

## An Outbreak of Vancomycin-Dependent *Enterococcus faecium* in a Bone Marrow Transplant Unit

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Outbreaks of vancomycin-resistant enterococci (VRE) are well described. The presence of mutants of VRE, such as vancomycin-dependent enterococci (VDE), in individual patients has been documented, but their potential to spread nosocomially has not been known. We present the first cluster of patients who acquired VDE nosocomially. Five bone marrow transplantation patients were infected or colonized by a genotypically indistinguishable multiantibiotic-resistant strain of *Enterococcus faecium*. Vancomycin dependence in 3 of the 5 isolates was demonstrated. All cluster patients had received protracted prophylactic treatment with vancomycin (mean, 22.6 days), and specimens from  $\geq 2$  body sites were repeatedly culture-positive for the outbreak strain. The outbreak was controlled with aggressive infection control strategies, and prophylactic antibiotic policies were revised. Awareness of the potential for nosocomial spread of multiantibiotic-resistant VDE is vital for the care of immunocompromised patients, especially those receiving prophylactic antibiotics.

Vancomycin-resistant enterococci (VRE), described in 1986, have emerged as important nosocomial pathogens. Numerous outbreaks have shown that the organisms are transferred from patient to patient on the hands of health care workers or by contaminated objects. The concern among health care professionals has been that organisms such as VRE, which are easily transmitted, could share resistance genes with other genera.

Vancomycin-dependent enterococci (VDE) were first isolated in 1993 [1], from the urine of a woman receiving long-term therapy with intravenous vancomycin. Derived from VRE, these mutants grow only in the presence of the glycopeptide antibiotic, and their clinical significance has not been well described. However, the ease of nosocomial spread of antibiotic-resistant VRE, coupled with the extensive use of vancomycin in immunocompromised populations, has led to concerns regarding the spread of VDE among patients treated with vancomycin.

We report the first nosocomial outbreak of multiantibiotic-resistant, vancomycin-dependent *Enterococcus faecium* involving neutropenic bone marrow-transplantation (BMT) patients receiving prophylactic vancomycin. Outbreak isolates were indistinguishable by DNA fingerprinting techniques. The strain,

including the vancomycin-dependent phenotype, was demonstrated to persist in surveillance cultures of specimens from the outbreak patients. New cases were not detected after changes in vancomycin utilization, and heightened infection control measures were implemented. This cluster reemphasizes the need for laboratory surveillance for VRE and VDE and the judicious use of prophylactic antibiotics.

### Methods

**Hospital and surveillance.** The Johns Hopkins Hospital is a 940-bed urban tertiary care center that serves the city of Baltimore and the surrounding area. The oncology center houses 64 patients on 4 nursing units. Two of these units (A and B) are geographically connected with 27 service beds (private rooms), which house BMT patients as well as some medical oncology patients. At the time of the outbreak, on the basis of oncology service protocol, all BMT patients received prophylactic antibiotics (vancomycin, norfloxacin, fluconazole, and acyclovir), beginning 2 days prior to BMT and continuing until resolution of neutropenia.

The prophylactic use of vancomycin in our oncology population began in 1989, as an effective method to prevent infection with viridans streptococci in BMT patients [2]. Penicillin replaced vancomycin as prophylaxis in 1993 because of the recognition of VRE and the desire to decrease the use of vancomycin. But a marked increase in bacteremia due to viridans streptococci following severe chemotherapy-induced mucositis, despite penicillin prophylaxis, prompted the reinstitution of vancomycin prophylaxis in 1994 (personal communication; C. Miller, Johns Hopkins Oncology Center, Baltimore, MD). Beginning in 1980, surveillance cultures of stool, throat specimens, and urine have been performed twice weekly on all leukemia and BMT patients housed in the oncology center. To

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detect resistant organisms in this population, use of culture media containing vancomycin and gentamicin began in 1983.

**Control of VRE.** At the time of the outbreak, hospital policy required placement of a chlorhexidine-based product at hand sinks in the rooms of patients colonized or infected with VRE, who were isolated. Equipment was dedicated to each patient's room, and all health care workers and visitors used gowns and wore gloves for patient contact. Rooms were terminally cleaned when a VRE colonized/infected patient was discharged. Environmental cultures were performed to identify environmental sources of outbreak organisms.

**Microbiology.** All environmental specimens and surveillance specimens from patients in the oncology center were plated onto VRE surveillance plates (selective trypticase soy agar with 5% sheep blood, vancomycin [10 µg/mL], and gentamicin [8 µg/mL]). Culture plates were incubated at 37°C in CO<sub>2</sub> and read 24 and 48 h later. Colonies that stained as gram-positive cocci or coccobacilli were tested for hydrolysis of PYR (L-pyrrolindoyl-betanaphthylamide; Murex Diagnostics, Darford, England). The species of PYR-positive organisms were then determined biochemically, according to the scheme of Fracklam and Collins [3].

Susceptibility to a panel of antibiotics, including vancomycin, was performed on Mueller-Hinton II agar by the agar dilution method (1, 2, 4, 16, and 64 µg/mL). Enterococci for which the MIC of vancomycin was >16 µg/mL but <64 µg/mL were further tested by Etest (AB Biodisk, Solna, Sweden). Enterococci for which the MIC of vancomycin was ≥32 µg/mL were considered resistant to vancomycin. In subcultures to blood agar media with and without vancomycin, isolates were considered VDE if growth occurred only in the presence of vancomycin. The presence of VDE was confirmed by growth around a standard vancomycin disk plated on Mueller-Hinton II agar containing 5% sheep blood (Kirby-Bauer method) [4, 5]. Antibiotic susceptibilities of VDE were also determined by the Kirby-Bauer method, with the addition of vancomycin (10 µg/mL). VRE isolates were stored at -70°C in trypticase soy broth with 5% glycerol.

Pulsed-field gel electrophoresis (PFGE) was performed to determine the molecular relatedness of the organisms. Organisms from frozen stock cultures were grown on blood agar media with vancomycin (10 µg/mL). To confirm purity of the isolates, 5 distinct colonies in each culture of VDE were individually streaked to blood agar media with and without vancomycin. Growth from media with vancomycin was sufficient for PFGE extraction. Because of insufficient growth of organisms in the absence of vancomycin, single vancomycin-independent colonies were subcultured back to vancomycin-containing media. DNA was then extracted and digested with the restriction enzyme *Sma*I, according to the method described by Murray et al. [6].

Macrorestriction fragments were separated on a 1% agarose gel in 0.5× Tris-Borate-EDTA with use of a CHEF-DR II apparatus (Bio-Rad, Richmond, CA), with a ramped pulse time from 5 to 30 s for 24 h at 200 V and 14°C. A molecular weight standard, strain *Enterococcus faecalis* OG1RF [7], was digested with 20 units of *Not*I restriction enzyme and was included on each gel. Controls representing the most prevalent hospital strain of VRE (vanA) and a distinct isolate of VRE detected on oncology unit A were also included on the gel. Following electrophoresis, gels were stained with ethidium bromide, photographed, and compared visually.

Vancomycin-resistance genotype was determined by PCR. Primer sequences for detection of *vanA* and *vanB* genes were selected from published sources [8, 9]. Primer sequences were as follows: *vanA*-1, 5'-AATGGGAAAACGACCATTGC; *vanA*-3, 5'-GGATAGGTAAACGTAGCTGC; *vanB*-1, 5'-CCCTATGTAGGCTGCGAT; and *vanB*-2, 5'-TATCGGGTGCTTGGATGC. These primer sets generated a 328-bp *vanA* product and a 504-bp *vanB* product. A 50-µL PCR reaction mix containing deoxyribonucleotides (200 µM), KCl (50 mM), Tris-Cl (10 mM, pH of 8.3), MgCl<sub>2</sub> (1.5 mM), *vanA* or *vanB* primers (5 µM), *Taq* polymerase (0.25 U/mL; Perkin-Elmer, Foster City, CA), and template DNA (20 µL) was used. Enterococcal template DNA was extracted with the In-stage DNA Purification Matrix (Bio-Rad).

PCR cycling conditions consisted of 3-min denaturing at 95°C, 35 1-min cycles of denaturing at 94°C, annealing at 50°C, and extension at 72°C. Cycling was completed with a final 5-min extension at 72°C. The *vanA*-containing strain *E. faecium* 228 [10], *vanB* strain *E. faecalis* V583 [9], and vancomycin-susceptible ATCC 29212 were included as controls.

PCR products were analyzed on 4% NuSieve (FMC Bioproducts, Rockland, ME) agarose gels containing ethidium bromide for ultraviolet illumination. To confirm the VanB phenotype, susceptibility to teicoplanin was determined by the broth macrodilution method, with 2-fold dilutions 0.5–64 µg/mL.

Serial dilution was performed to determine spontaneous reversion of vancomycin dependence back to vancomycin independence, by the method described by Dever et al. [11] and Fraimow and Jungkind [12]. The organisms were diluted from a 0.5 McFarland turbidity standard, equivalent to 10<sup>8</sup> organisms, and serially plated on trypticase soy agar plates with and without 10 µg/mL of vancomycin. The reversion rate was calculated by comparison of the numbers of colonies on plates with and without vancomycin.

## Results

**Outbreak description.** In the 8 months prior to the outbreak, no VRE or VDE were detected in surveillance cultures performed on oncology unit A. In February 1997 an organism consistent with VRE grew in a culture of blood from a BMT patient. Over the next 3 weeks, surveillance cultures of stool from 4 additional patients yielded similar VRE-like organisms (table 1). None of the patients had been colonized with VRE or VDE prior to the outbreak period.

All patients had been admitted for BMT for leukemia or lymphoma and were housed on the same hospital floor but not in adjacent rooms. Outbreak patients were on 1 nursing unit and were being cared for by a single medical team. Despite profound neutropenia in 4 of 5 patients, none of the patients experienced signs associated with an acute infection, including fever, hypotension, tachypnea, or tachycardia, at the time of culture positivity.

No graft-versus-host disease or bacteremia with a gram-positive organism secondary to mucositis was found. Complications related to antibiotic use, such as *Clostridium difficile* infection, were not found. All 5 BMT patients received prophylactic antibiotics on the basis of protocol. Apart from the

index patient, whose prophylactic vancomycin had been withdrawn 17 days before the positive culture, the remaining 4 patients were receiving vancomycin when their first surveillance culture for the outbreak strain was performed. All 5 patients received therapy with vancomycin, which began 2 days before BMT, for an average of 22.6 days (range, 5–46 days).

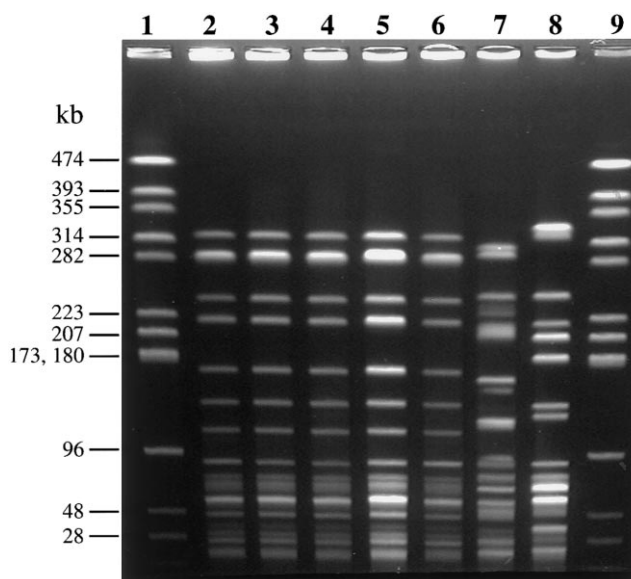
Three of the 5 patients also received broad-spectrum antibiotic therapy with a third-generation cephalosporin, imipenem, or ciprofloxacin before positive cultures were identified (table 1). As was the policy at the time, none of the patients had vancomycin withdrawn as a direct result of a surveillance culture positive for VRE. However, for 4 of 5 patients, vancomycin had been withdrawn before strict infection control precautions were instituted. All patients survived to be discharged, and no changes in clinical course due to infection or colonization with the outbreak strain were identified.

**Control measures.** For each patient, contact precautions were instituted with the first positive culture. After the first 3 cases, special precautions were instituted for contact with patients, which included handwashing, gowning, and gloving on room entry and handwashing on room exit. Patient, family, and staff education was undertaken, and strict enforcement of patient isolation was reemphasized. The entire unit A was isolated after recognition of 2 additional cases.

With the availability of microbiological data demonstrating an outbreak strain and the presence of vancomycin dependence in some isolates, the adjacent nursing unit (unit B) was also isolated in accordance with guidelines to prevent the spread of VRE. Medical equipment was cleaned between patients, and dedicated equipment was used for each patient. No further new isolates of the outbreak strain were identified after these stringent control measures were instituted.

Sixteen environmental cultures were performed on unit A, and none yielded an *Enterococcus* species. During this period, however, 1 additional patient whose surveillance culture yielded VRE was identified on unit A. The patient was known to be colonized with VRE before admission to the oncology ward. By PFGE, the isolate was found to be unrelated to the outbreak strain.

In addition to infection control measures and in conjunction with national guidelines, the policy to administer vancomycin



**Figure 1.** Pulsed-field gel electrophoretic profile of *Sma*I-digested *Enterococcus faecium* genomic DNA, stained with ethidium bromide. Lanes 2–6, oncology outbreak patients 1–5, respectively. Lane 7, non-outbreak case from oncology unit A. Lane 8, unrelated, highly prevalent hospital strain, shown as a control. Lanes 1 and 9, *Enterococcus faecalis* OG1RF, digested with *Not*I as a molecular weight standard. Molecular weights are shown in kilobase pairs.

prophylactically to BMT patients was reviewed by the hospital epidemiology and infection control, pharmacy, and oncology services, and such administration was limited to the first 7 days after BMT. In addition, the oncology service restricted vancomycin use to specific therapy for serious gram-positive infections or bacteremia, based on the guidelines of the Hospital Infection Control Practices Advisory Committee.

**Microbiology results.** All 5 cluster isolates were identified as *E. faecium* and had the same *Sma*I macrorestriction DNA fingerprinting pattern by PFGE (figure 1). Three of the 5 isolates (from patients 2–4; table 1) did not grow in agar-dilution susceptibility testing and were subcultured to media with and without vancomycin. On plates without vancomycin, all 3 showed minimal growth, small colonies, and varied colony mor-

**Table 1.** Characteristics of patients in the oncology center (February 1997).

Patient no.	Patient's sex, age (y)	Diagnosis	BMT type	Organisms isolated	Duration of vancomycin therapy, d <sup>a</sup>	Broad-spectrum antibiotics used	ANC	First positive specimen <sup>b</sup>	Other positive specimen(s) <sup>b</sup>	No. of positive cultures <sup>b</sup>
1	M, 55	SCL	Allogeneic	VRE	32	Ceftazidime, imipenem	2300	Blood	Urine	2
2	F, 26	ALL	Autogeneic	VDE	46	Ceftazidime, ciprofloxacin	100	Stool	Urine	9
3	F, 41	SCL	Allogeneic	VDE	5	None	140	Stool	Urine	8
4	F, 36	LCL	Autogeneic	VDE	22	Ceftazidime	73	Stool	Urine, Hickman exit site	9
5	F, 57	AML	Autogeneic	VRE	8	None	0	Stool	Urine, blood	12

NOTE. ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; ANC, absolute neutrophil count; BMT, bone marrow transplant; LCL, large-cell lymphoma; SCL, small-cell lymphoma; VDE, vancomycin-dependent enterococci; VRE, vancomycin-resistant enterococci.

<sup>a</sup> Prior to first positive culture.

<sup>b</sup> Outbreak strain-positive.

phology. On plates with vancomycin, the isolates grew at all concentrations (1–64  $\mu\text{g/mL}$ ) and showed vigorous growth in the presence of  $\geq 10\text{-}\mu\text{g/mL}$  concentration of vancomycin.

Heterodependent growth rather than pure vancomycin dependence was noted: vancomycin-independent colonies were consistently found on subculture of a single colony of VDE to media without vancomycin. To confirm that the VDE were not multiple strains, in each of the 3 cases with VDE, PFGE was performed with 10 individual colonies (5 from media with vancomycin and 5 from media without vancomycin). All 30 colonies tested yielded the same PFGE pattern, demonstrating the same genotype for vancomycin-dependent and -independent colonies from a single isolate.

Serial dilution of VDE was performed to determine their spontaneous reversion to vancomycin-independent VRE; an average reversion rate of 1 in  $5 \times 10^4$  colonies was determined for the 3 VDE isolates. The 2 remaining isolates (from patients 1 and 5; table 1) grew well in agar dilution and were identified as VRE without vancomycin dependence.

All isolates were consistent with the VanB phenotype in teicoplanin susceptibility testing. PCR analysis yielded a 504-bp product for each of the 5 isolates, confirming the *vanB* genotype (data not shown). All isolates had identical antimicrobial susceptibility patterns and were resistant to all antibiotics tested (ampicillin, oxacillin, penicillin, cefazolin, tetracycline, clindamycin, and erythromycin).

Subsequent to performance of the initial outbreak cultures, 35 cultures were performed for the 5 outbreak patients during their hospital courses for reasons pertaining to surveillance or patient care. All these cultures yielded *E. faecium*. Culture samples included stool, urine, blood, and a specimen from a Hickman catheter exit site. All these isolates demonstrated the same antibiotic resistance pattern as that of the outbreak strain and were presumed to represent ongoing colonization with the outbreak strain. Eight of the 35 isolates (from 3 of the 5 outbreak patients [patients 2, 4, and 5]; table 1) had been stored in the microbiology lab at  $-70^\circ\text{C}$ .

Genomic DNA from all 8 isolates was tested by PFGE and was found to have the same macrodigestion pattern as the outbreak strain (data not shown). Growth of the 8 isolates on Mueller-Hinton II agar with 5% sheep blood and a vancomycin disk demonstrated the persistence of vancomycin dependence in 1 isolate (from patient 2) 47 days after the original isolation of VDE. The VDE originally isolated from patient 4 were found to have lost vancomycin dependence when isolated from stool culture 2 months later. No nonoutbreak strains were isolated.

## Discussion

We present the first description of an outbreak of VDE suggesting nosocomial spread. The outbreak strain involved 5 BMT patients who had received extensive vancomycin prophylaxis; 4 patients initially colonized in the stool, and 1 patient

with bacteremia. In subsequent surveillance cultures, colonization with this strain persisted; it was recovered from the urine of all patients and from the blood and a central catheter exit site of 1 additional patient.

We suspect that, like other nosocomial pathogens, this organism was transmitted via hands or a contaminated environmental source (medical equipment or fomites). PFGE patterns of these isolates were indistinguishable, providing laboratory evidence of nosocomial transmission. The outbreak strain was distinct from the most prevalent hospital strain of VRE and from an additional case detected on oncology unit A that involved VRE (figure 1). In addition, the outbreak organism was of a VanB phenotype, rare in our hospital, further supporting the contention that nosocomial transmission occurred.

The first isolation of VDE was reported in 1993. The isolate grew in a culture of urine from a female patient with sepsis who had received intravenous vancomycin for  $\sim 120$  days [1]. We are aware of 11 cases that have been formally reported and 4 patients whose microbiological findings have been described (table 2). Prior exposure, often protracted, to vancomycin and other broad-spectrum antibiotics is a frequent finding in the VDE-related literature (tables 1 and 2). Patients exposed to "antibiotic pressure" with vancomycin and possibly extended-spectrum cephalosporins and antianaerobic agents are at increased risk for colonization and subsequent infection with VRE [19]. It is likely that antibiotic pressure also plays a role in promoting colonization or infection with VDE.

In 2 reported cases, VDE was considered clinically pathogenic: in association with fever and polymicrobial bacteremia [14] and in a culture of urine with WBCs and bacteria [1]. We were unable to conclude that the presence of VDE altered the clinical courses of our outbreak patients. However, the persistence of colonization, including that with organisms of the vancomycin-dependent phenotype, and growth of the outbreak strain in blood and urine raises concerns that clinically significant systemic infection could occur, particularly when vancomycin is used for a prolonged period and in immunocompromised populations.

Molecular mechanisms for the development of VDE have been recently described [14, 18]: vancomycin inhibits the growth of vancomycin-susceptible enterococci by binding to the normal bacterial cell wall dipeptide d-alanyl d-alanine ("d-ala d-ala") dipeptidoglycan. To permit survival and cell wall growth in the presence of vancomycin, both VDE and VRE have the ability to use ligases to make an alternative dipeptide, d-alanyl d-lactate ("d-ala d-lac"), to replace the normal cell wall "d-ala d-ala." If vancomycin is not present, VRE retain the ability to make the original dipeptide "d-ala d-ala" and grow normally. Because of amino acid substitutions or deletions in "d-ala d-ala" ligases, however, VDE are unable to make the original "d-ala d-ala" cell wall dipeptide. Its growth is thus dependent on the presence of vancomycin, which enables the bacteria to use "d-ala d-lac" as a cell wall constituent.

**Table 2.** Vancomycin-dependent isolates of *Enterococcus*: literature review.

Date, reference	Phenotype	<i>Enterococcus</i> species	Positive culture specimen(s)	Rate of reversion of dependence	Vancomycin used	Broad-spectrum antibiotic used	Characteristics of patients
1993 [1]	vanB	<i>E. faecalis</i>	Urine	$1 \times 10^7$	Yes	Yes, unspecified	Sepsis, intensive care
1994 [13]	Unknown	<i>E. faecium</i>	Stool	Unknown	Yes	Unknown	Renal failure
1994 [13]	Unknown	<i>E. faecium</i>	Stool	Unknown	Yes	Unknown	Renal failure
1995 [14]	vanB	<i>E. faecium</i>	Blood	$1 \times 10^{5-6}$	Yes	Imipenem, amikacin	Small bowel, liver transplant
1995 [11]	vanB	<i>E. faecium</i>	Stool	$1 \times 10^{6-7}$	Yes	Ceftazidime, ciprofloxacin	<i>Clostridium difficile</i> infection
1995 [12]	vanA	<i>E. faecium</i>	Urine	$1 \times 10^2$	Unknown	Unknown	Unknown
1995 [12]	vanB	<i>E. faecium</i>	Blood	$1 \times 10^6$	Unknown	Unknown	Unknown
1995 [12]	vanB	<i>E. faecium</i>	Abdominal drain	$1 \times 10^7$	Unknown	Unknown	Unknown
1995 [12]	vanA	<i>E. faecium</i>	Stool	$1 \times 10^2$	Unknown	Unknown	Unknown
1996 [15]	Unknown	<i>E. faecium</i>	Dialysis line, blood, urine	Unknown	No	Cefotaxime, imipenem	Esophageal rupture, intensive care
1996 [15]	Unknown	<i>E. faecium</i>	Blood, urine	Unknown	Yes	Cefotaxime, ciprofloxacin	Postoperative complications, intensive care
1997 [16]	Unknown	<i>E. faecium</i>	Blood	Unknown	Yes	Unknown	Renal failure
1998 [17]	Unknown	Undetermined	Blood	Unknown	Yes	Unknown	Renal transplant, peritonitis
1999 [18]	vanB	<i>E. faecalis</i>	Blood	Unknown	Yes	Unknown	Unknown
1999 [18]	vanB	<i>E. faecalis</i>	Urine	Unknown	Yes	Unknown	Unknown

For each VDE case in this outbreak, we observed the presence of vancomycin-dependent and -independent ("heterodependent") phenotypes in subcultures of the isolates. PFGE analysis confirmed that all colonies were of the same genotype and were not in a mixed or contaminated culture. Reversion of VDE to vancomycin independence occurs at varying rates, from 1 in  $10^2$  colonies (*vanA* isolates) to 1 in  $10^7$  colonies (*vanB* isolates), which results in a phenotypically mixed population. Our isolate (*vanB*) reverted rapidly (1 in  $5 \times 10^4$ ), which, in part, explains differences in colony phenotypes and the VRE/VDE patient-to-patient variability. Our findings are consistent with the findings of Van Bambeke et al. [18], who demonstrated an insertion in the *ddl* gene in vancomycin-resistant, teicoplanin-susceptible revertants of vancomycin-dependent *E. faecalis* that restored "d-ala d-ala" ligase activity and thus vancomycin-independent growth [18].

A well-established system of surveillance and the use of vancomycin-containing agar plates was essential in detection of this VDE outbreak. Control measures included heightened adherence to infection control practices, including isolation of all floor patients. In addition, we revised guidelines and decreased the prophylactic use of vancomycin for BMT patients. The outbreak isolate was not detected after institution of these policies. In addition, the overall incidence rates of VRE and bacteremia due to VRE have fallen in our oncology center after institution of these guidelines (data not shown).

The outbreak potential of VDE, as seen in our cluster, is a concern with regard to immunocompromised patients and those who receive long-term prophylaxis or treatment with vancomycin. Only ongoing laboratory surveillance with vancomycin-containing agar plates will identify the presence of VDE. Increased detection of the organism, with recognition of its potential for person-to-person spread, is essential for the institution of aggressive hospital infection control measures. Increased recognition of VDE is the first step in systematically

reassessing the benefits and risks of policies for antibiotic prophylaxis and in understanding the clinical significance of these organisms.

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