JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3166–3170 Vol. 35, No. 12
0095-1137/97/$04.00+0

Clinical and Epidemiologic Significance of Enterococci Intrinsically Resistant to Vancomycin (Possessing the *vanC* Genotype)

BALDWIN TOYE,[1,2]* JOSÉE SHYMANSKI,[3] MARIA BOBROWSKA,[2] WENDI WOODS,[1] AND KARAM RAMOTAR[1]

Departments of Pathology and Laboratory Medicine,[1] Medicine,[2] and Infection Control,[3] Ottawa General Hospital, University of Ottawa, Ottawa, Ontario, Canada

Received 19 June 1997/Returned for modification 20 August 1997/Accepted 24 September 1997

Constitutive low-level vancomycin resistance is found intrinsically in certain enterococcal species and is encoded by *vanC* ligase genes. These intrinsically vancomycin-resistant enterococci (VRE) will be referred to as VANC VRE. A prospective study to determine the clinical and epidemiologic significance of VANC VRE was conducted. VANC VRE were recovered from the stools of 34 of 601 (5.7%) patients, a rate similar to that obtained for the stools of 100 outpatients in the community (5%). VANC VRE were also isolated from the nonstool specimens of 9 of 538 patients (1.7%), including two patients with bacteremia. No VRE of the *vanA* or *vanB* genotypes were detected in nonstool specimens. Eighty-two hospital contacts of the first 23 patients found to be colonized or infected with VANC VRE were screened, and 6 contacts were found to be gastrointestinal carriers of VANC VRE. However, typing of isolates from these 6 contacts by pulsed-field gel electrophoresis with *Sma*I showed the isolates to be unique and different from those recovered from the index patients. In fact, all VANC VRE isolates from different patients in this study were unique. A case-control study with patients who were negative when screened for VANC VRE as controls failed to identify any risk factor associated with colonization or infection with this organism. VANC VRE were infrequently recovered from clinical specimens but were occasionally found as part of the normal stool flora. Since no transmission between patients was documented, additional isolation procedures may not be necessary for patients colonized or infected with VANC VRE.

Enterococci which are resistant to antimicrobials, especially vancomycin, have become important nosocomial pathogens. Vancomycin-resistant enterococci (VRE) have been recognized since 1988 and have been associated with hospital outbreaks and rapid dissemination in both Canada and the United States (15, 20). Infection due to VRE has been identified by the Centers for Disease Control and Prevention as an emerging infectious disease of the 1990s, and recommendations for preventing the spread of such infections have been published (6, 15).

Vancomycin resistance is the result of either intrinsic or acquired genes which code for a cell wall precursor with a reduced affinity for vancomycin. At least three classes of resistance have been described (1, 18). The *vanA* ligase gene results in high levels of vancomycin resistance as well as resistance to teicoplanin. The MICs of vancomycin for enterococci with the *vanB* ligase gene may be low to very high, but these enterococci remain susceptible to teicoplanin. Both these classes of vancomycin resistance (VANA and VANB VRE) have been detected mainly in *Enterococcus faecium* and *Enterococcus faecalis*. These types of resistance are acquired and encoded by genetic elements which are transferable. To date, all published outbreaks of VRE have been restricted to these two classes of vancomycin resistance.

Vancomycin resistance may also be found intrinsically in certain less-common species of enterococci, resulting in constitutive low-level resistance to vancomycin but susceptibility to teicoplanin. The *vanC* ligase genes, which encode this type of resistance, are not acquired or transferable. The *vanC1* ligase gene is specific for *Enterococcus gallinarum*, while the *vanC2/C3* ligase gene has been described in *Enterococcus casseliflavus*, *Enterococcus mundtii*, and *Enterococcus flavescens* (19, 24). These intrinsically vancomycin-resistant enterococci will be referred to in this study as VANC VRE.

The clinical and epidemiologic significance and the implications for infection control of the VANA and VANB VRE are well described (4, 15). The implications of VANC VRE, which have been recovered infrequently from clinical specimens, are not as clear. It has been suggested that VANC VRE are not a concern for infection control, primarily because no nosocomial outbreaks of these organisms have been reported (29, 30). However, the published data on the significance of VANC VRE are limited. Our objectives in the present study were to determine the clinical and epidemiologic significance of VANC VRE and the frequency of the nosocomial transmission of VANC VRE at our institution.

MATERIALS AND METHODS

Patient specimens. This study was conducted at the Ottawa General Hospital, Ottawa, Ontario, Canada, a 456-bed tertiary-care teaching center, which includes care for immunocompromised patients. From 1 November 1995 to 30 September 1996, all stools submitted for *Clostridium difficile* testing at our hospital were screened for VRE. Stools or rectal swabs were also obtained during a 1-week period from specific inpatient units in our institution where vancomycin was frequently used. These units included the nephrology, hematology, oncology, and bone marrow transplant inpatient units and the adult and neonatal intensive-care units. All ambulatory patients who received hemodialysis in the artificial kidney unit also submitted stools for VRE screening in January 1996. Stools from 100 different outpatients that were submitted to a private laboratory in the community (MDS Laboratories, Ottawa, Canada) for routine culture for enteric pathogens were also screened. In addition, all clinical isolates of enterococci recovered from patient specimens were screened for vancomycin resistance with a vancomycin screen plate.

* Corresponding author. Mailing address: Division of Microbiology, Ottawa General Hospital, 501 Smyth Rd., Ottawa, Ontario K1H 8L6, Canada. Phone: (613) 737-8323. Fax: (613) 737-8315. E-mail: btoye@ogh.on.ca.

Screening for VRE. Stool or rectal swabs were inoculated onto CNA agar (colistin [10 mg/liter], nalidixic acid [10 mg/liter], 5% sheep blood) supplemented with 6 mg of vancomycin per liter. Plates were incubated at 35°C in ambient air and examined at 24, 48, and 72 h. We determined that the sensitivity of detecting VRE with this medium was 100 to 500 CFU/g of stool (data not shown).

All clinical isolates of enterococci were screened for vancomycin resistance by inoculating 10 μl of a suspension equal to a 0.5 McFarland standard onto brain heart infusion agar containing 6 mg of vancomycin per liter. These plates were incubated in ambient air for 24 h at 35°C, and any growth was considered to be a positive test result (23, 26).

Microbial identification. Isolates recovered from stool or clinical specimens were presumptively identified as enterococci by colonial morphology, Gram's stain, the absence of catalase production, the presence of pyrrolidonylarylamidase by hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR test; Carr-Scarborough Microbiologicals Inc., Decatur, Ga.), and the presence of leucine aminopeptidase by hydrolysis of leucine-β-naphthylamide (LAP test; Murex Diagnostics Inc., Norcross, Ga.). Further identification of species was carried out with a test scheme based on carbohydrate fermentation, motility, and colony pigmentation (11).

Carbohydrate fermentation tests were performed in a heart infusion broth base (Difco Laboratories, Detroit, Mich.) containing 1% of the sugar being tested. Deamination of arginine was tested with Moeller arginine decarboxylase broth (PML Microbiologicals, Mississauga, Ontario, Canada). Motility was determined by inoculating motility test medium (PML Microbiologicals) and incubating it for up to 72 h at 30°C. Yellow pigmentation was observed after overnight growth on Trypticase soy agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and by taking a sweep off the plate with a cotton swab.

Susceptibility testing. MICs of vancomycin and teicoplanin were determined by the agar dilution method (23). Antimicrobials were tested in the range of 0.125 to 256 μg/ml with Mueller-Hinton II agar base (Becton Dickinson Microbiology Systems). Colony suspensions equal to a 0.5 McFarland standard were prepared and inoculated onto antibiotic containing medium with a Cathra Systems replicating device (MCT Medical Inc., St. Paul, Minn.) to yield a final inoculum of 10^4 CFU/spot. Plates were incubated in ambient air at 35°C for 24 h. The MIC was defined as the lowest antibiotic concentration showing no growth.

PCR-based detection of vancomycin resistance genes. A multiplex PCR assay was used to detect genes coding for vancomycin resistance in enterococci. A 1,429-bp fragment of the *vanA* ligase gene was amplified with a primer pair (5′-AGAATGAAGCAAGGAGCAT-3′ and 5′-CGGTGAAATTATCCCAAG T-3′; nucleotides 70 to 1498) selected from previously published sequences of this gene (10). Primer pairs previously reported were used to amplify fragments of the *vanB* (635 bp), *vanC1* (822 bp), and *vanC2*/*C3* (439 bp) ligase genes (9).

Four to five well-isolated colonies obtained after overnight growth on Trypticase soy agar were resuspended in lysis buffer (50 mM Tris-HCl [pH 8.0], 50 mM NaCl, 5 mM EDTA [pH 8.0]). Lysostaphin was added at a final concentration of 20 μg/ml, and the suspension was incubated at 37°C for 30 min. Proteinase K was then added to a final concentration of 1 mg/ml, and the suspension was incubated at 50°C for 60 min, followed by incubation at 100°C for 10 min. Processed samples were used immediately for PCR or stored at 4°C until they were used.

The PCR amplification mixture consisted of reaction buffer (final concentration, 2 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl [pH 8.3]), 0.2 mM (each) dATP, dCTP, dTTP, and dGTP, 0.1 μM *vanA* primer pair, 0.5 μM (each) *vanB*, *vanC1*, and *vanC2*/*C3* primer pairs, 1.20 U of *Taq* polymerase (Perkin-Elmer), and 1.0 μl of enterococcal DNA in a total volume of 10.0 μl. DNA amplification was carried with a GeneAmp PCR System 9600 thermal cycler (Perkin-Elmer). The cycling conditions consisted of an initial denaturation step at 94°C for 1 min and then 30 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min. In the final cycle, the extension step was carried out at 72°C for 10 min. A reagent blank (containing all the components of the reaction mixture with water instead of target DNA), positive controls for each *van* genotype (*E. faecium* 228 [*vanA*], *E. faecalis* ATCC 51299 [*vanB*], *E. gallinarum* [*vanC1*], and *E. casseliflavus* [*vanC2*/*C3*]) and a negative control (*E. faecalis* ATCC 29212) were run in every PCR procedure. Control strains were kindly provided by Gregory Tyrrell (University Hospital, Edmonton, Alberta, Canada).

Amplified PCR products were detected by agarose gel electrophoresis with 2% Nusieve gels which were stained with ethidium bromide. A ϕX174/*Hae*III marker (Gibco BRL, Gaithersburg, Md.) was run with each gel, and the VRE genotype was determined by the size of the amplified product.

Pulsed-field gel electrophoresis (PFGE) typing. Enterococcal genomic DNA was prepared in agar plugs as described by McCarthy et al. (21). *Sma*I (New England Biolabs, Beverly, Mass.) digests of genomic DNA were electrophoresed in 1.2% agarose gels in 0.5× TBE buffer (0.089 M Tris-HCl, 0.089 M boric acid, 0.0025 M EDTA) with a CHEF DR III System (Bio-Rad). Bacteriophage lamda concatemers (Lambda Ladder PFG Marker; New England Biolabs) were run as size controls. Electrophoresis was carried out for 20 h, with switch times ramped between 0.5 and 12 s at an applied voltage of 6 V/cm. The gels were stained in ethidium bromide, destained in distilled water as necessary, and photographed under UV illumination. Restriction fragment profiles were visually compared and interpreted based on guidelines recommended by Tenover et al. (27). Isolates with DNA restriction profiles that differed by 4 or more bands were considered to be different strains.

Screening of contacts. From November 1995 to June 1996, the infection control service identified hospital contacts of any patient colonized or infected with a VANC VRE. To document any secondary transmission, stool or rectal swabs were obtained from roommates and/or ward contacts for VRE screening whenever possible. Prior to this screening, the index cases had not received any additional infection control precautions beyond body substance isolation, which was the normal practice at our institution.

Case-control study. A case-control study was performed to determine if there were any risk factors associated with colonization or infection with VANC VRE. The cases included all patients infected or colonized with VANC VRE. Controls were randomly chosen patients who were negative for VANC VRE screening but who were from the same ward or outpatient area of the hospital as the cases. The controls were matched for the month screening took place and the type of specimen being screened. The hospital records of cases and controls were reviewed, and the following information was recorded: age, sex, hospital service, underlying disease, invasive procedures performed, duration of hospitalization prior to screening, clinical outcome, and antimicrobial agents received within the last 3 months. Colonization was defined as the isolation of VANC VRE from stool or from a nonstool site without signs or symptoms of infection. Infection was present if VANC VRE was isolated from a nonstool site in association with signs and symptoms of infection. VANC VRE was considered to be community acquired if cultures were positive upon admission or within the first 72 h of admission or if the patient was an outpatient.

Statistical analysis. Groups were compared by χ^2 analysis with Yates' correction or Fisher's exact test. Statistical comparisons of means were made by Student's *t* test.

RESULTS

VANC VRE were recovered from 30 of 444 (6.8%) patients whose stools were submitted for *C. difficile* testing and, during a 1-week period, from 4 of 157 (2.5%) patients in areas of the hospital with high vancomycin use. This resulted in an overall VANC VRE stool colonization rate of 5.7%. We also detected one patient colonized with a VANA VRE and one colonized with a VANB VRE. Both isolates were identified as *E. faecium* and were detected during the screening of a stool submitted for *C. difficile* testing. In comparison, 5 of 100 outpatients in the community who had submitted stools for routine culture carried VANC VRE in their gastrointestinal tracts, but none were positive for a VRE of either the *vanA* or the *vanB* genotype.

From 1 January to 30 September 1996, 724 nonstool isolates of enterococcus were recovered from clinical specimens from 538 patients. No VRE of the *vanA* or *vanB* genotype were detected in these clinical specimens. However, 10 (1.4%) VANC VRE isolates were recovered from nine patients (1.7%). This group included two patients with bacteremia, both cases occurring after a bone marrow transplant, with subsequent fatal outcomes. The other cases included three patients with polymicrobial wound infections, one patient with urosepsis complicating hydronephrosis, and one patient with spontaneous bacterial peritonitis who eventually died of hepatic failure. Of the other two patients, one was colonized with VANC VRE in the urine and one was colonized in a wound.

Overall, 38 patients at our hospital were identified as colonized or infected with VANC VRE (five patients had both a stool and a nonstool isolate). The first 25 of these 38 patients were identified during the period of November 1995 to June 1996. Two of the 25 patients were among the 90 hemodialysis patients from the artificial kidney unit whose stools were screened. The other 23 patients included 17 patients identified only by stool screening and 6 patients with nonstool isolates. Nine of these patients had no hospital roommate or a roommate who had died or been discharged from the hospital. Stools or rectal swabs from 82 roommates or ward contacts of the remaining 14 inpatients (10 of whom had diarrhea) were obtained and screened for VRE. The ward contacts included patients from the adult intensive-care, hematology, oncology, and bone marrow transplant units. From these 82 contacts, we detected six patients who were colonized with VANC VRE and one carrying a VANA VRE isolate.

TABLE 1. Characteristics of VANC VRE

Species (no. of isolates)	No. motile	No. pigmented	Genotype	MIC (mg/liter, range) of:	
				Vancomycin	Teicoplanin
Enterococcus gallinarum (37)	34	0	*vanC1*	2.0–8.0	<0.125–1.0
Enterococcus casseliflavus (5)	5	5	*vanC2/C3*	4.0	0.25–0.5
Enterococcus flavescens (3)	3	3	*vanC2/C3*	4.0	0.25–0.5
Enterococcus mundtii (1)	0	1	*vanC2/C3*	4.0	0.5
Probable *Enterococcus gallinarum*[a] (2)	2	0	*vanC1*	4.0	0.25–0.5

[a] These isolates would have been identified as *Enterococcus gallinarum* phenotypically, but they were raffinose negative.

To determine whether there was any genetic relatedness among the VANC VRE isolates, typing by PFGE was performed. All six VANC VRE isolates from the contacts were different from each other and the isolates recovered from the index patients. In fact, all VANC VRE isolates from different patients in this study were also unique. In contrast, 7 of 8 patients who had multiple isolates recovered from different stool specimens carried the same VANC VRE strain for periods of up to 157 days. Three different VANC VRE strains were recovered from the other patient's stools 28 to 30 days apart. In comparison, from November 1995 to March 1996, 14 patients in the intensive-care unit were infected or colonized with ampicillin-resistant enterococci (ARE). PFGE typing of 10 of these patient isolates showed that 9 were the same strain.

VANC VRE isolates from 48 of the 49 patients or contacts (including the 5 patients identified through screening of 100 outpatients in the community) were available for detailed analysis (Table 1). The other isolate was a motile, nonpigmented strain with a vancomycin MIC of 12 mg/liter by E test that was not saved by the laboratory. There were 37 *E. gallinarum* isolates, 5 *E. casseliflavus* isolates, 3 *E. flavescens* isolates, and 1 *E. mundtii* isolate. The other two isolates possessed the *vanC1* gene and would have been identified as *E. gallinarum* had they not been raffinose negative. The vancomycin and teicoplanin MICs for the isolates are summarized in Table 1.

The clinical features of the patients found to be colonized or infected with VANC VRE at our hospital are listed in Table 2. There was no significant difference in any of these features between cases and controls. There were trends toward a greater proportion of patients who had received a broad-spectrum cephalosporin (34 versus 16%, $P = 0.09$) or vancomycin (23 versus 9%, $P = 0.15$) and for admissions to the intensive-care unit during hospitalization (36 versus 18%, $P = 0.09$) among the cases compared to controls. Of the 40 stool carriers receiving care at our hospital, 13 had a positive stool culture for VANC VRE within 3 days of admission or were outpatients.

DISCUSSION

Vancomycin resistance in enterococci has become a major therapeutic and infection control challenge, especially in centers where the organism has become endemic among high-risk patients (22). In order to facilitate earlier efficient containment of the organism, the identification of colonized patients and certain measures have been recommended for use in hospitals where VRE have not yet been detected or remain rare. These measures include screening of all enterococci from clinical specimens for vancomycin resistance, the periodic surveillance of stools or rectal swabs from patients at risk (e.g., patients in the intensive-care unit), and the passive surveillance of stool samples submitted to the laboratory for *C. difficile* testing (15). Although the optimal method of screening stools for VRE has not yet been established, laboratories commonly utilize media supplemented with vancomycin (4). As the vancomycin MICs for some strains of VANB VRE may be in the susceptible or intermediate range, screening is frequently done with media supplemented with vancomycin concentrations as low as 6 mg/liter (20). This is the concentration of vancomycin that is recommended by the National Committee for Clinical Laboratory Standards for screening isolates of enterococci for vancomycin resistance (23). As a result, enterococci intrinsically resistant to vancomycin may be detected upon screening, and a decision must be made about the implications for infection control (14, 26).

Our results confirm that VANC VRE are infrequently recovered from clinical specimens. We were able to detect *vanC* genes in 1.4% of enterococcal isolates. Similarly, Ruoff reported that 2% of 302 consecutive isolates of enterococci from clinical specimens were identified as either *E. gallinarum* or *E. casseliflavus* (25) with a conventional test scheme. In another study, of 705 enterococcal isolates collected from eight tertiary-care hospitals, 1.1% were found to be either *E. gallinarum* or *E. casseliflavus* (12). The vancomycin MICs for all of these

TABLE 2. Comparison of clinical features of patients infected or colonized with VANC VRE and controls ($n = 44$)

Clinical feature	Cases (%)	Controls (%)[a]
Age in years (mean ± standard deviation)	55.6 ± 20.3	55.0 ± 18.5
Males/females	21/23	20/24
Infection	8 (18)	6 (14)
Community-acquired infection	15 (34)	NA[b]
Days hospitalized prior to screening (mean ± standard deviation)	18.7 ± 13.6	13.9 ± 13.3
Services		
Medical	32 (73)	31 (70)
Surgical	12 (27)	13 (30)
Underlying diseases		
Malignancy	20 (45)	16 (36)
Hematologic malignancy	8 (18)	6 (14)
Diabetes mellitus	3 (7)	3 (7)
Chronic renal failure	5 (11)	6 (14)
AIDS	5 (11)	2 (5)
Invasive procedures		
Central lines	9 (20)	8 (18)
Urinary tract	16 (36)	10 (23)
Abdominal or pelvic surgery	9 (20)	8 (18)
Admission to intensive-care unit during hospitalization	16 (36)	8 (18)
Death	8 (18)	4 (9)
Antibiotics received		
Any	38 (86)	33 (75)
Vancomycin	10 (23)	4 (9)
Narrow- or expanded-spectrum cephalosporin	10 (23)	17 (39)
Broad-spectrum cephalosporin	15 (34)	7 (16)
Penicillin group	15 (34)	12 (27)
Aminoglycosides	17 (39)	14 (32)
Quinolones	12 (27)	7 (16)

[a] P was >0.05 for all comparisons between cases and controls.
[b] NA, not applicable.

strains were between 2 and 8 mg/liter. However, although they are infrequently isolated, we and others have documented that VANC VRE can cause serious invasive infections, including bacteremia (14, 16).

It appears that approximately 5% of the outpatients in our community were colonized with VANC VRE in their gastrointestinal tracts. Thus, we found similar rates among outpatients in our community and patients who were hospitalized or receiving care at our hospital. Unfortunately, this study did not allow us to obtain more clinical information on the outpatients colonized with VANC VRE. Nevertheless, these results suggest that some patients in the community carry these organisms and bring them into the hospital upon admission. In fact, one-third (13 of 40) of the patients receiving care at our hospital who carried the organism in their stools were shown to have the organism upon admission or as an outpatient. In our hospital, less than 30% of the stools submitted for *C. difficile* testing come from outpatients or from inpatients within 72 h of admission (unpublished data). Perhaps a higher proportion of the patients at our hospital carrying the organism in their stools would have been shown to be colonized upon admission if they had been screened then rather than when *C. difficile* testing was requested.

It is possible that our results underestimate the actual carriage rate of VANC VRE. Blaimont et al. reported that 8.4% of patients carried *E. gallinarum* and 10.5% carried *E. casseliflavus* (3). We did not identify all of the enterococci isolated from stools to the species level but identified only isolates that grew on media supplemented with vancomycin. However, it has been shown that 90 to 100% of *E. gallinarum* isolates will grow on media supplemented with 6 mg of vancomycin per liter, including strains for which vancomycin MICs are as low as 2 mg/liter (5, 26, 30).

The source or reservoir of VANC VRE remains unknown. We did not investigate this, but a possibility is the food chain, since *E. gallinarum* and *E. casseliflavus* have been isolated from farm animals and fresh chickens obtained from grocery stores in the United States (7, 28). European studies have also reported the isolation of *E. faecium* with the *vanA* genotype from farm animals, especially poultry, although this finding has not been noted in studies from the United States (2, 7, 17, 28).

A risk factor for colonization or infection with VANC VRE could not be identified, again supporting the possibility that these organisms are part of the normal stool flora of the general population. It is interesting that although the MICs of vancomycin for these enterococci are normally higher than usual, the majority of patients with VANC VRE had not received any vancomycin during the previous 3 months; this contrasts with VANA or VANB VRE, where vancomycin use has been frequently reported as a risk factor for their acquisition (13, 15, 22).

Although most VANC VRE-colonized or -infected patients had diarrhea, we were unable to document any secondary transmission between patients. A number of contacts did carry the organism, but the strains they carried differed from the strains carried by the index patient. In contrast, during a 5-month period, 9 of 10 patients with ARE in the intensive-care unit were infected or colonized with the same strain, suggesting nosocomial transmission between patients. In fact, this strain was one of the two endemic ARE strains in our hospital that we have reported on previously (21).

There have not yet been any reported outbreaks involving VANC VRE, and the *vanC* genes are not known to be transferable. These facts and our findings collectively suggest that additional infection control precautions, such as those recommended for VANA and VANB VRE, may not be necessary in the management of patients with VANC VRE. The reason VANC VRE are not readily transmitted between patients, in contrast to VANA, VANB VRE, or even ARE, is not clear. Although we did not perform quantitative cultures, patients colonized with VANA or VANB VRE may shed higher numbers of enterococci than patients with VANC VRE and therefore may be more likely to have potential for nosocomial spread (7, 32). Other virulence factors also may enable VANA and VANB VRE to survive on environmental surfaces for longer periods of time than VANC VRE, thereby allowing dissemination of the organisms among patients.

The major implication of these results for the microbiology laboratory is the need to accurately and quickly differentiate VANC VRE from strains that carry either the *vanA* or the *vanB* ligase gene. This differentiation has major cost implications from an infection control perspective. Although PCR is probably the most accurate method of testing for these genes, it is not yet readily available or easily incorporated into a routine diagnostic laboratory. Determination of MICs of vancomycin by the E test would be helpful but would not discriminate between low-level VANB VRE and VANC VRE. Testing for motility is simple and can discriminate between these strains, but it may take up to 3 days and is medium dependent (33). Nonmotile strains of *E. gallinarum* or *E. casseliflavus* have also been reported, and we encountered three such strains (31, 33). Species identification by conventional methods would also be helpful, but few laboratories use these methods routinely, relying instead on commercial identification methods, which may misidentify the species (13). Recently, the use of a methyl-α-D-glucopyranoside reagent has shown promising results and may prove valuable (8). At present, a combination of these methods, such as an E test MIC with either motility or acidification of methyl-α-D-glucopyranoside, may be the best approach for laboratories which use routine procedures.

In summary, VANC VRE are infrequently recovered from clinical specimens but may cause serious invasive infections. They may colonize a minority of patients, but they do not appear to have the same implications for infection control as do the VANA or VANB VRE.

ACKNOWLEDGMENTS

We thank C. Oxley, B. Clark, and D. Leclerc for statistical data and other staff at the Microbiology Laboratory at the Ottawa General Hospital, A. Thibeault of MDS Laboratories, F. Chan for reviewing the manuscript, and F. Huot for secretarial support.

REFERENCES

1. **Arthur, M., and P. Courvalin.** 1993. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob. Agents Chemother. **37:**1563–1571.
2. **Bates, J., J. Z. Jordens, and D. T. Griffiths.** 1994. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J. Antimicrob. Chemother. **34:**507–516.
3. **Blaimont, B., J. Charlier, and G. Wauters.** 1995. Comparative distribution of Enterococcus species in faeces and clinical samples. Microb. Ecol. Health Dis. **8:**87–92.
4. **Boyce, J. M.** 1995. Vancomycin-resistant enterococci: pervasive and persistent pathogens. Infect. Control Hosp. Epidemiol. **16:**676–679.
5. **Cartwright, C. P., F. Stock, G. A. Fahle, and V. J. Gill.** 1995. Comparison of pigment production and motility tests with PCR for reliable identification of intrinsically vancomycin-resistant enterococci. J. Clin. Microbiol. **33:**1931–1933.
6. **Centers for Disease Control and Prevention.** 1994. Addressing emerging infectious disease threats: a prevention strategy for the United States. Morbid. Mortal. Weekly Rep. **43:**1–18.
7. **Coque, T. M., J. F. Tomayko, S. C. Ricke, P. C. Okhyusen, and B. E. Murray.** 1996. Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. Antimicrob. Agents Chemother. **40:**2605–2609.
8. **Devriese L. A., B. Pot, K. Kersters, S. Lauwers, and F. Haesebrouck.** 1996.

Acidification of methyl-α-D-glucopyranoside: a useful test to differentiate *Enterococcus casseliflavus* and *Enterococcus gallinarum* from *Enterococcus faecium* species group and from *Enterococcus faecalis*. J. Clin. Microbiol. **34:**2607–2608.
9. **Dutka-Malen, S., S. Evers, and P. Courvalin.** 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. **33:**24–27.
10. **Dutka-Malen, S., C. Molinas, M. Arthur, and P. Courvalin.** 1990. The VANA glycopeptide resistance protein is related to D-alanyl-D-alanine ligase cell wall biosynthesis enzymes. Mol. Gen. Genet. **224:**364–372.
11. **Facklam, R. R., and M. D. Collins.** 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27:**731–734.
12. **Gordon, S., J. M. Swenson, B. C. Hill, N. E. Pigott, R. R. Facklam, R. C. Cooksey, C. Thornsberry, Enterococcal Study Group, W. R. Jarvis, and F. C. Tenover.** 1992. Antimicrobial susceptibility patterns of common and unusual species of enterococci causing infections in the United States. J. Clin. Microbiol. **30:**2373–2378.
13. **Gordts, B., H. Van Landuyt, M. Ieven, P. Vandamme, and H. Goossens.** 1995. Vancomycin-resistant enterococci colonizing the intestinal tracts of hospitalized patients. J. Clin. Microbiol. **33:**2842–2846.
14. **Green, M., K. Barbadora, and M. Michaels.** 1991. Recovery of vancomycin-resistant gram-positive cocci from pediatric liver transplant recipients. J. Clin. Microbiol. **29:**2503–2506.
15. **Hospital Infection Control Practices Advisory Committee. Centers for Disease Control and Prevention.** 1995. Recommendations for preventing the spread of vancomycin resistance. Infect. Control Hosp. Epidemiol. **16:**105–113.
16. **Kaplan, A. H., P. H. Gilligan, and R. R. Facklam.** 1988. Recovery of resistant enterococci during vancomycin prophylaxis. J. Clin. Microbiol. **26:**1216–1218.
17. **Klare, I., H. Heier, H. Claus, R. Reissbrodt, and W. Witte.** 1995. VanA-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol. Lett. **125:**165–172.
18. **Leclercq, R., and P. Courvalin.** 1997. Resistance to glycopeptides in enterococci. Clin. Infect. Dis. **24:**545–556.
19. **Leclercq, R., S. Dutka-Malen, J. Duval, and P. Courvalin.** 1992. Vancomycin resistance gene *vanC* is specific to *Enterococcus gallinarum*. Antimicrob. Agents Chemother. **36:**2005–2008.
20. **Lior, L., M. Litt, J. Hockin, C. Kennedy, B. A. Jolley, M. Garcia, G. Gillis, A. Humar, I. Campbell, J. Brunton, H. Dedier, and J. Conly.** 1996. Vancomycin-resistant enterococci on a renal ward in an Ontario hospital. Can. Commun. Dis. Rep. **22:**125–128.
21. **McCarthy, A. E., G. Victor, K. Ramotar, and B. Toye.** 1994. Risk factors for acquiring ampicillin-resistant enterococci and clinical outcomes at a Canadian tertiary-care hospital. J. Clin. Microbiol. **32:**2671–2676.
22. **Morris, J. G., Jr., D. K. Shay, J. N. Hebden, R. J. McCarter, Jr., B. E. Perdue, W. Jarvis, J. A. Johnson, T. C. Dowling, L. B. Polish, and R. S. Schwalbe.** 1995. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Ann. Intern. Med. **123:**250–259.
23. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
24. **Navarro, F., and P. Courvalin.** 1994. Analysis of genes encoding D-alanine-D-alanine ligase-related enzymes in *Enterococcus casseliflavus* and *Enterococcus flavescens*. Antimicrob. Agents Chemother. **38:**1788–1793.
25. **Ruoff, K. L., L. De La Maza, M. J. Murtagh, J. D. Spargo, and M. J. Ferraro.** 1990. Species identities of enterococci isolated from clinical specimens. J. Clin. Microbiol. **28:**435–437.
26. **Swenson, J. M., N. C. Clark, M. J. Ferraro, D. F. Sham, G. Doern, M. A. Pfaller, L. B. Reller, M. P. Weinstein, R. J. Zabransky, and F. C. Tenover.** 1994. Development of a standardized screening method for detection of vancomycin-resistant enterococci. J. Clin. Microbiol. **32:**1700–1704.
27. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
28. **Thal, L. A., J. W. Chow, R. Mahayni, H. Bonilla, M. B. Perri, S. A. Donabedian, J. Silverman, S. Taber, and M. J. Zervos.** 1995. Characterization of antimicrobial resistance in enterococci of animal origin. Antimicrob. Agents Chemother. **39:**2112–2115.
29. **Van Horn, K. G., C. A. Gedris, and K. M. Rodney.** 1996. Selective isolation of vancomycin-resistant enterococci. J. Clin. Microbiol. **34:**924–927.
30. **Van Horn, K. G., C. A. Gedris, K. M. Rodney, and J. B. Mitchell.** 1996. Evaluation of commercial vancomycin agar screen plates for detection of vancomycin-resistant enterococci. J. Clin. Microbiol. **34:**2042–2044.
31. **Vincent, S., R. G. Knight, M. Green, D. F. Sahm, and D. M. Shlaes.** 1991. Vancomycin susceptibility and identification of motile enterococci. J. Clin. Microbiol. **29:**2335–2337.
32. **Whitman, M. S., P. G. Pitsakis, E. DeJesus, A. J. Osborne, M. E. Levison, and C. C. Johnson.** 1996. Gastrointestinal tract colonization with vancomycin-resistant *Enterococcus faecium* in an animal model. Antimicrob. Agents Chemother. **40:**1526–1530.
33. **Willey, B. M., W. Marshall, T. Mazzulli, P. Lo, B. Mustachi, A. Gill, M. Skulnick, R. Davidson, A. McGeer, and D. E. Low.** 1996. Evaluation of methods for the detection of enterococcal motility, abstr. C-378, p. 68. *In* Abstracts of the 96th General Meeting of the American Society for Microbiology, 1996. American Society for Microbiology, Washington, D.C.