Genomic analysis of teicoplanin resistance emerging during treatment of *vanB* vancomycin-resistant *Enterococcus faecium* infections in solid organ transplant recipients including donor-derived cases

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Objectives: We noted four cases of apparent *in vivo* emergence of teicoplanin resistance during failed therapy for initially teicoplanin-susceptible *vanB* vancomycin-resistant *Enterococcus faecium* (VREfm) infections in solid organ transplant recipients at our institution over a 12 month period. We investigated if *in vivo* emergence of resistance had occurred, if transplant-related vancomycin-resistant *Enterococcus* (VRE) infections had occurred and identified clinical predictors of resistance emergence.

Methods: Whole genome sequencing was performed on nine VREfm isolates for phylogenetic analysis and to identify determinants of teicoplanin resistance. Clinical treatment details were compared with other patients who received teicoplanin for confirmed *vanB* VRE infections but did not develop resistance during the same year at our institution.

Results: A high-resolution, core genome phylogeny was inferred for nine VREfm isolates and confirmed *in vivo* development of resistance during failed therapy in four cases. Four different non-synonymous single nucleotide polymorphisms (SNPs) were observed in the *vanRS* genes of teicoplanin-resistant strains compared with the index teicoplanin-susceptible strains, and these SNPs were predicted to confer teicoplanin resistance. VREfm within a cluster of early transplant-related infections were phylogenetically identical at the core genome level, indicating a common source donor. Focus eradication and absence of prosthetic material were characteristics of those patients treated successfully.

Conclusions: Clinicians should be cautious of resistance emerging during teicoplanin therapy for *vanB* VRE, particularly in immunosuppressed patients or where source control is difficult.

Keywords: whole genome sequencing, single nucleotide polymorphisms, *vanRS*

Introduction

We observed an unusual cluster of *vanB* vancomycin-resistant *Enterococcus faecium* (VREfm) infections in solid organ transplant recipients where teicoplanin resistance emerged during treatment. There also appeared to be a temporal relationship between transplantation and onset of *vanB* VREfm infection in several patients. We sought to determine the clinical factors associated with teicoplanin failure and used genomics to determine the molecular features of teicoplanin-resistant *vanB* VREfm, as well as investigate if there was a donor-related cluster of cases.

Case reports

Patient 1, a 57-year-old woman, had complications after orthotopic liver transplantation (OLTx), including anastomotic leak, and within 24 h of transplantation, *vanB* VREfm peritonitis and bacteremia. She received teicoplanin (12 mg/kg); however,
teicoplanin-resistant vanB VREfm emerged on day 18 despite multiple drainage procedures. She succumbed from persistent polymicrobial intra-abdominal sepsis 7 months later.

Patient 2, a 61-year-old man, developed urinary retention and a perinephric collection, and had positive urine cultures for vanB VREfm within 24 h of renal transplantation from the same donor as patients 1 and 2. Teicoplanin (9 mg/kg) was commenced, but cultures remained intermittently positive for >1 month. Teicoplanin resistance emerged on day 18 of therapy.

Patient 3, a 57-year-old woman, developed uncomplicated vanB VREfm cystitis within 24 h of renal transplantation from the same donor as patients 1 and 2, and was successfully treated with 2 weeks of teicoplanin (7 mg/kg).

Patient 4, a 55-year-old man with a previous renal transplant and vanB VREfm colonization, developed vanB VREfm bacteraemia and meningism. He completed 4 weeks of teicoplanin (9 mg/kg) for presumed meningitis. Ten days later he had bacteraemia with teicoplanin-resistant vanB VREfm and completed 6 weeks of linezolid without further recurrence.

Patient 5, a 39-year-old woman who was vanB VREfm colonized, developed vanB VREfm bacteraemia and intra-abdominal infection within 24 h of OLTx. Teicoplanin (13 mg/kg) was commenced and serum levels were appropriate [trough 21.7 mg/L (target 10–20 mg/L); peak 118.1 mg/L (target 40–60 mg/L)]. Despite multiple drainage procedures she had persistent vanB VREfm bacteraemia, and teicoplanin-resistant vanB VREfm emerged on day 16. She subsequently succumbed to persistent polymicrobial intra-abdominal sepsis.

The organ donor for patients 1, 2 and 3 had an out-of-hospital cardiac arrest. Prior to organ donation he had negative ante-mortem blood cultures as well as a negative rectal screening swab for vancomycin-resistant Enterococcus (VRE). The transplantation teams involved with patients 1, 2 and 3 were different.

Materials and methods

Laboratory identification and susceptibility testing of VRE

Enterococci were identified using routine methods. Species identification and van genotype were determined by PCR. Vancomycin and teicoplanin MICs were determined using Etest (bioMérieux, Marcy l’Etoile, France) according to the manufacturer’s instructions.

Genome sequencing and analysis of teicoplanin-resistant vanB VREfm strains

Whole genome sequencing (WGS) of nine E. faecium isolates from patients 1–5 was performed using ion torrent sequencing (Life Technologies, USA). The results from all genomes were aligned to the E. faecium reference strain Aus0085 (manuscript in preparation) using SHRiMP 2.0. Single nucleotide polymorphisms (SNPs) were identified using Nesoni v0.70 (www.bioinformatics.net.au). Phylogenetic analyses were performed using a distance method, based on pairwise comparisons of variable nucleotide positions present among all strains. Indels were excluded. A phylogeny was then inferred using the neighbour-joining method using uncorrected p distances with bootstrapping as implemented in SplitsTree4.

Analysis of patients successfully treated with teicoplanin for clinical vanB VRE infections

We searched the microbiology laboratory database to identify all patients with vanB VRE clinical isolates during the same calendar year as our patients (1 January 2009–31 December 2009). Positive vanB VRE rectal screening swabs were excluded. We used dispensing records from the pharmacy database to find which patients had also received teicoplanin treatment for more than 48 h. Patient demographics and treatment details were obtained using a retrospective chart review.

Results

Initial characterization of teicoplanin-resistant vanB VREfm strains

All isolates from patients 1–5 were reconfirmed to be E. faecium containing the vanB operon. Vancomycin and teicoplanin Etest MIC results performed contemporaneously on the original isolates, as well as antibiograms, are shown in Table S1 (available as Supplementary data at JAC Online).

Genome sequencing and analysis of teicoplanin-resistant vanB VREfm strains

Figure 1(a) shows the relationship of our sequenced strains compared with a reference E. faecium genome Aus0085 based on core genome comparisons and a resulting pool of 298 SNPs. The size of the SNP pool indicated that all strains were clonally related, and notably are related to the ST203 reference isolate AUS0085. This sequence type has been causing an outbreak of clinical E. faecium infection at our institution over recent years. The core genome sequences from patients 1, 2 and 3 were identical, confirming our suspicion of a donor-related transplant cluster. In contrast, isolates from patients 4 and 5 were not closely related to the transplant-derived cluster. Only a single SNP distinguished the teicoplanin-susceptible and teicoplanin-resistant isolates within each pair. The close relationship between the teicoplanin-susceptible and teicoplanin-resistant isolates from each pair indicates in vivo generation of teicoplanin resistance.

The mutations detected between isolates in each pair were further analysed. Figure 1(b) shows the codon location of these SNPs for each isolate within the vanB operon. Three of these SNPs were found in vanS9 and one in vanR9. All of these SNPs were predicted to result in an amino acid change (Figure 1b), and we believe these are the putative cause of teicoplanin resistance in our patients.

Analysis of patients successfully treated with teicoplanin for clinical vanB VRE infections

During the study period there were 18 patients with vanB VRE clinical infections treated with teicoplanin. Resistance to teicoplanin emerged during treatment in 4 (22.2%) of these patients, as described earlier, while the remaining 14 patients (including patient 3) were all treated without documented resistance. Clinical features of these teicoplanin-susceptible vanB VRE infections are shown in Table 1. The median duration of teicoplanin was 15 days and included dosing regimens up to 12 mg/kg.
We compared the clinical features of 14 patients who were successfully treated with teicoplanin for vanB VRE infections with four patients who developed teicoplanin resistance. A number of similarities between the two groups were noted, including two solid organ transplants, immunosuppression and the presence of invasive foci. However, patients with teicoplanin-resistant strains had greater immunosuppression or significantly more complex infections (e.g. intra-abdominal sepsis with multiple loculi not always amenable to drainage or complex urinary tract sepsis with post-operative collections).

**Discussion**

We report the in vivo emergence of teicoplanin resistance during failed therapy for vanB VRE clinical infections in solid organ...
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Comorbidities</th>
<th>Rectal colonization</th>
<th>Clinical organism</th>
<th>Site of infection</th>
<th>Duration of positive culture (days)</th>
<th>TEC dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TEC duration (days)</th>
<th>Prior VAN exposure&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>71</td>
<td>F</td>
<td>haemodialysis, forefoot amputation, diabetic osteomyelitis</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>osteoarticular</td>
<td>8</td>
<td>800 mg (11 mg/kg)</td>
<td>40</td>
<td>yes</td>
</tr>
<tr>
<td>B</td>
<td>65</td>
<td>M</td>
<td>peritonitis, total colectomy</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>intra-abdominal</td>
<td>8</td>
<td>720 mg (10 mg/kg)</td>
<td>29</td>
<td>yes</td>
</tr>
<tr>
<td>C</td>
<td>66</td>
<td>M</td>
<td>autologous haematopoietic stem cell transplant for multiple myeloma</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>BSI</td>
<td>1</td>
<td>800 mg (12 mg/kg)</td>
<td>22</td>
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<td>D</td>
<td>55</td>
<td>M</td>
<td>acute promyelocytic leukaemia</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>BSI</td>
<td>3</td>
<td>1200 mg (12 mg/kg)</td>
<td>16</td>
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<tr>
<td>E (patient 3)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>57</td>
<td>F</td>
<td>cadaveric renal transplantation for end-stage kidney disease</td>
<td>not colonized</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>urine</td>
<td>2</td>
<td>400 mg (7 mg/kg)</td>
<td>15</td>
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<tr>
<td>F</td>
<td>70</td>
<td>M</td>
<td>Parkinson's dementia</td>
<td>vanB VREfm&lt;sup&gt;e&lt;/sup&gt;, vanB VREfm&lt;sup&gt;f&lt;/sup&gt;</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>BSI</td>
<td>1</td>
<td>800 mg (12 mg/kg)</td>
<td>15</td>
<td>no</td>
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<td>G</td>
<td>47</td>
<td>F</td>
<td>relapsed acute myeloid leukaemia, febrile neutropenia</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>BSI</td>
<td>2</td>
<td>800 mg (12 mg/kg)</td>
<td>15</td>
<td>yes</td>
</tr>
<tr>
<td>H</td>
<td>65</td>
<td>F</td>
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<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>BSI, urine</td>
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<td>600 mg</td>
<td>15</td>
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<tr>
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<td>71</td>
<td>F</td>
<td>duodenal fistula and perforation</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>intra-abdominal</td>
<td>1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>800 mg</td>
<td>11</td>
<td>no</td>
</tr>
<tr>
<td>J</td>
<td>62</td>
<td>F</td>
<td>diffuse large B cell lymphoma prostate cancer with obstructive uropathy</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>urine</td>
<td>1</td>
<td>600 mg</td>
<td>8</td>
<td>no</td>
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<tr>
<td>K</td>
<td>63</td>
<td>M</td>
<td>relapsed acute myeloid leukaemia, febrile neutropenia</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>BSI</td>
<td>9</td>
<td>600 mg</td>
<td>5</td>
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<tr>
<td>L</td>
<td>47</td>
<td>M</td>
<td>progressive multiple sclerosis diabetic toe ulcer</td>
<td>not screened</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>SSSI</td>
<td>1</td>
<td>800 mg</td>
<td>3</td>
<td>yes</td>
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<tr>
<td>N</td>
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<td>F</td>
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<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>urine</td>
<td>1</td>
<td>800 mg</td>
<td>3</td>
<td>no</td>
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<td>O</td>
<td>84</td>
<td>F</td>
<td>progressive multiple sclerosis diabetic toe ulcer</td>
<td>vanB VREfm</td>
<td>vanB VREfm&lt;sup&gt;c&lt;/sup&gt;</td>
<td>urine</td>
<td>1</td>
<td>800 mg</td>
<td>3</td>
<td>no</td>
</tr>
</tbody>
</table>

TEC, teicoplanin; VAN, vancomycin; F, female; M, male; VREfs, vancomycin-resistant Enterococcus faecalis; VSEfs, vancomycin-susceptible E. faecalis; BSI, bloodstream infection; SSSI, skin and skin structure infection.

<sup>a</sup>Teicoplanin was administered every 12 h for three doses then 24 hourly in patients with normal renal function.

<sup>b</sup>Prior vancomycin exposure defined as vancomycin administered at least once during the preceding 30 days of clinical VRE infection.

<sup>c</sup>These patients had polymicrobial infections.

<sup>d</sup>Patient 3 received an organ from the same donor as patients 1 and 2 who developed teicoplanin-resistant strains during therapy (see main text).

<sup>e</sup>This isolate was a penicillin-susceptible vanB VREfs; however, the patient had a penicillin allergy necessitating treatment with teicoplanin.

<sup>f</sup>The patient was transferred from another hospital with clinical VRE infection; however, there was no subsequent clinical specimen at our hospital.
transplant recipients, despite adhering to and surpassing the manufacturer’s dosage recommendations. 5 Overall, resistance emerged in almost one-quarter of patients, raising questions about the utility of teicoplanin for the treatment of serious and complex vanB VRE infections. Agents such as linezolid, daptomycin or tigecycline are alternatives, although their use needs to be individualized to the patient’s clinical syndrome. Daptomycin is only licensed for complicated skin and skin structure infections or Staphylococcus aureus bacteraemia, and tigecycline is not recommended for bacteraemia. Linezolid is licensed for the treatment of VREfm; however, there are potentially treatment-limiting toxicities and resistance. 7

Teicoplanin-resistant vanB VRE has been previously reported from clinical specimens 8,9 and animal models of infection; 10,11 however, the genetics of teicoplanin resistance in vanB VRE are not completely understood. WGS demonstrated that the teicoplanin-susceptible and -resistant strains from our patients differed by only one SNP. These SNPs were predicted to result in four unique amino acid substitutions within vanR90, and were different from those described by Baptista et al. 12 or those summarized by Arthur et al. 13 However, the SNPs within vanS are predicted to alter signal transduction or phosphorylation. One SNP resulted in a P238S substitution; substitutions in the H box of vanS (codons 231–239) lead to impaired phosphatase activity and a constitutive phenotype. 13 The L28P mutation is located near the previously documented mutation A30G 14 in the sensor domain, which is associated with altered signal recognition or transduction, and has been associated with an inducible phenotype. The E221L mutation is located upstream of the H box in vanS and is possibly associated with impaired kinase activity. The allele change D11G in vanR is located at the N-terminal of vanR. vanR is a cytoplasmic response regulator, and we hypothesize that an amino acid substitution near the beginning of this coding sequence may have resulted in altered regulatory function (e.g. impaired phosphorylation). 13

In this study, we have demonstrated the power of WGS to determine relationships between isolates by confirming our suspicion that three patients (patients 1–3) appeared to have donor-related vanB VRE acquisition immediately following transplantation, a conclusion supported by their identical VREfm core genome sequences even though VRE was not isolated from the transplant donor. Post-harvest contamination is less likely with different transplant teams performing the harvesting and subsequent recipient transplantations. Transmission of multiresistant organisms is increasingly recognized as a major challenge in organ transplantation. 15,16 To our knowledge there is only one other report of donor-derived VRE transmission in two organ recipients; however, the donor was known to be bacteraemic prior to transplantation in that case. 17

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Transparency declarations

None to declare.

References


Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).