Prolonged Colonization with Vancomycin-Resistant *Enterococcus faecium* in Long-Term Care Patients and the Significance of "Clearance"

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Little is known about the persistence of colonization with vancomycin-resistant *Enterococcus faecium* (VRE) in the nononcologic, non–intensive care unit patient. We studied all patients who had VRE isolated on ⩾2 occasions of >1 year apart (Study A) and those who had been "cleared" of VRE colonization after 3 negative stool cultures (Study B). Twelve patients had stored VRE isolates recovered >1 year apart (Study A), and 58% of paired isolates were genotypically related according to pulsed field gel electrophoresis patterns. In Study B, stool samples were obtained weekly from 21 "cleared" patients for 5 weeks, which revealed that 24% were VRE positive. For these culture-positive patients, 72% of the cultures failed to detect VRE. Recent antibiotic use was significantly more common in the culture-positive patients, as compared with culture-negative patients ($P = .003$). Colonization with VRE may persist for years, even if the results of intercurrent surveillance stool and index site cultures are negative. Cultures for detection of VRE in stool samples obtained from patients declared "cleared" are insensitive.

Enterococci have become major nosocomial pathogens, being isolated from 12% of nosocomial cultures [1, 2]. They are intrinsically resistant to multiple antimicrobial agents and often acquire resistance to other drugs [3]. Vancomycin-resistant enterococci (VRE) were first identified as hospital-associated pathogens in Europe during the mid-1980s [4] and have since rapidly disseminated. Until recently, therapeutic options have been very limited [5–7]. Concern about the spread of this resistant determinant is heightened by the possibility of more virulent pathogens, such as *Staphylococcus aureus,* acquiring it [8].

In Europe, VRE have been found in the food chain and colonizing a variety of domestic and farm animals [9–12]. In addition, healthy individuals who live in those communities may carry VRE in their gastrointestinal tract without ill effect [13]. In the United States, however, VRE transmission is a perinosocomial process [14]. The gastrointestinal tract is the primary reservoir for enterococci, although skin colonization has been shown in severely ill patients with diarrhea [15–17]. Once an individual is colonized with VRE, there is no

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known effective means of definitive eradication [18, 19]. Colonization may persist for months [20, 21]. Given that VRE have not been found in the food chain in the United States, special emphasis has been placed on controlling nosocomial spread [22, 23].

The 1995 Hospital Infection Control Practices Advisory Committee guidelines suggest that patients colonized with VRE may be removed from isolation precautions when 3 consecutive surveillance cultures, performed ⩾1 week apart, did not yield VRE [22]. However, how effectively such data exclude colonization or predict the likelihood that a patient is no longer an environmental contamination risk has not been rigorously studied [24–26]. Of concern is the recent observation that barrier methods, such as gloves, are only partially protective in the prevention of hand colonization [27]. It is not clear what the optimal culturing technique is for VRE. Most microbiology laboratories use an agar-based system for VRE stool screening. However, one study that compared 5 selective media for the detection of VRE in stool samples found that a broth-enhanced technique detected 88% of positive cultures and was significantly more sensitive than are agar techniques [28].

Our Veterans Administration (VA) Medical Center provided a unique opportunity to study long-term gastrointestinal tract colonization in patients with VRE. In this institution, VRE isolates have been saved since 1993, and ~70% of patients are long-term residents of the nursing home or psychiatric long-term care services. This situation permitted us to study the clonal relationships of isolates recovered sequentially from patients colonized for >1 year (Study A) and to detect stool colonization by use of sensitive techniques in previously colonized patients who had been declared "clear" of VRE on the basis of repeatedly negative surveillance cultures performed using standard methods (Study B).

METHODS

Institution

Our VA medical center has 6 geographically separate structures used for patient care. From 1993 through 1997, the patient census decreased from ~850 to 500 patients, of which the acute-care patient population decreased from ~180 to 120. The majority of our patients require long-term care for such conditions as spinal cord injury or psychiatric disorders.

Case Detection

Study A. All VRE cultured from patients have been preserved since the first isolate was identified at our institution in 1993. All patients with 2 isolates of VRE recovered >1 year apart were identified from infection control records. These isolates had been collected either for clinical reasons or as part of a point prevalence survey by infection control staff. Data were available for each patient, including age, sex, comorbid illness, medications (including detailed antibiotic use), acute hospitalizations, and microbiologic information.

Study B. All patients deemed "cleared" (defined as 3 negative index site and 3 negative stool cultures obtained during a period of ⩾3 weeks) of VRE were identified from infection control and microbiology records. None of these patients had received specific therapy in an attempt to eradicate VRE. Cultures from this cohort of patients were performed once per week for 5 weeks from May to July 1998, by use of both the institutional standard VRE culturing technique and a broth-enrichment technique. Cultures were coded in the microbiology laboratory in order to blind the investigators and infection control personnel, given the unclear infection control significance if low-level colonization with VRE was found. In a blinded fashion, detailed patient information was obtained for the 12 months preceding VRE "clearance" and the 6 months preceding this study. Institutional Review Board approval was obtained.

Statistics

RRs, 95% CIs, and 2-tailed Fisher's exact tests were calculated for univariate comparisons within the cohort. Given the small number of subjects, no multivariate analyses were performed.

Laboratory Methods

For Study A, the initial and most-recent isolates of VRE and isolates from each intervening time period that a patient had a positive VRE culture were analyzed (up to 6 isolates per patient). For Study B, rectal swabs were obtained weekly for each "cleared" patient for 5 weeks. These swabs were plated on PV agar (phenylethylene alcohol [PEA] with 16 μg/mL of vancomycin), PVG agar (PEA with 16 μg/mL of vancomycin and 16 μg/mL of gentamicin), and the tips broken off into EVAN broth (Enterococcosel broth with 4 μg/mL of vancomycin, 64 μg/mL of aztreonam, 16 μg/mL of gentamicin, and 64 μg/mL of nalidixic acid; Becton Dickinson Microbiology Systems) [28, 29].

Bacterial identification was determined by means of biochemical properties (API 20 Strep system; bioMérieux Vitek), *aac(6′)-Ii* gene probe specific for *E. faecium,* or both [30, 31]. DNA was extracted by use of a modified colony lysis technique. Probes for *aac(6′)-Ii, vanA,* and *vanB2* were used as described elsewhere [32]. Antibiotic susceptibilities (MICs) were performed and interpreted according to the criteria of the National Committee for Clinical Laboratory Standards [33].

Pulsed Field Gel Electrophoresis (PFGE)

The preparation of the enterococcal DNA was adapted from the method described by Goering [34]. The prepared DNA was digested with *Sma*I at 24°C for 16 h. Electrophoresis was performed by use of a contour-clamped homogeneous field ap-

Table 1. The pulsed field gel electrophoresis gel clonal types of the 42 isolates recovered from the 12 patients in Study A. For an individual patient, a particular subclone of the 2 dominant genotypes was maintained.

Genotype	No. of patients (n = 12)	No. (%) of isolates (n = 42)
A[a]	7	29 (69)
B[b]	5	7 (17)
C	2	2 (5)
D	1	1 (2)
E	1	1 (2)
F	1	1 (2)
G	1	1 (2)
H	1	1 (2)

[a] Twelve subtypes.
[b] Four subtypes.

paratus (CHEF; BioRad). The gels were stained with ethidium bromide and imaged (BioRad Image System). *S. aureus* 519 (*Sma*I-digested DNA) was used as a molecular standard, in lanes 1, 8, and 15, on each gel, to facilitate across gel comparisons. PFGE criteria as described by Tenover [35] were used to determine clonality.

RESULTS

Study A

Two hundred seventy-nine patients became colonized with VRE from March 1993 through August 1997. Many patients resided in the VA long-term care facility or were long-term users of the acute-care service. We identified a group of 12 patients (4.3% of 279) who had at least 2 VRE-positive cultures >1 year apart. At the time of the initial positive VRE culture, these patients had a mean age of 69 years, had spent significant time using the acute-care service, and had received multiple courses of antibiotic therapy during the 6 months prior to VRE isolation. In addition, these patients typically had multiple comorbid illnesses, including diabetes mellitus (in 4 [33%] of 12 patients), chronic obstructive pulmonary disease (in 4 [33%]), chronic renal failure (in 2 [17%]), psychiatric illness (in 7 [58%]), and coronary artery disease (in 8 [66%]); also, they were often colonized with other resistant pathogens, such as methicillin-resistant *S. aureus* (in 7 [58%]) or *Clostridium difficile* (in 5 [42%]). Only 1 patient had an active oncologic process. Three of these 12 patients had been "cleared" of VRE at one time but subsequently became culture positive at 16, 18, and 19 months after "clearance."

Microbiology. From these 12 patients, 42 enterococcal isolates (2–6 isolates per patient) were evaluated from the stored collection. These 42 isolates were initially obtained from stool samples (27 isolates), urine samples (11), wound specimens (2), a soft-tissue biopsy specimen (1), and a blood sample (1). All were confirmed to be *E. faecium*. All isolates had an MIC of vancomycin of >256 μg/mL. All isolates carried the *vanA* gene and were resistant to teicoplanin (MIC_{50}, 64 μg/mL and MIC_{90}, 256 μg/mL) and ampicillin (MIC_{50}, 128 μg/mL and MIC_{90}, 256 μg/mL). Eighty-six percent of the isolates were highly resistant to gentamicin, and 64% demonstrated high-level resistance to streptomycin.

Molecular analysis. As shown in table 1, eight clonal groups were identified; 29 (69%) of the 42 strains belonged to 1 genotypic pattern with 12 subtypes. An additional 7 isolates (17%) belonged to a second clonal group. Molecular analyses of these 42 patient isolates confirmed that 7 (58%) of 12 patients remained colonized with an isolate genotypically related to their initial isolate. The time interval between the initial and most-recent VRE isolate in each of these 7 patients was 12 (for 3 patients), 16, 18, 27, and 38 months. Even though 69% of the isolates belonged to the same PFGE pattern, a given subclone was unique to a given patient (figure 1). None of the

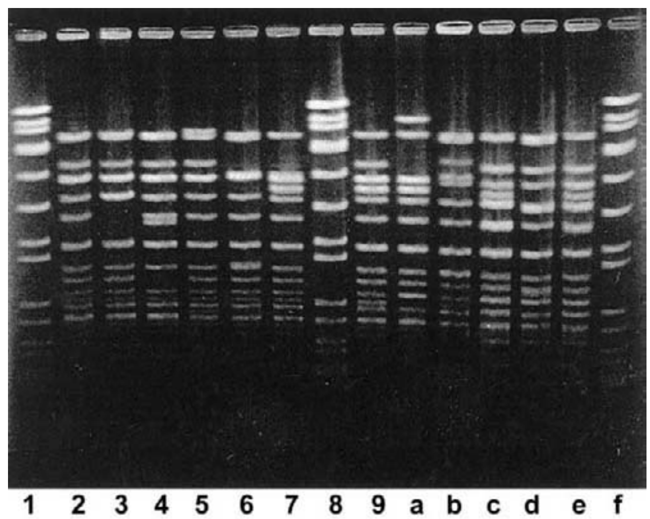

Figure 1. Pulsed field gel electrophoresis gel containing 12 strains of vancomycin-resistant enterococci (VRE) in chronological order from when they were identified, June 1993 *(lane 2)* to June 1996 *(lane e)* and 3 controls *(Staphylococcus aureus* 519, *lanes 1, 8,* and *f)*. The 12 strains of VRE are from 5 different patients, with the following strains isolated from the same patient, identified at different times: *lanes 2* and *3* (June and July 1993); lanes *6, 7,* and *b* (November 1993 and January and October 1994); and lanes *9, a, c, d,* and *e* (March and April 1994, February and May 1995, and June 1996). It is important to note that even though all 12 strains represent the same clonotype, strains from a given patient maintain a subclonal relationship. In addition, if the strains in *lanes a* and *d* were compared, they would be considered to be genotypically distinct; however, in the presence of the other isolates recovered from this patient *(lanes 9, c,* and *e)*, the relatedness is apparent. The isolates from *lanes 9* and *e* were recovered from the same patient 27 months apart.

patients with a persistent clone had evidence for a second clonal type. Of the 5 patients (41%) who did not maintain a particular clonal type over time, 2 had 3 different PFGE patterns, and 3 patients had 2 different PFGE patterns identified.

Study B

Fifty-two (19%) of the 279 colonized patients had previously been "cleared" of VRE. Of these 52 patients, 9 (17%) had died, 19 (37%) were outpatients, 3 (6%) had already had recrudescence and were discussed in study A (2 of these 3 recrudescing isolates were genotypically related to the patient's historical isolate), and 21 (40%) were available for further study. The initial sites of VRE detection in these patients were samples of urine (for 15 patients), stool (for 3), skin (for 2), and sputum (for 1). None of these 21 patients received any antibiotics in attempts to facilitate clearance of VRE. One patient did receive a short course of doxycyline during the 6 months that preceded VRE "clearance." Samples were obtained from this cohort of 21 patients for prospective culture once per week for 5 consecutive weeks, with only 1 patient missing 1 culture, for a total of 104 cultures. These patients had a mean age of 76 years and multiple comorbid medical conditions; however, only 4 patients (19%) had a remote history of malignancy (1 of whom received therapy for prostate cancer and 3 of whom had cancer in remission).

Five (24%) of these 21 patients were found to harbor VRE by stool culture that used the broth enrichment technique. One patient had a positive culture on 3 of the 5 samples, and the other 4 VRE-positive patients had VRE in only 1 of their 5 cultures. If we assume that these 5 patients did not become colonized with VRE during the 5 weeks of this study, then of the 25 cultures of samples obtained from these 5 VRE-positive patients, only 7 (28%) yielded VRE. The standard agar technique detected VRE in only 5 (71%) of 7 positive cultures and 3 (60%) of 5 patients who proved to be colonized by the more sensitive broth enrichment method.

During the 6 months prior to "clearance" of VRE, 18 (86%) of 21 patients had received antibiotics. In the month prior to clearance, 7 patients (33%) received antibiotics, 2 (10%) received broad-spectrum agents (ticarcillin-clavulanate or piperacillin), and 5 (24%) received narrow-spectrum antibiotics (such as trimethoprim-sulfamethoxazole, cephalexin, amoxicillin, erythromycin, or dicloxacillin).

In the 6 months prior to obtaining prospective cultures in Study B, there were no significant differences between the VRE relapsing and nonrelapsing groups with respect to age, sex, acute hospitalizations, antibiotic use, comorbid illness, time since first positive VRE culture, or time since they were deemed VRE "cleared," as shown in table 2. During that period, all 5 patients with relapsing VRE received antibiotics with significant gram-negative or anaerobic activity, compared with 14 (88%) of 16 patients with VRE that did not relapse. There was a significant difference between groups with respect to antibiotic use within 1 month of the study: 5 (100%) of 5 patients who relapsed and 3 (31%) of 16 patients who did not relapse had received antibiotics ($P = .003$; to enable mathematical computation, 1 was added to each group, given that none of the patients who relapsed received antibiotics; approximate RR, 8.8; 95% CI, 1.2–62.3).

Microbiology. The antibiotic susceptibilities of these isolates were similar to those found in Study A (MIC_{50} and MIC_{90} of ampicillin, 64 μg/mL; MIC_{50} and MIC_{90} of teicoplanin, 128 μg/mL; and all MICs of vancomycin, >256 μg/mL). High-level resistance to gentamicin or streptomycin occurred in 71% and 77% of strains, respectively. All isolates were *E. faecium;* phenotypically and genotypically, they were *vanA*.

Molecular analyses. Two of these 5 patients had isolates that were genotypically related to the patient's historical isolate (recovered 18 and 60 months previously), whereas the 3 other isolates appeared to be new to the respective patient (12, 18, and 21 months later). Thus, 2 (9.5%) of 21 VRE-"cleared" patients had evidence suggestive of persistent colonization for up to 5 years, and an additional 3 (14.3%) of 21 VRE-"cleared" patients were colonized with a new strain of VRE.

DISCUSSION

The VRE-colonized patient presents a significant challenge to the nosocomial environment. In addition to the morbidity and excess costs of caring for patients who are infected with these organisms, costs associated with infection-control practices are substantial [23, 36]. As an example, the culturing of samples obtained from patients in Study A was driven by clinical and infection-control considerations. The studies presented here provide insight into the colonization dynamics of VRE in medical patients receiving long-term care who have modest comorbid disease and who have been treated in accordance with current guidelines to minimize nosocomial transmission [22].

The first phase of our study revealed that at least 12 (4%) of 259 patients were culture positive >1 year after initial isolation of a VRE strain. Because the great majority of patients did not undergo a second culture, it is likely that the true prevalence of VRE culture positivity was substantially higher. In addition, our data indicate that the majority (58%) of patients who remain positive for VRE by stool culture for >1 year maintain a genotypically related strain. These observations extend what has been reported elsewhere, with persistent VRE carriage identified during the course of months in an oncologic population [21]. Although there was 1 predominant clone circulating at our institution at the time of the present study, it is unlikely that the patients in this report became reinfected over time for 2 reasons: (1) these patients were in geographically distinct wards, and (2) a given patient maintained a specific

Table 2. Characteristics of the cohort of patients "cleared" of their vancomycin-resistant enterococci (VRE) during the 6 months prior to the current study (Study B).

Characteristic	Patients who had relapse (n = 5)	Patients who did not have relapse (n = 16)	RR (95% CI)	P
Time after first positive VRE culture, mean days ± SD	775 ± 491	1070 ± 467		
Duration of clearance, mean days ± SD	650 ± 529	555 ± 567		
No. (%) of patients who received antibiotics				
During the 6 months prior to study period	5 (100)	14 (88)		
During the 1 month prior to study period	5 (100)	3 (19)	8.8 (1.2–62.3)[a]	0.003[a]

[a] The value of 1 was added to allow calculation of a RR, 95% CI, and P value.

VRE subclone population. We cannot exclude, however, recurrent reinoculation of individual patients with their own subclone excreted into their immediate environment.

Our analysis of these patients illustrates the limitations of PFGE criteria developed for the assessment of strain-relatedness in the outbreak setting [35] for evaluation of chronic carriage. By these criteria, isolates with 2–3-band differences are considered to be "closely related," those with 4–6-band differences are "possibly related," and a >6-band difference suggests that the strains are unrelated. As shown in figure 2, comparison of lanes *1* and *2* suggests that these 2 strains are unrelated (>6-band difference). However, in the context of the other VRE isolates recovered from this patient during a 27-month period, as seen in figure 1 (lanes *9, a, c, d,* and *e*), a molecular evolution and genomic relatedness of these isolates is suggested.

The results from Study B show that, once colonized with VRE, a significant proportion of patients either remain colonized or reacquire this organism (silently) but may be culture positive for at least 5 years after initial colonization. The cohort of 21 patients in Study B is only a subset of the 52 VRE-"cleared" patients, because the other 31 patients either died (9 [17%]), were discharged (19 [37%]), or spontaneously relapsed (3 [6%]); however, they do present the typical institutionalized medical patient who represents a significant infection-control challenge. Although this small cohort is poorly powered to detect differences between patients who once again became culture positive and those who did not, the significant difference between the VRE–culture-positive and -negative patients, with respect to recent antibiotic use, is intriguing and supports the observations of Tokars et al. [37] and Donskey et al. [38]. This suggests that antibiotic pressure increases the burden of VRE shed in the stool. The implications of this observation are that our measurements of colonization probably underestimate the true prevalence of colonization in those patients who did not receive antibiotics and that antibiotic use may exacerbate the infection-control risk of previously colonized patients who had been deemed "cleared" of gastrointestinal colonization. It is disconcerting that only 1 of the 5 VRE-positive patients in Study B was culture positive on >1 weekly sample. Although it is possible that the 4 patients with only 1 positive VRE culture were only transiently colonized, it is more likely that even our broth-enrichment technique failed to detect VRE 80% of the time and that the routine agar method was even less sensitive. Thus, surveillance cultures of asymptomatic patients receiving long-term care probably would fail to detect VRE the majority of the time. It may be possible to employ even more-sensitive detection methods than the ones we used, such as cultures of a large volume of stool, but such techniques would be impractical for routine clinical use.

What are the implications of failure to detect VRE stool colonization in patients like those we have described? It could be argued that colonization with an organism burden below the limit of detection poses such minimal risks of transmission that the insensitivity of detection methods is of little practical consequence. On the other hand, when such patients require antibiotics, become acutely ill, or develop diarrhea, the burden of organisms may increase. In those circumstances, individuals may become more efficient vectors of environmental contamination and transmission. We believe that this possibility should be kept in mind, even for patients deemed "cleared" of VRE on the basis of 3 negative stool surveillance cultures.

Finally, these data also provide insights into the evolution over time of the PFGE pattern of strains that colonize individual patients in the long-term care setting. In figure 1, lanes *9* and *a* demonstrate the impact of a single genetic event that leads to a 3-band change. Lanes *1* and *2* in figure 2 demonstrate the perils of comparing 2 isolates from a given patient separated by 13 months, because these 2 isolates have a >6-band difference; thus, they would be considered unrelated by the most commonly accepted criteria [35]. However, when placed in the context of the other VRE isolates from this patient (as seen in figure 1, lanes *a* and *d*), the genomic relationships become clear, and all of the isolates recovered from this patient appear to be related. This example highlights the difficulties of applying cri-

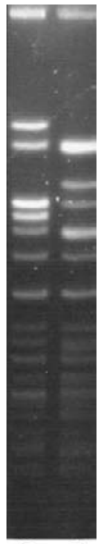

Figure 2. Molecular evolution of vancomycin-resistant enterococci during a 27-month period in a given patient. These isolates are the same as *lanes a* and *d* in figure 1. By traditional pulsed field gel electrophoresis criteria, these 2 isolates would be considered to be unrelated. In the context of the other isolates from this patient (*lanes 9, c,* and *e* in figure 1), these isolates are most likely genetically related.

teria designed for the acute outbreak setting (where time for genetic events to occur has not elapsed) as the de facto criteria for the assessment of relatedness in the long-term care environment, as emphasized by Tenover et al. [35]. Criteria for determining clonal relationships specific to the long-term care setting need to be developed. Possible solutions include changing the definition of "possibly related" strains to 9 bands (which increases the risk of identifying spurious genetic relationships), use of nucleoside sequence–based technologies, or perhaps the use of phylogenetic analyses.

These data demonstrate that (1) patients who receive long-term medical care, even without oncologic disease, may shed a genotypically related VRE strain for at least 5 years, (2) despite multiple negative VRE cultures (>3), approximately one-quarter of patients remain colonized with VRE, (3) current culture techniques may miss VRE detection the majority of the time, (4) recent antibiotic use leads to increased detection of VRE excretion, and (5) PFGE criteria for determining VRE genetic relationships in the long-term care setting must be developed, because the criteria that have been developed for acute outbreak settings may be misleading in the former situation.

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