EXTRAGENIC MOBILE ELEMENTS OF GLYCOPEPTIDE RESISTANCE IN ENTEROCOCCI AND STAPHYLOCOCCI

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Abstract

A wider knowledge of the spectrum of mobile genetic elements would allow a quicker identification and a more accurate definition of glycopeptide resistance determinants and strains as well as a better insight into their mechanisms of dissemination around the world. It would also permit swifter detection of lateral spread from humans to animals and vice versa. Insertion sequences (ISs) that create point mutations, deletions, and insertions introduce either enzymatic restriction sites or changes in the glycopeptide susceptibility patterns of isolates by disrupting some genes. Specific transposons of VanA elements are Tn*1546*, Tn*5281*, and Tn5482; those of VanB elements are Tn*1547*, Tn*5382*, and Tn*1583*. Tn*1546* has been detected on mobile Enterococci, *P. apiarius*, *B. circulans*, and *S. aureus*, among other genera, either by conjugation or transposition. ISs have been reported on intergenic regions *orf2-vanR* (IS*1542*, IS*1216V*, IS*1251*), *vanS-vanH* (IS*1251*, IS*1476*, IS IS*19*), and *vanX-vanY* (IS*1216V*, IS*1678*, IS*Efa5*). Detection of these ISs has prompted four different classifications of VanA elements: i) Four polymorphisms, A through IV, determined by enzymatic digestion; ii) 24 groups, identified as A through X, from using 10 pairs of *vanA* cluster oligonucleotides; iii) seven groups, A to G, obtained by RFLP; and iv) three types (I-III) depending on localization and number of IS copies into intergenic regions. IS elements are important not only for epidemiological studies but also for detection of potential promoters.

Key words: Tn*1546*, glycopeptides resistance, enterococci.

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The emergence of glycopeptide resistant enterococci occurs even without the antibiotic pressure of the hospital. In Europe it is difficult to find vancomycin resistant enterococci (VRE) associated to nosocomial infections. This is due to the use of avoparcin as a supplement in animal feed. VRE today is ubiquitous in community, sewage, and animal sources. In the past century, no VRE was isolated from animals in the United States (1-7).

Several human isolates are indistinguishable from those from nonhuman sources. The use of markers could identify the origin of the strains. The production of acid from rafinose in *E. faecium* could function as a marker, since it has been suggested that this test is positive in poultry isolates. The fermentation of rafinose in *E. faecium* could also be utilized as another possible marker of strains transmitted from chickens to humans (8). However, until this kind of biochemical identification is firmly established, the origin of the strains must be studied through the insertion sequence elements (IS elements) (9-11).

Enterococci have a great capacity to transfer antibiotic resistance determinants. One of the pathways is the conjugative transfer via plasmids or transposons. The conjugative transposon, Tn*916*, is considered one of the most promiscuous mobile elements, since it can be transferred to a wide variety of Gram positive and Gram negative species (12). Non–self–transferable transposons have also been described; they play a role in the dissemination of resistance determinants through intracellular transposition in conjugative plasmids. Enterococci have two kinds of transposons: compound and Tn*3*–like transposons (12).

An *E. faecium* isolate recovered from a Michigan dog with urinary tract infection presented two different conjugative plasmids: one, located in Tn*1546*, encoding high level glycopeptide resistance, and the other, in Tn*5281*, encoding high level aminoglycoside resistance. The high level of gentamicin resistance was due to the presence of the *aac6'–aph2"* gene flanked by IS*256* in the Tn*5281* transposon. It was the first report of canine *E. faecium* isolated in the United States with transposon Tn*1546* indistinguishable from those found in human isolates. Companion animals can be reservoirs of genes conferring clinically relevant resistance (13). IS*256* has been described in *Staphylococcus aureus*, coagulase negative staphylococci, and enterococci. Compound transposons such as Tn*4001*, Tn*4031*, Tn*5281*, and Tn*5384*, can carry a high degree of resistance to aminoglycosides (enterococci or staphylococci) (14-17).

The transposons and IS elements can act as targets for the insertion of other mobile elements (18-20). *E. faecalis* carries Tn*5385*, a transposon made up of several smaller mobile elements such as Tn*5381*, Tn*5384*, and Tn*552*–like elements stemming from *Staphylococcus*. This transposon evolved as a result of cointegration among wide host range enterococcal plasmids and smaller, mobile staphylococcal betalactamase plasmids (21). Methicillin–resistant *S. haemolyticus* harbors IS*1272*, which is quite similar to another from *E. hirae*, which suggests that IS*1272*-like elements can dwell in both genera and that genetic exchange can occur among their members (16, 22-24). ISs enterococcal similar to those found in *S. aureus* have been described, such as in the case of IS*1165* (25), and IS*1181* of *Leuconostoc mesenteorides* (26). Among some of the IS elements described in enterococci are: IS*6770*, IS*1542*, IS*1216V*, IS*1476*, IS*1251*, IS*19*, IS*1216V*-IS3-like, and IS*Efa5*. These elements have been described in VRE from North

America, rather than from isolates from Europe, and they are consistent with Brazilian VRE of American and Canadian origin (27-30).

vanA CLUSTER

Many glycopeptide resistant enterococci have been isolated worldwide. Vancomycin resistance encoded by the *vanRSHAX* gene cluster is carried by the prototype Tn*1546* element, located on a nonconjugative plasmid (9, 31), which is transferred by *in vitro* conjugation to susceptible strains of enterococci and staphylococci. The natural transfer from to *Staphylococcus* did not occur until 2002 (32-35). Tn*1546* is a member of the Tn*3* family, which is characterized for the presence of resolvase and transportase genes in addition to resistance determinants. They normally reside in plasmids (9, 20, 36).

Resistance to glycopeptides can be found in conjugative plasmids or related elements. A large number of studies on the epidemiology of VRE have been carried out in strains of different genetic origins. Several types of clonally related Tn*1546* have indicated instability within the Tn*1546*–related elements (9, 10, 37-42). The *vanA* operon is carried by Tn*1546* with IS elements (9, 10, 28, 43, 44).

The VanA elements have been detected in *E. faecium*, *E. faecalis*, *E. gallinarum*, *E. casseliflavus*, *E. avium*, *E. durans*, *E. mundtii*, *E. raffinosus*, *S. aureus*, *B. circulans*, *P. apiarius*, and *P. thiaminolyticus* (45). In enterococcal isolates in Northeastern United States the dissemination of *van* operons was found to occur by conjugation (29, 46), but in France and London, it has been demonstrated that such an acquisition occurs via Tn*1546* transposition (9, 39, 40).

The VanA type is the one most frequently detected among VRE human isolates (2, 5, 47-49). The prototype element, Tn*1546*, is heterogeneous. This diversity has been demonstrated by epidemiological studies on Tn*1546*, detecting point mutations due to insertion sequences, which have created heterogeneity among strains (28, 43, 44).

The largest diversity of elements in human enterococci reflects the selective pressure exerted by the hospital environment, which allows for a more rapid alteration of the elements than that occurring in nonhuman sources (42). These insertions or deletions were reported on the lengths from *vanS* to *vanH* and/or *vanX* to *vanY* (30). These deletions, insertions, and point mutations introduce changes in the glycopeptide susceptibility patterns of isolates.

The first report of an IS element in vancomycin resistant genes was IS*1476*, found disrupting the *vanY* gene in a Canadian strain of *E. faecium*. The IS*1476* insertion in the *vanY* gene produced a decrease of the functional levels of D,D-carboxypeptidase (27). IS*1476* has a remarkable likeness to IS*1181* of *S. aureus*, IS*1251* of *E. faecium*, ISL3 of *Lactobacillus delbruckei*, and IS*1165* of *Leuconostoc mesenteroides* (10, 25-27, 50). IS*1476* has been described in the intergenic region of *vanS–vanY* (51, 52). Until 1997, IS elements had been isolated outside of the realm of glycopeptide resistance genes; that is the case of IS*1251* in the intergenic *vanS–vanH* region and IS*1216V*–like in the intergenic *vanX–vanY* region. The alteration was detected by PCR screening. The mapping of the restriction endonucleases determined the localization of the insertion in the *vanY* gene. IS*1251* and IS*1251*–like elements have been reported almost exclusively in isolates of the United States (53). Exception to the above happened in both an isolate from Ireland and another from Norway (10, 53).

The polymorphism of glycopeptide resistance includes insertions or deletions of IS elements on the left (*orf1* side) and right (*vanZ* side) terminals of the transposon that includes the *orf1* and *vanZ* genes. IS element movement causes structural alterations in the VanA elements, including partial or complete loss of the transposition genes, *orf1* and *orf2*. The genetic rearrangement of *vanY* or *vanZ*, or the partial or complete deletion of genes following IS*1216V* insertion can be a common cause of VanB phenotype–*vanA* genotype VRE in Korea. Some VRE with these characteristics have been detected in Japan and Taiwan. Several researchers have proposed various explanations. Point mutations in the putative sensor domain of *vanS* could be responsible for the lack of teicoplanin resistance in VRE strains carrying the *vanA* operon. Another possible explanation for this atypical phenotype could be the deterioration of the accessory VRE proteins VanY and VanZ (53, 54, 58). VanD phenotype–*vanA* genotype VRE have surfaced in France. The VanD phenotype–*vanA* genotype of a French hospital outbreak was due to an IS*16* within the *vanY* gene that encodes the D,D-carboxypeptidase (57). No association have been found between the resistant phenotype and the genetic rearrangement on the left terminus of Tn*1546*, however, as has otherwise been demonstrated with both the IS*1542* or IS*1216V*–IS*1542* insertions in the *orf2–vanR* and the IS*1216V* insertions within or downstream of *vanX*. On the other hand, the genetic rearrangement of the right side of the transposon, including *vanX*, *vanY* and *vanZ*, was an associated resistance phenotype (53, 58, 59).

The first classification of VanA elements was made on the basis of the restriction sites of the *Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII, and *Pst*I enzymes. The groups were classified from I to IV. The restriction fragment profiles of groups II and III differ from one another and from group I isolates in the restriction fragment lengths spanning the

vanA–vanY region. Group IV isolates have a profile similar to that of group I excepting the *Eco*RV restriction site (9, 60).

Another classification derives from the use of 10 pairs of oligonucleotides of the VanA prototype element sequence, Tn*1546*, thus obtaining 24 groups designated from A to X. This classification is based on the size of the amplicons obtained, and permits differentiation between human and nonhuman enterococci sources: six, common in enterococci from all sources, eight from nonhuman origin, and ten unique to human isolates. Group H enterococci are related to nosocomial outbreaks, differing in three regions from Tn*1546*, and harboring IS*1542*, first seen in a study (42).

Another classification is based on the analysis of RFLP patterns and IS location in transposon Tn*1546*; seven different RFLP patterns, those from A to G, were identified. Type A was identical to the predicted Tn*1546* pattern. An insertion in B, D, E, and G bore an additional pattern. Deletion of fragments 1 to 6 became evident in types C through G at the left end. Types D and G lacked fragment 2 at the right end of the transposon. Type F did not harbor flanking fragments due to deletions at the left end of the transposon. The original fragment 4 was also also absent because of the insertion of IS*1251* in the *vanS–vanH* intergenic region (9, 10, 28). Type A is subdivided into four subtypes (A1 to A4); type B, into three subtypes (B1 to B3); type D, into four subtypes (D1 to D4); type E could be divided into subtypes E1 to E7, and type F into F1 and F2. The only ones that are not subdivided are types C and G. Tn*1546* types A1 and A2 were the most prevalent in The Netherlands, having been isolated from both human and farm animals. Tn*1546* type F2 has only been isolated in patients in the United States (28).

The A2 and B3 deletions here described due to insertions of IS*1251* in the *vanS–vanH* intergenic region have been previously characterized (28, 61, 62). Types A2, B3, C, D1 through D4, E1 through E7, F1, F2, and G have been shown to have deletions in the transposase and resolvase genes of the Tn*1546*–like transposon. The main transposon types isolated in hospitals in the United Kingdom and the United States (types D1 and F2) have no counterparts in animal strains (28, 63).

Studies revealed a 1,496 bp insertion between *vanS* and *vanH* (IS*1251*) at the beginning of the 17–nucleotide sequence near the stop codon of *vanS* (10). IS*1251* has been found in the *vanS–vanH* intergenic region, in human or nonhuman isolates (10, 52). IS*1251* has imperfect inverted repeats of 24 (IRL) and 23 (IRR) bp with a 16–bp conserved sequence at the termini. A diversity study of Scottish enterococcal strains revealed the lack of at least one IR. The loss of the left IR has been previously described as associated to the presence of IS*1216V* elements, either singly or in combination with a truncated IS*3*–like element (28, 46). The IS*3* family includes elements such as IS*150* and IS*600*, located in Gram negatives; IS*861*, in *S. agalactiae*; and IS*1223*, in *Lactobacillus* (19, 64-66).

Clinical isolates of *E. faecium* commonly contain IS*1251* in the genoma. IS*1216V* is present in the intergenic *vanX–vanY* region as types D1, D2, and D4. IS*1216V* was located upstream of Tn*1546* in strain types D, E, F, and G, being absent in type C. The IS*1216V*–IS*3*–like element insertion at the left end of the transposon has also been described in types A2 and B3. And so have the insertion of one or two copies of *IS1216V* in types B and D through G; deletions associated to IS insertions downstream of *vanX* in types D through G and at the left end of the transposon. The IS*1216V*

insertion increased the size of the *vanX–vanY* intergenic region in types B, D, E, and G (11, 28, 41, 46). The D1–, D2–, D3–, D4–, and G–type strains, which have undergone deletions of the *vanY* gene, are less resistant to teicoplanin, the *vanY* deletion perhaps having affected the *vanZ* transcription. Tn*1546* rearrangements are due to point mutations located in genes *orf1*, *vanS*, *vanA*, *vanX*, and *vanY* (7, 28, 43).

In yet another study, the B cluster yielded a different pattern, called b cluster. The changes observed in b require at least two genetic events, namely, the transposition of IS*1542* and the block duplication of the gene. IS*1542* is homologous to the IS*256* in *S. aureus*. Several copies of this element are present in the VRE genome with an *orf2–vanR* copy, as well as in some VRE which lack a copy of the latter. Furthermore, glycopeptide-sensitive enterococci reveal the presence of IS*1542*, which suggests that a transposition event in any part of the genome could have been responsible for this kind of isolates (42, 67). The presence of IS*1216V* in *vanX–vanY* and upstream of *vanR* indicates that it could be found in clinical isolates (28, 68). IS*1216V* is closely related to IS*S1*, an element that promotes chromosomal integration in *L. lactis*. An IS*S1*–like element was detected in the chromosome of *E. faecalis* (69, 70) IS*1216V* was present on the left side of Tn*1546*–like elements with or without the large deletions comprising the *orf1–orf2* region (30).

An English *E. faecium* strain, isolated from a hemoculture produced a larger than expected amplicon. Its enzymatic digestion indicated loss of a 418–bp fragment, suggestive of an intragenic insertion at the *vanS* 3’ terminus. Sequencing detected IS*1216V* flanked by 8–bp direct repeats. The sequence analysis revealed the loss of 11 amino acids at the C terminus of the VanS peptide, resulting in an altered sensor

peptide, despite which the strain bore a normal VanA. It has been demonstrated that VanS is not essential for the expression of resistance to glycopeptides (71, 72).

A recent Korean study classified VRE strains in three principal types according to the distribution of integrated ISs in the Tn*1546* elements. Type I is characterized by both the insertion IS*1542* in the *orf2-vanR* intergenic region and an insertion of IS*1216V* in the *vanX–vanY* intergenic region. Type II is characterized by two copies of IS*1216V* at the left end of Tn*1546*–like elements and in the *vanX–vanY* intergenic region, as well as IS*1542* in the *orf2–vanR* intergenic region. Type III is characterized by the presence of IS*19* in the *vanS–vanH* intergenic region, as well as IS*1542* in the *orf2–vanR* intergenic region and IS*1216V* in the *vanX–vanY* intergenic region (30).

In the VanD phenotype *E. faecium* BM4416 strain, the *ddl* ligase gene was disrupted by the insertion of an IS*19*, thus rendering the strain into a constitutive one with glycopeptide-resistance phenotype (73). The IS*19* element was also detected in the Canadian VanD3 strain (74). Type II has six subtypes: IIa, IIb, IIc:S12, IIc:A13, IIc:H7, and IIc:A12. The IIa subtype is characterized by the IS*1216V* insertion in the *orf1*, a copy of IS*1216V* in the *vanX-vanY* intergenic region, and an IS*1542* insertion in the *orf2–vanR* intergenic region. Type IIb is similar to IIa, although they differ in some bps nucleotides. Types IIc:S12 to IIc:A12 have two ISs (IS*1216V* and IS*1542*) in the *vanX–vanY* intergenic region. In type IIb and IIc isolates, IS*1216V* was inserted at the left ends of the VanA elements, thus introducing deletions into the *orf1–orf2* regions. The insertion of IS*116V* deleted the 3’ terminus of IS*1542* at various points in type IIc. This could be explained by late IS*1216V* expression rather than by IS*1542*. Although IS*1542* is still supposedly restricted to Europe, it was detected in Korean isolates (30). Type I

corresponds to type B in the European isolates, except for the IS*1542* insertion. Type II is very similar to type E. Type IIc seems to be identical to subtype E13 (28, 75). IS*1542*, located in the *orf2–vanR* intergenic region, and the IS*1216V*–like element, in the *vanX–vanY* intergenic region are responsible for the diversity (28, 42). The IS*1542* sequence is frequently found in clinical VRE isolates and in chickens from the UK and Ireland (42, 75).

The IS*1678* sequence in the intergenic region *vanX–vanY* was detected in a vancomycin resistant Korean strain of *E. faecium*, isolated from chickens. This was the first time that this was reported. An analysis of the sequences yielded the highest identity with the transposase in *Bacillus halodurans* and *Streptococcus pneunoniae*. It was the largest IS to be detected in the Tn*1546*–like element (76, 77). Almost all of the highly glycopeptide resistant Korean strains of *E. faecium* have IS*1216V* and IS*1542*.

The Tn*1546*–related non-prototypic elements have a considerable variation in the *orf1–orf2* region because of the insertion of an IS*1216V*–like element (44). This class of point mutation is typical of VanA VRE swine isolations (63). The IS elements are important not only for epidemiological studies (53, 56, 78), but also for the detection of hybrid forms of potential promoters attributed to the apparent presence of direct –35 regions within inverted repeats. The constitutive expression of the *van*– genes of hybrid promotors results in the elevated resistance to teicoplanin conferred by the non–prototypic Tn*1546* elements (79). Sequencing of the *orf2–vanR* intergenic region harboring IS*1251* identified a putative hybrid promoter. IS*1251* has a –35 conserved prokaryotic region in the right inverted repeat. A potential –10 conserved region separated by 16 bp from the –35 sequence is at the 3’ region in the IS*1251* located in the

vanS gene (10).

The US isolates differ from European isolates since the late 80s and may continue to evolve. The detection of IS elements allows identification of the source of the strains; some have been reported as having worldwide distribution, while others are more localized (10, 27). IS*1251* has restricted distribution and has been used to demonstrate the intercontinental dissemination of enterococci strains from the US to Norway and Ireland. IS*1542* was first found in the UK and IS*1476* in Canada. Korean isolates do not contain IS*1476* and IS*1251* elements (27, 30, 53, 80). IS*1476* is another IS–like element associated with Gram positive bacteria. It is homologous with IS*1181* of *Staphylococcus aureus*, IS*1251* of *E. faecium*, ISL*3* of *Lactobacillus delbruckei*, and IS*1165* of *Leuconostoc mesenteroides* (10, 25-27, 50, 81). IS1*216V* is not an adequate epidemiological tool for monitoring VanA VRE because it is widely disseminated in the VanA elements of enterococci of diverse geographic and ecological origins, and are always present in multiple copies (28, 44). The VanA IS*1251*–modified and IS*1476*–modified elements have been reported in Europe (82), and are more prevalent in US and Canadian isolates (27, 83). IS*1542* would seem to have more restricted distribution, and has been found in Asia and Europe (30, 80, 84).

In clinical isolates of *E. faecium* from the northeastern US, the *vanS–vanH* intergenetic region harbored in Tn*1546* was interrupted by IS*1251*. Strains from New York City have integrated Tn*5482* into the chromosome; supplementary studies on the strains show that clonally distinct enterococci contain an element similar but not identical to Tn*5482*. The sequence at the left end of Tn*5482* had a putative transposase similar to that of the ISS*1* element of *L. lactis* and almost identical to IS*1216* of *E. hirae*. Two IRs (ca.18 bp) were

flanking the transposase; another ORF was found to be homologous with various members of the IS*3* family, IS*150* of *E. coli* and IS*861* of *Streptococcus agalactiae*. The presence of IS*1216V* close to a putative terminus suggested that it could be a compound transposon (46, 85). Studies show that Tn*5482* is capable of being transferred by independent conjugation. This could be possible in the presence of a conjugative transposon with atypical structural characteristics (10).

IS*Efa5* is inserted between *vanX-vanY,* flanked by 8 bp GAAATATT direct repeats which is consistent with the target that is the duplication of the site followed by a transposition event. IS*1251* is more commonly present in *E. faecium* (80%) than in *E. faecalis* (14%). IS*Efa5* is detected 96% in *E. faecium* and only 52% in *E. faecalis*. IS*1542* has not been detected in Brazilian strains (86). IS*Efa5* was associated with the first outbreak of VRE in Brazil. These geographically restricted IS elements could be used as excellent markers to investigate the origin of *vanA* IS elements or of the strains that carry them. This is consistent with the considerable fluidity of the genome and the observed variation could reflect changes in plasmid transport. The prevalence of IS elements in the genomic DNA of VRE has been limited. IS elements could be used as molecular markers in local epidemiological studies of horizontal transmission and evolution of VanA elements, including cases in which the occurrence of IS elements within the genomes of enterococci have had limited efficiency for epidemiological studies, as it has been particularly demonstrated that they do not correlate with PFGE (86).

L-PCR is a successful epidemiological and evolutive tool to fingerprint and compare elements of resistance to glycopeptides by quickly identifying enterococci that have

insertions within the *vanRSHAX* cluster (44, 87). The RFLP assessment of the resulting amplicons could be used to identify the possible position of the insertion and to permit the relevant sequencing of the VanA elements (72). The lack of correlation between PFGE patterns of the isolates and the transport of the IS group means that the diversity could be mediated by the movement of IS elements. The IS elements play a role in the diversification as molecule markers in the horizontal transmission of Tn*1546*–like elements in glycopeptide resistant strains, thus affecting both the geographic distribution and the position of Tn*1546* in the genome (10, 27, 29, 30, 42, 68, 80).

vanB CLUSTER

In contrast to the *vanA* clusters, the *vanB* clusters have been reported to have heterogenity at the nucleotide level. Three subtypes of *vanB* cluster have been described, named *vanB*1, *vanB*2, and *vanB*3. Differences have been associated to sites of enzymatic restriction of *Hha*I and *Hae*II. Point mutations have created changes in the *vanB* gene, introducing an *Hha*I locus in the 5454 position and deleting same in the 5618 position in the *vanB2* type (88-90). *vanB* may either reside on large conjugative elements transferable from *E. faecium* to *E. faecalis* or illegitimately integrate into the chromosome by recombination (91). The *vanB* operons are transported by a larger element than that of the *vanA* cluster. Two distinct transposons have been described, Tn*1547* and Tn*5382*. The G+C content en the DNA of Tn*5382*–like transposons suggests another origin for these sequences. The *vanB* clusters seem conserved in the strains studied to date, and an IS element has been detected (92).

The IS sequences were previously described within the *vanB* cluster as well as within the Tn*5382* transposon of various isolates. These identical sequences could be detected

at both ends of the transposon. This transposon could be integrated in the chromosome or in a plasmid (56, 92). Tn*5382* transports the determinant of resistance to both gentamycin and glycopeptides (93). Tn*5382* has been reported to be inserted immediately downstream of the *pbp*5 gene in strains of *E. faecium* in the US. *vanB* clusters in isolates of *E. faecium* are associated with IS*Enfa110* and IS*Enfa200* elements. There are no reports that associate the *vanB* cluster with transposons as has occurred with other *van* clusters at low levels of resistance (producers of precursors of the D-Ala-D-Ser peptidoglycans) (93). IS*Enfa3* is a member of the IS*3* family, detected in Korean strains with inverted terminal repeats of 12 bp, but no direct repeats (56). Tn*5382*–like transposons have been shown to undergo conjugative transfer between enterococci and streptococci, and the same transposon was detected in the clostridia species, where the human colon could favor horizontal transfer events of vancomycine-resistant genes. Tn*5382* is a member of the conjugative transposons of the Tn*916* class and has potential of transference to many different Gram positive bacterial species present in the intestinal flora, serving as a reservoir of vancomycin–resistant genes. On the other hand, Tn*1583* produces circular intermediate molecules which are not transferable to either *E. faecalis* or to *E. faecium* (93-97). Tn*1547* is not involved in chromosome–chromosome conjugative transfer (90, 91, 93). The *vanB1* gene cluster is not part of the Tn*1546*, but rather part of the Tn*1547*, which has the insertion sequence elements IS*16* and IS*256*–like in *E. faecalis* BM4281.

The *vanB2* cluster is harbored by Tn*5382* and could also be transported by Tn*1549*. VanB elements have been detected in *E. faecium*, *E. faecalis, E. lenta*, *Clostridium* species, *S. mitis, S. gallolyticus*, *S. lutetiensis* and *S. bovis* (45, 93-95, 98-102). The *vanB2* cluster is commonly carried by both hospital–borne *E. faecium* and

nonenterococcal bacteria isolated from humans and fecal samples of veal calves (39, 92, 95, 100, 102, 103). *vanB1* to *vanB2* have sequence differences in $vanS_B$–$vanY_B$. Until now, *vanB1* has been associated with *E. faecalis,* and *vanB2* with *E. faecium*, even when these elements are not species–specific. VanB subtypes have been detected worldwide; the *vanB2* element is mostly isolated in Europe, while the *vanB3* has only been detected in one isolate in the US (88-90). The *vanB2* elements differ from the *vanB1* in nucleotide heterogeneity of genes (104).

GLYCOPEPTIDE RESISTANCE IN *Staphylococcus*

The natural transference of glycopeptide resistance from *E. faecalis* to S. *aureus* was reported in 2002, the analyses showing that the *vanA* sequence of the MI–VRSA and PA–VRSA strains were identical to that of the Tn*1546* transposon. The *orf1* gene of PA–VRSA could not be amplified. Larger than expected amplicons of *orf1–orf2* and *vanS–vanH* were obtained, suggesting the presence of insertion sequences. Analysis of the DNA sequence detected an IS*1216V*–like element (105).

This truncated insertion of the transposon affects its mobility (28) as well as the expression of vancomycin resistant genes due to the presence of the transposon in a very large plasmid in VRSA (35). In the same patient from whom this MI–VRSA strain was isolated, VRE and MRSA were also isolated (34, 106, 107). MI–VRSA is highly resistant to vancomycin and teicoplanin whereas PA–VRSA has lightly moderated resistance to vancomycin (33, 34). The VRSA strains are not methicillin–susceptible. Normally, all the clinical isolates of MRSA are under glycopeptide selective pressure. Tn*5384* is formed by repeated copies of IS*256* directly from staphylococci and enterococci; one of the copies is inserted into the *blaR1* gene regulator of the Tn*552*–

like element (70, 108), a configuration very similar to that of Tn*4001*, a transposon of *S. aureus*, which carries the genes of resistance to gentamycin (14). Tn*5384* is also characteristic of staphylococcal beta–lactamase plasmids, including the putative transposon and the regulation genes of the staphylococcal beta–lactamase transposon Tn*552*, which has an intact copy of the IS staphylococcal element IS*257* (17).

Even though the vancomycin resistant genes are in MI–VRSA and PA–VRSA isolates due to the acquisition of a plasmid that transports *vanA*, they have two different origins: MI–VRSA acquired the Tn*1546* element from the 58–kb pLW1043 plasmid (109); and PA–VRSA from a 127–kb plasmid (35). Differences in the first two VRSA isolates indicate that they came from independent genetic events (105). The IS*1251*–like element of the PA–VRSA isolate is flanked by the duplication of 8–bp of the target sequence (ATAATTTT), and is not oriented in reverse position. The sequence of the IS*1251*–like insertion was localized in the *vanS–vanH* intergenic region (105).

Comparison between genera is necessary because enterococci and staphylococci are together in the nosocomial environment, in humans and animals alike. Possible mechanisms of the transference of genes of antimicrobial resistance between staphylococci and enterococci remain poorly defined (21). *In vitro* plasmid transfer from a wide range of hosts such as pAMb1 (*E. faecalis*) and pIP501 (*S. agalactiae*) to other streptococci, staphylococci, and other Gram positive genera suggests a possible transfer mechanism of resistant genes in nature (109-111).

Variants of Tn*1546* have been seen to transport IS elements in the *orf2–vanR*, *vanS–vanH* and *vanX–vanY* intergenic regions. Various studies suggest epidemiological

relationships between animal and human reservoirs (10, 11, 30, 60).

The genetic isolation of unrelated VRE in nosocomial outbreaks means that the horizontal transfer of resistant genes among enterococci has a more significant impact on the dissemination of resistance to vancomycin than on the clonal dissemination of resistant enterococci. Monitoring the dissemination and evolution of glycopeptide resistance can be done by detecting the IS elements. The question involves not only whether ISs are from enterococci or staphylococci but also the origin of *van* clusters. *E. faecalis* and *E. faecium* have 38 and 39% of G+C, respectively, and the *vanA* and *vanB* clusters have 45 and 49% G+C, respectively (9, 112), which confirms the extragenus origin of resistance. Both transposons and operons are made up of genes and IS of different origins.

In conclusion, IS's are more frequent in *vanA* clusters than in *vanB* clusters. As Tn*1546*–like elements are responsible for the investigation of horizontal gene transfer variations, they play an important role in dissemination of *van* type VRE. The study of Tn*1546* heterogeneity may be successful as epidemiological molecular markers. Variations include IS integration with or without deletion in the site of insertion, point mutations, and deletions. The transposable VanA elements have been identified in community enterococci isolated of sewage, animal feces, and raw meat, suggesting that each one of these could act as a reservoir of resistant enterococci and *vanA* cluster.

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