Fecal carriage of vancomycin-resistant enterococci in hospitalized patients and those living in the community in the Netherlands


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Fecal Carriage of Vancomycin-Resistant Enterococci in Hospitalized Patients and Those Living in the Community in The Netherlands


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In order to determine the prevalence of vancomycin-resistant enterococci (VRE) in The Netherlands, 624 hospitalized patients from intensive care units or hemato-oncology wards in nine hospitals and 200 patients living in the community were screened for VRE colonization. Enterococci were found in 49% of the hospitalized patients and in 80% of the patients living in the community. Of these strains, 43 and 32%, respectively, were Enterococcus faecium. VRE were isolated from 12 of 624 (2%) and 4 of 200 (2%) hospitalized patients and patients living in the community, respectively. PCR analysis of these 16 strains and 11 additional clinical VRE isolates from one of the participating hospitals revealed 24 vanA gene-containing, 1 vanB gene-containing, and 2 vanC1 gene-containing strains. All strains were cross-resistant to avoparcin but were sensitive to the novel glycopeptide antibiotic LY333328. Genotyping of the strains by arbitrarily primed PCR and pulsed-field gel electrophoresis revealed a high degree of genetic heterogeneity. This underscores a lack of hospital-driven endemicity of VRE clones. It is suggested that the VRE in hospitalized patients have originated from unknown sources in the community.

Enterococcus spp. have recently emerged as important nosocomial pathogens (35). According to the data from the National Nosocomial Infections Surveillance System, enterococci are the fourth leading cause of nosocomial infections in the United States (12). Enterococcal infections that have frequently been reported include urinary tract infections, bactere mia, endocarditis, intra-abdominal infections, and surgical wound infections (27). Enterococcus faecalis is commonly isolated from the human gastrointestinal tract, whereas Enterococcus faecium is less frequently isolated from that site (31). This latter species, however, is noted for its antimicrobial resistance. Vancomycin-resistant E. faecium (VREF) strains have emerged in a setting of increasing high-level resistance of enterococci to penicillins and aminoglycosides (28). During the last few years, nosocomial outbreaks due to VREF have been described (17, 25). The emergence of VREF has raised serious concerns (28), and in response, the Hospital Infections Control Practices Advisory Committee (HICPAC), in collaboration with the Centers for Disease Control and Prevention, has developed recommendations for preventing the spread of vancomycin-resistant enterococci (VRE) (18). Given the concern that vancomycin resistance genes may transfer from enterococci to Staphylococcus aureus, a phenomenon that has been observed in vitro (31), control measures have already been proposed, should vancomycin-resistant S. aureus strains eventually arise (10).

The microbiology laboratory has an important role in the detection, reporting, and control of VRE. The HICPAC document emphasizes the need for routine susceptibility testing of all enterococci isolated from clinical specimens. Furthermore, in hospitals where VRE have not yet been detected, periodic culture surveys of stools or rectal swabs of patients at high risk for VRE infection or colonization are indicated (18). In The Netherlands, no systematic study has been done to evaluate the prevalence of VRE infection or colonization in hospitalized patients or patients living in the community. Therefore, the present study was started to determine the prevalence of fecal carriage of VRE in hospitalized patients with an increased risk for infection or colonization with VRE and in patients living in the community. We determined the susceptibility of VRE to vancomycin, teicoplanin, avoparcin (a glycopeptide available throughout Europe as an additive in animal feed [15]), and LY333328 (a new glycopeptide antibiotic [37]). In order to determine the genetic basis of the glycopeptide resistance phenotype, PCR assays aimed at the various resistance genes were performed. Moreover, the VRE were typed by pulsed-field gel electrophoresis (PFGE), arbitrarily primed PCR (AP-PCR), and ribotyping to determine the degree of genetic relatedness of this group of resistant microorganisms.

MATERIALS AND METHODS

Prevalence study. Five Dutch university hospitals in Rotterdam, Utrecht, Nijmegen, and Amsterdam and four regional teaching hospitals in Breda and Tilburg participated in the study. Six hundred twenty-four patients who were hospitalized in the following wards were screened for gastrointestinal carriage of VRE: medical and surgical intensive care unit (ICU), thoracic surgical ICU, neurological and neurosurgical ICU, pediatric ICU (either surgical, neonatal, or general pediatric), and hemato-oncology wards. The prevalence study was carried out in November 1995 and February 1996. In addition, 200 outpatients attending general practitioners for diarrhea were screened. For this latter group
of patients no information was available on prior antibiotic exposure. Early and empiric treatment of bacterial diarrhea in patients living in the community is uncommon practice in The Netherlands.

**Bacterial isolates.** Sixteen strains of VRE isolated during the prevalence study were included in this study. Fourteen of these isolates were isolated at Hospital A (hospital A) and one strain was obtained from the interval between the prevalence study, had the *vanE* genotype, and 2 had the *vanC1* genotype, and 2 had the *vanC2* [9] were used in the assays (50 pmol of each primer per reaction mixture). Amplification of DNA was performed in a Biomed model 60 thermocycler by using pre-denaturation at 94°C for 4 min, followed by 40 cycles of 1 min at 94°C, 1 min at 56°C, and 2 min at 74°C. Banding patterns were visualized after electrophoresis on a 1% agarose gel containing ethidium bromide in the presence of a 100-bp ladder. Banding patterns were interpreted by visual inspection. Different types were identified on the basis of even a single differentiating DNA fragment. Differences in ethidium bromide staining intensity were ignored.

**PFGE.** Ten colonies of an overnight culture grown on blood agar were suspended in 100 μl of EET buffer (100 mM sodium EDTA, 10 mM EGTA, 10 mM Tris-HCl [pH 8.0]). This suspension was mixed with 100 μl of 1% agarose (Incert agarose; FMC Bioproducts Corp., Rockland, Maine), and the mixture was transferred into sample plug molds (final agarose concentration, 0.5%). The plugs were incubated for 4 h at 37°C in 1 ml of EET buffer containing 100 mg/liter lysozyme (Sigma Chemical Co., St. Louis, Mo.). This lysis solution was replaced by a 1-ml EET buffer solution containing 1 mg of proteinase K and 1% sodium dodecyl sulfate for a further overnight incubation at 37°C. The plugs were washed six times (30 min each time at room temperature) in TE solution (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). To digest the DNA, a 5-mm slice of the sample plug was placed in a TE solution (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA) with 40 U of SmaI (Boehringer GmbH), and the mixture was incubated overnight at 25°C. The plugs were loaded onto a 1% agarose gel (SeaKem GTG agarose; FMC) in 0.5× TBE (Tris, borate, EDTA) (32). Electrophoresis was performed with a CHEF DR II apparatus (Bio-Rad, Richmond, Calif.) programmed in the auto-algorithm mode (block 1, run time of 8 h and switch time of 0.5 to 1.5 s, block 2, run time of 10 h and switch time of 15 to 30 s). The gels were stained with ethidium bromide for 15 min and were destained in distilled water for 1 h before photography. All gels were inspected visually by two different investigators. Patterns were designated by a single capital letter and numbered so that a distinct pattern (difference of four or more bands) was obtained. Isolates with identical profiles were assigned the same letter as the differences with the other glycopeptide agents tested.

Three hundred six (49%) of the 624 hospitalized patients and 161 (80%) of 200 diarrheic patients living in the community carried enterococci in their gastrointestinal tracts (P < 0.01). Of the 306 enterococci isolated from hospitalized patients, 132 (43%) were identified as *E. faecium*. Of 161 enterococci from patients outside the hospital, 52 (32%) were identified as *E. faecium* (P < 0.05). Thus, *E. faecium* was isolated from 132 of 624 (21%) of the hospitalized patients and 52 of 200 (26%) of the patients living in the community (P < 0.05). VRE were isolated from 12 (2%) of the 624 hospitalized patients and 4 (2%) of the 200 patients living in the community. Fifteen VRE were identified as *E. faecalis*. Fifteen (8%) of 184 *E. faecium* strains isolated in the prevalence study were vancomycin resistant. In addition, 11 strains of VRE were isolated in hospital A at times separate from the period of the prevalence study. Nine were identified as *E. faecium*, and two were identified as *E. gallinarum*. Thus, 27 strains of VRE were available for further studies.

The susceptibilities of the 27 strains of VRE to vancomycin, teicoplanin, avoparcin, and LY333328 and the resistance genotype are presented in Table 1. Complete cross-resistance between vancomycin and avoparcin was found. LY333328, however, was 250- to >1,000-fold more active than vancomycin against vanA VRE. Major discrepancies were observed between the MICs of LY333328 that were determined by the agar dilution method and those determined by the broth dilution method: on agar, the MIC of LY333328 at which 90% of isolates are inhibited (MIC90) for vanA VRE was 4 mg/liter (range, 0.25 to 4 mg/liter), whereas in broth the MIC90 was 0.5 mg/liter (range, 0.125 to 1 mg/liter). We did not observe such differences with the other glycopeptide agents tested. Four of the 27 strains of VRE, including all VRE from the prevalence study, had the vanA genotype; 1 had the vanB genotype, and 2 had the vanC1 genotype. For all vanA *E. faecium* isolates vancomycin MICs were >256 mg/liter and teicoplanin MICs were >64 mg/liter. For the vanB and vanC1
strains the vancomycin MIC was 8 mg/liter and the teicoplanin MIC was 0.5 mg/liter.

The restriction endonuclease patterns obtained by PFGE with SmaI for the 27 strains of VRE are presented in Fig. 1. An overview of all typing results is given in Table 2. The discriminatory power of AP-PCR with primers AP-7 and ERIC-1 was low compared to that with primers AP-1 and ERIC-2. Therefore, only the results of AP-PCR with primers AP-1 and ERIC-2 are presented in Table 2 (see also Fig. 2). Analysis of all 27 strains of VRE revealed 23 different patterns by PFGE.

TABLE 1. In vitro activities of four glycopeptide agents against 27 strains of VRE

<table>
<thead>
<tr>
<th>Strain group and no.</th>
<th>Species</th>
<th>MIC (mg/liter)</th>
<th>van genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Van</td>
<td>Tei</td>
</tr>
<tr>
<td>Clinical isolates</td>
<td></td>
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<td></td>
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<tr>
<td>10-a</td>
<td>E. faecium</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>10-b</td>
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<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>10-c</td>
<td>E. faecium</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>10-d</td>
<td>E. faecium</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>10-e</td>
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<td>&gt;256</td>
<td>128</td>
</tr>
<tr>
<td>10-f</td>
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<tr>
<td>10-g</td>
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<td>&gt;256</td>
</tr>
<tr>
<td>10-h</td>
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<td>&gt;256</td>
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<td>0.5</td>
</tr>
<tr>
<td>10-j</td>
<td>E. faecium</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>10-k</td>
<td>E. gallinarum</td>
<td>8</td>
<td>0.5</td>
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<td>Survey isolates</td>
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<td>&gt;256</td>
<td>&gt;256</td>
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<td>&gt;256</td>
<td>&gt;256</td>
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<td>&gt;256</td>
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<td>12-o</td>
<td>E. faecium</td>
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</tr>
<tr>
<td>62-α</td>
<td>E. faecium</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

a In vitro activities were determined by a standard NCCLS broth dilution method.
b Van, vancomycin; Tei, teicoplanin; Avo, avoparcin. The breakpoints for vancomycin and teicoplanin defined by NCCLS are 4 and 8 mg/liter, respectively. Breakpoints have not yet been defined for avoparcin and LY333328.
c Strains isolated in hospital A.

FIG. 1. Restriction endonuclease patterns obtained by PFGE with SmaI for 27 strains of VRE isolated from hospitalized patients and patients living in the community in The Netherlands. From left to right, the strains appear in the lanes in the same order in which they are listed in Tables 1 and 2. Each strain has a two-digit, one-letter code corresponding to the location (1, Rotterdam; 2, Amsterdam; 3, Breda; 4, Utrecht; 5, Nijmegen; 6, community), the screening (0, routine isolates from hospital A; 1, prevalence study of November 1996; 2, prevalence study of February 1997), and strain letter code corresponding to the order of the strains listed in Tables 1 and 2. A 50-kb ladder (Bio-Rad, Veenendaal, The Netherlands) is shown in the lane on the right as a molecular size standard.
17 by AP-PCR analysis with primers AP-1 and ERIC-2, and only 7 by ribotyping (see Fig. 3 for some examples). Some strains that were indistinguishable by AP-PCR were unrelated by PFGE (e.g., strains 21-p and 12-o). Vice versa, AP-PCR was able to distinguish strains that appeared to be highly related by PFGE (e.g., strains 32-u and 62-a).

PFGE of 15 strains from hospital A yielded 15 different patterns. PFGE of 16 strains of VRE from the prevalence study (including four strains from hospital A) yielded 12 different patterns. Five strains isolated from patients hospitalized in three different hospitals appeared to be closely related (Table 2). These five strains could be divided into two different subtypes by AP-PCR. Therefore, the combination of PFGE and AP-PCR demonstrated genetic unrelatedness for 13 of 16 strains of VRE from the survey.

DISCUSSION

The rapid emergence of resistance in enterococci and the increasing incidence of colonization and infection with VRE have become health care issues that have caused serious concern to physicians and health authorities alike (18).

This study documents the prevalence of intestinal colonization of selected patients from ICUs and hemato-oncology wards as well as of general practice patients in the Netherlands. Enterococci were found in 49% of the inpatients and 80% of the outpatients. This proportion of hospitalized patients who carry enterococci is lower than that found in previous studies, in which 75 to 90% of the patients carried these microorganisms (31, 36). These latter studies screened unscreened hospitalized patients. One can speculate as to whether greater use of penicillins like amoxicillin or the amoxicillin-clavulanic acid combination may have occurred in our selected group of patients and, thus, may have influenced the prevalence of enterococci isolated from the gastrointestinal tract.

We isolated \textit{E. faecium} from 21% of the inpatients and 26% of the outpatients, which is in agreement with previous findings of \textit{E. faecium} in 20 to 40% of stool cultures (3, 31). VRE were isolated from 2% of the patients living in the community. Several European studies have reported similar frequencies in the community (21, 22). However, a much higher frequency has been reported in a Belgian study (42). In the latter study, 11 (28%) of 40 volunteers living in the community who were healthy, who were not health care workers, and who had not received antibiotics for at least 1 year were colonized with...
VRE. The results of North American studies performed in the Houston, Texas, metropolitan area, however, contrast with the European data, since VRE appeared to be absent from healthy people in Houston (5). The level of colonization with VRE in people in the community in Europe parallels the level of colonization of animals with these resistant organisms (6). Several studies have now reported the absence of VRE from animals and people in the community in the United States, in contrast to the high frequencies in hospitals (5, 23, 39). Some investigators, however, have cautioned against comparing the results of the studies mentioned above, since differences in methodology could, at least in part, explain the observed differences in isolation rates (5).

Since ICU patients and patients in oncology wards were found to be at increased risk for infection or colonization with VRE (18), we decided to select these patients for our inpatient survey. The rate of isolation of VRE from these hospitalized patients was 2% and, therefore, was similar to the rate of isolation from outpatients. This is roughly in agreement with the results of a recent Belgian study, in which it was shown that 3.5% of hospitalized patients were carriers of VRE (16). In Finland, Suppola et al. (36) investigated hospitalized patients with hematological malignancies and reported a prevalence of VRE of 2%.

We analyzed the genetic relatedness of the 27 VRE strains by PFGE and AP-PCR. In previous studies PFGE has been shown to be the most discriminating technique for typing isolates of VRE, and this technique is now considered the “gold standard” (24, 25). Recently, however, AP-PCR has proven to be a powerful typing tool as well. Results of PFGE and AP-PCR are often in concordance (2). In our study, however, PFGE was more discriminatory than AP-PCR. Combining the data generated by the two methods, we demonstrated the genetic unrelatedness of 13 of 16 strains of VRE isolated during the survey and of all 15 strains that were isolated in hospital A. No evidence of major inter- or intrahospital spread of VRE in The Netherlands exists. This observation is remarkable since no special infection control measures for preventing the transmission of VRE were in place in the participating hospitals at the time of the survey. Together with the observed isolation rate of 2% for the patients living in the community, it is suggested that VRE in hospitalized patients may have originated from unknown sources in the community. The gastrointestinal tract is probably the major reservoir in humans, from which subsequent infection can eventually develop. This is in agreement with a recent report from New York City (32). Food has been proposed as a source (8, 28). Others have put forward pets and other domestic animals (6, 41). Furthermore, the use of antibiotics as feed additives for growth enhancement in animals may be associated with the emergence of VRE (22). An example of such a growth-promoting agent is avoparcin, a drug that has been used in The Netherlands for a long time. The production of pigs, poultry, and calves is an area of important economic activity in The Netherlands. To date, The Netherlands country is one of the leading exporters of consumer poultry at the retail level is colonized with VRE, possibly as a result of the unrestricted use of avoparcin in the poultry industry (43). Thus, the use of oral glycopeptide antibiotics in the animal production industry should be strongly discouraged. Recently, the European Community committed itself to a cautious approach and banned all use of avoparcin as feed additive in animals by 1 April 1997 (12a).

The emergence of VRE has resulted in an increase in the incidence of infections that are caused by these organisms and that cannot be treated with currently available antimicrobial agents (19). LY333328 is a new semisynthetic glycopeptide that has been reported to have increased activity against vancomycin-resistant gram-positive microorganisms (30). In our study, LY333328 was found to possess greatly enhanced activity against VRE. In general, the MICs of LY333328 were 25- to 1,000-fold lower than those of vancomycin. These data are in agreement with those presented in an earlier report (37). Surprisingly, the MICs of LY333328 obtained by an agar dilution method were four- to eightfold higher than those obtained by a broth dilution method, but we do not have an explanation for these discrepancies. This phenomenon has recently been reported by others (20). The results, however, indicate that LY333328 is a promising new drug that deserves further evaluation. In conclusion, we have shown in a multicenter study that, first, VRE can be isolated from hospitalized patients and patients living in the community in The Netherlands at a frequency of 2%. Second, these strains appear to be unrelated, and therefore, no evidence of major inter- or intrahospital spread of VRE strains in The Netherlands exists. Third, our data suggest that VRE are acquired outside the hospital environment. Further studies are warranted to elucidate the origin and the epidemiology of vancomycin resistance. In countries with large populations of animal livestock, including The Netherlands, where large quantities of feed additives are used, it seems wise to strongly discourage the use of oral glycopeptides not only in humans but in the animal production industry as well.

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REFERENCES


43. Vliegenthart, J. Personal communication.