

# Vancomycin-Variable Enterococcal Bacteremia

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**Vancomycin-variable enterococcus (VVE) is an emerging pathogen. VVE isolates initially appear phenotypically susceptible to vancomycin but possesses the *vanA* gene and can develop *in vitro* and *in vivo* resistance to vancomycin. We report a case of VVE bacteremia and describe how VVE poses diagnostic and therapeutic dilemmas.**

## CASE REPORT

In February 2014, a 77-year-old female with a history of coronary artery disease and dyslipidemia underwent an elective hepatic resection and Whipple's procedure for cholangiocarcinoma. Two weeks after the operation, she complained of increased abdominal pain, and a computed tomography (CT) scan demonstrated necrosis of her liver with associated gas, consistent with a leak from her duodenal stump. She was treated with empirical piperacillin-tazobactam for 8 days, and her condition stabilized.

Two months later, she developed tachycardia and leukocytosis. A repeat CT scan of her abdomen demonstrated evolving hepatic abscesses. Blood cultures were positive for *Escherichia coli* and *Bacteroides fragilis*, and she was treated again with piperacillin-tazobactam on the basis of susceptibility results. She went to the operating room 1 week later for closure of the duodenal stump leak and drainage of intraabdominal abscesses. The following day, she developed abdominal compartment syndrome and required two additional surgical procedures on consecutive days to evacuate an intraabdominal hematoma. Two sets of blood cultures were obtained because of ongoing fever and hemodynamic instability. Both sets grew an *Enterococcus faecium* strain reported as resistant to ampicillin (MIC, >32 mg/liter) and susceptible to vancomycin (MIC, 1 mg/liter) by Vitek 2 (bioMérieux, Marcy l'Etoile, France). Intravenous vancomycin was added to the antimicrobial regimen. Persisting hemodynamic instability and fever led to another blood culture being obtained on day 8 of vancomycin therapy (day 21 of therapy with piperacillin-tazobactam). Once again, *E. faecium* was isolated. On this occasion, Vitek initially failed to produce a susceptibility result; when the test was repeated, the result was a vancomycin MIC of >32 mg/liter. This isolate was sent to the provincial reference laboratory for confirmation of the susceptibility test result; the isolate was confirmed by agar dilution to be a vancomycin-variable enterococcus (VVE) susceptible to vancomycin (MIC, 1 mg/liter). The patient continued to deteriorate despite multiple surgical interventions and the use of broad-spectrum antibiotics. After discussions with the family, care was transitioned to comfort measures and the patient died.

Rectal swabs are routinely obtained from all of the adults admitted to our institution to screen for vancomycin-resistant enterococcus (VRE) by chromogenic agar VRESelect (Bio-Rad, Montreal, Canada). Testing of the index patient's admission rectal swab did not detect VRE. When VVE was clinically identified in the index patient, a total of three point prevalence screens were performed in the two units where the patient had resided; two point prevalence screenings were performed in the intensive care

unit (ICU; 12 patients were screened during the first point prevalence screening, and 15 were screened during a follow-up point prevalence screening), and one point prevalence screening was performed on a surgery ward (20 patients were screened). Rectal swabs obtained during the point prevalence screening were tested directly with GeneXpert *vanA/vanB* PCR (Cepheid). Standard methodology was used to isolate *E. faecium* in culture and confirm the VVE phenotype and genotype (1). VVE was isolated from the rectal swabs of two patients during the first ICU point prevalence screening, and these were included with three blood culture isolates from the case patient for analysis. Isolates were characterized by traditional biochemical testing, as well as by molecular assays to identify the presence of the *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, and *vanG* genes (2). In addition, the transposon Tn1546-like element was genetically characterized with previously described overlapping primer sets (2). Susceptibility testing was performed by agar dilution, and MIC results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (3). Isolates were also typed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) (2). Informed consent to publish the case report was obtained from the family.

The three blood culture isolates collected from the index patient before and during vancomycin therapy were identical by PFGE, suggesting persistent bacteremia despite effective antimicrobial therapy (Fig. 1). The isolates were resistant to ampicillin but susceptible to vancomycin, daptomycin, and linezolid (Table 1). Molecular assays confirmed that these isolates contained the *vanA* gene but were negative for the *vanB*, *vanC*, *vanD*, *vanE*, and *vanG* genes. Amplification and sequencing of the genes in transposon Tn1546 identified the presence of *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ*. However, none of the isolates contained the *orf1*, *orf2*, *vanR*, or *vanS* gene. On the basis of these results, it was confirmed that the patient was infected with the recently discovered VVE isolate (1, 2). With respect to the two colonized patients, one of the

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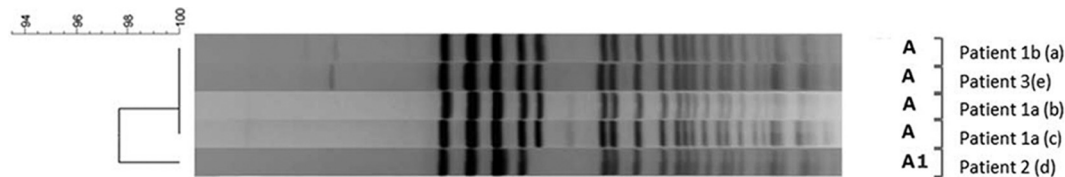
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**FIG 1** PFGE of isolates from the index patient bacteremic with VVE (patient 1; isolate 1a was obtained prior to vancomycin use, and isolate 1b was obtained while she was receiving vancomycin) and two patients colonized with VVE (patients 2 and 3). Isolates a, b, c, and e are labeled A since they are genetically identical to each other, whereas isolate d is labeled A1 because it is genetically closely related to the isolates above.

ICU patients was colonized with a strain identical to the index patient's VVE when PFGE analysis was performed and the other patient was colonized with a strain genetically closely related to the index patient's VVE, thus demonstrating transmission among patients (Fig. 1). These additional isolates had susceptibility profiles identical to that of the index patient's isolates, and all of the isolates were of sequence type 18 (ST18) by MLST (Table 1). Isolates recovered from the colonized patients were also positive for *vanHAXYZ* gene clusters but negative for the *orf1*, *orf2*, and *vanRS* genes. Patients colonized with VVE were placed on contact precautions (4, 5), and enhanced cleaning of high-touch surfaces with bleach was performed. The subsequent point prevalence screenings (one in the ICU and one on the surgical ward) did not identify additional cases. Neither of the two patients colonized with VVE developed a clinical infection.

VRE colonization and infection rates have increased over the last several decades (6, 7). Among VRE isolates, *E. faecium* is the most common species and causes both colonization and infection (8). Resistance to vancomycin in *E. faecium* is conferred primarily by the *vanA* gene, which encodes a ligase that changes the structure of terminal peptides in its cell wall from D-alanine–D-alanine to D-alanine–D-lactate, thereby reducing the binding affinity of glycopeptides (8). *vanA* is located on transposon Tn1546, along

with several other regulatory and structural genes necessary to confer resistance, i.e., *vanR*, *vanS*, *vanH*, and *vanX* (8).

A glycopeptide-susceptible, *vanA*-bearing *E. faecium* strain was first described in 2011 in Quebec (9). Six isolates were found to lack *vanR* and *vanS*, two components of the regulatory system involved in *vanHAX* gene expression. More recently, an outbreak of VVE was described in Ontario involving 44 patients with isolates once again lacking the *vanR* and *vanS* genes (2). More concerning was a case report published at the same time describing the emergence of vancomycin resistance in a patient colonized and infected with VVE after treatment with vancomycin (1). Currently, there is little guidance in terms of the management of patients colonized or infected with VVE.

To our knowledge, this is the first case report of bacteremia associated with VVE. This case raises several important questions. First, should vancomycin be avoided in patients with serious VVE infections? In our case, vancomycin failed to clear VVE from the blood, as the patient continued to be bacteremic after 8 days of vancomycin therapy. However, this may have been due to poor source control, given that the isolate was still susceptible to vancomycin on a repeat blood culture. A patient colonized with this organism was recently described, and it was shown in that case that the isolate became resistant to vancomycin *in vivo* over time during vancomycin therapy (1). A recent study reported that exposure of VVE strains to vancomycin *in vitro* leads to the development of vancomycin resistance by the constitutive expression of *vanHAXYZ* gene clusters (10). The use of vancomycin to treat VVE infections could provide selective pressure that may lead to the development of resistance on therapy and resultant treatment failure. Therefore, it seems prudent to avoid the administration of vancomycin to patients infected with VVE strains.

Second, if vancomycin should be avoided when treating VVE infections, should molecular testing be the standard of care for identifying VVE in all clinical specimens positive for phenotypically vancomycin-susceptible *E. faecium*? The isolate in this case would not have been identified as having the *vanA* gene if not for the Vitek system's failure to report a MIC on the repeat blood culture, triggering further testing. Our hospital now performs *vanA/vanB* PCR testing of any *E. faecium* isolates obtained from a sterile site. Further surveillance data are needed to better understand the prevalence of VVE in clinical specimens. If VVE becomes an established nosocomial pathogen, it may be appropriate to recommend routine genotypic testing of *E. faecium* clinical isolates, particularly those from sterile sites, to determine the presence of vancomycin resistance genes.

Last, should hospitals screen for VVE colonization? Routine VRE screening methods do not reliably identify VVE and did not detect the two colonized cases epidemiologically linked to the in-

**TABLE 1** Summary of susceptibility profiles, typing results, and the presence of *vanA* operon genes in VVE isolates recovered from three patients

Parameter	Result <sup>a</sup> for patient (isolate):				
	1a (a) <sup>b</sup>	1a (b) <sup>b</sup>	1b (c) <sup>c</sup>	2 (d)	3 (e)
Specimen type	Blood	Blood	Blood	Rectal swab	Rectal swab
MIC (mg/liter) of:					
Ampicillin	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)
Penicillin	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)
Daptomycin	4 (S)	4 (S)	4 (S)	4 (S)	4 (S)
Linezolid	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)
Vancomycin	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)
PFGE type	A	A	A	A1	A
ST	18	18	18	18	18
Presence of:					
<i>vanA</i> gene	+	+	+	+	+
<i>vanHAXYZ</i> genes	+	+	+	+	+
<i>vanRS</i> genes	–	–	–	–	–

<sup>a</sup> S, susceptibility; R, resistance.

<sup>b</sup> Isolate obtained before vancomycin use.

<sup>c</sup> Isolate obtained during vancomycin use.

dex case that we identified by molecular testing. A large hospital outbreak due to VVE has also been previously described in Ontario; thus, it is clear that VVE is able to spread within health care facilities and is circulating in the Toronto area (2). We suspect that our patient acquired this organism locally while in our hospital since the patient (and the two colonized patients subsequently identified by point prevalence screening) did not have recent hospitalizations in other health care facilities. In addition, we have shown that VVE can cause a clinically significant infection similar to that caused by VRE, suggesting that VVE should be managed from an infection control perspective as VRE; in our jurisdiction, screening and isolation for VRE are recommended (5). A prior study has shown that some VVE isolates can grow on Brilliance agar selective medium (Oxoid, Canada) and outlines an approach using Brilliance agar selective medium in combination with molecular detection methods (2). However, further research is required to determine the sensitivity of various selective agars in detecting VVE and whether this strategy in combination with molecular testing is cost-effective.

In conclusion, we report the first case of VVE bacteremia. This organism has been shown to develop resistance to vancomycin and raises new questions about screening for VVE and the diagnosis and treatment of VVE infections.

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