Chapter

Mobile Genetic Elements in Vancomycin-Resistant *Enterococcus faecium* Population

Gastón Delpech, Leonardo García Allende and Mónica Sparo

Abstract

Horizontal gene transfer constitutes a key driving force in bacterial evolution. The ability to acquire mobile genetic elements encoding antimicrobial resistance has contributed to the emergence of *Enterococcus faecium* as one of the main human nosocomial opportunistic pathogens. The deep analysis of the vancomycin-resistant *E. faecium* (VREfm) population's mobilome, as the architecture and evolution of the core genome enables to observe VREfm plasticity and power of adaptation in animals, plants, environment and food. The persistence of VREfm is facilitated by the exchange of plasmids, phages and conjugative transposons that have allowed them to achieve a rapid adaptation to changes in environmental conditions. They can acquire resistance determinants from several species and transfer resistance genes to other potentially pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* strains.

Keywords: *E. faecium*, vancomycin, resistance, mobile genetic elements

1. Introduction

Enterococcus faecium is a main bacterial agent of healthcare-associated infections in immunocompromised as well as severely ill patients, with a worldwide distribution [1, 2].

In 1988, vancomycin-resistant *E. faecium* (VREfm) was reported for the first time. Along the 1990s, a fast increase of infectious diseases due to this bacterium was detected in the United Kingdom hospitals, linking its emergence with the employment of the glycopeptide avoparcin in animal husbandry for food industry. In addition, at U.S hospitals it was observed the emergence of VREfm, but with not proved association to the use of avoparcin in animals [3–5].

The World Health Organization's global priority pathogens list of antibiotic-resistant bacteria has categorized VREfm as of high priority. For infectious diseases produced by VREfm, it has been reported that the therapeutic options are more limited, altogether with higher mortality rates and financial costs for the Health system when compared with vancomycin-susceptible enterococci [6, 7].

Food chain can be considered as one possible way of VREfm spread or for the transfer of its antimicrobial resistance genes to humans, as it has been reported for cattle, pork and poultry meat [5, 8].

In the European Union, despite the avoparcin ban 18 years ago, VREfm circulation in the environment has continued. A likely cause of vancomycin-resistance

plasmid genes persistence is the co-selection of other antimicrobials used in animals, such as macrolides or narasin, as it has been suggested by the presence of *ermB* type transporter genes (macrolide-lincosamide-streptogramin B resistance), as well as ABC type transporter genes and the presence of a toxin/anti-toxin system. Other possibilities which can relate with VREfm spread is their persistence in food farms, slaughterhouses or their environments due to poor hygienic conditions or through avian transmission [9–11].

It is important to highlight that, enterococci, as part of human and animal intestinal microbiota, are able to acquire resistance genes from other commensal bacteria, which can be spread as well to other pathogenic bacteria [12, 13].

Evolution of *E. faecium* from intestinal commensal bacteria to opportunistic pathogen is a complex and sequential process, in which seem to have been involved different factors, such as resistance and virulence determinants acquisition and persistence. Their expression is assumed to give an adaptive advantage since these factors facilitate the colonization of different epithelial cells (urinary, oral or intestinal), and at the same time, the bacterial adhesion to a wide variety of extracellular matrix proteins.

2. VREfm: natural and acquired antimicrobial resistance

E. faecium is intrinsically resistant to penicillin, ampicillin, cephalosporins and other β-lactams by mutations in the penicillin-binding protein PBP5 that is encoded by a horizontally transferred gene. Globally, enterococci are *in vivo* resistant to clindamycin (efflux pumps), trimethoprim-sulfamethoxazole (missing target) and the majority of aminoglycosides (enzymatic degradation). Furthermore, *E. faecium* has been acquiring resistance to quinolones, rifampicin and chloramphenicol, through mutations or by horizontal gene transfer [14–18].

In regard with vancomycin resistance, only the *vanA* and *vanB* genotypes are epidemiologically relevant in clinical isolates. In this sense, the *vanA* cluster is the most prevalent glycopeptide resistance determinant in clinical settings. Recently, the presence of *vanB* cluster has increased in Europe, while is the main vancomycin resistance mechanism in Australia. These genotypes are associated with mobile genetic elements. The *vanA* gene cluster is generally part of the Tn*3*-family transposon Tn*1546*. Among *vanB* cluster, $vanB_2$ is the most frequent subtype and constitutes an integral part of the integrative conjugative element Tn*1549*/*5382* [19–23].

2.1 The VREfm-mobilome

The mobilome is defined as all the mobile genetic elements (MGEs) able to move around within or between genomes. MGEs contribute to genome plasticity and dissemination of antimicrobial resistance and pathogenicity bacterial genes. In *E. faecium*, the acquisition of exogenous DNA is involved in the change of a commensal bacterium for becoming a pathogenic strain [24].

Horizontal gene transfer (HGT) allows the exchange of genetic material between bacteria. The most important HGT mechanism is conjugation, where the type IV secretion systems create channels between bacterial cells for transferring DNA.

The others mechanisms involved in HGT are transformation, in which bacteria are able to internalize naked DNA located in their immediate environment, and transduction, in which DNA is trapped within bacteriophages that have infected a bacterial cell and, then, is released and inserted into the genome of a new cell after bacteriophage transmission. Other gene transfer mechanisms

as nanotubes, micro-vesicles and gene-transfer agents have not been described in enterococci yet [25, 26].

There have been described three mechanisms of attack and defense interacting with HGT, toxin-antitoxin (T/A) systems, restriction/modification (R/M) systems and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas enzymes.

- T/A systems are small elements conformed by a toxin gen and its related antitoxin. Plasmid-encoded TA elements are important for plasmid maintenance. There are five types of T/A systems but only type 2 is prevalent in enterococci. In *E. faecium*, type 2 T/A systems comprise Axe/Txe and omega/epsilon/zeta. These plasmid-located T/A systems are enriched in clinical multi-drug resistant isolates [26–28].

- R/M systems, in which a restriction enzyme cleaves in a specific unmethylated DNA site and other enzyme links a methyl group to the same site; thus, DNA cleavage is blocked. This system contributes with the regulation of gene exchange in *E. faecium* [29].

- CRISPR-Cas systems constitute endogenous barriers to HGT in bacteria. A set of genes (*cas*) encoding nucleases are located near the CRISPR. Nosocomial clade of *E. faecium* is in great measure deficient of the CRISPR-Cas systems [30]. This fact is associated with the increased presence of MGEs. Conversely, commensal *E. faecalis* contain type II CRISPR-Cas systems, but multi-drug resistant (MDR) strains not carry complete CRISPR systems. Thus, MDR *E. faecalis* are prone for acquiring antibiotic resistance genes [31].

Among enterococci, different types of DNA arrangements and/or MGEs can be found, such as insertion sequences (IS), pathogenicity islands (PAIs), transposons (Tn) and plasmids.

IS are DNA segments (0.5–2 kb) able to autonomously move and to be found integrated in any replicon, in chromosomes as well as in plasmids. When IS appear in the middle of genes, they can interrupt the encoding sequence and inactivate the gene expression.

PAIs are fractions of a microorganism's genomic DNA linked with encoding sequences for virulence traits, such as adhesins, host immune evasion factors, toxins, cell components lytic enzymes, among others. Usually, PAIs are included in plasmids and their origin is associated with horizontal transfer of genetic material.

Tn are genetic elements that are directly movable as DNA and can harbor adaptive functions such as an antimicrobial resistance mechanism.

Plasmids are small extrachromosomal DNAs that can replicate independently (replicons). In enterococci, these genetic elements are wide-spread. Plasmid size is variable and is reflected in the number of genes they contain and the range of encoded functions. Plasmids are able to include antimicrobial resistance genes, stability modules and conjugation modules. In addition, are termed conjugative plasmids when they encode the type IV secretion system (T4SS) and are mobilizable if they contain the origin of transfer (*oriT*) and the relaxase protein, type IV coupling protein T4CP. In enterococci, plasmid replication proteins may be classified by mode of replication, sequence similarity and subdomains present within the translated gene. Replication proteins replicate the plasmids by unidirectional leading strand Rolling Circle Replication (RCR) and by bi-directional Theta (q) replication. RCR plasmids are frequently small, cryptic and unstable over a 10–15 kb size.

A plasmid typing method based on the replication regions from various plasmid incompatibility groups was described in enterococci and other Gram-positive bacteria, and 19 replicon families (*rep*-family) and some unique replicons were found [32].

The q plasmids are subdivided into replicon families: Rep_3, Inc18 and RepA_N:

- Rep_3 plasmids: narrow host range of similar size to RCR plasmids and often cryptic.
- Inc18 plasmids: often conjugative (25–50 kb) broad host-range plasmids; most of them harbor resistance determinants.
- RepA_N plasmids (10–300 kb): prevalent in low G + C content Gram positive bacteria with a narrow host range.

This scheme can be modified by recombination, leading to mosaic structures [26, 33–36].

The pheromone-responsive plasmids have been described mainly in *E. faecalis*; pAD1 and pCF10 were the first described.

Different plasmid diversity between VREfm and *E. faecalis* strains producers of nosocomial infections can be observed. VREfm, mainly CC17, show many *rep* types as rep_{11} (pB82), rep_{14} (pRI1), rep_{18} (pEF418), rep_{unique} (pC1Z2) rep_1 and rep_2 (Inc18), rep_{17} (pRUM), rep_{unique} (pHTβ) were found. Vancomycin-resistant *E. faecalis* carry a lower diversity of plasmid, generally associated with rep_9 type (pheromone responsive pAD1), rep_1 and rep_2 (Inc-18 type) as well [34].

The presence of big transferable plasmids, also known as megaplasmids (>150 kb) is common among clinical isolates of *E. faecium*, and can have a role related with their virulence. Often, these plasmids contain genes linked with different carbohydrates metabolism, such as hyl_{Efm} gene. Initially, it was suggested that this gene encoded for a hyaluronidase. Nevertheless, more recent sequencing studies showed that, actually, this gene encodes for a glycosyltransferase which allows the utilization of complex carbohydrates. Furthermore, it has been proven that the transfer of these MGEs to non-carrying plasmid commensal strains of *E. faecium*, will increase their virulence and their gastrointestinal colonization capability [19, 33, 37].

Worldwide, most of the VREfm strains recovered in clinical settings were included into the clonal complex 17 (CC17). Afterwards, they were divided into three lineages (17, 18 and 78), using multilocus sequence typing studies (MLST). More recently, the Bayesian Analysis of Population Structure (BAPS), applied to MLST data established two nosocomial groups: 2–1 (lineage 78) and 3–3 (lineages 17/18). All CC17 *E. faecium* strains contain many exogenously acquired genes such as IS, phages, and Tn encoding antimicrobial resistance. Furthermore, hospital-adapted VREfm are ciprofloxacin and ampicillin-resistant, with virulence traits also found in theirs genomes. VREfm strains have cell surface protein genes, regulatory genes, putative PAIs, plasmids, IS and integrated phages, which promote their adaptation to the healthcare-associated environment. The IS*16* and the *esp* gene are carried by an integrative conjugative element (ICEEfm1) with the *intA* integrase gene, and are considered as markers of nosocomial *E. faecium* strains [5, 17, 29].

In *E. faecium* CC17, the location of hyl_{Efm} gene was described in a large conjugative plasmid, pLG1 (281.02 kb), in association with the *vanA* operon, the *ermB* gene (macrolide-lincosamide-streptogramin B resistance) and the *tcrYAZB* operon (heavy metal resistance). The hyl_{Efm} gene, an important factor involved in enterococcal colonization and adhesion, it has also been described as part of a genomic

island. The dissemination of the multi-resistant megaplasmid pLG1, carrying hyl_{Efm} could explain the spread of the so frequently isolated hospital-associated *E. faecium* CC17 genotype [33].

Transposable elements contribute with the genome plasticity by different mechanisms. They are substrates for homologous recombination within and between different DNA elements and rearrangements are carried out in chromosome and plasmid DNA [38].

In glycopeptide-resistant enterococci, vancomycin resistance is classified into eight acquired gene clusters: *vanA*, *vanB*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM* and *vanN*. VanA- and VanB-type vancomycin-resistant enterococci (VRE) constitute the majority of VRE in clinical settings. VanA-type VRE shows high-level resistance to vancomycin (Minimum Inhibitory Concentration, MIC = 64–100 mg/L) and teicoplanin (MIC = 16–512 mg/L), while VanB-type VRE is susceptible to teicoplanin (MIC = 0.5–1 mg/L) and expresses different levels of resistance to vancomycin (MIC = 4–1000 mg/L). Also, it can be mentioned the intrinsic *vanC* genotype, found in *E. gallinarum* and *E. casseliflavus* [39, 40]. The main phenotypic and genotypic features of glycopeptide resistant enterococci are shown in **Table 1**.

The *vanB* gene cluster consists of a two-component regulatory system (*vanRB*, *vanSB*) and five resistance genes (*vanYB*, *vanW*, *vanHB*, *vanB*, *vanXB*). Conversely to the highly conserved resistance genes, the amino acid sequences of VanSB and VanRB show less similarity to those of VanSA and VanRA. These differences could be responsible for the characteristics of VanB-type resistance [41].

Furthermore, low-level vancomycin resistant *E. faecium* can turn into high-level vancomycin resistant during antibiotic therapy. This variant was named vancomycin-variable *E. faecium* [20, 39, 42]. A schematic diagram of *van* operon is shown in **Figure 1**.

Tn*1546* carries the *vanA* gene, and is often located on a plasmid belonging to the broad host range Inc18 family, involved in the *vanA* transfer from enterococci to *Staphylococcus aureus*. Typically, the *vanA* operon is associated with Tn, such as Tn*1546*, implicating two genes for the transposition of the element (*orf1* and *orf2*), and one gene involved with teicoplanin resistance (*vanZ*). The *vanA* gene cluster includes seven open reading frames transcribed from two separate promoters. The regulatory apparatus is encoded by the *vanR* (response regulator) and *vanS* (sensor kinase) two-component system. Both are transcribed from one promoter, while the remaining genes are transcribed from other promoter. *vanH* (dehydrogenase that converts pyruvate to lactate) and *vanA* (ligase that forms D-Ala-D-Llac dipeptide) modify the production of peptidoglycan precursors. The production of the normal ending D-Ala-D-Ala of the pentapeptide does not continue. The products of *vanX* (encodes a dipeptidase that cleaves D-Ala-D-Ala) and *vanY* (encodes a D, D-carboxypeptidase) genes hydrolyze, interrupt the production of the pentapeptides and cleave the pentapeptides that can still be produced. The variations in the composition of this vancomycin resistance operon is due to the insertion of IS elements. The *vanB* operon is carried by Tn*1547*, Tn*1549* and Tn*5382*. Tn*1549* conjugative Tn, is mainly located in large conjugative chromosomal elements and less frequently integrated in conjugative plasmids. This conjugative *vanB* Tn is widely prevalent among *VanB* type enterococci and other Gram-positive bacteria. The *vanB* operon has a similar genetic organization to the *vanA* because *vanB* operon contains two distinct promoters transcribing seven open reading frames. But *vanB* encodes a two-component signaling system (named *vanRB* and *vanSB*) that is considerably different from that encoded in *vanA*. Furthermore, *vanB* encodes homologs of *vanH* and the D-Ala-D-Ala ligase, and the peptidases (*vanX* and *vanY*). In addition, *vanB* lacks a homolog of *vanZ*, and instead encodes a protein *vanW*, with a role not totally explained. *vanB* gene (ligase) has been divided in three subtypes,

Phenotype	**VanA**	**VanB**	**VanD**	**VanE**	**VanG**	**VanL**	**VanM**	**VanN**	**VanC**
Resistance	Acquired	Acquired	Acquired	Acquired	Acquired	Acquired	Acquired	Acquired	Intrinsic
MICvan	16–>1000	4–>1000	16–128	8–32	16	8	>256	16	2–32
MIC_{tei}	16–512	0.25–2	2–64	0.5	0.5	0.5	96	0.5	0.12–2
Expression	I	I	C	I	I	I	I	C	I, C
Mobiltiy	Yes	Yes	No	No	Yes	No	Yes	Yes	No
Precursor	Ala-Lac	Ala-Lac	Ala-Lac	Ala-Ser	Ala-Ser	Ala-Ser	Ala-Lac	Ala-Ser	Ala-Ser
Operon	*vanA*	*vanB*	*vanD*	*vanE*	*vanG*	*vanL*	*vanM*	*vanN*	*vanC*
Subtypes	N/A	B1-B3	D1-D5	N/A	G1-G2	N/A	N/A	N/A	C1-C4
Required genes for expression	*vanH*, *vanA*, *vanX*	$vanH_B$, *vanB*, $vanX_B$	$vanH_D$, *vanD*, $vanX_D$	*vanE*, $vanXY_E$, $vanT_E$	*vanG*, *vanXYG*, *vanTG*	*vanL*, $vanXY_1$, $vanT_m$, $vanTr_1$	$vanH_M$, *vanM*, $vanX_M$	*vanN*, $vanXY_N$, $vanT_N$	*vanC*, $vanXY_C$, *vanT*

MIC, minimum inhibitory concentration (mg/L); van, vancomycin; tei, teicoplanin; I, inducible; C, constitutive; N/A, not applies. Adapted from [39, 40].

Table 1.
Main phenotypic and genotypic features of glycopeptide-resistant enterococci.

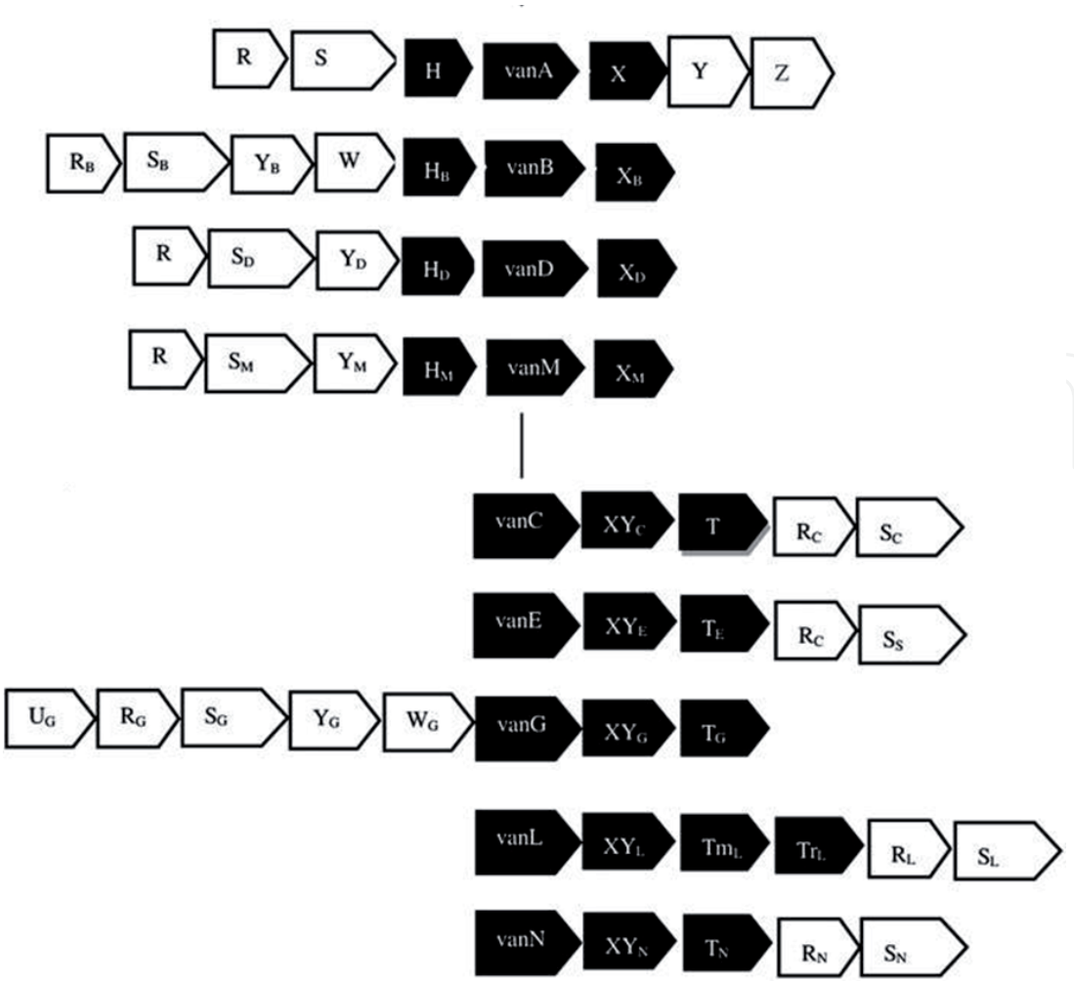

Figure 1.
Schematic diagram of van operon. Adapted from [40].

vanB1-3, based on nucleotidic sequence differences. *vanB2* subtype is the most commonly spreaded in clinical enterococci. Also, is part of conjugative Tn, Tn*1549*/Tn*5382*-like. The first description of a *vanB2*-Tn*1549*-like element in pheromone responsive plasmids (pCF10-like) carried by *E. faecalis* was reported at Japan. *vanB1* has only been described for certain isolates as part of composite Tn or an integrative conjugative element [21, 43–48].

It has been described that some *vanA* genotype isolates had a new type F Tn*1546* Tn associated with two insertion sequences: IS*1216V* and IS*1251* [49].

The *vanM* cluster is similar to *vanA*, *vanB*, and *vanD*, while *vanL* and *vanN* are similar to *vanC*. The *vanM* operon has been described in VREfm isolates and showed a close genetic arrangement to *vanD* and *in vitro* transferable resistance by conjugation. The *vanN* operon is the most recently identified gene cluster described in *E. faecium* and is a similar operon to *vanG*, but can be transferred by MGEs only in this enterococcal species. The IS elements can produce structural alterations in the genes, and as a consequence leads to changes of resistance phenotype. The *vanA* gene cluster is prone to IS-mediated alterations, modifying sometimes the vancomycin resistance phenotype, as being susceptible to glycopeptides but with possibility to revert to a resistant phenotype. These bacteria were named vancomycin-variable enterococci (VVE), which could cause serious clinical issues because of their possibility to escape of detection and surveillance as well as facilitating the horizontal transfer of vancomycin resistance [50–52].

An additional operon (*vanF*) was described but only in *Paenibacillus popilliae*. This *vanF* cluster has a high similarity to the amino acid sequences

of the *vanA* operon, and *P. popilliae* has been proposed as a possible origin for vancomycin resistance in enterococci [53].

3. Conclusions

The massive use of glycopeptides (vancomycin and teicoplanin) and non-glycopeptide agents such as extended-spectrum cephalosporins in clinical settings have been implicated in the emergence of VREfm. Delayed effective antimicrobial therapy more than 48 h after the beginning of VRE bacteremia is associated with higher mortality rates.

The core genes bring a phylogenomic reconstruction of the *E. faecium* population structure; the main contribution of accessory genes includes the adaptation of this species to nosocomial environments. It was observed that the plasmid component drives host specificity, while their whole genome and chromosome share a common evolutionary history.

The clinical isolates' mobilome are quite different from the other hosts. In VREfm the plasmid component of the pan-genome plays an important role in adaptation and its emergence as a nosocomial pathogen.

Conflict of interest

Authors declare no conflicts of interest.

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