Vancomycin variable Enterococcus faecium (VVE): in vivo emergence of vancomycin resistance in a vancomycin susceptible isolate

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Word Count: Abstract: 38
Text: 1030

Keywords: Enterococcus faecium, vancomycin, vanA

Running title: vancomycin variable Enterococcus faecium
Here, we report the emergence of vancomycin resistance in a patient colonized with a \textit{vanA}-containing, \textit{vanRS}-negative isolate of \textit{Enterococcus faecium} which was initially vancomycin-susceptible. This is a previously undescribed mechanism of drug resistance with diagnostic and therapeutic implications.

Vancomycin resistant \textit{Enterococcus faecium} is widely disseminated [1-3]. The predominant mechanism of resistance is encoded on transposon Tn1546, which is commonly carried on a plasmid, and includes a two component regulator gene \textit{vanRS} and a gene cluster including \textit{vanHAXYZ} encoding the resistance mechanism [3]. The \textit{vanA} resistance gene encodes a ligase that catalyzes the linkage of D-alanine and D-lactate, which replaces the typical D-alanine D-alanine precursor for peptidoglycan, thereby decreasing the affinity of glycopeptide antibiotics for their target site [2, 4].

Despite the tendency for insertion sequences to cause structural alterations within \textit{vanRS} and \textit{vanAXYZ}, all isolates of \textit{E. faecium} containing the Tn1546 plasmid reported before 2011 were resistant to glycopeptide antibiotics, and only a few hetero-resistant isolates had been identified [5-7]. Because of the correlation of \textit{vanA} gene carriage and glycopeptide resistance, and because molecular methods offer high sensitivity and faster turn-around-time, many clinical microbiology laboratories have introduced the detection of \textit{vanA} gene as a surrogate marker of vancomycin resistance.

Glycopeptide susceptible, \textit{vanA} bearing \textit{E. faecium} was described for the first time in six isolates obtained from routine patient screening samples from a single hospital.
in Quebec, Canada in 2011 [8]. Of the six isolates reported, one carried a modified Tn1546 plasmid with a deletion of \( \text{vanR} \), and five carried a modified Tn1546 plasmid with deletions of \( \text{vanR} \) and \( \text{vanS} \). Despite the presence of \( \text{vanHAXYZ} \), the isolates were susceptible to vancomycin on phenotypic testing [8].

Here, we report a shift in vancomycin susceptibility from susceptible to resistant in \( \text{E. faecium} \) bearing \( \text{vanA} \) gene in a modified Tn1546 plasmid isolated from a hospitalized patient in Toronto, Ontario, Canada following exposure to vancomycin.

**CASE REPORT**

A 69 year old man with a past medical history of type II diabetes mellitus, urinary retention, and colon adenocarcinoma with hepatic metastases causing portal hypertension and chronic ascites was admitted to hospital with \( \text{E. coli} \) sepsis and bleeding esophageal varices. He was known from an admission for spontaneous bacterial peritonitis three weeks previously to be colonized with an \( \text{E. faecium} \) positive for \( \text{vanA} \) by PCR (Xpert \( \text{vanA/vanB} \) assay, Cepheid, Sunnyvale, CA), but susceptible to vancomycin (MIC=1 µg/ml), and his admission rectal swab (obtained on day 0) yielded the same organism.

He received piperacillin-tazobactam for 2 days, then 8 days of ciprofloxacin for treatment of his bacteremia. Because of suspected recurrent sepsis on day 12, he received piperacillin-tazobactam from day 12-17 and intravenous vancomycin from day 12-14, Urine and ascitic fluid cultures yielded vancomycin susceptible \( \text{E. faecium} \) and a Tenckhoff catheter which had been inserted for control of ascites was removed. Other specimens submitted for culture were negative. On day 20 daily ceftriaxone was initiated
as prophylaxis for spontaneous bacterial peritonitis. He died from bleeding esophageal varices and hepatorenal syndrome on day 29.

Screening rectal swabs obtained on days 10, 12, 22 and 24, and specimens of ascitic fluid obtained on days 2 and 14 and urine obtained on day 10 all yielded *E. faecium* positive for the *vanA* gene by PCR. Vancomycin MICs, as determined by broth microdilution according to Clinical Laboratory Standards Institute (CLSI) guidelines (9), and by E-test according to manufacturer’s specifications, and teicoplanin, daptomycin and linezolid MICs as determined using E-test and the Vitek® 2 (bioMérieux), are shown in Table 1. There was no evidence of heteroresistance in the susceptible isolates. The pulsed-field gel electrophoresis patterns of all isolates were indistinguishable.

Plasmids were isolated from the vancomycin susceptible isolate taken on day 2 (Pr0) and the vancomycin resistant isolate taken on day 24 (Ps24) and sequenced using a 454 sequencer (Roche, Basel, Switzerland). In both the vancomycin susceptible and resistant isolates, plasmids contained *vanHAXY* but lacked *vanRS*. Whole genome sequencing did not reveal any chromosomal insertion of an additional Tn1546 transposon, nor the presence of *vanRS* or *vanRS* homologues or another known vancomycin resistance operon (9).

**DISCUSSION**

Herein, we report a novel phenotype of a *Enterococcus faecium* with variable resistance to vancomycin. This initially susceptible *E. faecium*, which contained a modified Tn1546 plasmid bearing *vanHAXY* but not *vanRS*, acquired vancomycin resistance in vivo following exposure to vancomycin. The potential for vancomycin
resistance to arise following vancomycin exposure creates a risk of treatment failure. 

This situation would be complicated by the fact that traditional culture and susceptibility testing would not be able to identify the risk; both phenotypic and molecular methods are needed to detect this strain.

Several features of this isolate require further investigation. The distribution of this phenotype is not known. It has now been detected in at least 13 hospitals in Ontario and Quebec, with more than 95 patients identified as colonized or infected with vanA containing vancomycin susceptible strains of *E. faecium* in 2012 [8,10,11]. The strain has also been shown to readily transmit from one patient to another in a hospital setting, with 7 Ontario hospitals reporting from 1 to 42 affected patients in 2012 [10,11]. While the phenotype of vancomycin susceptibility changing may require specific host strain characteristics not yet elucidated, the potential of this plasmid to pass to other species or strains clearly exists. The mechanism of resistance for these isolates is not established. Further phenotypic and genetic characterization of these strains and the modified plasmid both *in vitro* and *in vivo* is necessary to ensure that a novel *vanA*-independent resistance mechanism is not present, or to identify the genes responsible for regulating expression of the *vanAHXY* complex.

It is not clear whether the development of vancomycin resistance upon vancomycin exposure is a unique phenomenon in this isolate in this patient or common among isolates of this strain; nor can we be certain from this one case whether the development of vancomycin resistance was causally associated with vancomycin exposure. In light of this case, however, it seems prudent to consider therapy with antibiotics other than vancomycin when managing patients with infection due to this type
of *E. faecium* until more data are available to determine whether and how frequently resistance may arise on therapy.

This report represents the first isolate of vancomycin susceptible *Enterococcus faecium* known to become resistant to vancomycin following drug exposure. We propose the name ‘vancomycin variable Enterococcus (VVE)’ to describe such isolates. They potentially represent an important clinical and microbiologic challenge.
References


Table 1. Clinical and microbiologic details of vanA bearing VRE isolate before and after patient exposure to vancomycin*

<table>
<thead>
<tr>
<th>Day of Admission</th>
<th>Specimen</th>
<th>Source</th>
<th>vanA PCR</th>
<th>Vancomycin MIC (µg/ml)</th>
<th>Teicoplanin MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to vancomycin treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 Pr0</td>
<td>Rectal swab</td>
<td>+</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>2 Pr2</td>
<td>Ascitic fluid</td>
<td>+</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>10 Pr10</td>
<td>Urine</td>
<td>+</td>
<td>1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>12 Pr12</td>
<td>Rectal swab</td>
<td>+</td>
<td>1</td>
<td>Nt</td>
<td></td>
</tr>
<tr>
<td>Concurrent with vancomycin treatment (day 12-14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Co14a</td>
<td>Ascitic fluid</td>
<td>+</td>
<td>1</td>
<td>Nt</td>
<td></td>
</tr>
<tr>
<td>14 Co14b</td>
<td>Rectal swab</td>
<td>+</td>
<td>1</td>
<td>Nt</td>
<td></td>
</tr>
<tr>
<td>After vancomycin treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 Ps22</td>
<td>Rectal swab</td>
<td>+</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td></td>
</tr>
<tr>
<td>24 Ps24</td>
<td>Rectal swab</td>
<td>+</td>
<td>&gt;256</td>
<td>&gt;256</td>
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</tbody>
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*all isolates tested were susceptible to linezolid and quinupristin-dalfopristin
+ = positive PCR for vanA gene; nt=not tested