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MICs of Mutacin B-Ny266, Nisin A, Vancomycin, and Oxacillin against Bacterial Pathogens

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Peptide antibiotics, particularly lantibiotics, are good candidates for replacing antibiotics to which bacteria have become resistant. In order to compare two such lantibiotics with two antibiotics, the MICs of nisin A, mutacin B-Ny266, vancomycin, and oxacillin against various bacterial pathogens were determined. The results indicate that nisin A and mutacin B-Ny266 are as active as vancomycin and oxacillin against most of the strains tested. Furthermore, mutacin B-Ny266 remains active against strains that are resistant to nisin A, oxacillin, or vancomycin. The wide spectrum of activity of mutacin B-Ny266, its low MICs against bacterial pathogens, and its activity against bacteria resistant to other inhibitors support the development of this substance for therapeutic use.

The discovery of antimicrobial agents has created a new era of medicine (1). The control of infectious diseases is now based on the choice and careful use of a large group of low-molecular-weight inhibitors with diverse mechanisms of action and various spectra of antibacterial and antifungal activities (1). On the other hand, microorganisms have developed a variety of defense mechanisms against antibiotics (1, 21, 27, 35). In past years, hundreds of tons of antimicrobial agents have been released on bacterial populations. However, bacteria not only have survived but even have flourished in such a hostile environment (1). Antibacterial drug resistance has been reported for most of the predominant pathogenic bacteria (2, 5, 21, 27) and, more recently, this problem was shown to be on the rise worldwide (10). New antimicrobial substances will thus have to be developed in order to treat bacterial infections. New and more efficient antibiotics will have to be sought continually because of the capacity of microorganisms to survive their action. Many different strategies for finding new antimicrobial agents are actually proposed (13, 35, 38), and the area of antibacterial peptides is under intense investigation (13, 38). Among the most promising antibacterial peptides are bacteriocins (15), such as lantibiotics (17, 33).

Bacteriocins are defined as proteinaceous bactericidal substances produced by bacteria (15). Some of these antimicrobial substances were found to contain unusual amino acids, lanthionines, and were thus classified as lantibiotics (14, 17, 16, 30, 33). These lantibiotics were further subdivided into two types (A and B) on the basis of their different ring structures and primary sequence similarities in both the propeptide and, depending on available information, the leader peptide segments (17, 33). As lantibiotics are small peptides, it is possible to chemically or genetically modify their structures in order to increase or diversify their properties (33).

Bacteriocins produced by *Streptococcus mutans* are termed mutacins (12). The mutacins produced by some strains were shown to be small peptides active against many gram-positive bacteria (25, 26, 30, 32). Twenty-four different groups of mutacins were defined according to their activity spectra and their resistance to the other mutacinogenic strains (25). So far, three mutacins (J-T8, B-Ny266, and B-JH1140) have been found to be lantibiotics (14, 26, 30). Mutacin B-Ny266 was purified and characterized and found to be active against more than 98% of the gram-positive bacteria tested (25, 32). This substance was identified as a type A lantibiotic having an amino acid sequence very similar to those of epidermin, gallidermin, and mutacin B-JH1140 (14, 26). Mutacin B-Ny266 inhibits many pathogens, such as actinobacilli, bacilli, clostridia, corynebacteria, enterococci, listeriae, mycobacteria, neisseriae, staphylococci, and streptococci (25, 32).

The purpose of this study was to compare the efficiency of mutacin B-Ny266 with those of nisin A, vancomycin, and oxacillin against various bacterial pathogens, including strains that are antibiotic resistant.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The bacterial strains designated with ATCC numbers were obtained from the American Type Culture Collection (ATCC), Manassas, Va. Strains obtained from the Centre Hospitalier de l'Université Laval (Laboratoire d'Infectiologie) were as follows: *Enterococcus faecalis* EF-Chul; *Neisseria gonorrhoeae* 007, 013x, 016, 017, 022, 071940, 141, 167, 265, 31540, INF2, and INF4; *Staphylococcus aureus* R621, R629, R630, R650, R678, R694, and R695; *Streptococcus pneumoniae* ULM; and *Streptococcus pyogenes* ULM. Strains obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) were as follows: *Staphylococcus epidermidis* DSM 3095 and *Staphylococcus gallinarum* DSM 4616. Strains obtained from the Food Research and Development Center (Agriculture and Agrifood Canada, St. Hyacinthe, Québec, Canada) were as follows: *Listeria monocytogenes* FRDC 1089, FRDC 88171, FRDC 8853, FRDC 8856, FRDC Lm 04, and FRDC Lm 21. The strain obtained from Faculté de Médecine Vétérinaire, Université de Montréal (St. Hyacinthe, Québec, Canada) was as follows: *Streptococcus suis* serotype 2. Strains obtained from Hôpital Laval (Québec, Québec, Canada) were as follows: *E. faecalis* HL 900 and HL 1148; *S. epidermidis* HL 1656 and HL 3176; and *Staphylococcus haemolyticus* HL 2344. Strains obtained from the Health Protection Branch (Health Canada, Ottawa, Ontario, Canada) were as follows: *Listeria grayi* HPB 29, *Listeria innocua* HPB 13, *Listeria ivanovii* HPB 28, *L. monocytogenes* Scott A HPB 3, *Listeria murrayi* HPB 30, *Listeria seeligeri* HPB 62, and *Listeria welshimeri* HPB 89. Strains obtained from the Laboratoire de Santé Publique du Québec (Ste. Anne de Bellevue, Québec, Canada) were as follows: *Bordetella bronchiseptica* LSPQ 2021; *Corynebacterium diphtheriae* LSPQ 3076; and streptococcus group C LSPQ 3377, group F LSPQ

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3374, and group G LSPQ 3375. The strain obtained from the National Collection of Type Cultures (Central Public Health Laboratory, London, United Kingdom) was as follows: *Helicobacter pylori* NCTC 11638. A. Delisle provided *S. mutans* BHT (7), S. Fujimura (Department of Oral Microbiology, Matsumoto Dental College, Shiojiri City, Nagano Prefecture, Japan) provided *Propionibacterium acnes* EXC-1, D. Grenier (Dental School, Université Laval) provided *P. acnes* UD, J. F. Hillman provided *S. mutans* JH1140 (14), T. Kurita provided *S. mutans* 32K (20), J. Lapointe (Department of Biochemistry, Université Laval) provided *Bacillus subtilis* 168T, and J. S. Van der Hoeven (Department of Preventive Dentistry, University of Nijmegen, Nijmegen, The Netherlands) provided *Actinomyces viscosus* Ny1 and *S. mutans* Ny266 (ATCC 202022). The other strains were our own isolates from different studies (11, 24, 37): *Clostridium bifermentans* 2D1.04; *E. faecalis* 2D4.22, 2L5.07, 78.4, D2.20, L2.4, M2.01, and S1.17; *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* UL719; *Pediococcus acidilactici* UL5, R1, R1M, and T5; *S. aureus* D2.5 and M3.18; *Staphylococcus saprophyticus* BALB/c; *Staphylococcus xylosus* BALB/c; and *Streptococcus agalactiae* BALB/c.

Precultures of the genera *Actinomyces*, *Bordetella*, *Campylobacter*, *Corynebacterium*, and *Gardnerella* were prepared from frozen vials with brain heart infusion (BHI; Difco, Detroit, Mich.) and then incubated at 37°C. Strains of *Actinomyces* and *Bordetella* were grown under anaerobic conditions (80% N_2, 10% H_2, 10% CO_2) in a model 12467 glove box (Coy Laboratory Products Inc., Ann Arbor, Mich.), strains of *Campylobacter* and *Gardnerella* were grown in a CO_2 (5%) incubator (Napco Controlled Environment 6100; National Appliance, Portland, Oreg.), and the strain of *Corynebacterium* was grown under aerobic conditions in a standard incubator. The strains of *Haemophilus* and *Neisseria* were grown and tested in a CO_2 (5%) incubator at 37°C with BHI to which 1% supplement B (Difco) had been added. For strains of *Helicobacter*, 5% fetal bovine serum was added to BHI. *P. acidilactici* was grown and tested in MRS broth (BDH, Darmstadt, West Germany), and the remaining strains were grown and tested in tryptic soy broth (Difco) enriched with 0.3% yeast extract (Difco). The strains of *Bacillus*, *Enterococcus*, *Lactococcus*, *Listeria*, *Mycobacterium*, *Pediococcus*, and *Staphylococcus* were grown and tested in a standing incubator; those of *Bordetella*, *Clostridium*, *Peptostreptococcus*, *Propionibacterium*, and *Streptococcus* were grown and tested in an anaerobic chamber; and the strains of *Micrococcus* were grown and tested under aerobic conditions with low agitation. All strains were grown and tested at 37°C, except for *Bacillus stearothermophilus* (55°C) and *Mycobacterium smegmatis* (25°C).

Antimicrobial agents. Mutacin B-Ny266 was purified from the cell pellet of *S. mutans* Ny266 by ethanol extraction at pH 2.0 followed by reversed-phase chromatography on Sep-Pak cartridges and by high-pressure liquid chromatography on a C_{18} column as previously described (26). Pure nisin A (Ambicin N) was kindly provided by Aplin & Barrett Ltd. (Beaminster, Dorset, United Kingdom), and vancomycin hydrochloride and oxacillin sodium salt monohydrate were obtained from Sigma (St. Louis, Mo.). Stock solutions consisted of 0.8 mg of pure mutacin B-Ny266 or 2.1 mg of pure nisin A per ml in sterile Clark-Lubs buffer (0.2 M KCl, 0.2 M HCl) at pH 2.0 (6) and 3.2 mg of oxacillin or 3.0 mg of vancomycin per ml in sterile double-distilled water. The working solutions were dilutions of these stock solutions in sterile double-distilled water. For mutacin B-Ny266, the concentrations were 0.8, 8.0, 16, 32, and 64 μg/ml; for nisin A, they were 2.09, 20.9, 41.8, 83.6, and 167.2 μg/ml; for vancomycin, they were 20.07, 37.6, 75.25, 150.5, and 301 μg/ml; and for oxacillin, they were 21.13, 39.6, 79.25, 158.5, and 317 μg/ml.

Determination of the MICs. The MICs were determined by use of a microplate assay routinely used in our laboratory to test bacteriocin activity (31). The tested bacteria were grown to mid-log phase in appropriate medium and conditions. The optical density (OD) of the cultures was adjusted to 0.1 with appropriate fresh medium by use of a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.) at a wavelength of 625 nm. This OD corresponds approximately to a 0.5 McFarland standard (28). The number of viable cells in the inoculum was not determined for these standard suspensions, and it probably varied depending on the species tested, but it was the same for the four substances tested simultaneously.

Twofold dilutions of the working solutions of mutacin B-Ny266, nisin A, vancomycin, and oxacillin were freshly prepared with microtiter plates (Falcon Microtest III tissue culture plates 3072; Becton Dickinson Laboratories, Franklin Lakes, N.J.), the wells finally containing 100 μl of culture medium with or without (control) an inhibitor. Twenty-five microliters of OD-standardized bacterial suspension was added to each well to a final volume of 125 μl. The plates were incubated under the appropriate conditions according to the strain tested until the control (tested strain grown without an antibacterial substance) attained stationary phase, as judged by stabilization of the OD, thus adjusting for the slower growth of some species. After the incubation period, which varied from 1 to 5 days, the OD was measured at 630 nm with a microplate reader (model MR 5000/7000; Dynatech Laboratories Inc., Chantilly, Va.). The blank was the medium alone incubated under the same conditions. The MIC was calculated from the highest dilution showing complete inhibition of the tested strain (OD equals OD of the blank). The MIC determinations were repeated independently 3 to 12 times, always with *Micrococcus luteus* ATCC 272 as a control. The results are presented as the median and the range for the different repetitions.

Hemolytic activity. The hemolytic activities of mutacin B-Ny266 (16 to 64 μg/ml), nisin A (21 to 84 μg/ml), oxacillin (21 to 317 μg/ml), and vancomycin (20 μg/ml) were tested against defibrinated sheep erythrocytes (Quelab, Montréal, Québec, Canada) suspended in 0.2 M phosphate-buffered saline (PBS) at pH 7.0 in flat-bottom microtiter plates (Falcon). Twofold dilutions of the tested substances were prepared in 0.2 M PBS (pH 7.0) in triplicate wells, to which 25 μl of blood was added. After 15 min at room temperature, the plates were observed for hemolysis and read at 630 nm on a microplate reader (Dynatech). Double-distilled water was used as the hemolytic positive control, and 0.1 M PBS and 0.2 M PBS (pH 7.0) were used as negative controls.

Antibiograms. Antibiograms were determined for strains of neisseriae, enterococci, and staphylococci by use of antimicrobial disk susceptibility test M2-A4 according to the National Committee for Clinical Laboratory Standards (NCCLS) (28). Strains of *Neisseria* were tested on GC agar base (Difco) containing 1% supplement B, and enterococci and staphylococci were tested on Mueller-Hinton agar (Difco) as described by the NCCLS (28). The following antibiotic disks (Sensi-Disc; Becton Dickinson Microbiology Systems, Cockeysville, Md.) were used: ampicillin (AM 10), bacitracin (B 10), cephalothin (CF 30), chloramphenicol (C 30), erythromycin (E 15), gentamicin (GM 10), kanamycin (K 30), lincomycin (L 2), nalidixic acid (NA 30), neomycin (N 30), oxacillin (OX 1), penicillin (P 10), polymyxin B (PB 300), rifampin (RA 5), streptomycin (S 10), sulfisoxazole (G 25), tetracycline (T 5), trimethoprim (TMP 5), and vancomycin (VA 30). The zone diameters were interpreted according to the tables of the NCCLS (28) after 24 h of incubation at 37°C in 5% CO_2 for neisseriae and under aerobic conditions for the other organisms. Only results indicating that a strain was resistant were used to determine the antibiotic resistance patterns; for this purpose, the intermediate strains were considered sensitive.

RESULTS

Neither mutacin B-Ny266 (64 μg/ml), nisin A (84 μg/ml), oxacillin (317 μg/ml), nor vancomycin (20 μg/ml) showed hemolytic activity after 15 min at 25°C against sheep erythrocytes. Mutacin B-Ny266 was generally more active than nisin A, vancomycin, and oxacillin (except for strain ATCC 9341) against all *M. luteus* strains tested (Table 1). *M. luteus* ATCC 272 showed the highest sensitivity toward mutacin B-Ny266 (MIC range, 0.03 to 0.05 μg/ml) and was subsequently selected as an indicator strain for determining mutacin activity with the microtiter assay.

Mutacin B-Ny266 was slightly more active than nisin A and less active than vancomycin and oxacillin against enterococci (Table 1), except against strain 78.4, for which the activity of mutacin B-Ny266 (MIC range, 1.6 to 3.2 μg/ml) was comparable to that of vancomycin (MIC range, 2.0 to 2.0 μg/ml).

Against *Listeria* strains, the activity of mutacin B-Ny266 was comparable to that of vancomycin, and both were generally more effective than oxacillin and nisin A (Table 1).

Against all the tested staphylococci, the MIC of mutacin B-Ny266 was equal to or lower than that of nisin A (Table 1). It was also lower than or equal to that of vancomycin, except for one strain (R695), and it was lower than or equal to that of oxacillin, except for four strains (ATCC 25923, ATCC 6538, D2.5, and ATCC 12228).

The four antimicrobial substances were generally active against the streptococcal strains tested. Nisin A was the least active and oxacillin was the most active of the four substances (Table 1).

Against spore-forming gram-positive bacilli, mutacin B-Ny266 consistently had lower MICs than nisin A (Table 1) and generally had lower MICs than vancomycin, except for strain ATCC 2 of *Bacillus cereus*.

The four antimicrobial substances were active against the gram-positive strict anaerobes tested, except for nisin A against *Actinomyces viscosus* (Table 1). They were also active against *C. diphtheriae* and *Gardnerella vaginalis* but were not very active against the strains of *M. smegmatis*, which were resistant to mutacin B-Ny266 and oxacillin (Table 1).

The tested strains of *H. pylori*, *Campylobacter jejuni*, and *Neisseria* spp. were sensitive to mutacin B-Ny266 and nisin A, with mutacin B-Ny266 being the most active of the four substances (Table 2). The *Haemophilus influenzae* strain tested was resistant to nisin A, vancomycin, and oxacillin and slightly more sensitive to mutacin B-Ny266 (Table 2). The strain of *B.*

TABLE 1. Activities of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against gram-positive bacteria

Organism (no. of strains)	MIC[a] (μg/ml) of:			
	Mutacin B-Ny266	Nisin A	Vancomycin	Oxacillin
Micrococcus spp. (4)	0.05 (0.03–0.08)	1.1 (0.3–16.7)	1.0 (0.5–2.0)	2.1 (0.01–4.2)
Enterococcus spp. (12)	12.8 (1.6–25.6)	16.7 (8.4–33.4)	3.9 (1.9–>120)	11.9 (4.2–63.4)
Listeria spp. (23)	0.8 (0.4–2.0)	4.2 (1.1–16.7)	1.0 (0.3–2.1)	4.2 (0.5–15.9)
Staphylococcus spp. (19)	1.6 (0.1–18.1)	4.2 (1.5–83.6)	3.8 (0.3–15.1)	7.9 (0.1–>127)
Streptococcus spp. (17)	0.4 (0.03–6.4)	8.4 (0.3–83.6)	0.5 (0.3–1.0)	0.1 (0.02–1.1)
Bacillus spp. (5)	0.8 (0.01–6.4)	4.2 (0.01–10.5)	0.5 (0.1–0.5)	0.3 (0.002–>127)
Clostridium spp. (4)	0.2 (0.01–3.2)	1.1 (0.1–4.2)	0.5 (0.3–4.0)	1.1 (0.1–4.2)
Actinomyces viscosus (2)	0.4 (0.4–0.8)	83.6 (41.8–83.6)	0.5 (0.5–1.0)	0.5 (0.5–0.8)
Peptostreptococcus spp. (2)	0.4 (0.2–0.8)	2.1 (1.1–2.1)	0.5 (0.5–1.0)	0.3 (0.1–0.5)
Corynebacterium diphtheriae (1)	0.4 (0.4–0.8)	4.2 (4.2–8.4)	1.0 (0.5–1.0)	2.1 (1.1–2.1)
Gardnerella vaginalis (1)	0.03 (0.01–0.05)	1.1 (1.1–2.1)	0.4 (0.3–0.5)	0.1 (0.07–0.1)
Mycobacterium smegmatis (2)	32 (25–70)	8.4 (7.4–15)	8.0 (8.0–15)	95.1 (63.4–126.8)
Propionibacterium acnes (2)	1.2 (0.8–1.6)	2.1 (1.1–4.2)	1.0 (0.5–1.0)	0.5 (0.5–1.1)

[a] The MIC data are expressed as the median (range) obtained from at least three independent repetitions for each strain.

bronchiseptica was the most resistant gram-negative pathogenic strain tested. Nevertheless, the MIC range of mutacin B-Ny266 against this strain was lower than those of nisin A, vancomycin, and oxacillin (Table 2).

Clinical isolates of *N. gonorrhoeae* which were more resistant to vancomycin and oxacillin remained sensitive to mutacin B-Ny266 and, to a lesser extent, to nisin A (Table 2).

As judged by the antimicrobial disk susceptibility test, most of the clinical and mouse isolates demonstrated resistance to more than one antibiotic (Table 3). Bacitracin inhibited all the staphylococci, streptococci, and *Neisseria* strains tested. Only one strain was resistant to chloramphenicol (*S. haemolyticus* HL 2344), two strains were resistant to gentamicin (*E. faecalis* EF-Chul and HL 900), and three strains were resistant to rifampin (*E. faecalis* 2D4.22 and HL 900 and *S. epidermidis* HL 1656). Cephalothin was very active against neisseriae and staphylococci but slightly less active toward enterococci, most of the strains being moderately sensitive to resistant. Four strains of *Neisseria* spp. and one enterococcal strain were resistant to vancomycin. Oxacillin resistance was observed for 9 strains of *N. gonorrhoeae*, all 10 strains of enterococci, and 8 strains of staphylococci.

Mutacin B-Ny266 was the most active antimicrobial agent against most of the multidrug-resistant strains tested, except for enterococci (Table 3). It was, however, the most active (MIC range, 6.4 to 6.4 μg/ml) against the vancomycin-resistant strain EF-Chul, and it was also active against oxacillin-resistant enterococcal strains (2L5.07, 78.4, EF-Chul, and HL 1148) (Table 3). Furthermore, mutacin B-Ny266 was active against oxacillin-resistant strains of *Neisseria* spp. and staphylococci (Table 3) and *B. cereus* ATCC 2, also oxacillin resistant.

Mutacin B-Ny266 was active against nisin-resistant mutants obtained from *P. acidilactici* and *L. monocytogenes* (Table 4).

In order to verify if the production of another lantibiotic could confer cross-immunity toward mutacin B-Ny266 and nisin A on the producing strain, we tested the four antibiotics against several lantibiotic-producing strains. While many of the tested strains were resistant to nisin A, only *S. epidermidis* DSM 3095, producing epidermin, appeared less sensitive to mutacin B-Ny266 (Table 5).

DISCUSSION

The method used here for MIC determination differs from the standard method recommended by the NCCLS (28). It was adopted to compare the activities of the four substances because it was already in use in our laboratory for bacteriocin titer determinations (31) and the results were not intended for immediate clinical intervention, as is often the case in hospital laboratories. Although the suggested reference strains (28) were not used in our studies, the MICs obtained for oxacillin against *S. aureus* ATCC 6538 and ATCC 25923 varied from 0.1 to 1.1 μg/ml, within twofold of the acceptable quality control range (0.2 to 0.5 μg/ml) recommended by the NCCLS (28). Similarly, with *E. faecalis* ATCC 27275, the MICs of oxacillin varied from 7.9 to 8.4 μg/ml, while the acceptable quality control range is 8 to 32 μg/ml (28). For vancomycin, the MICs were 1.0 to 8.0 μg/ml against *S. aureus* and 2.0 to 4.0 μg/ml against *E. faecalis*, while the corresponding proposed acceptable quality control ranges are 0.5 to 2 and 1 to 4 μg/ml, respectively (28).

In drug discovery, antimicrobial agents are first identified and tested to determine whether or not they have clinically relevant antimicrobial activity, then tested for toxicity, and finally tested for their ability to be delivered to the site of infection. Mutacin B-Ny266 was previously characterized (26)

TABLE 2. Activities of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against gram-negative pathogens

Organism (no. of strains)	MIC[a] (μg/ml) of:			
	Mutacin B-Ny266	Nisin A	Vancomycin	Oxacillin
Bordetella bronchiseptica (1)	49.2 (25.6–80)	>836	>120	>127
Campylobacter jejuni (1)	0.07 (0.01–0.1)	1.1 (0.3–1.5)	1.0 (1.0–2.0)	0.1 (0.05–1.5)
Haemophilus influenzae (1)	13 (13–26)	66.9 (33.4–83.6)	>120	63.4 (44.8–63.4)
Helicobacter pylori (2)	0.07 (0.03–0.4)	0.3 (0.07–2.1)	1.0 (0.7–1.0)	0.5 (0.3–1.1)
Neisseria spp. (13)	1.6 (0.8–6.4)	8.4 (4.2–16.7)	30.1 (7.5–>120)	11.2 (0.2–>127)

[a] The MIC data are expressed as the median (range) obtained from at least three independent repetitions for each strain.

TABLE 3. Activities of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against antibiotic-resistant clinical and mouse isolates

Organism(s)	Antibiotic resistance pattern[a]	MIC[b] (μg/ml) of:			
		Mutacin B-Ny266	Nisin A	Vancomycin	Oxacillin
Neisseria meningitidis ATCC 13077	LIN-TMP-**VAN**	1.6 (1.6–3.2)	6.3 (4.2–8.4)	>120	2.1 (2.1–4.2)
Neisseria gonorrhoeae					
013x	AMP-LIN-**OXA**-PEN-TMP	1.6 (0.8–3.2)	4.2 (4.2–8.4)	30.1 (30.1–42.6)	127 (127–127)
016	AMP-LIN-**OXA**-PEN	3.2 (1.6–3.2)	8.4 (8.4–8.4)	30.2 (15.1–30.2)	>127
017	AMP-LIN-NEO-**OXA**-PEN-POB-TMP	2.3 (1.6–3.2)	8.4 (4.2–8.4)	30.6 (30.1–42.6)	>127
022	LIN-NEO-**OXA**-PEN-POB-SFX-TMP-**VAN**	3.2 (2.2–6.4)	12 (8.4–17)	60 (30–120)	32.7 (22.4–63.4)
071940	LIN-**OXA**-POB	1.6 (1.6–1.6)	4.2 (4.2–4.2)	30.1 (30.1–60.2)	9.8 (8.5–11.2)
141	LIN-NEO	0.8 (0.8–1.6)	5.9 (4.2–8.4)	15.1 (8.0–30.1)	0.6 (0.2–0.8)
167	AMP-LIN-**OXA**-PEN-TMP	1.6 (0.8–1.6)	7.1 (5.9–8.4)	25.7 (15.1–60.2)	95.1 (63.4–127)
265	POB-TMP	1.6 (1.1–2.3)	5.9 (5.9–8.4)	21.3 (15.1–30.1)	13.5 (11.2–15.9)
31540	**OXA**-POB-TMP	3.1 (1.6–3.2)	8.4 (5.9–11.8)	72.7 (30.1–85.1)	54.1 (31.7–63.4)
INF2	LIN-**OXA**-POB-TMP-**VAN**	2.3 (2.3–2.3)	8.4 (8.4–8.4)	60.2 (30.1–60.2)	31.7 (15.9–31.7)
INF4	LIN-NEO-**OXA**-POB-STR-TMP-**VAN**	1.9 (1.1–3.2)	7.1 (4.2–16.7)	30.1 (30.1–30.1)	15.9 (7.9–15.9)
Enterococcus faecalis					
ATCC 27275	LIN-NAL-NEO-**OXA**-POB-STR-SFX-TET	12.8 (12.8–25.6)	14.3 (8.4–16.7)	4.0 (2.0–4.0)	8.4 (7.9–8.4)
2D4.22	LIN-NAL-NEO-**OXA**-POB-RIF-SFX	15.5 (12.3–25.6)	18.8 (16.7–33.4)	2.0 (2.0–2.0)	15.9 (15.8–31.7)
2L5.07	CEF-LIN-NAL-NEO-**OXA**-PEN-POB-STR-SFX	6.4 (6.4–12.8)	8.4 (8.4–16.7)	1.9 (1.9–2.0)	31.7 (15.8–63.4)
78.4 and S1.17	LIN-NAL-NEO-**OXA**-POB-STR-SFX	3.2 (1.6–25.6)	8.4 (8.4–23.6)	2.0 (2.0–2.0)	10.2 (8.4–11.9)
EF-Chul	ERY-GEN-KAN-LIN-NAL-NEO-**OXA**-POB-STR-SFX-TET-TMP-**VAN**	6.4 (6.4–6.4)	8.4 (8.4–16.7)	>120	10.2 (7.9–15.9)
D2.20, L2.4, and M2.01	NAL-NEO-**OXA**-POB-STR-SFX-TET	12.8 (12.8–25.6)	9.4 (8.4–20.9)	4.0 (2.0–4.0)	8.4 (4.2–15.9)
HL 1148	LIN-NAL-**OXA**-POB-STR-SFX-TET	6.4 (6.4–12.8)	16.7 (16.7–33.4)	4.0 (3.8–7.5)	31.7 (22.4–44.8)
HL 900	GEN-KAN-LIN-NAL-NEO-**OXA**-POB-RIF-STR-SFX-TET	12.8 (8.0–25.6)	9.4 (8.4–20.9)	4.0 (4.0–4.0)	11.9 (8.4–15.9)
Enterococcus hirae ATCC 8043	CEF-LIN-NAL-NEO-**OXA**-POB-STR-SFX	12.8 (6.4–16.0)	16.7 (16.7–20.9)	4.0 (4.0–4.0)	8.4 (8.4–7.9)
Staphylococcus aureus					
ATCC 43300	AMP-PEN	3.2 (3.2–6.4)	10.1 (8.4–17)	2.8 (1.9–8.0)	7.9 (4.0–15.9)
M3.18	AMP-PEN	3.2 (1.6–3.2)	8.4 (4.2–8.4)	3.8 (3.8–5.3)	1.0 (0.5–2.0)
R621	AMP-ERY-**OXA**-PEN	3.2 (3.2–3.2)	8.4 (5.9–8.4)	2.3 (1.9–3.8)	23.8 (15.9–31.7)
R629, R630, and R694	AMP-**OXA**-PEN	1.6 (1.1–3.2)	2.5 (2.1–8.4)	2.3 (1.9–3.8)	31.7 (22.4–63.4)
R650	AMP-KAN-NAL-**OXA**-PEN-STR-TET	3.2 (3.2–4.5)	8.4 (4.2–8.4)	3.8 (3.8–3.8)	44.8 (15.9–63.4)
R678	AMP-ERY-KAN-LIN-NAL-**OXA**-PEN	3.2 (3.2–3.2)	8.4 (4.2–8.4)	2.7 (1.9–3.8)	15.9 (15.9–63.4)
R695	AMP-**OXA**-PEN-TMP	3.2 (3.2–3.2)	5.9 (5.9–8.4)	2.7 (1.9–2.7)	31.7 (15.9–63.4)
Staphylococcus epidermidis					
ATCC 12228	AMP-PEN-SFX-TET	0.8 (0.8–1.6)	2.1 (2.1–6.0)	2.0 (2.0–4.0)	0.3 (0.1–0.4)
HL 3176	AMP-ERY-KAN-LIN-**OXA**-PEN	1.6 (0.8–1.6)	3.0 (2.1–4.2)	3.8 (2.0–4.0)	>127
HL 1656	AMP-ERY-KAN-LIN-**OXA**-PEN-RIF-STR-TET	1.6 (1.1–3.2)	3.0 (2.1–4.2)	4.0 (2.0–4.0)	8.4 (7.9–15.9)
Staphylococcus haemolyticus HL 2344	AMP-CHL-ERY-KAN-LIN-NAL-**OXA**-PEN-STR-TET-TMP	0.8 (0.8–0.8)	4.2 (2.1–4.2)	2.8 (1.9–4.0)	>127
Staphylococcus xylosus BALB/c	NAL	0.2 (0.2–0.4)	8.4 (8.4–16.7)	0.3 (0.3–0.5)	0.5 (0.5–0.8)
Staphylococcus saprophyticus BALB/c	NAL	0.2 (0.1–0.4)	2.1 (2.1–2.1)	2.0 (2.0–4.0)	2.1 (2.1–2.1)

[a] Established by the antimicrobial disk susceptibility test. AMP, ampicillin; CEF, cephalothin; CHL, chloramphenicol; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; LIN, lincomycin; NAL, nalidixic acid; NEO, neomycin; OXA, oxacillin; PEN, penicillin; POB, polymyxin B; RIF, rifampin; STR, streptomycin; SFX, sulfisoxazole; TET, tetracycline; TMP, trimethoprim; VAN, vancomycin.

[b] The MIC data are expressed as the median (range) obtained from at least three independent repetitions.

TABLE 4. Activities of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against nisin-resistant mutants

Antimicrobial substance	MIC[a] (μg/ml) for:						
	Listeria monocytogenes Scott A			*Pediococcus acidilactici*			
	HPB 3	Nisin-resistant mutants[b]		UL5	Nisin-resistant mutants[c]		
		ATCC 700301	ATCC 700302		R1	R1M	T5
Mutacin B-Ny266	1.6 (0.8–1.6)	1.0 (0.8–1.6)	1.4 (0.8–1.6)	0.4 (0.2–0.4)	0.8 (0.8–0.8)	3.2 (3.2–3.2)	1.6 (1.6–1.6)
Nisin A	5.9 (4.2–10.5)	16.7 (16.7–16.7)	16.7 (16.7–16.7)	0.03 (0.02–0.07)	6.8 (3.7–8.4)	41.8 (23.6–47.3)	41.8 (41.8–41.8)
Vancomycin	1.4 (1.0–2.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	>120	>120	>120	>120
Oxacillin	7.9 (5.6–8.5)	1.5 (1.5–2.1)	2.6 (2.1–3.0)	7.9 (7.9–15.9)	4.0 (2.8–4.2)	2.8 (2.0–3.0)	2.1 (2.0–4.0)

[a] The MIC data are expressed as the median (range) obtained from at least three independent repetitions.
[b] Nisin-resistant mutants were obtained from Crandall and Montville (3) and Mazzotta et al. (23).
[c] Nisin-resistant mutants were obtained from Goulhen et al. (11).

and was shown here to be active in vitro against many important human bacterial pathogens. Indeed, mutacin B-Ny266 inhibited most of the gram-positive pathogens tested (enterococci, listeriae, staphylococci, streptococci, bacilli, clostridia, actinomyces, corynebacteria, peptostreptococci, and propionibacteria) at low concentrations (<4.0 μg/ml), except for the enterococci, for which the MIC ranged from 1.6 to 25.6 μg/ml.

Vancomycin and oxacillin are often used when other antibiotics have failed (9). However, strains resistant to these antibiotics are now appearing (18, 21). Enterococci have acquired plasmid-mediated resistance to vancomycin (21) and penicillin (27), seriously compromising the efficacy of the antibiotic treatment of enterococcal infections (9). Plasmids conferring resistance to chloramphenicol, macrolides, and tetracyclines have been found in several clinical isolates of *L. monocytogenes* and have raised concern for the future (36). Antibiotic-resistant strains of staphylococci represent a major clinical and epidemiological problem in hospitals, even more since the appearance of methicillin-resistant strains (18). Drug resistance in *Mycobacterium tuberculosis* has become a serious concern (29). The efficiency of treatments of *H. pylori* infections has decreased with the emergence of metronidazole-resistant strains (16). Antimicrobial resistance is now widespread among strains of *N. gonorrhoeae* and *N. meningitidis* (2, 19).

The results presented in this paper indicate that mutacin B-Ny266 is generally as active as vancomycin and oxacillin against most of the important bacterial pathogens tested and is even active against strains that have become resistant to vancomycin or oxacillin or both. Multidrug-resistant pathogens are also sensitive to nisin A, in agreement with the data reported by Severina et al. (34), but they are even more sensitive to mutacin B-Ny266. Mutacin B-Ny266 could thus be considered a candidate for eventual use against infections caused by such antibiotic-resistant pathogens.

Although lantibiotics are not usually active against most gram-negative bacteria (17, 33), nisin A was found to be active against *Neisseria* spp. (22) and *H. pylori* (8). Preliminary tests (32) showed that most gram-negative bacteria were resistant to mutacin B-Ny266, except for *Neisseria subflava* and *Flavobacterium capsulatum*. In this study, the list of gram-negative pathogens that are susceptible to mutacin B-Ny266 was extended. Mutacin B-Ny266 and nisin A remained active against vancomycin- and oxacillin-resistant strains of *Neisseria* spp., making them good potential candidates for use against *Neisseria* infections.

H. pylori has been strongly associated with peptic ulceration and gastric cancer (16). Certain antimicrobial agents are inactivated in the acidic environment of the stomach (16). In contrast, lantibiotics, such as nisin A, are very resistant to an acid pH (8) and are thus good candidates as potential antibacterial agents of choice against *H. pylori*. Indeed, nisin A (Ambicin N) is currently being tested for this application (8). We found here that mutacin B-Ny266 is slightly more active than nisin A, vancomycin, and oxacillin against *H. pylori*. Furthermore, as mutants resistant to nisin A, vancomycin, and oxacillin are still sensitive to mutacin B-Ny266, as shown in this paper, the latter could be a good replacement in case *H. pylori* strains develop resistance to the former antibiotics.

As antibiotic resistance genes may originate from the antibiotic-producing strain, we tested the resistance of known lantibiotic-producing strains for their sensitivity to mutacin B-Ny266 and nisin A. Most of the lantibiotic (nisin A, nisin Z, epidermin, and mutacins B-JH1140 and B-Ny266)-producing strains are resistant to nisin A, although it is not known

TABLE 5. Activities of mutacin B-Ny266, nisin A, vancomycin, and oxacillin against other lantibiotic-producing strains

Producing strain	Lantibiotic produced	MIC[a] (μg/ml) of:			
		Mutacin B-Ny266	Nisin A	Vancomycin	Oxacillin
Bacillus subtilis ATCC 6633	Subtilin	1.6 (1.0–1.6)	8.4 (8.4–10.5)	0.5 (0.3–0.5)	0.5 (0.5–0.5)
Lactococcus lactis subsp. *lactis* ATCC 11454	Nisin A	1.0 (0.8–1.6)	27.2 (20.9–33.4)	0.5 (0.5–1.0)	2.1 (1.1–4.2)
Lactococcus lactis subsp. *lactis* biovar *diacetylactis* UL719	Nisin Z	0.8 (0.8–1.6)	33.4 (33.4–41.8)	1.0 (0.5–1.0)	4.0 (3.0–4.2)
Staphylococcus epidermidis DSM 3095	Epidermin	12.8 (8.7–18.1)	83.6 (66.9–83.6)	8.0 (7.5–15.1)	0.2 (0.1–0.3)
Staphylococcus gallinarum DSM 4616	Gallidermin	1.6 (1.6–1.6)	2.1 (2.1–3.0)	2.0 (2.0–2.0)	0.8 (0.8–1.1)
Streptococcus mutans BHT	Mutacin b	0.8 (0.4–0.8)	16.7 (8.4–16.7)	0.5 (0.5–0.5)	0.2 (0.2–0.4)
Streptococcus mutans 32K	Mutalipocin	0.8 (0.8–1.6)	83.6 (41.8–83.6)	0.8 (0.5–1.0)	0.1 (0.1–0.3)
Streptococcus mutans JH1140	Mutacin B-JH1140	1.6 (0.8–1.6)	41.8 (41.8–83.6)	1.0 (1.0–1.0)	0.05 (0.05–0.1)
Streptococcus mutans Ny266 (ATCC 202022)	Mutacin B-Ny266	4.8 (3.2–6.4)	33.4 (16.7–33.4)	1.0 (1.0–1.0)	0.1 (0.03–0.1)

[a] The MIC data are expressed as the median (range) obtained from at least three independent repetitions.

whether genes coding for immunity are involved. This resistance could eventually spread among bacterial populations and impair the efficiency of nisin as an antibiotic. Although the strain (*S. mutans* Ny266) that produces mutacin B-Ny266 is about 10 times more resistant to its own lantibiotic than the *S. mutans* type strains, it is susceptible to 5 μg of mutacin B-Ny266 per ml, thus slightly alleviating the problem. *S. epidermidis* DSM 3095, which produces epidermin, is about 16- and 40-fold more resistant to mutacin B-Ny266 and nisin A, respectively, than the *S. epidermidis* type strain (ATCC 12228). If the genes responsible for this resistance eventually spread among bacterial pathogens, the efficiencies of both mutacin B-Ny266 and nisin A will be impaired. However, more studies are needed to evaluate the real threat of the dissemination of lantibiotic resistance genes.

The wide spectrum of activity of mutacin B-Ny266, the fact that it is active against antibiotic-resistant strains and nisin-resistant mutants, and the fact that no stable mutants resistant to mutacin B-Ny266 could be obtained (4; H. Morency and M. C. Lavoie, unpublished data) while nisin-resistant mutants are easily obtained (3, 11, 23, 34) are all factors supporting the development of mutacin B-Ny266 as an eventual clinical antibiotic. However, although there are many type A lantibiotics (13, 15, 17, 33), only nisin A is actually being tested for clinical use (8), as far as we know, perhaps because these peptides may be toxic when administered systemically. Mutacins produced by *S. mutans*, which are found in the mouths of nearly 50% of people, do not appear to be hemolytic and may be less toxic. However, further studies are needed to assess the toxicity of mutacin B-Ny266 and then to evaluate its ability to be delivered to the site of infection.

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