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Transferable vancomycin resistance in clade B commensal-type *Enterococcus faecium*

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Objectives: Vancomycin-resistant *Enterococcus faecium* is a leading cause of MDR hospital infection. Two genetically definable populations of *E. faecium* have been identified: hospital-adapted MDR isolates (clade A) and vancomycin-susceptible commensal strains (clade B). VanN-type vancomycin resistance was identified in two isolates of *E. faecium* recovered from blood and faeces of an immunocompromised patient. To understand the genomic context in which VanN occurred in the hospitalized patient, the risk it posed for transmission in the hospital and its origins, it was of interest to determine where these strains placed within the *E. faecium* population structure.

Methods: We obtained the genome sequence of the VanN isolates and performed comparative and functional genomics of the chromosome and plasmid content.

Results: We show that, in these strains, VanN occurs in a genetic background that clusters with clade B *E. faecium*, which is highly unusual. We characterized the chromosome and the conjugative plasmid that carries VanN resistance in these strains, pUV24. This plasmid exhibits signatures of in-host selection on the *vanN* operon regulatory system, which are associated with a constitutive expression of vancomycin resistance. VanN resistance in clade B strains may go undetected by current methods.

Conclusions: We report a case of vancomycin resistance in a commensal lineage of *E. faecium* responsible for an atypical bacteraemia in an immunocompromised patient. A reservoir of transferable glycopeptide resistance in the community could pose a concern for public health.

Introduction

Enterococcus faecium, natively a gastrointestinal (GI) tract commensal organism, emerged as a leading cause of MDR hospital-acquired infection in the 1980s.[1] Epidemiological and recent comparative genomic studies reported the existence of distinct subpopulations of *E. faecium* and the features that distinguished epidemic hospital-adapted isolates [named clade A and including the so-called clonal complex 17 (CC17)] and commensal-type strains (clade B).[2–5] The hospital-adapted epidemic strains diverged from the clade B commensal strains by a distance usually associated with speciation and, importantly, clade A strains are frequently resistant to high levels of aminoglycosides, ampicillin and vancomycin, which undoubtedly contributes to their spread among hospitalized patients worldwide.[4–7] By contrast, the clade B strains are usually susceptible to most antibiotics, including vancomycin, and rarely cause infection aside from sporadic cases in severely immunocompromised patients.[4]

In the USA, between 35% and 75% of speciated enterococcal infections are caused by *E. faecium*, of which a majority are vancomycin resistant.[8,9] Glycopeptide antibiotics (namely vancomycin and teicoplanin) are widely used for the treatment of severe infections due to Gram-positive bacteria and act by inhibition of peptidoglycan synthesis.[10] Since the early descriptions of vancomycin-resistant enterococci in 1988,[11,12] nine variations of gene clusters conferring resistance to glycopeptides have been identified, distinguished by sequence differences in either D-alanyl-D-lactate ligase (VanA, VanB, VanD and VanM) or D-alanyl-D-serine ligase (VanC, VanE, VanG, VanL and VanN).[10,13–15] In addition to the ligase, the genes in the resistance cluster encode enzymes involved in the synthesis of modified peptidoglycan precursors,

with lower affinity for glycopeptides, as well as enzymes that hydrolyse the competing, natively produced D-Ala-D-Ala precursor.[10] The resistance genes are regulated by two-component regulatory systems (VanR and VanS) that, when functional, induce the expression of the resistance in the presence of glycopeptide.[10]

In addition to the threat posed by VRE, MDR enterococci serve as a reservoir for horizontal gene transfer (HGT) of antibiotic resistance to other pathogens, as illustrated by reports of *vanA* transfer to *Staphylococcus aureus*.[16] Aside from the VanC-type resistance, which is ordinarily intrinsic to species *Enterococcus gallinarum* and *Enterococcus casseliflavus*, *van* clusters have been acquired through HGT.[10] The *vanA* and *vanB* gene clusters are generally located on transposons (Tn*1546* and Tn*1549*/Tn*5382*) often found in conjugative plasmids.[10] In addition, both *vanE* and *vanD* resistance clusters have been shown to be part of putative conjugative elements, although transferability could not be demonstrated.[17,18]

The *vanN* resistance cluster, which we previously showed to be transferable by conjugation of a large plasmid of unknown structure and origin, has been found in two clonal strains isolated from the blood and the faeces (strains UCN71 and UNC72) of a hospitalized patient in France,[15] from the faeces of a hospitalized patient in Canada and also in five clonal strains isolated from domestic chicken meat in Japan.[19,20] Interestingly, routine molecular typing by MLST indicates that the chromosomes of the three groups of strains are not clonally related, all being outside of the CC17 of *E. faecium*.[15,19,20]

To understand the genomic context in which VanN occurred, the risk it posed for transmission in the hospital and its origins, it was of interest to determine where these strains placed within the *E. faecium* population structure. Here, we show that in UCN71 and UCN72, this VanN type of resistance occurs in strains that cluster with clade B commensal *E. faecium*, a population in which vancomycin resistance had not been previously reported. Because of the low level of resistance (16 mg/L) conferred by the WT *vanN* cluster, this phenotype may go undetected.

Materials and methods

Bacterial strains

The strains used in this study are listed in Table S1 (available as Supplementary data at *JAC* Online). Comparative genomic analysis was performed on VanN-type strains UCN71 and UCN72; a VanN transconjugant, UCN73;[15] and comparator *E. faecium* strains (Table S1), representing the breadth of the *E. faecium* population and previously used to distinguish and characterize clade A and clade B lineages.[4] Specifically, these strains are from 51 distinct STs carefully chosen to maximize the diversity and representation of MLST types and clusters.[4]

Genome sequencing

For Illumina sequencing, total DNA was purified (DNeasy DNA extraction; Qiagen, Valencia, CA, USA), quantified (Qubit dsDNA High-Sensitivity; Invitrogen, Carlsbad, CA, USA) and sequencing libraries were prepared from 50 ng of DNA per sample (Nextera DNA Sample Preparation; Illumina, San Diego, CA, USA). Libraries were normalized to 2 nM, multiplexed and subjected to 200 bp paired-end sequencing on the MiSeq platform at the Massachusetts Eye and Ear Infirmary Ocular Genomics Institute (Boston, MA, USA). On average, 5 million high-quality paired-end reads were collected for each strain, representing >250-fold coverage of the ~3 Mb genomes. For 454 sequencing, a DNA fragment library was prepared using the GS DNA Library Preparation Kit (Roche, Indianapolis, IN, USA) followed by emulsion-based PCR amplification. Sequencing was performed using a 454 Life Sciences (Roche) GS-FLX system. For UCN72 pyrosequencing, 164 965 reads (read length between 250 and 300 nt) were collected. Draft genomes and raw reads of the strains UCN71, UCN72, UCN73 and BM4107 are accessible in GenBank: Bioproject number PRJNA245745.

Assemblies, PCR for plasmid closure, annotation and orthogrouping

Sequence reads were assembled *de novo* (CLC Genomics Workbench; Cambridge, MA, USA). Gene prediction, annotation and orthoclustering were conducted as previously described.[4] For strain UCN72, Illumina and 454 reads were pooled and assembled *de novo* to produce a high-quality hybrid assembly of 12 supercontigs, ranging in length from 8063 to 1 094 650 bp ($N_{50} = 3$ contigs, mean coverage >250-fold). Contig #8 (154 330 bp) from the UCN72 genome assembly contains the vancomycin resistance cluster and was putatively identified as a plasmid. In order to determine its entire sequence, we designed specific primers pUV24F (5′-GCACTAGGGCTATACGCCTGT-3′) and pUV24R (5′-TCTCAACTTCTGGTGCTG GATCTG-3′) reading outward from the ends, sequenced the PCR product and filled the 6 nt gap to obtain the circularized plasmid, named pUV24 (154 336 bp).

Phylogeny, SNP identification, whole-genome alignment and mobilome identification

A phylogenetic tree (1344 single-copy core orthogroups, mid-point rooted) was determined using PhyML with 1000 bootstrap replicates.[21] The UCN72 high-quality assembly was used as the reference for mapping of *E. faecium* UCN71 sequence reads. Quality-based variant detection, based on the Neighborhood Quality Standard algorithm,[22] was used to identify polymorphisms. High-confidence variants, using a combination of quality filters and thresholds for coverage (>100×) and frequency (>95%), were identified. Contigs of UCN72, UCN73 and BM4107 were ordered and oriented to the genome of reference strain *E. faecium* Aus0004 using progressiveMauve,[23] and plotted using GenoPlotR.[24] The prevalence of glycopeptide resistance in strains of a given ST was obtained by analysing all strains ($n = 3077$) from the MLST database as well as all strains ($n = 764$) for which the genome was available in the GenBank WGS database (last accessed October 2017). Plasmids and phage-related genes were predicted using PlasmidFinder (http://cge.cbs.dtu.dk/services/PlasmidFinder/) and PHAST (http://phast.wishartlab.com/).

D,D-Peptidase activity

Cultures were grown in the absence or presence (8 mg/L) of vancomycin, cells were pelleted and lysed, and the cytoplasmic fraction was assessed for D,D-peptidase activity as described previously.[15]

Results

VanN is a rare occurrence of vancomycin resistance in clade B *E. faecium*

VRE *E. faecium* UCN71 and UCN72 (16 mg/L) were isolated from the same patient, in France.[15] The patient was diagnosed with Burkitt's lymphoma (the primary GI non-Hodgkin's lymphoma) and intravenous vancomycin therapy was started on day 4 and ended on day 29 (Figure S1A). UCN71 was isolated from the blood on day 33 and UCN72 was isolated from the stool on day 55.

Notably, the patient developed VRE bacteraemia on day 33, 1 day after a negative screen for VRE in the faeces.

Comparison of nucleotide polymorphisms in the 1344 core genes of UCN71 and UCN72 to a diverse set of *E. faecium* genomes places the VanN-type isolates within clade B (Figure 1a). When all *E. faecium* strains within the MLST database were searched for the occurrence of glycopeptide resistance, it was found only in the clade A hospital-adapted strains, making VanN unique in its occurrence within clade B *E. faecium* (Figure 1a). In addition, when 764 publically available *E. faecium* genomes were searched and assigned to a clade (Figure S1B), 64% ($n = 486$ vancomycin-resistant *E. faecium*) were found to harbour a vancomycin resistance cluster (Figure 1b). Of 486 vancomycin-resistant *E. faecium*, >99% belong to the MDR hospital-adapted clade A, leaving only three clade B vancomycin-resistant *E. faecium* (Figure 1b), including UCN71 and UCN72 VanN isolates. The only other occurrence of vancomycin resistance outside of clade A was found in EnGen0263 (accession number NZ_AJAD00000000), an isolate that possesses a *vanA* cluster on a *rep7* family conjugative plasmid (data not shown).

VanN strains lack the accessory genome enriched in hospital-adapted E. faecium

In addition to distance between the clades stemming from genetic drift, MDR epidemic clade A and clade B commensal *E. faecium* strains also differ in gene content.[4] We thus compared the genome of commensal strain UCN72 (as representative of both clonal strains) with the complete 3.0 Mb genome of *E. faecium* Aus0004, as representative of the hospital-adapted epidemic clade (Figure S2). Aus0004, a bacteraemia isolate, contains various mobile genetic elements including four prophages, two genomic islands, as well as three circular plasmids.[25] Genome alignment revealed that none of the chromosomally integrated mobile genetic elements identified in *E. faecium* Aus0004 is present in UCN72 (Figure S2), which otherwise contain only a single chromosomally integrated element (a putative prophage predicted as incomplete by PHAST) (Figure S2). It has been postulated that the paucity of mobile genetic elements in commensal enterococci correlates with the presence of clustered, regularly interspaced, short palindromic repeat (CRISPR) loci, which provide a type of acquired immunity.[26] Unlike Aus0004, UCN72 contains both CRISPR1 and CRISPR2 loci and functional CRISPR-associated genes (*cas*). The genome of strain UCN72 was assembled into 12 scaffolds; 4 of these were putatively identified as plasmid fragments and named pUV24, pUV25, pUV26 and pUV27 (Figure S2).

vanN is carried by a conjugative plasmid related to those in MDR epidemic E. faecium

In previous work,[15] we obtained vancomycin-resistant transconjugant UCN73 by mating UCN71 with recipient *E. faecium* strain BM4107. Alignment of UCN73 and BM4107 genomes showed that the gain of vancomycin resistance by UCN73 was associated with the acquisition of conjugative plasmid pUV24 (Figure S2). This large plasmid (154 336 bp, GC content 35.7%) carrying the *vanN* cluster was closed and circularized using targeted PCR and further

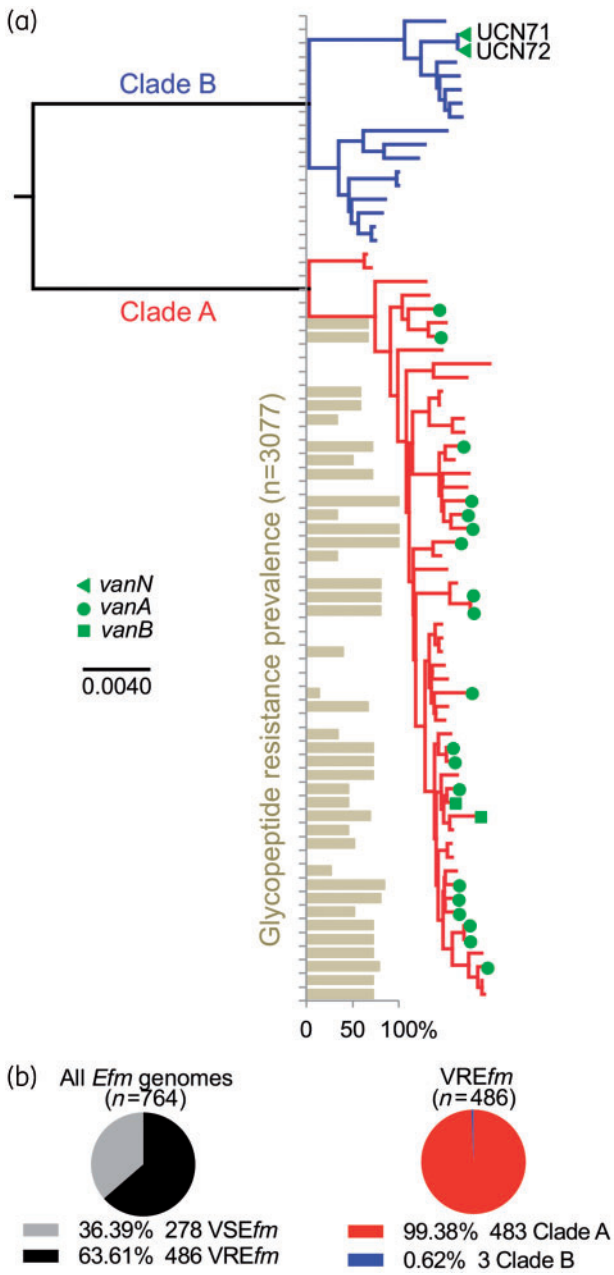

Figure 1. (a) VanN-type vancomycin resistance occurs in clade B *E. faecium*. Phylogenomic tree of a collection of *E. faecium* strains, including the VanN-type UCN71 and UCN72 isolates. The clade structure of the population is shown: clade B commensal strains (blue); and clade A hospital-adapted MDR strains (red). The vancomycin resistance genotype of each strain is shown. The prevalence of glycopeptide resistance of *E. faecium* strains from the MLST database for each particular ST is shown. Additional strain information is available in Table S1. (b) Prevalence of *E. faecium* strains ($n = 764$ publicly available genomes) harbouring (vancomycin-resistant *E. faecium*) or not (vancomycin-susceptible *E. faecium*) a vancomycin resistance cluster and distribution of vancomycin-resistant *E. faecium* within clades A and B. *Efm*, *E. faecium*; VRE*fm*, vancomycin-resistant *E. faecium*; VSE*fm*, vancomycin-susceptible *E. faecium*.

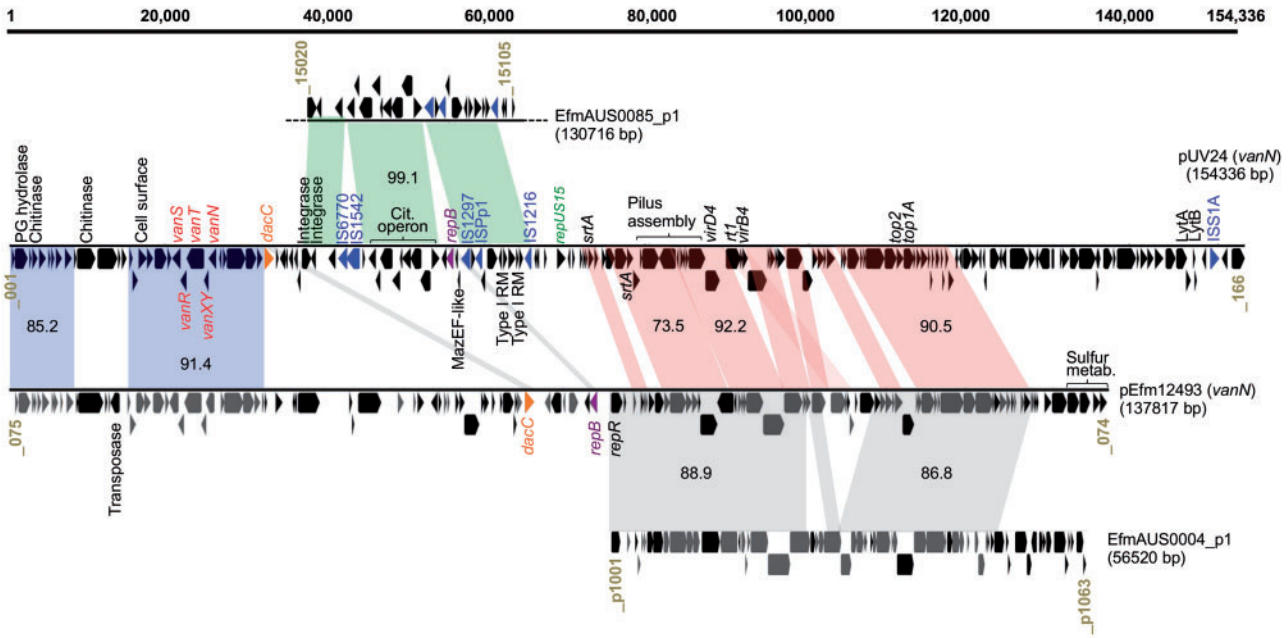

Figure 2. Schematic diagram of plasmid pUV24 from strain UCN71. Where relevant, genes or groups of genes are indicated by putative function or gene name. Syntenic regions with identity to plasmid pEfm12493 (accession no. KP342511), plasmid p1 of *E. faecium* AUS0004 (accession no. CP003352) and plasmid p1 of *E. faecium* AUS0085 (accession no. CP006622) are projected (grey) and average nucleotide identities are indicated. Locus tags are provided at the beginning and end of each linearized plasmid. A scale bar, in bp, is provided. Dotted lines indicate a truncation to avoid representation of non-related material.

compared with the sequence of pEfm12493,[19] the only known *vanN*-carrying plasmid, found in an isolate from the abscess of a patient in Canada (Figure 2). Interestingly, pUV24 (154 kb) is significantly larger than pEfm12493 (138 kb) and is predicted to harbour 166 ORFs. Two main segments (representing ∼80 kb) show an overall synteny and share between 73% and 92% of nucleotide identity between both *vanN*-carrying plasmids (red and blue syntenic blocks; Figure 2). First, a region of ∼50 kb in pUV24 (red blocks; Figure 2) includes conjugation genes [i.e. a predicted fully functional type IV secretion system (TIVSS)], genes encoding two sortases and genes encoding two topoisomerases. As described previously,[19] this region also shares extensive sequence identity (87% overall) and synteny with plasmid Aus0004_p1 from the MDR and epidemic Aus0004 strain (grey blocks; Figure 2). Importantly, pUV24 contains a *repA* gene [99.9% nucleotide identity with *repUS15* (from PlasmidFinder) and 98% identity with *repA*-pLG1 (accession number ADO66907.1) plasmid families[27]] instead of the replication gene shared by pEfm12493 and Aus0004_p1. The RepA_pLG1 family groups large plasmids and is highly prevalent in both clades of *E. faecium* (>78%; 57/72 strains in this study). Other studies have repeatedly associated this family of conjugative plasmid with various resistance genes, including vancomycin and aminoglycosides.[27] Besides differences in the *rep* gene, it is also worth noting the rearrangements between pUV24 and pEfm12493 in the genes coding for the TIVSS. Boyd and co-authors[19] noted that, in pEfm12493, the *virB4* ATPase gene was truncated and interrupted by the *rt1* gene and thus conjugative transfer functions may be compromised. In light of our data, this would explain the conjugative nature of pUV24, which contains a full-length *virB4*, compared to pEfm12493.

The second region (blue syntenic blocks; Figure 2) shared between pUV24 and pEfm12493 includes the *vanN* cluster, several cell surface protein genes (including a putative peptidoglycan hydrolase) and a putative chitinase. To investigate in detail the evolution of the *vanN* resistance cluster, we compared the sequences from the four *vanN*-VRE reported so far (Figure 3a). Clusters from UCN71, UCN72 (France) and GU121-1 (Japan) share 99.9% nucleotide identity. By contrast the *vanN* resistance cluster from N12-0493 (Canada) is more distantly related (91% identity). Interestingly, *vanN* is harboured on a larger segment shared by plasmids pUV24 and pEfm12493 (blue blocks; Figure 2). A predicted transposase sits upstream of this element in pEfm12493 and could be responsible for the movement of the *vanN* resistance cluster. However, sequence analysis of the boundaries of the shared element did not reveal any obvious signatures of transposition in pUV24 or pEfm12493. Plasmid pUV24 diverges from pEfm12493 by two regions. One shares very high identities (99.1%) with a segment from plasmid EfmAUS0085_p1 in the vancomycin-resistant epidemic *E. faecium* Aus0085 and contains a complete citrate operon and two putative type I restriction systems (green syntenic blocks; Figure 2). The second region (35 kb at the 3′ end of pUV24) is of unclear origin and BLAST analyses return no significant hits. We identified six insertion sequences (IS*6770*, IS*1542*, IS*1297*, IS*1216*, IS*Pp1* and IS*S1A*) harboured by pUV24 but none seemed directly associated with the movement of VanN. Finally, pUV24 possesses one complete type II toxin–antitoxin (TA) loci (MazEF-like) that may act as plasmid addiction systems to ensure stable maintenance and inheritance. A total of 24 pUV24 ORFs (not shown) encode surface-associated proteins of unknown function or peptidoglycan synthesis-associated proteins,

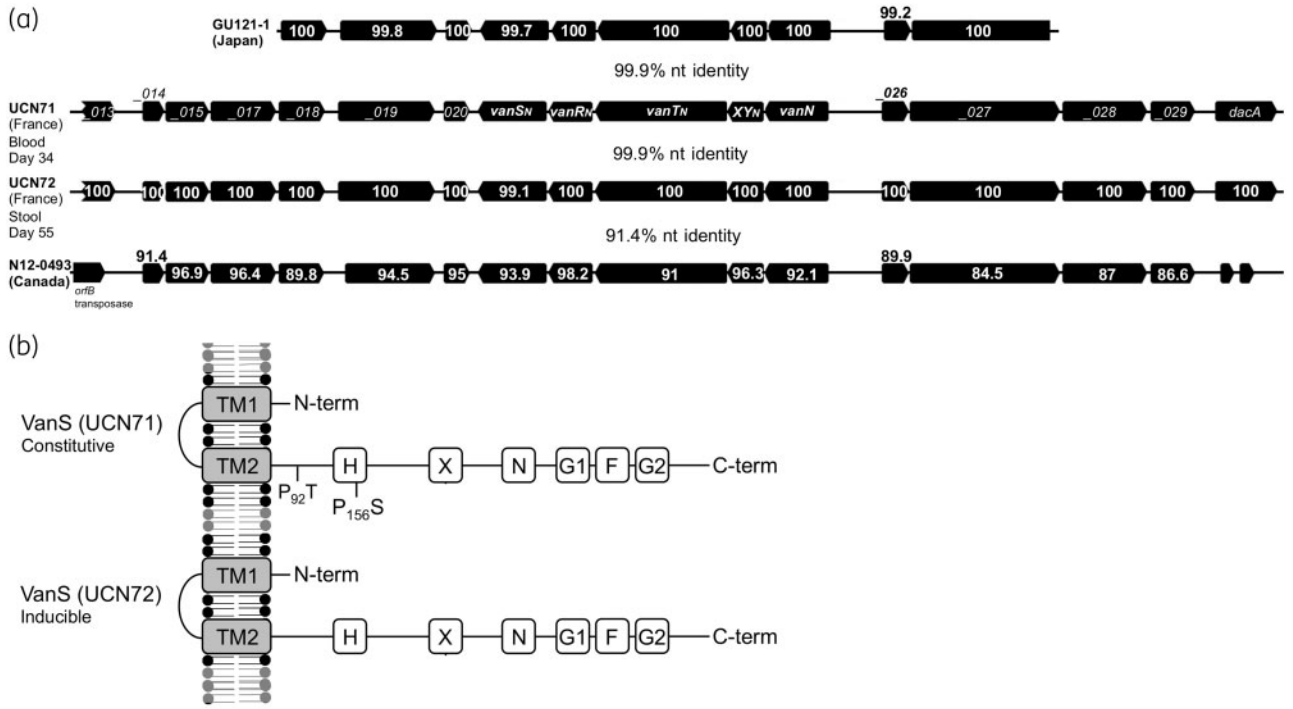

Figure 3. (a) Comparison of the *vanN* cluster in strains UCN71 and UCN72 from France, strain GU121-1 from Japan and strain N12-0493 from Canada. Locus tags are provided for the *vanN* resistance cluster in UCN71. For other resistance clusters, the amino acid identities are indicated for each gene. The overall nucleotide identities of the syntenic regions for each pairwise comparison are provided. (b) Comparison of the VanS protein in strains UCN71 and UCN72. TM1 and TM2 represent the putative transmembrane (TM)-associated sensor domains. The putative catalytic ATP binding domain containing amino acid motifs (H, X, N, G1, F and G2) that are conserved in histidine kinases are indicated, as well as amino acid substitutions. P, proline; S, serine; T, threonine.

indicating some remodelling of the cell surface in strains carrying pUV24. Among these surface-related proteins are the LytA and LytB *N*-acetylmuramoyl-L-alanine amidases (Figure 2), which participate in peptidoglycan biosynthesis, with LytB homologues having been described to have autolytic activity and to be involved in cell separation.[28] Additionally, a DacC homologue occurs that is thought to possess D-alanyl-D-alanine carboxypeptidase activity, removing the C-terminal D-alanyl residues from sugar-peptide cell wall precursors,[29] potentially supplementing the activity of VanXY.

In vivo selection affects expression of vancomycin resistance

Since UCN71 and UCN72 were isolated from the same patient 21 days apart, it was of interest to determine whether the strains had diverged. We therefore performed SNP analysis to identify polymorphisms between their genomes. Across the whole genome, a total of three high-confidence SNPs were identified and confirmed by PCR (Table 1). One SNP is synonymous and would not change the amino acid sequence of the corresponding putative ATPase. However, two SNPs surprisingly occurred within the same gene, which encodes VanS, and both are non-synonymous substitutions (Table 1).

We previously hypothesized that amino acid substitutions in VanS of UCN71 were responsible for the constitutive expression of vancomycin resistance in this isolate.[15] With the finding of polymorphisms in VanS between UCN71 and UCN72, it was of interest to investigate the resistance expression in strain UCN72. Expression of vancomycin resistance in *E. faecium* UCN72 was studied by analysing the D,D-dipeptidase activity of $VanXY_N$, with (8 mg/L) or without vancomycin induction (Table 2). Induction of resistance expression in strain UCN72 increased intracellular D,D-dipeptidase activity in cytoplasmic extracts from 8 ± 7 to 37 ± 9 nmol/min/mg of protein. No such increase was noted for strain UCN71 ($P < 0.05$). Alignment of the $VanS_N$ protein sequences of UCN71 (constitutive) and UCN72 (inducible) suggests that either the P → T or P → S substitutions (at positions 92 and 156, respectively) might be responsible for the constitutive expression of the resistance in UCN71 (Figure 3b).

Discussion

The results of our genomic and phylogenetic analyses indicate that the VanN-type strains studied here constitute a very unusual case of vancomycin resistance in the clade B community-associated *E. faecium* population, contrasting sharply with the clade A hospital-adapted strains that are frequently associated with such resistance (70% prevalence in 694 clade A *E. faecium* genomes; Figure S1C).

VanN vancomycin resistance was reported in Japan in five *E. faecium* strains (ST669) isolated from chicken meat.[20] Although these strains are not clonally related to UCN71 and UCN72 (ST240), they independently acquired a *vanN* cluster nearly identical to that in UCN71, suggesting a recent HGT. VanN resistance

Table 1. Whole-genome SNP comparison between UCN71 and UCN72 serial isolates

SNP #	Function	Nucleotide (position)	Amino acid (position)
UCN71_2511	ATPase putative	T→A (321)	R→R (107)
UCN71p_021	VanS histidine kinase	C→A (276)	P→T (92)
UCN71p_021	VanS histidine kinase	C→T (466)	P→S (156)

Table 2. D,D-Dipeptidase enzymatic activities in UCN71 and UCN72 *E. faecium* strains

Strain	Vancomycin (mg/L)	Activity (nmol/min/mg of protein)	Resistance
UCN71	0	49 ± 6	constitutive
UCN71	8	52 ± 10	
UCN72	0	8 ± 7	inducible
UCN72	8	37 ± 9	

was also recently reported in a strain (ST955) in Canada isolated in 2008,[19] and sequence analyses indicate an independent acquisition. Although these strains were not part of our genome phylogeny, we predict, based on their ST designation (Figure S1D), that strains from Japan are also clade B *E. faecium*, while the strain from Canada belongs to the hospital-adapted lineage (though does not belong to CC17). This indicates that the *vanN* vancomycin resistance already has spread within both clade B and clade A MDR *E. faecium* in different parts of the world. The association with agriculture in Japan highlights the prospect that entry of VanN resistance into the human ecology might have occurred through the food chain. A plasmid of similar length to pUV24 was described to carry the resistance cluster in the Japanese VanN strains.[20] By contrast, the plasmid found in the VanN strain from Canada was significantly different from the one in UCN71, again suggesting distinct acquisitions and subsequent rearrangements in both strains. Considerable sequence similarity was noted between fragments of pUV24 and plasmids circulating in MDR epidemic strains (in fact the *repA*_pGL1 plasmid family is highly prevalent in both clades of *E. faecium*[27]), suggesting the absence of a barrier to plasmid transfer between hospital endemic and clade B strains. In fact, vancomycin plasmid transfer has been experimentally observed *in vivo* in the gastrointestinal tract of mice.[30]

In the antibiotic era, vancomycin-resistant *E. faecium* bacteraemia has become a vexing problem because of the lack of effective alternative treatment options.[8] Highly hospital-adapted MDR lineages have emerged and account for most of the problem.[4,8] The infection model that emerges is that initial administration of antibiotics to a patient results in elimination of key elements of the microbiota that otherwise limit entry of new members into the consortium, leading to large numbers or even predominance of VRE in the gut.[31–35] This is then followed by translocation of VRE into the bloodstream and bacteraemia. In addition to antibiotic resistance, hospital-adapted clade A strains acquired new metabolic capabilities which have been shown to enhance their ability to colonize antibiotic-treated mice.[4,36] This led to the proposition that clade A MDR strains colonize a microniche in the antibiotic-perturbed GI tract of patients in a manner that is non-competitive with commensal clade B strains.[31] This, together with the comparatively low level of vancomycin resistance (16 mg/L) conferred by the WT operon, may account for the inability of the clade B VanN-type *E. faecium* to grow to large enough numbers in the GI tract to be easily detected in surveillance prior to the onset of bacteraemia. In fact, gut colonization by VanN-type *E. faecium* appears to have been more insidious (the weakened intestinal barrier in the patient treated for a gastrointestinal lymphoma might have allowed recurrent leakage of the commensal flora into the blood) and only afterward was it found in rectal swab cultures, perhaps influenced by the expectation that the presence of this microbe in the gut usually presages bacteraemia.

Expression of resistance is a transient need for bacterial cells, as they face inhibitory concentrations of drugs only temporarily. Vancomycin resistance is mediated by a sophisticated mechanism that relies on a complex operon and combines synthesis of modified cell wall peptidoglycan precursors and elimination of the normal target precursors.[10] Thus, constitutive expression of vancomycin resistance has been shown to have a high fitness cost for the cell.[37] However, the same study showed that tight regulation of resistance expression drastically reduces the biological cost associated with vancomycin resistance in *Enterococcus.* The VanN resistance in UCN71 is one of the few cases, in addition to VanD isolates,[38,39] of constitutive vancomycin resistance expression found in clinical settings.[15] Interestingly, we found that the clonal UCN72 strain, isolated 21 days after UCN71, showed an inducible vancomycin resistance phenotype, providing strong evidence that it represents the genotype originally acquired in the gut preceding its translocation into the bloodstream. Overall, we found a strong in-host selection that resulted in the accumulation of two (out of three total genome wide) non-synonymous SNPs in the *vanS* gene that likely affect the phosphatase activity of VanS and thus account for constitutive expression of resistance.

In conclusion, we report a case of vancomycin resistance in a commensal lineage of *E. faecium* responsible for an atypical bacteraemia in an immunocompromised patient. Because this strain eluded detection prior to its occurrence in the blood, we believe that the prevalence of vancomycin resistance in the community may be underestimated and that such a reservoir of glycopeptide resistance could pose a serious concern for public health.

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Transparency declarations

None to declare.

Supplementary data

Table S1 and Figures S1 and S2 are available as Supplementary data at *JAC* Online.

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