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Emergence of vancomycin-resistant enterococci from vancomycin-susceptible enterococci in hospitalized patients under antimicrobial therapy

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ABSTRACT

Objectives: Enterococci are opportunistic pathogens with plastic genomes that evolve, acquire, and transmit antimicrobial-resistant determinants such as vancomycin resistance clusters. While vancomycin-resistant enterococci (VRE) have emerged as successful nosocomial pathogens, the mechanism by which vancomycin-susceptible enterococci (VSE) transform to VRE in hospitalized patients remains understudied.

Methods: Genomes of *Enterococcus faecium* from two critically ill hospitalized patients subjected to multiple antibiotic therapies, including broad-spectrum antibiotics, were investigated. To identify mechanisms of resistance evolution, genomes of vancomycin-susceptible and -resistant isolates were compared.

Results: While VSE isolates were initially identified, VRE strains emerged post-vancomycin therapy. Comparative genomics revealed horizontal transmission of mobile genetic elements containing the Tn*1549* transposon, which harbours the *vanB*-type vancomycin resistance gene cluster. This suggests that broad-spectrum antibiotic stress promoted the transfer of resistance-conferring elements, presumably from another gut inhabitant.

Conclusion: This is one of the first studies investigating VSE and VRE isolates from the same patient. The mechanism of transmission and the within-patient evolution of vancomycin resistance via mobile genetic elements under antibiotic stress is illustrated. Our findings serve as a foundation for future studies building on this knowledge which can further elucidate the dynamics of antibiotic stress, resistance determinant transmission, and interactions within the gut microbiota.

1. Introduction

Enterococcus faecium are commensals in the gastrointestinal tract of many animals, including humans, but can also survive in a wide range of environments including hospital care settings [1], and they can cause endocarditis, urinary tract infection, bacteraemia, and surgical wound infections [2]. The success of *E. faecium* as a critical nosocomial pathogen is linked to its ability to acquire, evolve, and successfully transfer antibiotic resistance genes through plasmids and other mobile genetic elements (MGEs) [3]. Therapeutic options for serious infections with *E. faecium* are usually limited to glycopeptide or oxazolidinone [4], since penicillins are ineffective in the majority of clinical *E. faecium* isolates, mostly due to mutations near the active site of penicillin-binding protein 5, giving a lower affinity towards ampicillin [2,5]. Vancomycin was successfully used for treatment until enterococci developed resistance (vancomycin-resistant enterococci (VRE)) [1].

Vancomycin-susceptible enterococci (VSE) can evolve into VRE

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through horizontal transfer of MGEs containing *van* gene clusters. Presently, there are nine different acquired *van* gene clusters, namely *vanA, B, D, E, G, L, M, N*, and *P* [3], but only *vanA* and *B* clusters are widespread, presumably because they are often part of successful MGEs [3,6]. High-level vancomycin and teicoplanin resistance characterize VanA-type VRE, whereas VanB-type VRE expresses resistance to vancomycin only [7]. Vancomycin resistance level (MIC) in *vanB*-type VRE varies considerably (MIC 1 to >256 mg/L) in clinical enterococci, challenging current laboratory detection methods [8].

Three allelic variants of the *vanB* gene cluster (*vanB1–3*) have been identified, of which *vanB2* is the most prevalent as an integrated part of the integrative and conjugative elements (ICE) Tn*1549* [9,10]. Other MGEs occasionally carrying *vanB* are composite transposons (Tn*1547*), other ICEs (ICE*Efa*V583, ICE*Slu*van), and conjugative plasmids. Additionally, chromosomal transfer of *vanB* has been described [6].

Rarely, *vanB* genes have been found in enterococcal species other than *E. faecium* and *E. faecalis*, as well as streptococci and staphylococci. However, anaerobic Gram-positive human gut colonizers belonging to the genus *Clostridium*, as well as representatives of *Atopobium minutum, Eggerthella lenta*, and *Ruminococcus* sp., carry *vanB* MGEs relatively frequently and act as a reservoir for transfer of *vanB* to enterococci [6].

In Europe, an overall increase in vancomycin resistance in *E. faecium* has been reported [11], while in Norway the incidence has varied over the last 10 years, with *vanA* and *vanB* representing the dominant VRE gene clusters [12]. In Germany, a sharp increase in *vanB*-type resistance was reported in recent years, but this trend is not reported in neighbouring countries [13].

With recent medical advancements, hospitals take care of increasing numbers of elderly and immune-compromised patients who are receptive to infections and treated with a wide range of antimicrobials. Multiple antibiotics create selective stress, leading to selection pressure on antibiotic resistance genes [14]. In this paper, we report two cases of VRE emerging from VSE in patients who were hospitalized with severe complicated medical histories, including serious infections, and hence were treated with multiple antimicrobials including several broad-spectrum antibiotics and vancomycin.

2. Materials and methods

2.1. Background case 1

A 51-year-old woman was admitted to a hospital in northern Norway with an acute myocardial infarction in the inferior territory of the heart, treated with thrombolytics prehospitally. The patient had a medical history of insulin-dependent diabetes mellitus from the age of 16 months, and she also had a chronic decubitus ulcer on her right leg for the last 10 years. Immediately after admission, angiography revealed a total occlusion of the proximal part of the left descending coronary artery. During the percutaneous coronary intervention procedure, the patient had two cardiac arrests which required heart-lung resuscitation, intubation with insertion of an intra-aortic balloon pump, extracorporeal membrane oxygenation treatment, and further insertion of a pacemaker. The patient stayed in the intensive care unit for about seven weeks. During this stay, her state further worsened with multiple infections, which required several types of antimicrobial treatments as shown in Fig. 1a.

On day 4 after admission, she was treated with cefotaxime intravenously for eight days because of ventilator-associated pneumonia. Bronchoalveolar lavage culture revealed *Haemophilus influenzae* susceptible to ampicillin and cefotaxime. At day 10, she was detected with septicaemia, and her blood cultures showed *Candida albicans* susceptible to fluconazole and *E. faecium* (KresVSE0001) resistant to ampicillin (MIC >256 mg/L) and with high-level resistance to gentamicin (MIC > 1024 mg/L) but susceptible to linezolid and vancomycin. Thus, she was treated with both fluconazole and vancomycin.

Because of the multiple invasive procedures, the patient had several wounds, including one in her right groin and sternum, apart from her leg wound. Culturing of wound samples from these locations at day 20–25 showed growth of *E. faecium* with the same resistance pattern as in the blood cultures, *Enterobacter cloaca* complex with a depressed AmpC, and *Acinetobacter* sp. Therefore, her antimicrobial treatment was reinforced with ciprofloxacin. On day 32, new cultures from her wounds showed *Bacteroides thetaiotaomicron* in addition to the earlier bacterial findings, for which she was treated with metronidazole. About two weeks later, on day 45, culturing of her wounds from the sternum and right groin and a new decubitus from the sacrum revealed a vancomycin-resistant (MIC 6 mg/L) *E. faecium* (KresVRE0004) that was *vanB* PCR positive. Despite all the advanced treatment, the patient's health condition showed no improvement, and she eventually died from multiorgan failure and multiple cerebral infarctions.

2.2. Background case 2

A 79-year-old man was admitted to a local hospital in northern Norway because of a total atrioventricular block stage III and acute renal insufficiency with a creatinine of 261 µmol/L (60–105 µmol/L). A pacemaker with atrial and ventricular electrodes was inserted. The patient was preoperatively treated with cloxacillin on the day of surgery. Because of paroxysmal atrial fibrillation, he was started with a thromboembolic prophylactic treatment of the anticoagulant warfarin.

After the implantation of the pacemaker, the patient had diffuse symptoms of sub-febrile temperatures for unknown reasons. Under suspicion of possible erysipelas, the patient was treated with penicillin and gentamicin intravenously with a change to clindamycin one day later. The patient was treated for a total of 10 days before the antibiotic treatment was stopped. A record of the patient's antimicrobial treatments during the case description period is shown in Fig. 1b.

Again, about a month later, the patient repeatedly experienced macroscopic haematuria with urine retention, which required rinsing and evacuation of blood from his bladder. One of these evacuations led to perforation of his bladder wall, resulting in an explorative laparotomy. This procedure revealed a 3.5-centimeter-long perforation in the top of the bladder wall that was sutured. Postoperatively, the patient was treated with piperacillin/tazobactam for seven days. A few days later, a new episode of macroscopic haematuria and urine retention caused septicaemia. Blood cultures revealed growth of *E. faecium* (KresVSE0002) resistant to ampicillin (MIC >256 mg/L), low level resistant to gentamicin (MIC 4 mg/L), and susceptible to linezolid and vancomycin. Because of this septicaemia, the patient was treated with vancomycin, but only one dose of 1 gram intravenously with a change to linezolid caused by his renal insufficiency. Because of his paroxysmal atrial fibrillation and repeated episodes of macroscopic haematuria followed by urinary retention and infections, the anticoagulation treatment with warfarin was changed to the blood platelet inhibitor acetylsalicylic acid. However, one month later, he again experienced macroscopic haematuria with urine retention, fever with a temperature of 40°C, and chills.

Blood cultures then showed growth of *vanB*-positive vancomycin-resistant *E. faecium* (KresVRE0005) with the resistance pattern: ampicillin (MIC >256 mg/L), gentamicin (MIC = 4 mg/L), vancomycin (MIC = 8 mg/L), and susceptibility to linezolid. In addition, the anaerobic bacteria *Veillonella* sp. was detected.

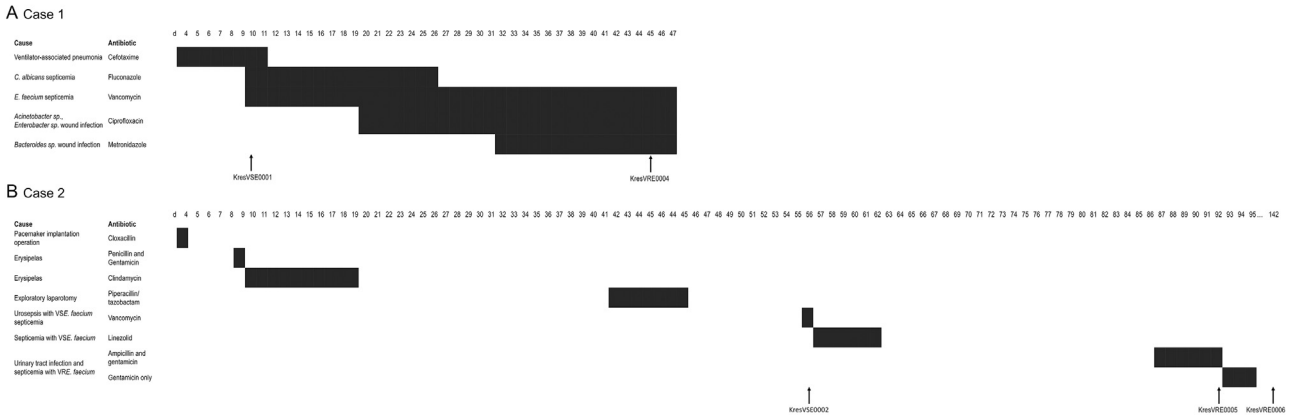

Fig. 1. Timeline of antimicrobial treatment, causes for treatment, and collected isolates. (A) Timeline case 1. (B) Timeline case 2.

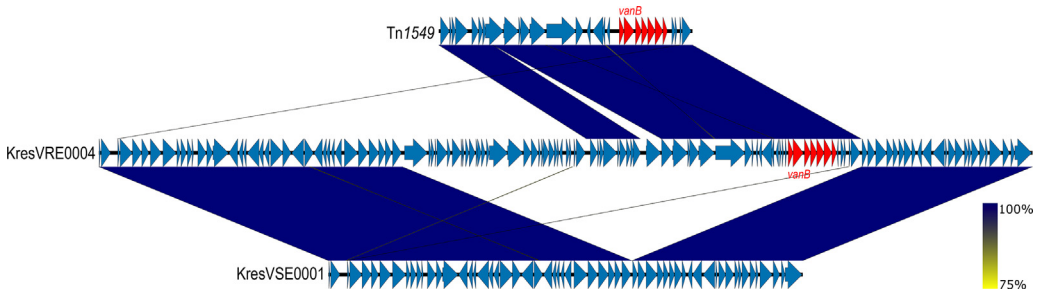

Fig. 2. Comparison of the *vanB*-containing contig in case 1. Comparison of the prototypic Tn*1549* (AF192329.1) (top) and the contig containing the inserted region with *van* cluster, and comparison of the contig containing the inserted region with *van* cluster against the *van*-susceptible genome from case 1 (bottom). CDS are indicated in blue arrows along with their directions. The *van* cluster is indicated in red colour. Blue to yellow gradient bands between sequences represent forward matches from 100% to 75% as indicated.

Culturing of his urine sample also showed vancomycin-resistant *E. faecium* with the same resistance pattern as seen in the blood cultures. Despite the resistance pattern of the *E. faecium* isolate, the patient was treated with ampicillin and gentamicin for 6 days with clinical effects probably due to rinsing of the urinary tract and insertion of a new urinary catheter. VRE screening from the rectum revealed that the patient was colonized with vancomycin-resistant *E. faecium* with the same resistance pattern as the isolates found in his blood and urine. The patient was then discharged from the hospital. After discharge, his rectum was screened for VRE colonization, and *E. faecium* (KresVRE0006) was isolated and analysed for vancomycin resistance both *in vitro* and *in silico*. Ten months later, the patient had another rectal screen for VRE colonization due to additional hospital admission. This screening was negative.

2.3. Growth conditions, resistance testing, and initial typing

The *E. faecium* isolates were grown on blood agar or brain heart infusion (BHI) broth or agar at 37°C. MIC values were obtained using MIC test strips (Liofilchem) according to the manufacturer's instructions. The presence of the *vanB* gene was confirmed by *vanAB* real-time PCR with primers and TaqMan probe (Applied Biosystems) [15] in qPCR Mastermix PLUS-LOW ROX (Eurogentec) according to the manufacturer's instructions.

2.4. Sequencing and assembly

For short-read sequencing (KresVSE0001, KresVSE0002, KresVRE004, KresVRE0005, KresVRE0006), genomic DNA was isolated with the Qiagen MagAttract HMW DNA isolation kit (Qiagen) from the *E. faecium* isolates before sequencing at the Genomics Support Centre Tromsø, University of Tromsø, with NextSeq500 using the Nextera XT DNA library preparation kit and the Mid Output 300 cycles cell according to standard protocols (Illumina, San Diego, CA, USA). Illumina reads were trimmed based on their quality scores, and adapters were removed with Trimmomatic-0.39 in paired-end mode [16]. Reads were error corrected and assembled with Shovill 1.0.9 with a minimum contig length of 200 bp [17].

Long-read sequencing (KresVSE0002, KresVRE0005) was performed at the Norwegian Sequencing Centre (University of Oslo). Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) and quantified with a Qubit fluorometer. To prepare multiplexed microbial libraries, SMRTbell Express Template Prep Kit 2.0 was used according to Pacific Biosciences protocol. Fragmentation of DNA was carried out using g-tubes (Covaries) resulting in 10–16 kb sized fragments. BluePippin with an 8 kb cutoff was used to select the final library. Libraries were sequenced on ~90% of 8M SMRT cell on Sequel II using Sequel II Banding kit 2.0 and Sequencing Chemistry v.2.0. Demultiplex Barcodes pipeline was carried out using SMRT Tools (SMRT Link v.9.0.0.92188) to demultiplex the reads (minimum barcode score 26). Finally, the circular consensus sequencing (CCS) sequences were produced for demultiplexed data using CCS pipeline (SMRT Link v.9.0.0.92188). PacBio reads were trimmed and quality filtered using Filtlong v.0.2.0 [18] and Illumina reads as reference for trimming bases from the start and end of reads which did not match a k-mer in the reference. The worst 10% of read bases and reads shorter than

1 kb were discarded. Trimmed Illumina and PacBio were hybrid assembled using Unicycler v.0.4.8 [19]. Multilocus sequencing typing (MLST) was used to characterize all five assemblies [20].

2.5. Data availability

Sequences of all five isolates were submitted to NCBI under BioProject PRJNA486083, and GenBank accession numbers were obtained (KresVSE0001: SAMN09836113, KresVSE0002: SAMN09836114, KresVRE004: SAMN09836111, KresVRE0005: SAMN09836112, KresVRE0006: SAMN36083373).

2.6. Comparative genomics

Contigs of KresVRE0004 was compared against KresVSE0001 and contigs of KresVRE0005 and KresVRE0006 were compared against KresVSE0002 using blastn searches [21]. The comparisons were visualized in the Artemis Comparison tool. Comparison files were inspected for large indels, and in the case of KresVSE0002 and KresVRE0005, they were also scanned for recombinations as they are closed genomes. All five assembled genomes were scanned for resistance genes by ABRicate v.0.6 [22] against the Bacterial Antimicrobial Resistance Reference Gene Database with an accession ID of PRJNA313047. The same program was also used to identify plasmid types using the PlasmidFinder database [23] with default settings. Comparison figures were drawn using Easyfig v.2.2.2 [24]. BLAST searches against the nonredundant (nr) database were used to investigate the homology of the newly inserted region.

The starting point of the circularised KresVRE0005-*vanB*-plasmid from the Unicycler hybrid assembly was identified and set to be rep2_1_orf1 (pRE25) gene using Abricate 1.0.1 with the PlasmidFinder database 2021-Mar-27 [22] and circlator fixstart 1.5.5 [25], respectively. The plasmid was annotated with NCBI Prokaryotic Genome Annotation Pipeline using PGAP-6.4 docker image pgap:2022-12-13.build6494 [26]. BLAST Ring Image Generator (BRIG) [27] was used to visualize the comparison of KresVRE0006, KresVSE0002 and the *vanB*-containing *E. faecalis* transposon Tn*1549* (AF192329.1) to the reference KresVRE0005-*vanB*-plasmid.

2.7. Variant prediction

To generate minimum spanning trees, cgMLST was performed using SeqSphere+ software v.6.0.2 (Ridom GmbH, Münster, Germany [28]). For *E. faecium* isolates, the scheme includes 1423 core genes, and a threshold of ≤20 allelic differences was used for cluster calculation and determination of the clonal relatedness [29]. To check their variations, Snippy [30] with default parameters was run for case 1 and case 2 separately along with SnpEff for the functional effect of the observed variations [31].

3. Results

3.1. Within patient chromosomal insertion of a vanB carrying Tn1549 ICE led to the emergence of ST192 VRE

Both isolates from case 1, KresVSE0001 and KresVRE0004, belong to ST192, which is one of the major hospital-associated dominant clones of *E. faecium* [32]. KresVSE0001 and KresVRE0004 show identical plasmid profiles as shown in Table 1. The two genomes are highly similar, with only one allelic difference in the MS (Minimum Spanning) tree (Supplementary Fig. S1) and 13 core genome SNP differences, 12 in intergenic regions and one in the *queH* epoxyqueuosine reductase, which we do not expect to play a role in the resistance phenotype (Supplementary Table S1).

The only major difference between KresVSE0001 and KresVRE0004 is the presence of the *vanB* gene cluster region. The isolates also show an identical resistance gene profile in exhibiting resistance genes towards macrolide, lincosamide, aminoglycoside, and trimethoprim (Table 1). It is thus likely that KresVSE0001 evolved to KresVRE0004 upon *vanB* cluster acquisition.

A 61-kb chromosomal insertion enclosing the *vanB* cluster is seen when comparing KresVRE0004 to the susceptible KresVSE0001 (Fig. 2). The inserted region has an elevated GC content of 48.8% against the overall genomic GC content of 37.9%, which indicates the insertion of non-enterococcal foreign DNA, and it is flanked by the nucleotides 5′-TTTTAT-3′. AT-rich sequences are known as the preferred insertion sites of many MGEs including Tn*1549* [33].

The source of the inserted DNA containing Tn*1549* which harbours the *vanB* cluster could not be determined with absolute certainty but is likely to be a gut anaerobe. BLAST search of the region gave hits to *Lachnospiraceae, Lactococcus lactis, Clostridium difficile, Clostridium perfringens, Erysipelotrichaceae,* and *Streptococcus pasteurianus* (26%–40% query coverage, 91.6%–100% identity, top 10 blast hits); however, none of the hits covered the *vanB* region. Thus, we hypothesize that the inserted region either comes from an unidentified gut anaerobe or represents a mosaic with elements from several sources.

3.2. A vanB-containing rep2-plasmid led to within-patient VRE emergence

KresVSE0002 and KresVRE0005 of case 2 belong to ST80, another hospital-associated globally dominant sequence type of *E. faecium* [34]. However, KresVRE0006, which was isolated after the second patient was discharged is of ST117, is yet another hospital-dominant sequence type [35]. The relatedness of the isolates is illustrated in Supplementary Fig. S1. Of note, the patient was only treated with vancomycin for 1 day (Fig. 1). KresVRE0005 and KresVRE0006 harbour some of the plasmid replicons found in the susceptible isolate from the same patient (KresVSE0002) as shown in Table 1. All three genomes show resistance genes towards tetracycline, macrolide, aminoglycoside, and trimethoprim as shown in Table 1. Both the genomes of KresVRE0005 and KresVRE0006 possess the *vanB* gene cluster. The genomes KresVSE0002 and KresVRE0005 are highly similar with no allelic differences in the MS tree (Supplementary Fig. S1) and only a difference of eight core genome SNPs, four in intergenic regions and four in hypothetical proteins (Supplementary Table S1). Since the genomes KresVSE0002 and KresVRE0006 belong to different STs, they have 309 allelic differences in the MS tree (Supplementary Fig. S1) and 6466 core genome SNPs in total.

Interestingly, KresVRE0005 and KresVRE0006 possess the same *rep2* plasmid, in which several IS-element transposase genes localize in a GC-low region, while KresVSE0002 does not possess this plasmid. The plasmid carries GC-rich *vanB* containing Tn*1549* (Fig. 3). The GC percentage shift indicates the insertion of a MGE (Tn*1549*) from a different source. While the average GC percentage for the KresVRE0005-*vanB*-plasmid is 47.2%, the average GC percentage for non-Tn*1549* in that plasmid is 35.9% and 53.0% for Tn*1549* (base range 5719–39 110).

This is an interesting case, as the plasmid persists in the patient's gut even after discharge and is found in KresVRE0006. However, whether the plasmid or Tn*1549* first entered a susceptible ST80 or ST117 strain cannot be ultimately concluded, since no vancomycin-susceptible ST117 strain or the original gut microbe was isolated. Still, we do show that the ST80 and ST117 strains share the *vanB* containing plasmid.

Table 1
Characteristics of the isolates used in this study.

Genome (phenotypic profile)	MLST	Replicon type	AMR genes					
			Tetracycline	Macrolide	Lincosamide	Aminoglycoside	Vancomycin	Trimethoprim
KresVSE0001 (amp^R, HLGR)	192	rep2_1_orf1(pRE25) rep11a_1_repA(pB82) rep18b_1_BO23315710(pE1p13) repUS15_2_repA(pNB2354p1) rep17_2_repA(AUS0004p1) rep22_1b_repB(pAMalpha1)		*msr(C)* *emr(B)*	*lnu(B)* *lsa(E)*	*ant(6′)-Ia* *aph(3′)-IIIa* *aph(2′′)-Ii* *aacA-ENT1*		*dfrF*
KresVRE0004 (amp^R, HLGR, van^R)	192	rep2_1_orf1(pRE25) rep11a_1_repA(pB82) rep18b_1_BO23315710(pE1p13) repUS15_2_repA(pNB2354p1) rep17_2_repA(AUS0004p1) rep22_1b_repB(pAMalpha1)		*msr(C)* *emr(B)*	*lnu(B)* *lsa(E)*	*ant(6′)-Ia* *aph(3′)-IIIa* *aph(2′′)-Ii* *aacA-ENT1*	*vanR* *vanS* *vanY* *vanW* *vanH* *vanB* *vanX*	*dfrF*
KresVSE0002 (amp^R)	80	repUS43_1_CDS12738(DOp1) rep22_1b_repB(pAMalpha1) repUS15_2_repA(pNB2354p1) rep11a_1_repA(pB82) rep18b_1_BO23315710(pE1p13)	*tet(L)* *tet(M)*	*msr(C)* *emr(T)*		*aph(2′′)-Ii* *aacA-ENT1*		*dfrG*
KresVRE0005 (amp^R, van^R)	80	repUS43_1_CDS12738(DOp1) rep22_1b_repB(pAMalpha1) repUS15_2_repA(pNB2354p1) rep2_1_orf1(pRE25) rep11a_1_repA(pB82) rep18b_1_BO23315710(pE1p13) rep14b_4_EMQU3221(pQY003) rep14a_3_EFAU085p5001(AUS0085p5)	*tet(L)* *tet(M)*	*msr(C)* *emr(T)*		*aph(2′′)-Ii* *aacA-ENT1*	*vanR* *vanS* *vanY* *vanW* *vanH* *vanB* *vanX*	*dfrG*
KresVRE0006 (amp^R, van^R)	117	rep2_1_orf1(pRE25) rep14b_4_EMQU3221(pQY003) rep14a_3_EFAU085p5001 (AUS0085p5) repUS15_1_repA(DO3) rep11a_1_repA(pB82) rep18b_1_BO23315710(pE1p13)	*tet(L)* *tet(M)*	*msr(C)* *emr(B)* *mef(H)*		*aph(3′)-IIIa* *aacA-ENT1*	*vanR* *vanS* *vanY* *vanW* *vanH* *vanB* *vanX*	*dfrG*

amp^R, ampicillin-resistant; HLGR, high-level gentamicin resistant (MIC >128 mg/L); van^R, vancomycin-resistant.

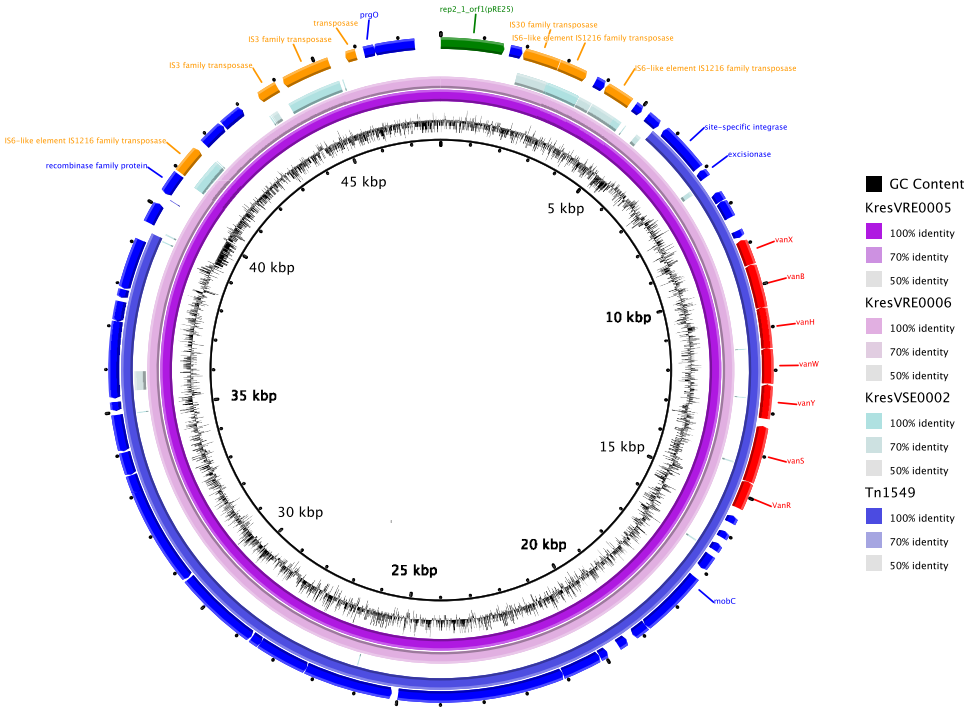

Fig. 3. Visualization of the *rep2*-plasmid. BRIG representation of the circularized KresVRE0005-*vanB*-plasmid (purple circle) in comparison to KresVRE0006 (pink circle), KresVSE0002 (turquoise circle) and the *vanB*-containing *Enterococcus faecalis* transposon Tn*1549* (AF192329.1) (blue circle). The innermost circle represents the GC content (black) of the KresVRE0005-*vanB*-plasmid. The colour intensity is proportional to the BLASTn identity. KresVRE0005-*vanB*-plasmid features are annotated: *rep2_1_orf1(pRE25)* in green, IS-element transposase genes in orange, *vanB* cluster in red, and other CDS in blue.

4. Discussion

In this study, we have analysed two different cases of vancomycin-resistant *E. faecium* emerging from successful *E. faecium* hospital clones which were originally susceptible to vancomycin in two different patients. VRE emerges with the administration of vancomycin out of VSE within a short span of time. In recent decades *E. faecium* has evolved into a successful pathogen in health care settings, with the emergence of multiple resistances and virulence factors [36]. In our case, both patients were treated with multiple antibiotics, which presumably altered the gastrointestinal tract microbiota and thus favoured colonization by drug-resistant enterococci and gut anaerobes with *vanB* [1]. Administration of broad-spectrum antibiotics alters the intestinal microbiota diversity and lowers the abundance of Gram-negatives, which results in an increased population of Gram-positives [37,38].

In addition, antibiotic stress accelerates MGE mobilization. In the GI tract, the transposon Tn*1549* that carries the *vanB* cluster has also been reported in *Clostridium, Eggerthella*, and other gut microbes [39,40]. In our case, we hypothesize that Tn*1549* has transferred horizontally from another gut microbe. It has been shown that Tn*1549* can transfer from commensal gut anaerobes to *E. faecium* in a mouse model [41]. The element Tn*1549* has host flexibility and adaptability, making it a successful MGE [33].

The collected samples do not allow us to conclusively reconstruct all mobilization and transmission events of the *vanB* cluster. In future studies, it might thus be necessary to characterize multiple enterococcal isolates. We suspect that in case 2, ST80 and ST117 might have coexisted but were not detected at an earlier timepoint.

In conclusion, we have reported two cases of *vanB* resistance acquisition through MGE transfer to vancomycin-susceptible *E. faecium*. We hypothesize that the acquisition of the *vanB* cluster is via horizontal transfer from the patient's gut microbiota.

Of note, VRE is not a life-threatening condition on its own. Yet, it is still important to understand the evolution of VRE in hospital care settings, where the patients are easily susceptible to the colonization of resistant bacteria, limiting treatment options. Accurate and appropriate choice of antimicrobials will aid in the prevention of VRE, and healthy maintenance of gut microbiota helps eradicate VRE upon colonization. Studies like ours shed light on the consequences of VRE colonization of healthy bowel microbiota and horizontal transfer to other clinically important strains such as *E. faecium* in health care settings.

Competing interests: None declared.

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Ethical approval: This study has been approved by Data Protection Officer representing the Northern Norway hospital board. The consent of the patients' closest relative was obtained to investigate, analyse, and publish the work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.12.010.

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