes were lower than those of other drugs, and some strains showed resistance to clindamycin and/or rokitamycin, whereas YM133 inhibited the rokitamycin- and clindamycin-resistant strains.

In accordance with the classification of macrolide-resistant strains of Mitsuhashi and Inoue (10), *S. aureus* strains belonging to group A were constitutively resistant to all macrolides. Strains belonging to group B were resistant to erythromycin and oleandomycin but became resistant to all macrolides after induction with erythromycin or oleandomycin. A group C strain was only erythromycin resistant but became resistant to all macrolides after induction with erythromycin. For the group A strains of *S. aureus*, the MICs of rokitamycin and josamycin were $\geq$100 μg/ml. YM133 inhibited these strains at a concentration of 1.56 to 12.5 μg/ml. Of the set of macrolide-resistant *S. aureus* strains, MS15009, MS15026, MS15009/pMS97, and MS15027 were isogenic strains. The MICs of YM133 were 1.56 μg/ml for MS15026 (group A) and 0.39 μg/ml for other strains. So the MIC of YM133 for the group A strain was fourfold higher than those against susceptible strain and the groups B and C strains. Therefore, it is likely that there is weak cross-resistance between YM133 and other 16-membered macrolides.

Another important property of YM133 is its bactericidal activity. Erythromycin and almost all of other macrolides are bacteriostatic (6), and rokitamycin is known to have killing activity (4). In this study, the MBCs of YM133 for almost all strains tested were lower than those of rokitamycin and were equal to or less than 32-fold of the MICs. Hardy et al. reported that rokitamycin showed bactericidal activity against *H. influenzae*, but it was bacteriostatic against *S. aureus* (4). In this study, the MBCs of YM133 against *S. aureus* strains were lower than those against *S. epidermidis* and were similar to those for *S. pyogenes*. Since the strains used in the MBC determination were picked out randomly from susceptible strains of clinical isolates, other strains of *S. aureus* might have a higher MBC. At least, it is likely that the killing activity of YM133 for *S. aureus* is not weaker than those for *S. pyogenes*. In contrast, the MBCs of rokitamycin for *S. pyogenes* were lower than those for *S. aureus* and *S. epidermidis*, which is similar to the result of Hardy et al. (4). In the time-kill curve study, the reduction of viable cells by YM133 started by 2 h, and the decrease of CFU was large. The data suggested that the killing of bacteria by YM133 began immediately after contact between the drug and bacteria.

It was reported that the plasma level and the tissue levels of YM133 were higher than those of rokitamycin and that the half-life of YM133 was longer than that of rokitamycin in rats and dogs (5). Thus, YM133 would be therapeutically useful because of its excellent activity against macrolide-resistant strains and against anaerobes.

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