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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

Genetic description of VanD phenotype *vanA* genotype in vancomycin-resistant *Enterococcus faecium* isolates from a Bone Marrow Transplantation Unit

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Abstract

Background Vancomycin-resistant *Enterococcus faecium* (VREfm) is an important agent of hospital-acquired infection. VanA phenotype is characterized by resistance to high levels of vancomycin and teicoplanin and is encoded by the *van*A gene, whereas VanD phenotype is characterized by resistance to vancomycin and susceptibility or intermediate resistance to teicoplanin; however, some isolates carry a VanD phenotype with a *vanA* genotype, but there are many gaps in the knowledge about the genetic mechanisms behind this pattern.

Objective To characterize the genetic structure, clonality, and mobile genetic elements of VRE isolates that display a VanD-*van*A phenotype.

Results All *vanA* VRE-fm isolates displayed minimum inhibitory concentration (MIC) for vancomycin > 32µg/mL and intermediate or susceptible MIC range for teicoplanin (8–16µg/mL). The isolates were not clonal, and whole-genome sequencing analysis showed that they belonged to five different STs (ST478, ST412, ST792, ST896, and ST1393). The absence of some *van* complex genes were observed in three isolates: Ef5 lacked *vanY* and *vanZ*, Ef2 lacked *vanY*, and Ef9 lacked *orf*1 and *orf*2; moreover, another three isolates had inverted positions of *orf1*, *orf2*, *vanR*, and *vanS* genes. *IS*1542 was observed in all isolates, whereas *IS*1216 in only five. Moreover, presence of other hypothetical protein-encoding genes located downstream the *vanZ* gene were observed in six isolates.

Conclusion VRE isolates can display some phenotypes associated to *vanA* genotype, including VanA and VanB, as well as VanD; however, further studies are needed to understand the exact role of genetic variability, rearrangement of the transposon Tn1546, and presence of insertion elements in isolates with this profile.

Keywords *Enterococcus faecium* · Whole-genome sequence · Resistance · Molecular characterization

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Introduction

Vancomycin-resistant *Enterococcus faecium* (VREfm) is an important agent of hospital-acquired infection [1]. Among six glycopeptide-resistant phenotypes described, VanA is the most common, characterized by resistance to high levels of both vancomycin and teicoplanin, and usually encoded by the *vanA* gene [2]. VanD phenotype is characterized by resistance to vancomycin and susceptibility or intermediate resistance to teicoplanin [3].

Genetic variability can determine the phenotype. The VanD phenotype in VREfm is usually justified by the presence of *vanD*. However, some isolates presenting VanD phenotype carry the *vanA* gene, and some genetic phenomena have been associated with this pattern, such as impairment of accessory genes (*vanY* or *vanZ*) due to the insertion of IS16 [4]. However, studies investigating VanD phenotype *vanA* genotype are rare and explore but a few strains. Thus, there are many gaps in the knowledge about genetic mechanisms behind this pattern, such as the mobile genetic elements and bacterial strains involved.

The aim of this study was to characterize the genetic structure, clonality, and mobile genetic elements of the VRE isolates that display a VanD-*vanA* phenotype.

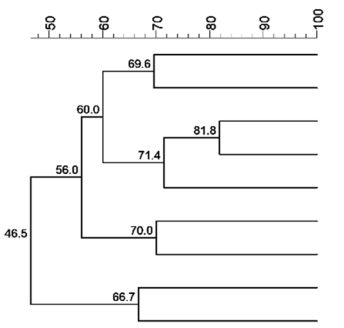
Material and methods

Nine clinical VREfm isolates from different patients, described in a previous study, were selected for molecular characterization based on their glycopeptide susceptibility profile (vancomycin-resistant and teicoplanin-susceptible, or teicoplanin-intermediate), confirmed by broth microdilution methods, interpreted according to Clinical and Laboratory Standards Institute [5], and based on the presence of the *vanA* gene after polymerase chain reaction (PCR) confirmation [6]. They were isolated from clinical samples of inpatients of a Bone Marrow Transplantation Unit in a teaching hospital in São Paulo, Brazil, between 2005 and 2014. These isolates were submitted to pulsed field gel electrophoresis (PFGE) to evaluate the clonality using enzyme FastDigest SmaI (Thermo Fisher Scientific, Waltham, MA, USA), and a tolerance of 1.5% and optimization of 0.5% were used for comparison. Isolates with similarity < 80% were considered different [7]. Whole-genome shotgun project was deposited at the DDBJ/EMBL/GenBank: QHLB00000000 (Ef1), MXAT00000000 (Ef2), QHLC00000000 (Ef3), QNUP00000000 (Ef4), MVGF00000000 (Ef5), MVGH00000000 (Ef6), QNUQ00000000 (Ef7), QHLD00000000 (Ef8), and MVGJ00000000 (Ef9).

Whole-genome sequencing of all VREfm isolates was performed using MiSeq Illumina™ (Illumina Inc., San Diego, CA, USA) or Ion Torrent technologies [8, 9]. The *Enterococcus faecium* BM4147 (GenBank accession number M97297) strain was used as a reference for molecular characterization. MLST, virulome, and resistome analysis were performed using MLSTFinder 2.0 [10], VirulenceFinder 2.0 [11], and ResFinder 4.0 [12], respectively. Additionally, the genes related to *van* transposon were searched in the genomes using local BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [13]. The draft genomes were used for alignment of the transposon region and visualized using EasyFig [14].

Results

All *van*A-positive VRE-fm isolates displayed minimum inhibitory concentration (MIC) for vancomycin of 128 µg/mL (Ef1, Ef2, Ef4, Ef5, Ef6, Ef7, Ef9) or 256 µg/mL (Ef3, Ef8). They were intermediate (MIC 16 µg/mL: Ef3, Ef6, Ef1, Ef9, Ef4, Ef7, Ef8) or susceptible (MIC 8 µg/mL: Ef2, Ef5) for teicoplanin, compatible with VanD phenotype. PFGE analysis showed that they are not clones and belong to nine different clusters (Fig. 1). Whole-genome sequencing analysis showed that they belong to five different lineages: ST478



Sample	Isolation site	Isolation Date	ST	MIC (µg/mL) Vancomycin	MIC (µg/mL) Teicoplanin
*Ef*3	Colonization	2013	792	256	16
*Ef*6	Colonization	2014	896	128	16
*Ef*1	Infection	2007	412	128	16
*Ef*9	Colonization	2014	896	128	16
*Ef*2	Infection	2006	478	128	8
*Ef*4	Colonization	2013	896	128	16
*Ef*5	Colonization	2013	896	128	8
*Ef*7	Colonization	2014	1393	128	16
*Ef*8	Colonization	2014	896	256	16

Fig. 1 Clonality of nine VanD phenotype *Enterococcus faecium*

Table 1 Phenotypic and genotypic features of the isolates from this study

Sample	Isolation site	Isolation date	Susceptible profile		Resistome			Virulome	
			Vancomycin MIC µg/mL	Teicoplanin MIC µg/mL	Aminoglycoside	Glycopeptide	MLS	Adherence	Colonization
Ef1	Blood	2007	128	16	*aph(3')-III, ant(6)-Ia*	*vanH-A, vanA, vanX-A, vanZ-A, vanY-A, vanR-A, vanS-A*	*msr(C), erm(B)*	*acm, efaAfm, esp, asa1*	*gelE*
Ef2	Blood	2006	128	8	*aph(3')-III, ant(6)-Ia*	*vanH-A, vanA, vanX-A, vanZ-A, vanR-A, vanS-A*	*msr(C), erm(B), lnu(B)*	*acm, efaAfm, esp, asa1*	*gelE*
Ef3	Rectal swab	2013	256	16	*aph(3')-III, ant(6)-Ia*	*vanH-A, vanA, vanX-A, vanZ-A, vanY-A, vanR-A, vanS-A,*	*msr(C), erm(B)*	*acm, efaAfm, esp, asa1*	*gelE*
Ef4	Feces	2013	128	16	*aph(3')-III, aac(6')-aph(2'')*	*vanH-A, vanA, vanX-A, vanZ-A, vanY-A, vanR-A, vanS-A*	*msr(C), erm(B), lnu(B)*	*acm, efaAfm, esp, asa1*	-
Ef5	Feces	2013	128	8	*aph(3')-III, ant(6)-Ia*	*vanH-A, vanA, vanX-A,vanR-A, vanS-A*	*msr(C), erm(B)*	*acm, efaAfm, esp, asa1*	-
Ef6	Feces	2014	128	16	*aph(3')-III, ant(6)-Ia*	*vanH-A, vanA, vanX-A, vanZ-A, vanY-A, vanR-A, vanS-A*	*msr(C), erm(B)*	*acm, efaAfm, esp*	-
Ef7	Nasal swab	2014	128	16	*aph(3')-III*	*vanH-A, vanA, vanX-A, vanZ-A, vanY-A, vanR-A, vanS-A*	*msr(C), erm(B)*	*acm, efaAfm*	-
Ef8	Feces	2014	256	16	*aph(3')-III, ant(6)-Ia*	*vanH-A, vanA, vanX-A, vanZ-A, vanY-A, vanR-A, vanS-A*	*msr(C), erm(B)*	*acm, efaAfm, esp*	-
Ef9	Feces	2014	128	16	*aph(3')-III, ant(6)-Ia*	*vanH-A, vanA, vanX-A, vanZ-A, vanY-A, vanR-A, vanS-A*	*msr(C), erm(B)*	*acm, efaAfm, esp*	-

MIC minimum inhibitory concentration, *MLS* macrolide, lincosamide, and streptogramin

($n=1$), ST412 ($n=1$), ST792 ($n=1$), ST896 ($n=5$), and ST1393 ($n=1$). Virulome analysis showed that the isolates harbor genes related to adherence (*acm*, *efaAfm*, *esp*, and *asa*1) and colonization (*gel*E), but the *asa*1 gene is present in only five isolates (Ef3, Ef6, Ef1, Ef9, and Ef2), and *gelE* in only three isolates (Ef3, Ef6, and Ef1). Resistome analysis identified that the isolates harbor resistance genes related to aminoglycoside, glycopeptide, macrolide, lincosamide, and streptogramin (Table 1). Three isolates did not harbor some of the *vanA* genotype complex genes: Ef5 lacked *vanY* and *vanZ*, Ef2 lacked *vanY*, and Ef9 lacked *orf*1 and *orf*2 (Fig. 2). Three isolates (Ef3, Ef4, and Ef7) had inverted positions of *orf1*, *orf2*, *vanR*, and *vanS* genes. The insertion sequence *IS*1542 was present in all isolates, and in seven of them, it was duplicated in different regions. Isolate Ef9 (ST896) presented a different pattern compared with others,

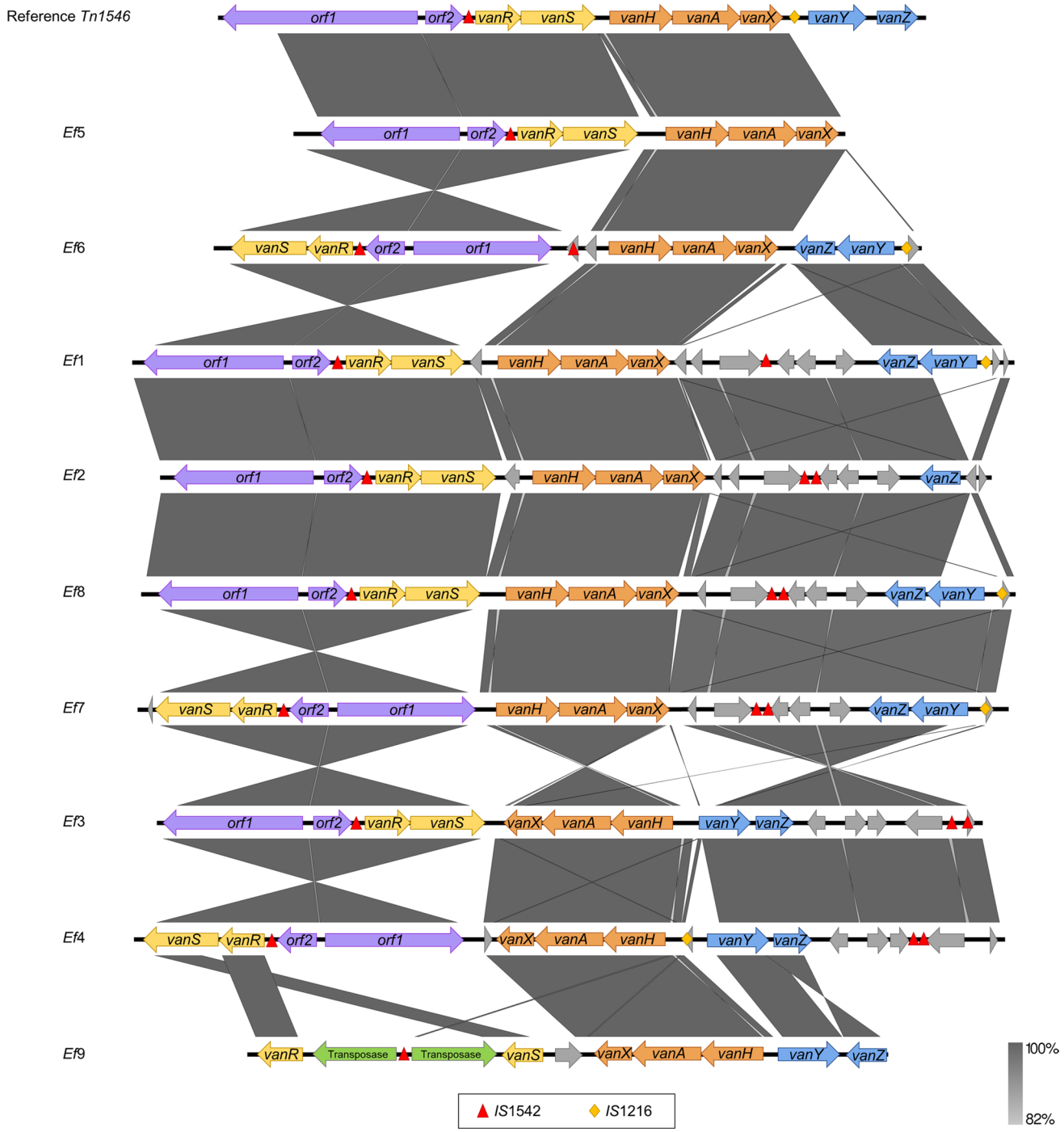


Fig. 2 Structures and diversity of *van* transposons in nine VanD phenotype *Enterococcus faecium*

with *IS*1542 between two transposases that were situated between *vanR* and *vanS* genes. The *IS*1216 was also present in five isolates (Ef6, Ef1, Ef8, Ef7, and Ef4), located downstream of the *vanY* gene. When compared to the reference, six isolates (Ef1, Ef2, Ef8, Ef7, Ef3, and Ef4) presented insertion of other hypothetical protein-encoding genes located downstream the *vanZ* gene (Fig. 2).

Discussion

This study describes the phenotypic and genotypic characteristics of nine *Enterococcus faecium* clinical isolates with vancomycin resistance profile and susceptibility or intermediate to teicoplanin; however, this profile has been previously described in other studies in Brazil [15, 16].

The isolates were not clonal, harbored *vanA* gene, and, although the isolates belonged to clonal complex (CC) 17, the STs were distinct, despite ST896 being the most prevalent among them. However, the STs are different to previous studies that reported isolates with VanD-*vanA* profile and belonged to the ST203, ST192, ST17, ST80, ST78, ST18, ST205, and ST206 [17, 18].

The most common mechanism of vancomycin and teicoplanin resistance is through the *vanA* gene that confers a VanA phenotype and is carried by transposon Tn*1546*. However, studies have reported isolates with genotype *vanA*, resistant to vancomycin, but exhibit susceptibility or intermediate to teicoplanin characterizing a VanD phenotype and were previously described in Asia and Europe [2, 4, 17, 19]. In the present study, all nine isolates carried the *vanA* gene and had the susceptibility profile consistent with previous reports.

Studies suggest that some characteristics may be related to the VanD-*vanA* phenotype, such as point mutations in *vanS*, presence of insertion elements along the transposon or in the *vanY*, and impairment of the accessory genes *vanY* and *vanZ* [17–19]. In our isolates, we did not observe mutations in *vanS* or the presence of insertion elements in the *vanY* gene, but rather, presence of the insertion sequence IS1216 was found in three isolates, and *IS*1542, in different positions along of transposons. Moreover, absence and rearrangement of accessory genes *vanY* and *vanZ* as well as the presence of uncharacterized proteins in most of the isolates were observed. Although inversions were found in three genomes (Ef3, Ef4, and Ef7), we could not find data in the literature supporting this phenomenon. All together, these results are in agreement with other studies that describe that ISs have an important role in the transposon formation, which could lead to genetic alterations as well as the deletion of *vanY* or *vanZ* or both in the loss of teicoplanin resistance [20–22].

Conclusion

Some VRE isolates can display phenotypes associated to *vanA* genotype, including the most commonly observed VanA and VanB, as well as VanD. However, further studies are needed to understand the exact role of genetic variability, rearrangement of the transposon Tn1546, and presence of insertion elements in isolates that exhibit the VanD-*vanA* profile.

Author contribution All authors have reviewed and approved the manuscript.

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Declarations

Ethics approval These experiments were approved by the Ethical Committee of Hospital das Clínicas of the University of São Paulo, Brazil.

Conflict of interest The authors declare no competing interests.

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