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Clinical and Microbiologic Characteristics of Pediococci

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Over a 43-month period, 23 separate isolates of nonenterococcal alpha- and nonhemolytic streptococci were reported by our clinical microbiology laboratory to be resistant to vancomycin. This constituted 0.32% of nonenterococcal alpha- and nonhemolytic streptococci reported and 4.4% of such streptococci upon which susceptibility testing was performed. Of 13 isolates which were available for further study, all were highly resistant to vancomycin (MIC ≥ 1,024 μg/ml), but none were actually streptococci. Three were clearly gram-positive rods by Gram stain and were found to be homofermentative lactobacilli. Two strains with elongated gram-positive cocci from colonies on agar showed small gram-positive rods when grown in thioglycolate broth and were physiologically identified as *Lactobacillus confusus*. Two isolates with lenticular gram-positive cocci appeared to be *Leuconostoc mesenteroides* subsp. *mesenteroides*. Six gram-positive isolates with round cells from growth on agar and from broth were arranged in tetrads in broth and closely resembled *Pediococcus acidilactici*. Twelve additional strains of pediococci that were not of human origin were also found to be highly resistant to vancomycin. These findings confirm published reports of clinical isolation of organisms resembling pediococci and suggest that clinically isolated, vancomycin-resistant bacteria which superficially resemble streptococci are probably other lactic acid bacteria.

Clinical microbiology laboratories often report catalase-negative, gram-positive cocci, the colonies of which produce either no hemolysis or a green hemolytic pattern on blood agar plates, as alpha- or nonhemolytic streptococci or as viridans group streptococci, if tests exclude pneumococci or enterococci. Despite growing evidence to the contrary, the oral streptococci are often thought to be predictably susceptible to most antimicrobial agents. Therefore, even when isolated from normally sterile sites, susceptibility testing may not be done on these organisms.

When performed upon bacteria reported to be alpha- or nonhemolytic streptococci, antimicrobial susceptibility testing occasionally shows resistance to vancomycin. Although recent studies have found that vancomycin resistance in enterococci (15, 29, 33, 35, 49) and coagulase-negative staphylococci (43) can be clinically shown, the true incidence of vancomycin resistance in organisms reliably classified as viridans group streptococci has been suggested to be low, if it occurs at all (15, 27). A vancomycin-resistant *Streptococcus sanguis* II isolate (46) has been recently found to be a *Leuconostoc* species or a heterofermentative *Lactobacillus* species on subsequent testing (David M. Shlaes, personal communication).

Our interest in this issue prompted a retrospective review from our clinical microbiology laboratory of vancomycin-resistant, alpha- and nonhemolytic streptococci which are neither pneumococci nor enterococci. Particular attention was paid to organisms superficially resembling oral streptococci by both colony morphology and broth Gram stain. The clinical setting surrounding isolation of these bacteria was also reviewed. Isolates studied appeared to be members of genera loosely related to *Streptococcus*: *Lactobacillus*, *Leuconostoc*, and *Pediococcus*.

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MATERIALS AND METHODS

All isolates that had been identified as vancomycin-resistant, alpha- or nonhemolytic streptococci which were neither pneumococci nor Lancefield serogroup B or D were sought by manual and computer searching of records of isolates detected from specimens submitted to the clinical microbiology laboratory of the Cleveland Clinic Foundation for the 43-month period from October 1983 through April 1987. Pneumococci, enterococci, and Lancefield group B and D streptococci were identified by a variety of common physiologic procedures during this period, but serogroup confirmation was infrequently performed. This review was facilitated by laboratory policies during much of this period that allowed for frequent susceptibility testing and storage of fastidiously growing isolates. Blood and body fluid isolates were characterized particularly thoroughly, including those from intra-abdominal or abscess material for which anaerobic processing had been ordered. Isolates which did not grow in Mueller-Hinton broth were sent to a separate laboratory, where a variety of media were used to perform microdilution susceptibility testing, after which the isolates were stored. For this search vancomycin resistance was defined as growth in the presence of ≥4 μg of vancomycin per ml. All such vancomycin-resistant isolates that could be recovered were further studied.

The incidence of streptococci that were resistant to vancomycin was determined from computer records. Numbers of vancomycin-resistant alpha- or nonhemolytic streptococci, which were neither pneumococci nor group B or D, were compared with the total number of such organisms reported for a 28-month period between January 1985 and April 1987. A similar search for vancomycin-resistant lactobacilli was also performed, as noted in the Discussion.

Physiologic and biochemical reactions. Various growth and colonial characteristics, as well as biochemical reactions, were examined. Gram staining was performed after 24- to 48-h growth on sheep blood agar and in thioglycolate broth. Spot catalase reactions were performed with colonies grown on blood agar. Further tests for cytochrome oxidases were

done by the benzidine test (10) and by Wong's modification of the porphyrin test of Kilian (30, 52) on isolates grown on blood-free media. Temperature and atmosphere preference were determined by growth on blood agar in ambient air and under anaerobic conditions at 23, 30, 35, 40, and 50°C. Growth on blood agar at 35°C in 5% CO_2 was also compared. Colonial morphology and hemolytic patterns under these conditions were noted at 24 and 48 h and after 7 days. Growth was also attempted on MacConkey agar and Rogosa SL acetic acid-acetate agar (Difco Laboratories, Detroit, Mich.). The presence of polysaccharide production was noted on 5% sucrose agar (53). The formation of gas from glucose was detected by using lactobacillus DeMan, Rogosa, and Sharpe (MRS) broth (Difco) with petrolatum overlay and Durham tubes. Arginine hydrolysis was performed in Moeller decarboxylase medium (14).

Additional tests were performed upon the isolates resembling pediococci. Tolerance to salt was noted at concentrations of 4, 6.5, and 10% in bromcresol purple heart infusion broth (Difco), thioglycolate broth, tryptic soy broth, and MRS broth and APT broth (Difco). Rapid bile-esculin (14) tests and 1-h litmus milk (32) reactions were done. L-Pyrrolidonyl-β-naphthylamide (PYR) was hydrolyzed with Streptococcus A-E (Scott Laboratories, Inc., West Warwick, R.I.). Growth on bile-esculin agar (14) and determination of esculin hydrolysis were attempted at 24 h (14). The presence of streptococcal group antigens A, B, C, D, F, and G on the clinical isolates resembling pediococci (strains no. 1 to 6) was sought by latex agglutination after extraction by using Streptex (Wellcome Diagnostics, Temple Hill, Dartford, England) and for group D antigen by coagglutination for all pediococci by using Phadebact (Pharmacia Diagnostics, Piscataway, N.J.).

Carbohydrate fermentation and esculin reactions were performed at least twice on all of the studied organisms by the Minitek procedure described by Setterstrom et al., using supplemental phenol red (44). Production of acid from the following carbohydrates was recorded: arabinose, cellobiose, glucose, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. Starch utilization was determined by growth in purple broth (Difco) with 1% starch for the pediococci. Additional carbohydrate utilization methods were used for individual carbohydrates that were deemed critical to identification or were not typical of reported results. Disks impregnated with carbohydrates (differentiation disks; Difco) were added to heavily inoculated cystine-tryptic agar, which was incubated in air for 1 to 5 days. Pediococci were tested for maltose, rhamnose, lactose, xylose, sucrose, trehalose, and arabinose utilization with this method. The *Leuconostoc* strains (no. 7 and 8) and *Lactobacillus* strains (no. 9 and 10) were tested for arabinose, lactose, maltose, mannitol, melibiose, raffinose, salicin, and trehalose utilization by this method. Additional confirmation was accomplished by using a lightly inoculated basal carbohydrate broth with Andrade indicator (Carr-Scarborough Microbiologicals, Stone Mountain, Ga.) after 1- to 5-day growth in air for arabinose, glucose, lactose, maltose, mannitol, raffinose, and xylose for the two strains resembling lactobacilli. This medium was also used to confirm salicin utilization and the failure to utilize maltose in the pediococci. Reproducibly positive carbohydrate utilization by any method was recorded as positive in the results.

Antimicrobial agent susceptibilities. Because of insufficient growth in cation-adjusted Mueller-Hinton broth by most strains, microdilution susceptibility tests were performed in Mueller-Hinton broth (Difco) supplemented with 5% lysed sheep erythrocytes. Inocula were checked at final well concentrations of between 10^4 and 10^6 CFU/ml. Endpoints were determined by the absence of visible growth after aerobic incubation at 35°C for 24 and 48 h. An anaerobic atmosphere generally produced more rapid growth but similar MICs. Tests were done at least in triplicate, with the median MIC reported. Macrodilution MICs, using inoculum densities similar to those in the microdilution tests, were determined in triplicate in Todd-Hewitt broth (Difco). Subcultures were performed after 24 h, and MBCs were noted at growth concentrations yielding ≥99.9% killing. Additionally, the presence of any zone of inhibition about 0.04-U bacitracin disks was noted (Taxo A; BBL Microbiology Systems, Cockeysville, Md.).

Reference strains. Twelve strains of pediococci were kindly provided by Dallas G. Hoover, Department of Food Science, University of Delaware, Newark. These strains were subjected to susceptibility testing and physiologic classification as described above. Organisms (and their sources) were as follows: *Pediococcus acidilactici* ATCC 33314 (NCDO 1859, from M. Daeschel, U.S. Department of Agriculture, Raleigh, N.C.), NCDO 521 (ATCC 8042 from the National Collection of Dairy Organisms [NCDO], National Institute for Research in Dairying, Reading, United Kingdom), ABC-PC (from ABC Research, Gainesville, Fla.), ABC-P02 (from ABC Research), ABC-91 (from ABC Research), and NRRL B5627 (from the Northern Regional Research Center [NRRL], U.S. Department of Agriculture, Peoria, Ill.); *Pediococcus pentosaceus* NCDO 990 (ATCC 33316, from NCDO), NCDO 813 (ATCC 8081, from NCDO), NRRL B14009 (from NRRL), E66 (from J. Stamer, Geneva, N.Y.), and 3885 (DSM 20206, from M. Sasser, University of Delaware); and *Pediococcus cerevisiae* NRRL B1153 (from NRRL). Species identities have been confirmed by published DNA homology studies for strains NCDO 990, NCDO 813, NCDO 521, and apparently E66 (3, 11). However, the type strain of *P. acidilactici*, ATCC 33314, has been shown to be homologous to strains of *P. pentosaceus* (3, 11).

Clinical information. Patient records pertaining to the clinical isolates were obtained and reviewed. Attention was paid to the following: Gram stain results for the cultured specimen, amounts and types of organisms isolated from the specimen, clinical symptoms and signs pertaining to the possibility of infection at the site from which the specimen was taken, the opinions of the clinician regarding clinical significance of the isolate at the time of isolation, any antimicrobial agent or surgical treatments administered, and the response to treatments. Clinical importance was classified as not significant, of unclear significance, or possibly significant.

RESULTS

Isolates. Twenty-three clinical isolates of vancomycin-resistant, alpha- or nonhemolytic streptococci that were neither pneumococci nor Lancefield serogroup B or D were reported by the clinical microbiology laboratory over the 43-month period. During a 28-month period, 14 (0.32%) vancomycin-resistant streptococci of this description were noted among 4,418 total similar streptococci reported. Since susceptibility testing was performed on only 315 such isolates during this period, vancomycin resistance was present in 4.4% of isolates tested.

Of the 23 strains reported, 15 had been stored and 13 were successfully reconstituted. Gram stains of colonies on agar and from thioglycolate broth clearly showed that three

strains were gram-positive rods. This finding and the absence of formation of gas from glucose suggested that these strains were homofermentative lactobacilli (16, 28); they were not studied further. The 10 remaining strains were studied in more detail. Similarities allowed them to be divided into three groups.

Six strains (no. 1 to 6) showed round, gram-positive cocci from colonies on plates. Smears from thioglycolate broth showed variable-size round cocci that tended to form tetrads in a gram-intermediate but predominantly gram-positive fashion. Colonial growth was generally pinpoint size at 35°C in O_2 at 24 h, 1 to 2 mm at 48 h, and 2 to 4 mm after 4 days, although one strain grew more slowly than this, with both large and small colonies (no. 3). Colonies were white to gray, smooth, and nonhemolytic on blood agar, although a greening reaction was slightly visible around colonies after prolonged incubation. The optimum growth temperature was 40°C, although 50°C was tolerated. Anaerobic and 5% CO_2 growth atmospheres produced more rapid growth than did ambient air at 23, 35, and 40°C. All strains were catalase negative and cytochrome negative by both the benzidine test and the porphyrin test of Kilian. Rapid growth was also noted on Rogosa SL acetic acid-acetate agar. Pinpoint growth of generally noncolored colonies was supported by MacConkey agar in ambient air for most strains. Although all strains grew well in thioglycolate and heart infusion broths, no growth occurred in the presence of 4 to 10% NaCl in these media. However, good growth was found after 1 to 2 days of incubation in 6.5% NaCl-supplemented MRS and APT broths for all strains. Two strains (no. 3 and 5) grew in 10% salt in MRS and APT broths after 3 to 7 days of incubation. Nonmucoid, nonadherent colonies were present on 5% sucrose agar. Gas was not formed from glucose. All strains grew rapidly and hydrolyzed esculin on bile-esculin medium, although only two strains darkened greater than 50% of the medium. All strains produced negative rapid bile-esculin reactions at 30 min with an inoculum adjusted to match the density of a 0.5 McFarland standard, but two strains were positive at 30 min with a heavy inoculum and more strains were positive at longer incubation times. No strains were positive in litmus milk at up to 4 h incubation. Group D antigen was detected by latex agglutination in two strains and by coagglutination in five strains, with the sixth strain weakly positive by coagglutination. Although utilized in the Minitek system by only three strains, salicin was utilized by all strains in Andrade broth, but only after 3 to 7 days of incubation.

The 12 reference strains of pediococci were morphologically and physiologically similar to the 6 clinically isolated strains (Table 1). For the sake to tabulation, two reference strains were reclassified on the basis of their properties. Strain ATCC 33314 failed to grow at 50°C and was maltose positive; therefore, it was more typical of *P. pentosaceus*, a finding supported by DNA homology studies (3, 11). Strain NRRL B1153 was typical of *P. acidilactici*.

The six clinically isolated organisms in this group appeared to be pediococci on the basis of their physiologic properties (15, 18). The identification to species level of four strains was entirely consistent with *P. acidilactici* (15, 18). Two strains (no. 3 and 5) grew slowly in 10% NaCl; however, they tolerated 50°C, preferred 40°C, and were unable to utilize maltose. These strains seemed most consistent with *P. acidilactici* as well.

Antimicrobial susceptibilities of the 6 clinically isolated strains resembling pediococci and the 12 reference strains of pediococci are shown in Table 2. High-level vancomycin resistance was present in all strains (MIC ≥ 2,048 μg/ml).

TABLE 1. Some properties of clinical and reference isolates of pediococci

Test[a]	No. of positive *P. acidilactici* strains		No. of positive *P. pentosaceus* reference strains (n = 6)
	Clinical (n = 6)	Reference (n = 6)	
Tetrads	6	6	6
Greening reaction	3	1	4
Spot catalase	0	0	0
Cytochromes	0	0	0
40°C preference	6	6	4
Anaerobic preference	6	6	6
Group D antigen	5	3	5
50°C tolerance	6	4	0
40% bile tolerance	6	6	6
6.5% NaCl tolerance	6	6	6
10.0% NaCl tolerance	2	4	1
Rogosa AcA[b] agar	6	6	6
MacConkey agar	4	4	4
Arginine hydrolysis	6	6	6
Esculin hydrolysis	6	6	6
PYR[c] hydrolysis	0	0	0
Acid formation from:			
Arabinose	6	6	6
Cellobiose	6	6	6
Glucose	6	6	6
Glycerol	0	0	0
Lactose	1	1	5
Maltose	0	0	6
Mannose	6	6	6
Mannitol	0	0	0
Raffinose	0	0	1
Rhamnose	3	2	2
Salicin	6	6	6
Sorbitol	0	0	0
Sucrose	0	3	1
Trehalose	5	5	6
Xylose	6	6	2

[a] See text for description of tests.
[b] AcA, Acetic acid-acetate.
[c] PYR, L-Pyrrolidonyl-β-naphthylamide.

The remaining isolates fell into two groups of two isolates each. In Gram-stained smears of colonies on agar, two strains (no. 7 and 8) showed gram-positive cocci with frequent, lenticular elongation; a lenticular shape was more consistent on smears from thioglycolate broth. These strains grew on blood agar with a greening reaction at a rate and with a colonial appearance comparable to those of alpha-hemolytic streptococci, although no colonial central umbilication was present. The preferred incubation was in O_2 or CO_2 at 35°C. These two strains fit the description of *Leuconostoc mesenteroides* subsp. *mesenteroides* (16, 17). High-level resistance to vancomycin was noted in these two *Leuconostoc* strains (Table 2): MICs and MBCs were ≥1,024 μg/ml.

Two strains (no. 9 and 10) had elongated gram-positive cocci with infrequent small-rod formation on smears from colonies on plates. Smears from thioglycolate broth showed small, gram-positive rods. These two strains were heterofermentative or class III lactobacilli (28). Identification to the species level was consistent with *Lactobacillus confusus* (15, 16, 28).

Identification to species level by physiologic criteria for all clinical strains of pediococci, lactobacilli, and leuconostocs and by DNA homology for the two strains of *L. confusus*

TABLE 2. Susceptibilities of pediococci to 11 antimicrobial agents[a]

Antimicrobial agent	MIC (μg/ml)[b] for:	
	Clinical strains (n = 6) of *P. acidilactici*	Reference strains (n = 12) of *P. acidilactici* and *P. pentosaceus*
Vancomycin[c]	2,048 (2,048–4,096)	2,048 (2,048–4,096)
Penicillin	1 (0.5–1.0)	1 (0.5–2.0)
Ampicillin	2 (2–4)	2 (2–8)
Erythromycin	<0.25	<0.25
Clindamycin	<0.25	<0.25
Tetracycline	>4	>4
Oxacillin	>2 (<2–>2)	>2
Cefazolin	8 (8–>8)	8 (8–>8)
Trimethoprim-sulfamethoxazole	>2/80	>2/80
Gentamicin	4 (2–4)	1 (1–4)
Norfloxacin	>16	>16

[a] Results for vancomycin determined by macrodilution in Todd-Hewitt broth; others determined by microdilution in 5% sheep blood-Mueller-Hinton broth.

[b] Median MICs are listed; range is given in parentheses.

[c] The MBC of vancomycin for the clinical strains of *P. acidilactici* was 4,096 μg/ml (range, 4,096 to >4,096 μg/ml), and that for the reference strains of *P. acidilactici* and *P. pentosaceus* was >4,096 μg/ml (range, 4,096 to >4,096 μg/ml).

was confirmed by Richard R. Facklam (personal communication).

Clinical information. The six isolates resembling pediococci (no. 1 to 6) were all recovered in mixed culture from abdomen-related sites (Table 3). Three isolates (no. 1, 5, and 6) came from peritoneal fluid after elective colorectal surgery. Since postoperative infections did not develop, no clinical significance could be attached to these isolates. Two isolates (no. 2 and 4) of unclear significance were found in mixed culture with members of the family *Enterobacteriaceae* and *Bacteroides fragilis* in material from enterocutaneous fistulae with associated cellulitis or abscess formation in patients with Crohn's disease. One isolate (no. 2) was obtained in a moderate amount along with a rare amount of penicillin-susceptible alpha-hemolytic streptococci from a small, asymptomatic, abdominal-wall abscess which was unexpectedly encountered during an elective colostomy revision in an afebrile, obese, diabetic patient. A postoperative wound infection due to *Staphylococcus aureus* further obscured analysis of the significance of this isolate.

One of the two isolates identified as *Leuconostoc mesenteroides* subsp. *mesenteroides* was isolated from one of two blood cultures taken 1 h apart in a continuously febrile, immunosuppressed, ventilator- and steroid-dependent patient with disseminated *Mycobacterium avium-Mycobacterium intracellulare* infection. Six days previously, a central venous catheter had been changed and vancomycin had been started for a coagulase-negative staphlococcal, central-venous-catheter-related bacteremia. Higher fevers and hypotension prompted another central-line change on the date of this single positive blood culture. The catheter tip and blood cultures taken on each of the next 2 days showed no growth. Even though the clinical situation stabilized after the catheter was changed, erythromycin was subsequently given for 14 days because of the isolation of the organism. The second isolate was obtained in a mixed culture with *Enterobacter cloacae*, a *Kluyvera* species, and *Candida tropicalis* from a contaminated heparin infusate in a febrile, postoperative orthopedic patient with *E. cloacae* bacteremia. No clinical significance could be attached to this isolate.

The two *L. confusus* isolates were found in mixed culture and were abdomen related. One (no. 9) was a peritoneal fluid isolate from a specimen taken after colonic surgery from which no infection resulted. The other (no. 10) was isolated in a moderate amount along with moderate growth of *Klebsiella pneumoniae* and very rare enterococci from a febrile patient with a tender, fluctuant, abdominal-wall abscess adjacent to a gastrostomy tube inserted 1 month previously. Drainage and administration of cephalosporin antibiotics resulted in prompt cure.

DISCUSSION

Aerobic bacteria that utilize carbohydrates as an energy source in various glycolytic pathways and form lactate as a major end product in these reactions have been called lactic

TABLE 3. Clinical isolation of vancomycin-resistant lactic and acid bacteria

Organism and strain	Age (yr)/sex[a] of patient	Underlying disease	Specimen source	No. of other bacterial isolates	Clinical significance
Pediococcus acidilactici					
1	77/M	Rectal carcinoma	Peritoneal fluid after colon surgery	12	None
2	65/M	Rectal carcinoma	Subfascial abscess	1	Possible
3	46/M	Crohn's disease	Abdominal-wall abscess, fistula	4	Unclear
4	46/M	Crohn's disease	Perirectal abscess, fistula	4	Unclear
5	28/M	Crohn's disease	Peritoneal fluid after protectomy	2	None
6	32/F	Ulcerative colitis	Peritoneal fluid after colectomy	2	None
Leuconostoc mesenteroides subsp. *mesenteroides*					
7	22/M	Immunosuppressed central line	Blood	0	Unclear
8	65/M	Prosthetic hip surgery	Intravenous infusate	3	None
Lactobacillus confusus					
9	71/M	Cecal carcinoma	Peritoneal fluid after hemicolectomy	2	None
10	12/F	Vegetative state, gastrostomy	Abdominal wall	2	Unclear

[a] M, Male; F, female.

acid bacteria. Bacteria that share these properties are frequently found in fermenting organic materials. The production of lactic acid and other by-products is beneficial in some commercial situations, such as dairying and sausage-making, but is often associated with spoilage in many food-related industries. The term lactic acid bacteria emphasizes these characteristics and is commonly used in the food sciences (16–18, 28, 45).

Lactic acid bacteria are catalase-negative, nonsporeforming, gram-positive cocci, coccobacilli, or rods. Genera that fit the description of lactic acid bacteria are *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Pediococcus*. The genus *Aerococcus* has been mentioned in reference to lactic acid bacteria but is generally not included in this group (45).

The concept of the grouping of lactic acid bacteria, originally attributed to Orla-Jensen, based upon these physiologic and ecologic similarities (28), has served microbiology well. Similarities between members of these genera have recently been shown by phylogenetic studies of isofunctional enzymes (34) and 16S rRNA relatedness (48). However, these studies have suggested that pediococci and leuconostocs, which appear closely related to streptococci by morphologic and physiologic criteria, are more closely related to lactobacilli. Streptococci appear phylogenetically more distantly related to the other lactic acid bacteria (48).

Genus differentiation among the lactic acid bacteria can usually be achieved in the microbiology laboratory by analysis of Gram stain results and determination of whether glucose is utilized strictly heterofermentatively and whether arginine can be hydrolyzed (16, 45). MRS medium is probably preferred for detection of the formation of gas from glucose. It is one of several media which are sufficiently complex to support the growth of leuconostocs, pediococci, and lactobacilli (16). We found an 8- to 24-h period of incubation of MRS broth with Durham tubes more convenient to perform and easier to read than MRS broth overlaid with petrolatum. The 2% glucose concentration of MRS medium produced a stronger and more easily detectable gas-from-glucose reaction than the 1% glucose concentration of Andrade broth. Differences in ciprofloxacin and imipenem susceptibilities sufficient to assist in genus classification have recently been noted in most strains of these three genera (48a).

Although genus differentiation of the lactic acid bacteria is usually possible by broth Gram stain, production of gas from glucose, and arginine hydrolyis, further physiologic testing may be useful to determine whether a given isolate fits a recognized species. For this purpose, determination of carbohydrate utilization by using miniaturized systems has been shown to be reliable compared with broth macrodilution methods for streptococci (44), pediococci (12), and lactobacilli (19). However, sufficient overlap exists that occasional isolates will prove difficult to identify to the genus level. It may not be possible to distinguish arginine-negative, gram-positive coccobacilli that produce gas from glucose as a *Leuconostoc* species, *Lactobacillus veiridescens*, or *L. confusus* (16), an organism whose name implies its physiologic similarity to *Leuconostoc* sp. (28). Likewise, differentiation of an arginine-negative, gram-positive coccobacillus that does not produce gas from glucose between *Streptococcus* and *Lactobacillus* species may be difficult.

Of the eight *Pediococcus* species currently recognized (18), only *P. acidilactici* and *P. pentosaceus* have been isolated from clinical sources (7, 15, 42). These species are closely related by DNA homology (3, 11); individual isolates may not be physiologically distinguishable (18). Reported differentiating physiologic features are as follows: maltose fermentation (*P. acidilactici*, negative; *P. pentosaceus*, positive) (2, 4, 15, 18, 31, 51); saccharose (2, 4) and methylglucoside (4) fermentation (*P. acidilactici*, negative; *P. pentosaceus*, variably positive); ability to grow on MacConkey agar (*P. acidilactici*, able to grow; *P. pentosaceus*, variably able to grow) (15); 10% NaCl tolerance (*P. acidilactici*, intolerant; *P. pentosaceus*, tolerant [18], although this has been reported variable in both species [4]); 50°C tolerance (*P. acidilactici*, tolerant; *P. pentosaceus*, intolerant) (18); and optimal growth temperature (40°C for *P. acidilactici*, 30°C [31] or 35°C [18] for *P. pentosaceus*). Xylose fermentation (*P. acidilactici*, positive; *P. pentosaceus*, variably negative) has been reported to be possibly useful (15, 18), but this has been disputed (4). Discrepant results for other potentially useful differentiating fermentations have also been noted for arabinose, cellobiose, glycerol, inulin, maltotriose, mannitol, salicin, and sucrose (4, 15, 18, 31). Some discrepancies may be method dependent (glycerol and salicin) (15), while others could be due to transmissible plasmids (sucrose, raffinose, and α-galactosidase degradation) (22, 39), but most are likely due to the relatively few isolates whose species identity has been reliably confirmed. Therefore, the disputed results of these fermentation reactions, as well as agreed-upon variations in lactose, raffinose, rhamnose, and trehalose (4, 15, 18, 31), do not as yet assist differentiation.

Identical API Rapid Strep (Analytab Products, Plainview, N.Y.) profiles have been found for a limited number of *P. acidilactici* and *P. pentosaceus* strains (42). Properties of these species not reported here or in other review articles (4, 15, 18, 31) include positive reactions for Voges-Proskauer, β-galactosidase, and leucine arylamidase. Negative reactions were noted for alkaline phosphatase, α-galactosides, β-glucuronidase, and hippurate (42).

Among the features studied here, maltose fermentation was the most useful in confirming the species identities of the reference strains of pediococci. All methods of detecting maltose fermentation tried here were reproducible and the results were in agreement. Although tolerance of 50°C was helpful when it was present, tolerance of 10% salt, the ability to grow on MacConkey agar, and the optimal growth temperature did not seem to be reliable. Failure to ferment xylose was found only in *P. pentosaceus*. Arabinose fermentation was not found to be a useful marker with the methods used here, in contrast to findings with the API system (15). The reported failure of pediococci to tolerate 6.5% salt (15) may have been due to medium selection, since the strains of *P. acidilactici* and *P. pentosaceous* studied here failed to grow in salt-supplemented heart infusion broth and tryptic soy broth but grew well in salt-supplemented MRS and APT broths.

The role of the pediococci in causing infection was unclear. In this study, pediococci were found on three occasions when an infection was present but only once in predominance. In that case, the infection was of such a mild nature that it was asymptomatic and only inadvertently recognized. The role of pediococci in mixed culture in the fistulous tracts of patients with Crohn's disease is unclear, but it can be speculated that their presence implies that the fistulous milieu was acidic, since pediococci produce so much lactic acid and are so acid tolerant.

The ecologic niche of pediococci in humans appears to be within the enteral tract. When selective media have been used, organisms that resemble what would be currently classified as either *P. acidilactici* or *P. pentosaceus* have

been found in saliva in 1.9% of volunteers (47) and in 3% of clinically submitted stool specimens (42). The six isolates in this study were found in association with procedures or drainage from the small or large intestine.

Two additional vancomycin-resistant strains, physiologically similar to *P. acidilactici* (i.e., maltose negative, preferring 40°C, and tolerant of both 50°C and 6.5% NaCl but not 10% NaCl), have been subsequently isolated from gastrointestine-related sites by our clinical microbiology laboratory. One isolate was recovered with a vancomycin-resistant, homofermentative *Lactobacillus* strain from a surveillance rectal swab in a transplant recipient. The other was found in mixed culture with *Pseudomonas aeruginosa*, *Candida albicans*, and an *Enterococcus* strain from a percutaneous drain leading to a biliary stent in a patient with sclerosing cholangitis who was receiving ceftazidime, vancomycin, and amphotericin B for apparent stent-related biliary sepsis.

Both of the clinical isolates that were identified as *L. confusus* were also found in association with the gastrointestinal tract. *L. confusus* has been previously recovered from saliva (28).

The human ecologic niche of the genus *Leuconostoc* is as yet not fully determined. *Leuconostoc mesenteroides* is possibly a part of mouth flora, since it has been recently isolated from an odontogenic buccal-masseteric space infection (50). One isolate was found in spinal fluid in an adolescent with meningitis (8). Organisms that are probably *Leuconostoc* spp. have also been found among the vaginal flora (40). Coovadia et al. have suggested that residual skin colonization, which is probably derived from the maternal genital tract, is responsible for the isolation of *Leuconostoc* sp. in infants and newborns (9). These authors reported the isolation of *Leuconostoc* strains as contaminants from blood cultures of two newborns and noted the fairly high reported frequency of newborns and infants as sources for *Leuconostoc* isolates (7 of 21, including isolates reported here) (6, 8, 9, 13, 24, 26, 27, 35, 41, 42, 50). The 2 *Leuconostoc* isolates found in this study, as well as 11 other isolates of which we are aware, have been associated with peripheral or central venous catheters or other percutaneous invasive devices, such as peritoneal catheters or gastrostomy tubes (6, 13, 24, 26, 27, 41, 42). In these settings, infection may have occurred from environmental sources, such as tube feeding material (27), intravenous infusates, or the skin-device interface.

Although the *Leuconostoc* isolates in this study were of unclear significance, *Leuconostoc* isolates have been associated with infection (6, 8, 9, 13, 24, 26, 27, 41, 50). Due to easy confusion with streptococci and the theoretical potential for causing endocarditis, particularly with the polysaccharide-producing *Leuconostoc* and *Lactobacillus* strains, determination of the vancomycin susceptibility of organisms resembling streptococci isolated from patients with serious infections, such as endocarditis, seems particularly warranted.

In addition to problems with reliable genus identification, methodologic problems in susceptibility testing of lactic acid bacteria exist (15, 48a). Strains of nonenterococcal streptococci previously reported by the Centers for Disease Control, Atlanta, Ga., to be resistant to vancomycin were more recently noted to be susceptible on repeat testing using current methodologies (R. R. Facklam and C. Thornsberry, Antimicrob. Newsl. **1**:63–64, 1984). Most of the remaining reports of vancomycin-resistant streptococci have noted only rare isolates with low-level resistance (5, 23); therefore, the occurrence of clinically isolated, vancomycin-resistant streptococci has been felt to be unusual or nonexistent (15, 27; Facklam and Thornsberry, Antimicrob. Newsl.). Lactococci are also thought to be routinely susceptible (15, 37), but clinical isolates of several species of enterococci have been recently found to be resistant to vancomycin (15, 29, 35, 49). However, the degree of vancomycin resistance among enterococci has been generally moderate compared with the extreme resistance found in pediococci, leuconostocs, and some lactobacilli (15, 33, 35, 49). All 18 strains of pediococci tested in this study were highly resistant to vancomycin. Vancomycin resistance has been noted in at least six other clinically isolated strains of pediococci (7, 42), as well as in 25 isolates referred to the Centers for Disease Control (15, 48a).

Vancomycin resistance has been reported in at least 25 clinically isolated *Leuconostoc* strains (6, 8, 9, 13, 24, 26, 27, 35, 41, 42, 50), as well as in at least 44 clinically isolated strains of *Leuconostoc* species reported by Facklam et al. (15). High-level vancomycin resistance has also been common in *Leuconostoc* strains that were not clinically isolated (6, 36).

Although lactobacilli are usually susceptible to vancomycin, resistance of both heterofermentative and homofermentative species has been occasionally reported (20, 25, 48a). Of 37 isolates of organisms reported to be lactobacilli that were tabulated in one collection, 4 were resistant to vancomycin (1). In order to estimate the frequency of vancomycin resistance among lactobacilli in our laboratory, a 31-month computer search was performed. For six *Lactobacillus* isolates from different patients, the vancomycin MICs were found to be >16 μg/ml. If the four strains of lactobacilli that were misidentified as streptococci from this study during this time period are included, vancomycin-resistant lactobacilli constituted 1.4% of all lactobacilli reported and 10% of lactobacilli upon which susceptibility testing was performed.

The mechanism of vancomycin resistance among nonenterococcal lactic acid bacteria is unclear. Since transferable, plasmid-mediated resistance to vancomycin has been demonstrated in enterococci (33), and since intergeneric transfer of antibiotic-resistance plasmids has been shown to occur among *Leuconostoc*, *Pediococcus*, *Enterococcus*, *Streptococcus*, and *Lactococcus* species (21, 33, 38), plasmid-mediated resistance to vancomycin must be considered for all lactic acid bacteria. However, plasmids probably do not mediate vancomycin resistance in all leuconostocs or pediococci. Of 14 vancomycin-resistant *Leuconostoc* species in one series, 5 contained no plasmids of the size commonly associated with resistance to antimicrobial agents; transfer of vancomycin resistance could not be demonstrated (36). Two of the reference strains of pediococci from this study (ATCC 33314, NRRL B14009) contain no plasmids (Dallas G. Hoover, personal communication). It has also been shown that vancomycin is not inactivated after in vitro growth of strains of vancomycin-resistant pediococci, lactobacilli, and leuconostocs (27, 36, 42).

Vancomycin resistance in nonenterococcal, clinically isolated organisms resembling oral streptococci may, therefore, be a marker for identification of other genera of lactic acid bacteria. Our results suggest this is true; all 13 strains of such bacteria studied here proved to be pediococci, lactobacilli, or leuconostocs.

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