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Occurrence of insertion sequences within the genomes and Tn*1546*-like elements of glycopeptide-resistant enterococci isolated in Brazil, and identification of a novel element, IS*Efa5*

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Abstract

Insertion sequences (IS) occur widely within the Tn*1546*-like elements responsible for VanA glycopeptide resistance in enterococci from several countries. As such insertions can be used as epidemiological markers and for studying horizontal transfer of gene clusters, we investigated the distribution of IS*6770*, IS*1542*, IS*1216V*, IS*1476*, and IS*1251* elements in 26 VanA *Enterococcus faecium* and 21 VanA *Enterococcus faecalis* from Brazil. PCR, using genomic DNA as a template, indicated that most of the isolates contained IS*6770* (97%), IS*1216V* (87%) and IS*1476* (72%) elements. IS*1251* was also detected, but at a higher frequency in *E. faecium* (80%) than in *E. faecalis* (14%). None of the isolates harboured IS*1542*. Only two of 47 isolates had IS elements within their Tn*1546*-like elements; one possessed IS*1251* between *vanS* and *vanH*, as reported in the United States; another possessed a novel IS element, designated IS*Efa5*, located between *vanX* and *vanY*. This novel element was found in the genomic DNA of 25 (96%) *E. faecium* and 11 (52%) *E. faecalis*. In stability studies, no IS-mediated changes were detected in the Tn*1546*-like elements of 25 vancomycin-resistant enterococci (VRE) monitored over 11 months. These results suggest that the occurrence of IS in Brazilian isolates is similar to that reported in American isolates, but that these elements occur rarely within the *vanA* gene clusters. As patterns of IS carriage did not correlate with the PFGE type of the VRE, the prevalence of IS elements in genomic DNA of VRE is not a useful epidemiological marker. However, the presence of IS-modified Tn*1546*-like elements, which appear to be rare in Brazil, could be a useful molecular marker in local epidemiological studies to monitor the evolution and horizontal transmission of VanA elements.

Keywords: IS*Efa5*; IS elements; VRE; *Enterococcus faecium*; *Enterococcus faecalis*; Glycopeptide resistance

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Introduction

Enterococci are a frequent cause of infection in patients hospitalised for long periods or in patients receiving multiple courses of antimicrobial therapy (Mundy et al., 2000). In Brazil, *Enterococcus faecalis* is responsible for 90% of enterococcal infections and *Enterococcus faecium* causes most of the remainder. High-level aminoglycoside resistance and glycopeptide resistance are most common among *E. faecium* isolates in Brazil (Cereda et al., 1997).

There are currently five acquired forms of glycopeptide resistance in enterococci, VanA, -B, -D, -E and -G; VanA and VanB are most common (Woodford, 2001). Vancomycin-resistant enterococci (VRE) emerged in Brazil in 1996, when a VanD *E. faecium* was isolated in Curitiba, Parana state (Dalla Costa et al., 1998, 2000). In 1997, the first confirmed VanA *E. faecium* occurred in a hospital in Sao Paulo city (Zanella et al., 1999). The first recorded nosocomial outbreak of vancomycin-resistant *E. faecium* and *E. faecalis* occurred in the same hospital 1 year later (Zanella et al., 2003). Subsequently, a vancomycin-resistant *E. faecalis* was isolated from a patient who had received a bone marrow transplant in another hospital in Sao Paulo city. As such, VRE have become a concern in Brazilian hospitals (Cereda et al., 2001).

The majority of VRE in Brazil possess the *vanA* gene cluster as part of transposon Tn*1546*-like elements (Zanella et al., 2003). Insertion sequences (IS elements) have been reported within the *vanA* gene clusters of VRE isolated in Europe, the United States, Canada, and Korea (Huh et al., 2004). The IS elements distributed among different enterococcal species represent more than nine different IS families, groups or isoforms (Mahillon and Chandler, 1998; Darini et al., 1999a, b). Those within *vanA* gene clusters have been used as molecular markers in short-term epidemiological studies (Simonsen et al., 2000; Lee and Kim, 2003; Camargo et al., 2004); some have been described as widespread throughout the world, whereas others have more restricted distributions (Handwerger and Skoble, 1995; Handwerger et al., 1995; MacKinnon et al., 1997). IS*1251*, which has a restricted distribution has been used to demonstrate intercontinental spread of an enterococcal strain from the USA to Norway and Ireland (Simonsen et al., 2000).

The potential value of IS elements as molecular markers of VRE has not been investigated widely in Brazil, and there are few data on the occurrence of IS elements in Tn*1546* of Brazilian isolates. For this report, we investigated the occurrence of five IS elements, IS*6770*, IS*1542*, IS*1216V*, IS*1476*, and IS*1251*, that occur commonly in enterococci (Donabedian et al., 2000), in 47 VanA isolates from Brazil. During this study, we identified a new IS element, designated IS*Efa5*, and also investigated its distribution.

Materials and methods

Strains

Forty-seven VRE isolates, 26 *E. faecium* and 21 *E. faecalis*, from four Brazilian states (Sao Paulo, Rio de Janeiro, Parana, and Rio Grande do Sul) were used in this study. All 47 isolates harbored the *vanA* gene, detected as described previously (Woodford et al., 1993) (data not shown). Three *E. faecalis* and 14 *E. faecium* were isolated during the first nosocomial outbreak in Brazil (Zanella et al., 2003). The remaining 30 isolates were not related to this outbreak; two *E. faecium* were collected from Rio de Janeiro, two *E. faecalis* from Parana, two *E. faecalis* from Rio Grande do Sul, and 24 were from other hospitals in Sao Paulo (10 *E. faecium* and 14 *E. faecalis*). All isolates were stored in glycerol at −70 °C.

E. faecium A (Palepou et al., 1998), which carries a prototype Tn*1546* element, was used as a control in long-range PCR studies. Three strains, *E. faecium* H, which carries IS*6770*, IS*1542* and IS*1216V* (Palepou et al., 1998), *E. faecium* (IS*1251*) (Demertzi et al., 2001) and *E. faecium* (IS*1476*) (Mackinnon et al., 1997) were used as positive controls in PCR reactions to amplify IS elements.

Pulsed-field gel electrophoresis (PFGE)

SmaI-digested genomic DNA was prepared from VRE and separated by PFGE as described previously (Bannerman et al., 1995). The resulting DNA fingerprints were compared visually using the criteria of Tenover et al. (1995).

Characterisation of VanA elements

The VanA elements of VRE were amplified by long-range PCR using a single primer located in the terminal inverted repeats of Tn*1546*-like elements (Expand Long Template PCR System; Roche, Indianapolis, USA), and compared with Tn*1546* (10.8 kb) (Arthur et al., 1993) on agarose gels (Woodford et al., 1998). Amplicons longer or shorter than Tn*1546* were analysed by overlapping PCR (Palepou et al., 1998; Darini et al., 1999b) with published primers (Woodford et al., 1998) to locate the insertion or deletion event.

DNA sequencing of new element, IS*Efa5*

Primers 5f (5′-AATTCATCTACATTGGT-3′) and 5r (5′-TTCCTGAGAAAACAGTGCTTCA-3′) are complementary to regions flanking the novel IS element IS*Efa5* in strain *E. faecium* 172/98 (see Results) and were used to amplify a fragment of ~1700 bp. The amplified