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Recovery of Vancomycin-Resistant Gram-Positive Cocci from Children

MICHAEL GREEN,[1,2]* ROBERT M. WADOWSKY,[3,4] AND KAREN BARBADORA[1]

Departments of Pediatrics,[1] Surgery,[2] and Pathology,[3] Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, and Department of Infectious Disease Microbiology, Graduate School of Public Health, University of Pittsburgh,[4] Pittsburgh, Pennsylvania 15213

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A cross-sectional survey of vancomycin-resistant gram-positive cocci (VRGPC) in the feces of children was initiated after several bacteremic infections with these organisms occurred at our hospital. A selective medium consisting of colistin-nalidixic acid agar, 5% sheep blood, vancomycin (5 mg/liter), and amphotericin B (8 mg/liter) was developed to isolate VRGPC. A single stool specimen submitted to the clinical microbiology laboratory from each of 48 patients was inoculated onto the medium. Plates were incubated at 35°C with 5% carbon dioxide and examined at 24, 48, and 72 h. Susceptibilities were determined by broth microdilution. A total of 14 isolates from 11 of 48 (22%) children were recovered. The density of growth ranged from a single colony to 2+. The VRGPC were identified as *Leuconostoc lactis* (*n* = 2), *Lactobacillus confusus* (*n* = 4), *Enterococcus* species (*n* = 5), and *Lactococcus lactis* (*n* = 3). One strain of *Lactobacillus confusus* was recovered from both the stool and the blood of one of these patients. The MICs of vancomycin were 4 μg/ml for one of the isolates, 8 μg/ml for four of the isolates, and more than 16 μg/ml for the remaining eight isolates. All isolates were susceptible to both penicillin and ampicillin. Only 1 of the 11 children had received prior treatment with vancomycin. We conclude that low concentrations of VRGPC may be common in the gastrointestinal tracts of children.

Resistance to vancomycin among gram-positive cocci was previously thought to be rare. Several recent reports, however, suggest the emergence of this resistance (1, 7, 9, 12, 15, 18). Shlaes et al. (15) described an episode, in an oncology patient, of bacteremia caused by *Streptococcus sanguis* II which was resistant to vancomycin. Another case of bacteremia caused by a vancomycin-resistant bacterium was reported by Rubin et al. (12). The isolate was initially identified as a *Streptococcus salivarius*-like bacterium, but more detailed testing revealed the isolate to be *Leuconostoc mesenteroides*. Several additional reports have also documented episodes of bacteremia associated with vancomycin-resistant *Leuconostoc* spp., several of which have occurred in children (1, 2, 5–7, 18). Furthermore, mechanisms of resistance have been suggested. Leclercq et al. (9) reported that vancomycin resistance was associated with a plasmid in an enterococcal isolate, confirming that such resistance occurs.

The actual incidence of infections caused by vancomycin-resistant gram-positive cocci (VRGPC) is unknown, since many clinical laboratories neither routinely employ identification schema to differentiate *Leuconostoc* species from viridans group streptococci nor routinely determine the susceptibilities of clinical isolates of viridans group streptococci to vancomycin. Furthermore, the frequency of representation of these bacteria in the indigenous normal flora of humans has not been established. Ruoff et al. (13) reported that 6 of 10 *Leuconostoc* isolates were cultured from sites contiguous to the gastrointestional tract. This finding suggests that the gastrointestional tract may serve as a reservoir of colonization and, by means of bacterial translocation (17), may also serve as a source of infection. The observation of bacteremia caused by VRGPC in three pediatric patients at the Children's Hospital of Pittsburgh prompted the following cross-sectional survey to determine the prevalence and biochemical profiles of VRGPC in the feces of children.

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MATERIALS AND METHODS

Selective medium. A selective medium (VRGPC medium) which inhibits a wide variety of gram-negative bacilli, gram-positive cocci, and yeasts was developed for the isolation of VRGPC from feces. Columbia CNA agar (BBL Microbiology Systems, Cockeysville, Md.) served as the basal medium and was prepared according to the directions of the manufacturer. The autoclaved basal medium was cooled to 47°C and supplemented with sheep blood (50 ml/liter), vancomycin (5 mg/liter), and amphotericin B (8 mg/liter) prior to pouring plates.

Isolation of VRGPC from stool specimens. From 48 children, consecutive fresh stool specimens were submitted in Amies transport medium to the clinical microbiology laboratory of the Children's Hospital of Pittsburgh, inoculated onto approximately one-sixth of a plate of the selective medium, and streaked for isolation with a sterile bacteriologic loop. The plates were incubated at 35°C in the presence of 5% carbon dioxide and were inspected for the presence of colonies at 24, 48, and 72 h. The amount of growth of VRGPC was determined to be 1+ to 4+ on the basis of whether growth was observed in the first, second, third, or fourth quadrant as described previously (8). Cellular morphology was determined from Gram stains of cells from those cultures positive for growth. No further tests were performed on those isolates identified as gram-negative rods, gram-positive rods, or fungi.

Bacteriological studies. Isolates of gram-positive cocci

* Corresponding author.

TABLE 1. Characteristics of 15 isolates of VRGPC

Characteristic	Identification and laboratory designation of isolate														
	Leuconostoc lactis		*Lactobacillus confusus*					*Enterococcus faecalis*		*Enterococcus gallinarum*[a]			*Lactococcus lactis*		
	36	56	27[b]	28A	41	49	53	7	38	1	46	47	28	32	57
Glycerol	−	−	−	−	−	−	−	+	+	−	−	V[c]	−	−	−
L-Arabinose	+	+	−	−	−	+	−	−	−	+	+	+	−	−	+
Ribose	−	−	+	+	+	−	−	+	+	+	+	+	+	+	+
D-Xylose	−	−	+	+	+	+	+	−	−	−	+	+	V	−	+
Galactose	−	−	+	+	+	−	+	+	+	+	+	+	+	V	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−
Inositol	−	−	−	−	−	−	−	−	V	−	−	−	−	−	−
Mannitol	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−
Sorbitol	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−
α-Methyl-D-glucoside	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−
N-Acetylglucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amygdalin	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−
Arbutin	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−
Esculin	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−
Salicin	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−
Cellobiose	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Lactose	+	−	−	−	−	−	−	+	+	+	+	+	−	+	−
Melibiose	+	+	−	−	−	−	−	−	−	+	+	+	−	−	−
Saccharose	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−
Trehalose	−	−	−	−	+	−	−	+	+	+	+	+	+	+	+
Melezitose	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−
D-Raffinose	+	+	−	−	−	−	−	−	−	+	+	+	−	−	−
Starch	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−
β-Gentiobiose	+	−	−	−	+	−	+	+	+	+	+	+	+	−	+
D-Turanose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Taganose	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−
Gluconate	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−
Leucine aminopeptidase	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
Alkaline phosphatase	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
β-Galactosidase	+	+	+	+	+	−	+	−	−	+	+	+	−	+	−
Acetoin production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hippurate hydrolysis	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
γ-Galactosidase	+	+	−	−	−	−	−	−	−	+	+	+	−	−	−
Arginine dehydrolase	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
PRYase	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−
Gas production in MRS broth	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
NaCl tolerance	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−
Vancomycin MIC[d] (μg/ml)	128	128	128	128	128	128	128	4.0	8.0	8.0	8.0	8.0	128	128	128

[a] Positive motility.
[b] Blood isolate obtained simultaneously with stool isolate 28A.
[c] V, Variable reaction.
[d] MIC at 48 h in CSMHB.

were subjected to a battery of identification tests including catalase, gas production in MRS broth overlaid with 2% agar (7), growth in 6.5% NaCl, and growth on bile-esculin agar. Isolates that produced gas in MRS broth were initially classified as either *Lactobacillus* or *Leuconostoc* species and subjected to the API CHL50 system (Analytab Products, Plainview, N.Y.) according to the recommendation of the manufacturer. These isolates were also subjected to the following tests in order to confirm their identities to the species level: production of slime on 5% sucrose agar and growth at 15 and 42°C. All other isolates were presumed to be streptococci and were subjected to both the API CHS50 system and the API Rapid Strep system. Isolates presumptively identified as streptococci were tested for the presence of group D antigen by a latex agglutination test (Burroughs Wellcome Co., Research Triangle Park, N.C.) after extraction by the autoclave method (12). Motility was then determined on those strains identified as *Enterococcus* (16) species. Group N antigen determination (Wellcome Reagents Limited, Beckenham, England) was performed when specified after consultation with the API reference laboratory. Presumptive identifications and their assigned qualities (e.g., excellent, good, or doubtful) were determined on the basis of the above tests in consultation with API and their reference data base.

Susceptibility testing. Testing of the susceptibility of VRGPC isolates was performed by broth microdilution, using the SENSITITER system (Radiometer, Copenhagen, Denmark). Isolates were evaluated in both cation-supplemented Mueller-Hinton broth (CSMHB) and Todd-Hewitt broth (THB). Isolates identified as *Lactobacillus*, *Leuconostoc*, or *Lactococcus* species were also tested in CSMHB

with lysed horse blood. MICs were read at 24 and 48 h. An MIC of vancomycin of 4 μg/ml or less was considered susceptible, 8 to 16 μg/ml was considered moderately susceptible, and more than 16 μg/ml was considered resistant (11).

Because of the poor growth of many of the VRGPC in both CSMHB and THB as well as the lack of growth of several strains of lactic acid bacteria in CSMHB with lysed horse blood, we assessed the feasibility of performing susceptibility testing, using MRS broth as we used standard broths (CSMHB and THB). The pHs of MRS broth and the standard broths were determined before and after incubation with bacteria. Quality control was performed by testing American Type Culture Collection organisms. MICs were determined and compared in the three broths.

RESULTS

Overall, 29 of 48 plates exhibited some growth after 72 h of incubation. A total of 14 isolates of gram-positive cocci were recovered from 11 of 48 children (22%) on VRGPC medium. The density of growth ranged from a single colony to 2+. Of the 48 plates, 18 (37.5%) exhibited growth of organisms other than VRGPC. Growth of ≥2+ of organisms other than VRGPC occurred on only seven (14.5%) of the plates. Organisms other than VRGPC included gram-negative rods in four, yeasts in two, and gram-positive rods in one of these patients. One patient, a multivisceral transplant recipient, was treated with an oral selective decontamination regimen including vancomycin; she developed bacteremia concurrent with colonization by the identical isolate found in the stool (see below).

The biochemical profiles of the 14 isolates of gram-positive cocci are shown in Table 1. In addition, all of the isolates had negative results with the following tests: erythritol, D-arabinose, L-xylose, adonitol, β-methyl-xylsoside, L-sorbose, dulcitol, α-methyl-D-mannoside, inulin, glycogen, xylitol, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-keto-gluconate, 5-keto-gluconate, and β-glucuronidase. Six of the isolates produced gas in MRS broth, indicating that they belonged to either the *Lactobacillus* or *Leuconostoc* genus. Isolates 36 and 56 were identified as *Leuconostoc lactis* (excellent and doubtful, respectively). Both isolates grew at 15°C in MRS broth and did not produce slime on 5% sucrose agar or grow at 42°C in MRS broth; these occurrences are all characteristic of *Leuconostoc* species. Isolate 56 received a doubtful rating because unlike most *Leuconostoc lactis* strains, it failed to produce acid from lactose, galactose, and trehalose. Isolates 28A, 41, 49, and 53 were identified as *Lactobacillus confusus* (isolates 28A, 41, and 53, excellent; isolate 49, doubtful). Isolate 49 did not produce slime on 5% sucrose agar, a finding which is inconsistent with the identification of *Lactobacillus confusus*, and thus received a doubtful rating. Isolates 27 and 28A were recovered simultaneously from the blood and stool of the multivisceral transplant recipient and had identical biochemical profiles.

TABLE 2. Ranges of MICs at 24 and 48 h in CSMHB and THB for five strains of enterococci

Antibiotic	MIC (μg/ml) range					
	CSMHB		THB		MRS broth	
	24 h	48 h	24 h	48 h	24 h	48 h
Ampicillin	0.5–2.0	1.0–2.0	1.0–2.0	1.0–4.0	0.5	0.5
Penicillin	1.0–2.0	1.0–4.0	1.0–2.0	1.0–4.0	1.0	1.0–4.0
Vancomycin	4.0–8.0	4.0–8.0	4.0–16.0	4.0–16.0	1.0–8.0	2.0–8.0

TABLE 3. Ranges of MICs at 48 h in CSMHB and THB for three strains of *Lactococci lactis*

Antibiotic	MIC (μg/ml) range			
	CSMHB	CSMHB-LHB[a]	THB	MRS broth
Ampicillin	2.0–4.0	2.0	0.5	1.0–4.0
Penicillin	0.5	0.5	0.06–0.5	0.25–0.5
Cephalothin	2.0–4.0	2.0–4.0	<0.50–2.0	2.0–4.0
Chloramphenicol	8.0	<4.0	<4.0	<4.0–8.0
Clindamycin	<0.12–0.5	<0.12	<0.12–0.5	<0.12
Erythromycin	<0.12–0.5	<0.12	<0.12–0.5	0.5–1.0
Tetracycline	64.0	8.0	16.0–32.0	16.0–32.0
Vancomycin	>128	>128	>128	>128

[a] CSMHB with lysed horse blood; one strain of *Lactococcus lactis* did not grow in CSMHB-LHB.

Five of the isolates were identified as belonging to the *Enterococcus* genus. Two were identified as *Enterococcus faecalis* (isolates 7 and 38, excellent) and three were identified as *Enterococcus gallinarum* (isolates 1, 47, and 46, excellent). Isolates 57 and 28 were identified as *Lactococcus lactis*; in both of these, identification was confirmed by growth at 10°C, lack of growth at 42°C, and the presence of a group N antigen. Isolate 32 was presumptively identified as *Lactococcus lactis*. All reactions were typical for this identification, except that this isolate did not react with group N antisera.

The results of testing of susceptibility to a variety of antibiotics are shown in Tables 2 to 4. The MIC at 48 h in CSMHB for all isolates is shown in Table 1; the range of MICs for the isolates of enterococci, lactococci, and *Leuconostoc* or *Lactobacillus* species are shown in Tables 2 to 4. No difference was seen in the susceptibilities of the five isolates of enterococci when results for incubation time used (24 versus 48 h) or broth used (CSMHB versus THB) were compared. Four of the five isolates were moderately susceptible to vancomycin; the MIC for the fifth isolate was 4 μg/ml.

The susceptibilities of the three *Lactococcus* isolates and seven *Leuconostoc* or *Lactobacillus* isolates could not be reliably determined at 24 h because of poor growth in both CSMHB and THB. Enhanced growth was seen at 24 h when CSMHB with lysed horse blood was used for these isolates; however, one strain of lactococcus and another of lactobacillus failed to grow in this broth. MICs for the lactococci were slightly higher in CSMHB and CSMHB with lysed

TABLE 4. Ranges of MICs at 48 h in CSMHB and THB for seven strains of *Leuconostoc* or *Lactobacillus* species

Antibiotic	MIC (μg/ml) range			
	CSMHB	CSMHB-LHB[a]	THB	MRS broth
Ampicillin	0.5–2.0	0.5–1.0	0.5–1.0	0.5–1.0
Penicillin	0.5–2.0	0.25–2.0	0.5–2.0	0.12–1.0
Cephalothin	<0.5–16.0	1.0–16.0	2.0–8.0	1.0–8.0
Chloramphenicol	<4.0–8.0	<4.0–8.0	<4.0–8.0	8.0–32.0
Clindamycin	<0.12–4.0	<0.12	<0.12	<0.12–2.0
Erythromycin	<0.12–4.0	<0.12	<0.12	0.25–0.5
Tetracycline	4.0–16.0	<2.0–8.0	2.0–4.0	8.0–16.0
Vancomycin	16.0–>128	>128	>128	>128

[a] CSMHB with lysed horse blood; one strain of *Lactobacillus confusus* did not grow in CSMHB-LHB.

horse blood than in THB; no difference was seen for the *Leuconostoc* or *Lactobacillus* strains. The MICs of vancomycin for all isolates of both the lactococci and *Leuconostoc* or *Lactobacillus* groups were greater than 128 μg/ml.

The charts of the 48 patients were reviewed. Of the 48 children, 22 had been seen in the outpatient department (either in the emergency department, ambulatory clinics, or pediatric gastroenterology clinic). Only limited information was available on these 22 patients. Diarrhea was present in all 48 patients. Although a few of the patients had underlying chronic diseases, the vast majority did not have important medical conditions besides the presence of diarrhea. Information concerning the types of food, formula, or nutritional supplements in the diets of the outpatients was not available. In the areas of age, race, and hospitalization at the time of stool culture, patients with positive cultures were similar to those not colonized with VRGPC. Because no difference in the rate of colonization between inpatients and outpatients was noted, no attempt to analyze the role of dietary factors was made for the five colonized inpatients. Only 1 of the 11 colonized patients, the multivisceral transplant recipient, had received both oral and parenteral vancomycin prior to having a stool cultured.

The pHs of the MRS broth, CSMHB, and THB prior to growth of bacteria were 6.3, 7.2, and 7.7, respectively. The pHs of these broths after 48 h of incubation were 4.0, 6.2, and 5.6 for lactococci; 4.6, 6.9, and 5.6 for enterococci; and 4.5, 7.1, and 6.2 for *Leuconostoc* or *Lactobacillus* spp.

The susceptibilities of *Streptococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were determined at 24 and 48 h in all three broths. No appreciable difference was seen between 24 and 48 h for either isolate. Susceptibilities of the staphylococcal isolate to cefazolin, clindamycin, ampicillin, and tetracycline were identical in all three broths. A difference was seen for erythromycin (MICs, 0.5, 0.25, and 2 μg/ml) and vancomycin (MICs, 1, 1, and 4 μg/ml) for CSMHB, THB, and MRS broth, respectively. No difference was seen in the final MIC of ampicillin, penicillin, or vancomycin for the *Streptococcus faecalis* isolate. Only the susceptibility of the staphylococcal isolate to erythromycin tested in MRS broth was outside the range expected by the National Committee for Clinical Laboratory Standards (11).

DISCUSSION

Until recently, vancomycin resistance among gram-positive cocci had been thought uncommon. Three episodes of bacteremia due to VRGPC at our institution and several recent reports of similar infections prompted this epidemiologic evaluation of the frequency of gastrointestinal colonization with VRGPC. The use of a VRGPC-selective medium offered a useful screening tool for conducting this limited cross-sectional survey of the prevalence of VRGPC. The selective medium was simple to prepare and had a relatively high specificity.

Low concentrations of VRGPC were found in 22% of the stool specimens evaluated in this study. These findings are similar to those of Ruoff et al. (13), who also found a 22% colonization prevalence of VRGPC in stools. The vast majority of isolates in that study were identified as *Lactobacillus* isolates. In contrast, in the current study there was a broader distribution of bacterial species including *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and *Lactococcus* species. Only 1 of the 11 colonized patients in the current study had received vancomycin prior to the isolation of VRGPC in the stool. Ruoff et al. (13) also evaluated the role of prior exposure to vancomycin among individuals colonized with VRGPC in the gastrointestional tract and found prior vancomycin treatment in 5 of 10 patients from whom vancomycin-resistant gram-positive organisms were recovered. The lack of consistent previous exposure to vancomycin suggests that low-level colonization with VRGPC may be fairly common. Furthermore, only one of four clinically significant isolates in the study by Ruoff et al. (13) was associated with prior vancomycin therapy. Additional studies are necessary to determine the risk factors for infection with VRGPC.

The identification of VRGPC, and lactic acid bacteria in particular, is extremely difficult. With conventional testing, pediococci and enterococci may be difficult to separate, since both groups of bacteria may grow on bile-esculin, be tolerant of 6.5% NaCl, and react with Lancefield group D antiserum. However, they may be easily separated with the PRYase test provided in the API Rapid Strep system (3). Facklam and colleagues (3) also compared the use of some of the API tests to conventional tests using *Leuconostoc*, *Lactobacillus*, and *Pediococcus* species and found good agreement, with the exception of the Voges-Proskauer test. For the differentiation of *Leuconostoc*, *Lactobacillus*, *Lactococcus*, *Enterococcus*, and *Streptococcus* species, Facklam et al. (3) reported the following tests to be the most useful: gas from glucose, streptococcal group antigen, bile-esculin, PRYase, and leucine aminopeptidase. The reactions to these tests obtained with the isolates identified in our study completely agree with the proposed criteria of Facklam et al. (3) for the identification of VRGPC to the genus level. In using the API Rapid Strep and CHS system for enterococci, the only contradiction found between API identification and that obtained by using the identification scheme of Facklam and associates (4) was that the three isolates of *Enterococcus gallinarum* were identified as *Enterococcus faecium* by API. A positive motility test differentiates these two species, and we therefore suggest the addition of a motility test to the API Rapid Strep for the identification of enterococci. For *Leuconostoc*, *Lactobacillus*, and *Lactococcus* species, the API data base provided a good tentative identification when compared with the proposed standard scheme of Facklam and associates. However, too few isolates were tested to make an accurate assessment of the reliability of API products in the identification of lactic acid bacteria to the species level.

Three isolates, all with high-level vancomycin resistance (MIC, ≥128 μg/ml), were identified as *Lactococci lactis*. There are no previous reports of vancomycin resistance among this species. Although Leclercq et al. were able to transfer a plasmid associated with vancomycin resistance to *Lactococcus lactis*, the recipient strain did not exhibit high-level vancomycin resistance (10). We are currently evaluating our isolates for plasmid-associated resistance to vancomycin.

Leuconostoc or *Lactobacillus* species were found in six of our patients, including the patient with bacteremia. Although all six of these isolates appeared as cocci on Gram stain, three were identified as *Lactobacillus* spp. Others have noted the difficulty in differentiating streptococci from either *Lactobacillus* or *Leuconostoc* species by microscopic morphology alone (3). Observations may be more accurate when determined from broth culture, which was not routinely done in our study. Two additional episodes of bacteremia due to *Lactobacillus confusus* (with identical biochemical profiles) have recently occurred at our institution. In both cases, the identical organisms were found in both the stools and throats of these patients, only one of whom had received

prior therapy with vancomycin. Both of these patients, as well as the multivisceral transplant recipient, had central vascular lines.

The enterococcal isolates identified in this study were moderately susceptible to vancomycin (MIC range, 4 to 16 μg/ml). This pattern of susceptibility differs from the high-level resistance of ≥128 μg/ml found by Leclercq et al. (9) and may be attributed to a mechanism of resistance which is not plasmid mediated. The MIC for *Enterococcus gallinarum* (8 μg/ml) seen in our three strains is identical to that found by Swenson et al. (16) for six strains of this species. They speculated that *Enterococcus gallinarum* is inherently more resistant to vancomycin than are other species of enterococci. It is also possible that this pattern of intermediate susceptibility may be inducible to high-level resistance upon exposure to vancomycin. Williamson et al. (19) have recently reported an initial MIC for an isolate of *Enterococcus faecium* which was 16 μg/ml, with inducible high-level resistance to ≥128 μg/ml. As in the isolates of Leclercq et al., the low-level resistance could be cured but was associated with a different-molecular-weight protein than that seen in isolates with high-level resistance (14).

Susceptibility testing of isolates of VRGPC can be performed with a commercial microtiter system. The slow growth of *Leuconostoc* or *Lactobacillus* spp., as well as that of the lactococci, may require delaying the visual reading of susceptibilities until 48 h. The use of CSMHB with lysed horse blood enhanced growth in most isolates, but no growth was seen in two of nine isolates repeatedly tested with this broth. Alternatively, all of our isolates grew extremely well in MRS broth, with clear, readable endpoints at 24 h. The increased acidity of this medium may cause some difficulty with certain antibiotics. Although the effect of increased acidity is not known for all antimicrobial agents, higher MICs of erythromycin, gentamicin, and trimethoprim-sulfamethoxazole were noted. However, no difference in the interpretation of the susceptibility test with either MRS broth or CSMHB or THB for antibiotics that the National Committee for Clinical Laboratory Standards suggests testing was found, in either our isolates or the American Type Culture Collection strains that we evaluated. Although further experience with susceptibility testing in this broth is needed, these data suggest that MRS broth may be an alternate medium for use in testing of the susceptibilities of these slow-growing organisms when conventional media provide insufficient growth.

Finally, the identification of our bacteremic isolates of VRGPC would not have occurred if testing of susceptibility to vancomycin had not been performed. Clinical laboratories should perform routine tests of susceptibility to vancomycin for clinical isolates which resemble the viridans group streptococci. Furthermore, the laboratory should consider differentiating *Leuconostoc* or *Lactobacillus* spp. from streptococci in cases in which resistance to vancomycin is identified so that the importance of VRGPC in clinical disease can be determined.

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