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In Vitro Activities of Three Semisynthetic Amide Derivatives of Teicoplanin, MDL 62208, MDL 62211, and MDL 62873

FRANCESCA BIAVASCO,* REMO LUPIDI, AND PIETRO E. VARALDO
Institute of Microbiology, University of Ancona Medical School, 60131 Ancona, Italy

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MDL 62208, MDL 62211, and MDL 62873 are three semisynthetic amide derivatives of teicoplanin (MDL 62208 is an amide of teicoplanin aglycone, MDL 62211 is an amide of the teicoplanin A2 complex, and MDL 62873 is the corresponding derivative of peak A2-2 of the complex). The three semisynthetic glycopeptides were evaluated for in vitro antibacterial activity in comparison with the parent drug (teicoplanin) and vancomycin. A variety of gram-positive bacteria of clinical origin, whose species were carefully determined and that included 428 staphylococci (207 methicillin susceptible and 221 methicillin resistant), 41 streptococci, 82 enterococci, 43 strains of *Listeria monocytogenes*, 10 JK coryneform bacteria, and 67 anaerobes belonging to the genera *Clostridium*, *Propionibacterium*, *Peptostreptococcus*, and *Eubacterium*, were tested. The only resistances to MDL 62208, MDL 62211, and MDL 62873 were encountered with vancomycin- and teicoplanin-resistant enterococci. All of the other test strains, including some teicoplanin-resistant coagulase-negative staphylococci of the species *Staphylococcus haemolyticus* and *Staphylococcus epidermidis*, were highly susceptible to the three teicoplanin amides. Only minor differences in activity were observed among MDL 62208, MDL 62211, and MDL 62873, whereas the three experimental compounds were usually found to be more potent than teicoplanin or vancomycin (especially against staphylococci, with differences mostly ranging from 2- to 16-fold). The MBC-to-MIC ratios varied depending on the organisms, with the highest ratios usually observed for enterococci and listeriae. Overall, the MBC-to-MIC ratios yielded by the teicoplanin analogs were slightly greater than those yielded by teicoplanin or vancomycin.

Vancomycin, the first glycopeptide antibiotic, was introduced into clinical practice in the late 1950s essentially to deal with those serious infections caused by penicillinase-producing strains of *Staphylococcus aureus*, which were raging out of control at that time. Even though highly effective in the treatment of such infections, the drug soon lost favor because of its toxicity (especially oto- and nephrotoxicities) and adverse reactions during administration (44) and was quickly overshadowed by the new drugs methicillin and cephalothin. Unlike ristocetin (another glycopeptide antibiotic which proved to be toxic to bone marrow and to cause platelet aggregation and was thus soon withdrawn [28]), vancomycin, although virtually unused for many years, was nevertheless kept on the market.

A renewal of interest in vancomycin began in the late 1970s. This new trend arose for a variety of concurrent reasons, including (i) the progressive increase in infections caused by gram-positive bacteria, after 2 decades during which the proportion of such infections had substantially dropped under pressure from gram-negative organisms; (ii) the emergence, especially in hospital-associated infections of compromised patients, of highly and often multiply resistant but vancomycin-susceptible, gram-positive pathogens (e.g., methicillin-resistant staphylococci, enterococci, or JK corynebacteria); (iii) the proposal of new uses for vancomycin, such as its oral administration as a topical agent in the treatment of pseudomembranous colitis or its use in prophylactic regimens; and (iv) the improved control of vancomycin toxicity resulting from both the greater purity of modern drug formulations and the clinical monitoring of levels in serum.

The same factors leading to the revival of vancomycin prompted research programs in the pharmaceutical industry aimed at developing new glycopeptide antibiotics. Teicoplanin, which became commercially available in Europe in the late 1980s, demonstrated greater activity than vancomycin in vitro (25, 27, 37) and favorable pharmacokinetics (43) associated with ease of administration and safety in clinical practice (41). New glycopeptides, including both natural (12, 31) and semisynthetic (18, 22, 26, 34) molecules, are currently being investigated for future development. In particular, a large number of compounds resulting from the condensation of the carboxyl group of teicoplanin with amines carrying various functional groups and chains have been synthesized and investigated for structure-activity relationships (22).

In this study, we have evaluated in vitro three amide derivatives of teicoplanin (MDL 62208, MDL 62211, and MDL 62873). They were generally found to be more active than the parent structure (teicoplanin) and vancomycin against a variety of clinical strains of gram-positive bacteria. In particular, MDL 62208, MDL 62211, and MDL 62873 were active against a few coagulase-negative staphylococci resistant to teicoplanin, but they remained ineffective against enterococci resistant to both vancomycin and teicoplanin.

MATERIALS AND METHODS

Bacterial strains. A total of 428 staphylococci, 41 streptococci, 82 enterococci, 43 listeriae, 10 corynebacteria, 55 clostridia, and 12 anaerobic gram-positive bacteria from other genera were studied. With the exception of five selected enterococci (see below), the test organisms were unrelated, randomly collected strains recently isolated from clinical specimens in various Italian hospitals. Most isolates were initially identified by using commercial and automated biochemical test systems, but the identification of many

* Corresponding author.

isolates was confirmed by determining additional distinguishing characters relevant to the laboratory determination of species of staphylococci (38), streptococci and enterococci (9), listeriae (30), coryneform bacteria (20), and anaerobic gram-positive organisms (14).

The staphylococci included 201 strains of *S. aureus*, 113 strains of *S. epidermidis*, 39 strains of *S. haemolyticus*, 20 strains of *S. simulans*, 18 strains of *S. saprophyticus*, 14 strains of *S. hominis*, and lower numbers of strains (from 1 to 7) of each of the following coagulase-negative species: *S. auricularis*, *S. capitis*, *S. cohnii*, *S. lugdunensis*, *S. schleiferi*, *S. warneri*, and *S. xylosus*. Based on oxacillin MICs determined by the microdilution broth method with the recommended precautions (24), staphylococcal strains were preliminary differentiated as methicillin susceptible (with oxacillin MICs of ≤2 μg/ml) or methicillin resistant (with oxacillin MICs of ≥4 μg/ml).

The streptococci included 15 strains of *Streptococcus pneumoniae*, 14 strains of *S. pyogenes*, and lower numbers of strains (from 2 to 5) of the species *S. agalactiae*, *S. bovis*, *S. mutans*, and *S. sanguis*.

Randomly collected enterococci included 61 strains of *Enterococcus faecalis*, 12 strains of *E. faecium*, and 4 strains of *E. durans*. Five additional enterococci already known to be resistant to vancomycin and teicoplanin were expressly procured as such: one was isolated in Italy at the Institute of Microbiology of the University of Catania Medical School and was identified as *E. faecium*, and four (two each of *E. faecalis* and *E. faecium*, isolated in Great Britain) were obtained from the National Collection of Type Cultures, London, England (NCTC 12201, NCTC 12202, NCTC 12203, and NCTC 12204).

All listeriae tested (43 strains) belonged to the species *Listeria monocytogenes*, and all corynebacteria (10 strains) were multiresistant group JK organisms.

Most anaerobic gram-positive bacteria tested belonged to the species *Clostridium difficile* (48 strains). Lower numbers of strains (from 1 to 4) belonged to *Clostridium perfringens*, *Clostridium septicum*, *Clostridium novyi*, *Propionibacterium acnes*, *Peptostreptococcus anaerobius*, *Peptostreptococcus indolicus*, *Peptostreptococcus magnus*, *Peptostreptococcus micros*, and *Eubacterium lentum*.

Antimicrobial agents. Vancomycin was supplied by Eli Lilly Italia, Sesto Fiorentino, Italy. Teicoplanin and its three semisynthetic amide derivatives (MDL 62208, MDL 62211, and MDL 62873) were obtained from the Lepetit Research Center, Gerenzano, Italy. MDL 62208, also known as TD-A3, is the same compound as that indicated as no. 62 in the series of amide derivatives of teicoplanin aglycone reported by Malabarba et al. (22). In the same study, MDL 62211, also known as CTA-A1, was reported as compound 21 in the series of amide derivatives of the teicoplanin A2 complex. The preparation of MDL 62873 used was at least 75% MDL 62873 (the amide of peak A2-2) and not more than 25% amides of other components of the complex.

Assessment of inhibitory activity. MICs were determined essentially according to the standard microdilution procedures recommended by the National Committee for Clinical Laboratory Standards. The five glycopeptide antibiotics were tested at final concentrations (prepared from serial twofold dilutions) ranging from 0.03 to 128 μg/ml. The MIC was defined as the lowest concentration which yielded no visible growth.

With aerobic bacteria (24), the test medium was cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 5% lysed horse blood when streptococci, enterococci, and listeriae were tested and with 5% rabbit serum when JK corynebacteria were tested. The inoculum was 10^6 CFU/ml (10^5 CFU/0.1-ml well), i.e., slightly higher than the recommended value of 5×10^5 CFU/ml (24). This modification, which preliminary comparative trials proved to yield substantially the same results as the standard inoculum (data not shown), made the subsequent determination of the 99.9% killing endpoint from the same trays more reliable (see below). The inoculated trays were incubated at 35°C for 18 h (in an atmosphere containing 5% CO_2 in the case of pneumococci and JK coryneform bacteria). *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as quality control strains.

With anaerobic bacteria (23), the test medium was Wilkins-Chalgren anaerobe broth (Oxoid Ltd., Basingstoke, England) supplemented, when needed, with 5% horse serum. The inoculum was 10^6 CFU/ml (10^5 CFU/0.1-ml well). The inoculated trays were incubated for 48 h at 35°C in GasPak jars (Becton Dickinson Microbiology Systems, Cockeysville, Md.). *C. perfringens* ATCC 13124 was used as a control.

The following MIC susceptibility breakpoints were considered for vancomycin (24): susceptible, ≤4 μg/ml; intermediate, 8 to 16 μg/ml; resistant, ≥32 μg/ml. Teicoplanin is not reported in the latest documents published by the National Committee for Clinical Laboratory Standards, but this same committee has very recently approved the following breakpoints for this drug (11, 17): susceptible, ≤8 μg/ml; intermediate, 16 μg/ml; resistant, ≥32 μg/ml. We tentatively applied these same MIC breakpoints to the teicoplanin derivatives as well, considering their close structural relationship to teicoplanin.

Assessment of bactericidal activity. MBCs were established by extending the MIC procedure to the evaluation of bactericidal activity. This approach was applied not only to aerobic organisms, but, because of a lack of standardized alternatives, also tentatively to anaerobic bacteria. After the MIC was read, 0.025-ml volumes were drawn with an Eppendorf pipette from the wells showing no growth and were spread onto suitable agar plates (over at least a quarter of the surface to avoid drug carryover effects). These plates were incubated (aerobically or anaerobically, depending on the organisms) at 35°C for 24 to 48 h. The MBC was read as the lowest concentration of antibiotic which resulted in ≤0.1% survival in the subculture.

RESULTS

MIC tests. A comprehensive comparison of the activities of MDL 62208, MDL 62211, MDL 62873, teicoplanin, and vancomycin is shown in Table 1 (staphylococci), Table 2 (streptococci, enterococci, listeriae, and group JK corynebacteria), and Table 3 (anaerobic gram-positive bacteria). The results obtained with those isolates which were found to be resistant or intermediate to at least one of the five glycopeptide antibiotics examined are detailed in Table 4.

The three semisynthetic derivatives of teicoplanin were highly active against both methicillin-susceptible and methicillin-resistant staphylococci. This was also true for those coagulase-negative isolates (15 of *S. haemolyticus* and 2 of *S. epidermidis*, all methicillin resistant except one methicillin-susceptible isolate in each species) which proved to be resistant to teicoplanin. The MICs of MDL 62208 did not usually exceed 0.5 μg/ml, a value of 1 μg/ml being recorded

TABLE 1. Comparative activities of MDL 62208, MDL 62211, MDL 62873, teicoplanin, and vancomycin against 428 staphylococci

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (105)	MDL 62208	<0.06–0.5	0.125	0.25
	MDL 62211	0.06–1	0.125	0.25
	MDL 62873	0.06–0.25	0.125	0.25
	Teicoplanin	0.125–2	0.25	1
	Vancomycin	0.5–2	0.5	1
Staphylococcus aureus, methicillin resistant (96)	MDL 62208	0.06–0.5	0.125	0.25
	MDL 62211	0.06–1	0.25	0.5
	MDL 62873	0.06–1	0.125	0.25
	Teicoplanin	0.125–8	1	2
	Vancomycin	0.25–4	1	2
Staphylococcus epidermidis, methicillin susceptible (55)	MDL 62208	0.06–0.5	0.125	0.25
	MDL 62211	0.06–1	0.25	0.5
	MDL 62873	0.06–1	0.25	0.5
	Teicoplanin	0.125–8	1	2
	Vancomycin	0.25–4	1	2
Staphylococcus epidermidis, methicillin resistant (58)	MDL 62208	0.06–0.5	0.125	0.25
	MDL 62211	0.125–0.5	0.125	0.25
	MDL 62873	0.06–2	0.25	0.5
	Teicoplanin	0.125–32	4	8
	Vancomycin	0.25–4	1	2
Staphylococcus haemolyticus, methicillin susceptible (10)	MDL 62208	0.06–0.25	0.125	0.25
	MDL 62211	0.125–2	0.25	1
	MDL 62873	0.125–2	0.25	1
	Teicoplanin	0.25–32	1	4
	Vancomycin	0.5–2	1	2
Staphylococcus haemolyticus, methicillin resistant (29)	MDL 62208	0.06–1	0.125	0.25
	MDL 62211	0.125–4	0.25	2
	MDL 62873	0.125–4	0.5	2
	Teicoplanin	0.5–128	8	32
	Vancomycin	0.5–8	1	2
Staphylococcus simulans[b] (20)	MDL 62208	0.06–0.125	0.06	0.125
	MDL 62211	0.06–0.25	0.125	0.25
	MDL 62873	0.125–0.5	0.125	0.25
	Teicoplanin	0.125–4	0.5	1
	Vancomycin	0.25–2	1	1
Staphylococcus saprophyticus[c] (18)	MDL 62208	<0.06–0.5	0.06	0.125
	MDL 62211	0.06–0.5	0.125	0.25
	MDL 62873	0.06–0.5	0.125	0.25
	Teicoplanin	0.125–2	0.5	1
	Vancomycin	0.5–2	1	1
Staphylococcus hominis[d] (14)	MDL 62208	0.06–0.25	0.125	0.25
	MDL 62211	0.06–1	0.125	0.25
	MDL 62873	0.06–0.5	0.125	0.25
	Teicoplanin	0.125–4	0.25	2
	Vancomycin	0.25–2	0.5	1
Other *Staphylococcus* spp.[e] (23)	MDL 62208	<0.06–0.5	0.06	0.25
	MDL 62211	0.06–1	0.125	0.25
	MDL 62873	0.06–0.5	0.125	0.25
	Teicoplanin	0.06–2	0.25	2
	Vancomycin	0.125–1	0.5	1
Total methicillin-susceptible coagulase-negative staphylococci (102)	MDL 62208	<0.06–0.5	0.125	0.25
	MDL 62211	0.06–2	0.25	0.5
	MDL 62873	0.06–2	0.25	0.5
	Teicoplanin	0.06–32	0.5	2
	Vancomycin	0.125–4	1	1
Total methicillin-resistant coagulase-negative staphylococci (125)	MDL 62208	0.06–1	0.125	0.25
	MDL 62211	0.06–4	0.125	0.25
	MDL 62873	0.125–4	0.125	0.5
	Teicoplanin	0.125–128	2	8
	Vancomycin	0.125–4	1	2

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively.
[b] Including 5 methicillin-susceptible and 15 methicillin-resistant strains.
[c] Including 12 methicillin-susceptible and 6 methicillin-resistant strains.
[d] Including six methicillin-susceptible and eight methicillin-resistant strains.
[e] Including 14 methicillin-susceptible strains (5 of *S. capitis*, 3 of *S. xylosus*, 2 of *S. warneri*, and 1 each of *S. auricularis*, *S. cohnii*, *S. lugdunensis*, and *S. schleiferi*) and 9 methicillin-resistant strains (4 of *S. warneri*, 2 of *S. capitis*, 2 of *S. cohnii*, and 1 of *S. xylosus*).

TABLE 2. Comparative activities of MDL 62208, MDL 62211, MDL 62873, teicoplanin, and vancomycin against 41 streptococci, 77 enterococci, 43 strains of *L. monocytogenes*, and 10 JK coryneform bacteria

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Streptococcus pneumoniae (15)	MDL 62208	0.06–0.125	0.125	0.125
	MDL 62211	0.06–0.125	0.06	0.125
	MDL 62873	0.06–0.125	0.06	0.125
	Teicoplanin	<0.06–0.06	0.06	0.06
	Vancomycin	0.25–0.5	0.25	0.5
Streptococcus pyogenes (14)	MDL 62208	<0.06–0.125	0.06	0.06
	MDL 62211	<0.06–0.25	0.06	0.125
	MDL 62873	<0.06–0.125	<0.06	0.06
	Teicoplanin	<0.06–0.25	<0.06	0.06
	Vancomycin	0.25–0.5	0.25	0.5
Other *Streptococcus* spp.[b] (12)	MDL 62208	0.06–0.25	0.06	0.125
	MDL 62211	<0.06–0.25	0.06	0.125
	MDL 62873	<0.06–0.125	0.06	0.125
	Teicoplanin	<0.06–0.25	0.06	0.25
	Vancomycin	0.25–2	0.5	2
Total streptococci (41)	MDL 62208	<0.06–0.25	0.06	0.125
	MDL 62211	<0.06–0.25	0.06	0.125
	MDL 62873	<0.06–0.125	0.06	0.125
	Teicoplanin	<0.06–0.25	0.06	0.125
	Vancomycin	0.25–2	0.25	0.5
Enterococcus faecalis (61)	MDL 62208	0.06–0.25	0.06	0.25
	MDL 62211	<0.06–0.25	0.06	0.125
	MDL 62873	<0.06–0.25	0.06	0.25
	Teicoplanin	0.06–2	0.125	0.25
	Vancomycin	0.25–4	1	2
Enterococcus faecium (12)	MDL 62208	<0.06–0.25	0.06	0.125
	MDL 62211	<0.06–0.25	0.06	0.06
	MDL 62873	<0.06–0.25	0.06	0.06
	Teicoplanin	<0.06–0.25	0.125	0.25
	Vancomycin	0.5–4	2	2
Enterococcus durans (4)	MDL 62208	<0.06–0.06		
	MDL 62211	All <0.06		
	MDL 62873	All <0.06		
	Teicoplanin	<0.06–0.06		
	Vancomycin	0.5–1		
Total enterococci (77)	MDL 62208	<0.06–0.25	0.06	0.25
	MDL 62211	<0.06–0.25	0.06	0.125
	MDL 62873	<0.06–0.25	0.06	0.125
	Teicoplanin	<0.06–2	0.125	0.25
	Vancomycin	0.25–4	1	2
Listeria monocytogenes (43)	MDL 62208	0.06–0.125	0.06	0.125
	MDL 62211	0.06–0.125	0.06	0.125
	MDL 62873	0.06–0.125	0.06	0.125
	Teicoplanin	0.06–0.5	0.125	0.25
	Vancomycin	0.06–2	0.25	1
JK coryneform bacteria (10)	MDL 62208	<0.06–0.06	0.06	0.06
	MDL 62211	0.06–0.25	0.06	0.25
	MDL 62873	0.06–0.125	0.06	0.125
	Teicoplanin	0.125–0.25	0.125	0.25
	Vancomycin	0.25–0.5	0.5	0.5

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively.
[b] Including five strains of *S. agalactiae*, three of *S. sanguis*, two of *S. bovis*, and two of *S. mutans*.

only for a methicillin-resistant strain of *S. haemolyticus* for which the teicoplanin MIC was 128 μg/ml. The MICs of MDL 62211 and MDL 62873 did not usually exceed 2 μg/ml, values of 4 μg/ml being recorded only for another methicillin-resistant *S. haemolyticus* isolate for which the teicoplanin MIC was 32 μg/ml and the vancomycin MIC was 8 μg/ml. The MICs of the teicoplanin derivatives were consistently lower (mostly 2- to 16-fold) than those of teicoplanin or vancomycin. However, even greater differences from the parent drug could be encountered with those strains of *S. haemolyticus* or *S. epidermidis* which proved to be intermediate (MIC, 16 μg/ml) or resistant (MIC, ≥32 μg/ml) to teicoplanin (Table 4).

For streptococci, the MICs of the teicoplanin derivatives were generally lower than for staphylococci and mostly overlapped with the MICs of teicoplanin. Vancomycin MICs were 2- to 16-fold higher.

The MICs of the three teicoplanin derivatives for the 77 randomly collected enterococci never exceeded 0.25 μg/ml and were generally identical to or 2-fold lower (but occasionally up to 16-fold lower) than the MICs of teicoplanin. Vancomycin MICs were 4- to 32-fold higher. Since we did not find any

TABLE 3. Comparative activities of MDL 62208, MDL 62211, MDL 62873, teicoplanin, and vancomycin against 67 strains of anaerobic gram-positive bacteria

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Clostridium difficile (48)	MDL 62208	0.125–0.5	0.125	0.5
	MDL 62211	0.125–0.25	0.25	0.25
	MDL 62873	0.06–0.125	0.125	0.125
	Teicoplanin	0.06–0.25	0.125	0.125
	Vancomycin	0.5–1	0.5	1
Other *Clostridium* spp.[b] (7)	MDL 62208	0.125–1		
	MDL 62211	0.125–0.5		
	MDL 62873	<0.06–0.25		
	Teicoplanin	<0.06–0.25		
	Vancomycin	0.5–16		
Other anaerobic gram-positive bacteria[c] (12)	MDL 62208	0.125–2	0.25	1
	MDL 62211	0.125–2	0.25	1
	MDL 62873	0.06–2	0.25	1
	Teicoplanin	0.06–2	0.125	1
	Vancomycin	0.25–4	1	4
Total anaerobic gram-positive bacteria (67)	MDL 62208	0.125–2	0.125	0.5
	MDL 62211	0.125–2	0.25	0.5
	MDL 62873	<0.06–2	0.125	0.5
	Teicoplanin	<0.06–2	0.125	0.5
	Vancomycin	0.25–16	0.5	2

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively.
[b] Including four strains of *C. perfringens*, two of *C. septicum*, and one of *C. novyi*.
[c] Including four strains of *Propionibacterium acnes*, two of *Peptostreptococcus anaerobius*, two of *P. indolicus*, one of *P. magnus*, one of *P. micros*, and two of *Eubacterium lentum*.

glycopeptide-resistant enterococcus similar to those recently described as an emerging clinical problem (6, 35), we expressly procured five strains already known to be vancomycin and teicoplanin resistant in order to see their responses to teicoplanin derivatives. All such strains proved to be highly resistant to all three teicoplanin analogs. In particular, the MICs of MDL 62208 were identical to or twofold higher than those of teicoplanin, whereas the MICs of MDL 62211 and MDL 62873 (consistently >128 μg/ml) were more similar to those of vancomycin (Table 4).

The teicoplanin derivatives were very active against the 43 test strains of *L. monocytogenes*, with MICs distributed

TABLE 4. Susceptibility data obtained with those isolates found to be resistant or intermediate to at least one of the five glycopeptide antibiotics examined

Strain[a]	MIC/MBC (μg/ml)				
	MDL 62208	MDL 62211	MDL 62873	Teicoplanin	Vancomycin
S. epidermidis MR 402	0.125/0.125	0.5/4	2/2	32/64	4/8
S. epidermidis MS 448	0.25/0.5	1/4	1/8	16/16	1/2
S. haemolyticus MR 601	0.25/1	1/4	2/4	32/128	2/4
S. haemolyticus MR 602	1/1	2/4	2/8	128/>128	2/4
S. haemolyticus MR 603	0.125/1	1/2	1/4	64/128	2/2
S. haemolyticus MS 609	0.25/2	0.25/2	0.25/1	32/64	2/4
S. haemolyticus MR 611	0.125/0.5	0.25/1	0.5/2	32/128	1/2
S. haemolyticus MR 615	0.125/1	0.25/1	0.25/1	16/64	1/4
S. haemolyticus MR 616	0.125/1	1/2	1/1	16/16	1/8
S. haemolyticus MR 620	0.125/1	1/4	1/4	16/128	4/8
S. haemolyticus MR 631	0.25/2	2/4	0.5/4	32/64	2/4
S. haemolyticus MR 676	0.125/1	1/2	2/4	16/32	2/2
S. haemolyticus MR 704	0.125/1	0.5/1	1/2	16/32	1/4
S. haemolyticus MR 801	0.125/2	1/2	1/2	32/64	1/4
S. haemolyticus MR 806	0.125/1	1/4	2/4	32/64	2/4
S. haemolyticus MR 908	0.125/1	0.25/1	0.5/2	16/32	1/2
S. haemolyticus MR 955	0.5/4	4/8	4/16	32/128	8/8
E. faecalis NCTC 12201	128/>128	>128/>/128	>128/>128	64/128	>128/>128
E. faecalis NCTC 12202	64/>128	>128/>128	>128/>128	32/64	128/>128
E. faecium NCTC 12203	64/>128	>128/>128	>128/>128	64/128	>128/>128
E. faecium NCTC 12204	64/>128	>128/>128	>128/>128	64/64	>128/>128
E. faecium ct 188	128/>128	>128/>128	>128/>128	64/>128	>128/>128
C. novyi ge A1	1/2	0.125/1	0.25/1	0.25/0.5	16/32

[a] With staphylococci, the strains designated MS were methicillin susceptible and those designated MR were methicillin resistant.

TABLE 5. Comparative distribution of MBC-to-MIC ratios for MDL 62208, MDL 62211, MDL 62873, teicoplanin, and vancomycin

Organism (no. tested)	Antimicrobial agent	% of strains with indicated MBC-to-MIC ratio					
		1	2	4	8	16	>16
Staphylococcus spp. (428)	MDL 62208	34	28	32	5	<1	<1
	MDL 62211	29	35	29	6	<1	
	MDL 62873	37	32	26	4	1	
	Teicoplanin	38	49	12	1		
	Vancomycin	53	36	11			
Streptococcus spp. (41)	MDL 62208	59	20	10	12		
	MDL 62211	63	32	2	2		
	MDL 62873	61	34	2	2		
	Teicoplanin	54	29	15	2		
	Vancomycin	54	39		7		
Enterococcus spp. (77)	MDL 62208			3	3	27	68
	MDL 62211			4	9	16	71
	MDL 62873			9	8	30	53
	Teicoplanin		3	5	30	19	43
	Vancomycin		1	19	5	26	48
Listeria monocytogenes (43)	MDL 62208	7	2	9	14	14	53
	MDL 62211	12	5	9	7	23	44
	MDL 62873	7	21	5	9	21	37
	Teicoplanin		30	5	12	14	40
	Vancomycin	5	26	9	9	26	26
JK coryneform bacteria (10)	MDL 62208		20	80			
	MDL 62211		30	60	10		
	MDL 62873		30	70			
	Teicoplanin		60	30	10		
	Vancomycin	20	60	20			
Anaerobic gram-positive bacteria (67)	MDL 62208	3	43	39	12	1	1
	MDL 62211	13	34	48	4		
	MDL 62873	4	49	39	6		1
	Teicoplanin	12	55	25	7		
	Vancomycin	15	43	37	3	1	

over a narrow range (0.06 to 0.125 μg/ml). The MIC for 90% of the strains was twice as high with teicoplanin as with the teicoplanin derivatives and 8-fold higher with vancomycin.

All 10 test strains of JK coryneform bacteria were highly susceptible to the five glycopeptides, with MICs not exceeding 0.06 μg/ml for MDL 62208, 0.125 μg/ml for MDL 62873, 0.25 μg/ml for MDL 62211 and teicoplanin, and 0.5 μg/ml for vancomycin.

The 48 strains of *C. difficile* were inhibited by concentrations not exceeding 0.125 μg/ml for MDL 62873, 0.25 μg/ml for MDL 62211 and teicoplanin, 0.5 μg/ml for MDL 62208, and 1 μg/ml for vancomycin. The teicoplanin derivatives were similarly active against the lower numbers of test strains of other clostridial species (which included a *C. novyi* isolate for which the vancomycin MIC was 16 μg/ml) and of *Propionibacterium acnes*. For the test strains of *E. lentum* and *Peptostreptococcus* spp., MICs did not exceed 2 μg/ml for teicoplanin and its amide derivatives and 4 μg/ml for vancomycin.

MBC tests. MBCs exceeded MICs to a variable extent, depending on the particular glycopeptide but especially on the organisms (Table 5). Overall, the MBC-to-MIC ratios yielded by the teicoplanin derivatives were slightly greater than those yielded by teicoplanin or vancomycin. With all antimicrobial agents, the highest MBC-to-MIC ratios were observed for enterococci, with values of ≥16 recorded for most isolates, and for *L. monocytogenes*, with values distributed over a wide range but being ≥16 for over half of the strains. Ratios were mostly in the range 1 to 2 for streptococci, and 2 to 4 for JK coryneform bacteria and anaerobic bacteria. For the majority of *Staphylococcus* isolates, the MBC-to-MIC ratios fell in the range 1 to 4 with the teicoplanin amides and 1 to 2 with teicoplanin and vancomycin, with no significant differences between either *S. aureus* and coagulase-negative staphylococci or methicillin-susceptible and methicillin-resistant isolates.

DISCUSSION

Glycopeptide antibiotics are active against a wide range of gram-positive bacteria, including those genera which are most commonly involved in human infections. Other gram-positive genera such as *Leuconostoc*, *Pediococcus*, *Lactobacillus*, and *Erysipelothrix*, all rare or uncertain human pathogens, seem inherently resistant to glycopeptides (16). Among those organisms within the spectrum of activity, there has been no trend towards vancomycin resistance during 30 years of clinical experience with this antibiotic (5), and a similar uniform susceptibility was apparently also the rule with teicoplanin (25, 27, 37). In order to explain this unique nonemergence of in vitro resistances, it was even hypothesized that mutations which would lead to a change in the D-alanyl-D-alanine target and so reduce glycopeptide binding would also affect the synthesis of a rigid peptidoglycan and hence be lethal for the cell (29).

During the last few years, however, strains resistant to vancomycin or teicoplanin or both have emerged among staphylococci and enterococci. Within staphylococci, resistant strains, usually more resistant to teicoplanin than to vancomycin, are largely confined to *S. haemolyticus* (1, 7, 33, 39, 42), a coagulase-negative species generally regarded as relatively uncommon in clinical practice but recently

reported as a potentially important nosocomial pathogen with a tendency to develop multiple resistances (10). Moreover, *S. haemolyticus* strains exposed to vancomycin or teicoplanin in vitro proved particularly prone to yielding surviving clones and high-level resistances, especially to teicoplanin (3, 32, 40). In recent French studies (8, 13), resistant strains have also been reported among *S. epidermidis* clinical isolates, and this finding is confirmed by the present study. Within enterococci, after initial reports of resistances (19, 21, 36), two major phenotypes of resistant strains have been described: one is characterized by inducible, high-level resistance to both vancomycin and teicoplanin, and one is characterized by lower-level resistance to vancomycin and susceptibility to teicoplanin (6, 35).

MDL 62208, MDL 62211, and MDL 62873 were highly active in vitro against staphylococci of all species, whether methicillin susceptible or resistant. In particular, the three amide derivatives of teicoplanin were consistently active against those coagulase-negative isolates, belonging to *S. haemolyticus* or to *S. epidermidis*, found to be resistant to the parent drug (one such *S. haemolyticus* strain was also intermediate to vancomycin). These findings are in keeping with other reports (2, 15, 17) and with a recent experimental study in which we have shown that not only did all the test strains of all major *Staphylococcus* species (including *S. haemolyticus* and *S. epidermidis*) fail to yield any resistant survivors when exposed to MDL 62208 or MDL 62211, but also all clones selected through exposure to vancomycin or teicoplanin remained highly susceptible to the two semisynthetic glycopeptides (3). The antistaphylococcal MICs and MBCs of the experimental glycopeptides were both generally lower than the respective values of teicoplanin or vancomycin; with many strains, however, the MBC-to-MIC ratios obtained for the teicoplanin amides were slightly greater than those for teicoplanin or vancomycin.

With respect to the values obtained with staphylococci, the MICs of teicoplanin analogs for streptococci, listeriae, JK coryneform bacteria, and anaerobic gram-positive bacteria were usually lower in absolute value and relatively closer to the MICs of teicoplanin. In particular, unlike Jones et al., who reported the occurrence of MDL 62873 resistance in two of three strains of *Peptostreptococcus* spp. (17), we did not find any glycopeptide-resistant strain among six *Peptostreptococcus* isolates of four different species. The results given by the teicoplanin derivatives against *C. difficile* strains substantially agree with previously reported data obtained using MDL 62208 and MDL 62211 (4) or MDL 62873 (2). As for the MBC tests, the results obtained with anaerobic bacteria should be considered with caution, since there is no reliable procedure to determine the bactericidal activity of such organisms and it is even questionable whether the bactericidal testing of anaerobes is justified (23). The marked differences between MBCs and MICs observed for *L. monocytogenes* isolates with all glycopeptides are in agreement with the results previously reported with vancomycin and teicoplanin (25).

Although having expectedly high MBC-to-MIC ratios (as for teicoplanin and vancomycin or even greater), the experimental glycopeptides were very active against all randomly collected enterococci, with MICs similar to or slightly lower than those of teicoplanin and considerably lower than those of vancomycin. However, the three teicoplanin derivatives were substantially devoid of activity against the five selected strains already known to be highly resistant to both vancomycin and teicoplanin, with MICs even higher than those of the parent drug. These were the only resistances to the teicoplanin derivatives we observed in this study. It is worth noting that when a few enterococci highly resistant to vancomycin and teicoplanin were tested for susceptibility to a number of experimental derivatives of vancomycin, they were reported to be cross-resistant to some such compounds but susceptible to others (26). In the present study, we did not have the opportunity to test any strain of another enterococcal phenotype which is reported to be resistant to lower levels of vancomycin and to remain susceptible to teicoplanin (6, 35). However, for an enterococcus of this phenotype (vancomycin MIC, 32 μg/ml; teicoplanin MIC, 0.5 μg/ml), Shlaes et al. (34) reported a higher MIC for MDL 62208 (4 μg/ml) and a lower one for MDL 62211 (0.13 μg/ml) than for the parent drug.

Even though the two currently available glycopeptides (vancomycin and teicoplanin) remain excellent antibacterial drugs, the development of new antibiotics of the same family is desirable because of the emerging, albeit rare, resistances in some coagulase-negative *Staphylococcus* species and in enterococci. Our present findings on three experimental amide derivatives of teicoplanin are encouraging. In vitro, these semisynthetic glycopeptides are generally more potent than vancomycin and teicoplanin against a wide range of gram-positive organisms and are highly active against those coagulase-negative staphylococci which respond poorly to the parent drug. Such greater potency might reflect the enhanced ability of teicoplanin amides to penetrate through the bacterial cell wall, an ability which has been reported to be likely related to the combined effects of their moderate basicity and their slightly increased lipophilicity compared with those of the parent drug (22). In anticipation of clinical trials, further investigations to elucidate the pharmacological and toxicological properties of these teicoplanin derivatives are indicated.

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