High prevalence of ST-78 infection-associated vancomycin-resistant *Enterococcus faecium* from hospitals in Asunción, Paraguay

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Abstract

Forty infection-associated VanA-type vancomycin-resistant *Enterococcus faecium* (VRE) strains obtained from five collaborating hospitals in Asunción, Paraguay were investigated. Genotyping using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing revealed the presence of 17 cluster types and four STs, with 93% (37/40) of isolates comprising ST type 78. Other ST types included ST-132, ST-210 and one new ST type (ST-438). All but one isolate (ST-438) were associated with clonal complex 17 (CC17), and 97% of the total isolates carried the *esp* gene. Three Tn*1546* variants were found, including a new lineage containing an IS*Efa5* insertion in an existing IS*1251* element.

Keywords: Bacterial genotypes, insertion sequences, multilocus sequence typing, transposons, vancomycin-resistant *Enterococci*

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Vancomycin-resistant *Enterococci* (VRE) are nosocomial pathogens widely disseminated within hospitals [1], with the *vanA* genotype being the most common type of acquired glycopeptide resistance found in these enterococci. Although the first clinical isolate of VRE was detected in Europe in 1986, studies have now shown that clinical VRE may be isolated in several world regions [2]. Further, it is currently accepted that a single focal group of *vanA*-type VRE (clonal complex 17 or CC17) is particularly associated with nosocomial infections [3]. However, there is some evidence to suggest that this grouping may be influenced by the high rates of recombination associated with *E. faecium* [4].

In 1988, French researchers identified a small mobile genetic element in VanA-type *Enterococcus faecium* called Tn*1546*, which is involved in vancomycin resistance [5], and there are currently five other acquired vancomycin resistance genotypes reported in enterococci, namely *vanB*, *vanD*, *vanE*, *vanG* and *vanL* [5,6].

Currently, there exist limited data regarding the incidence and types of clinical vancomycin-resistant *E. faecium* from countries in South America. Therefore, the objective of this study was to investigate the complexity of clinical *E. faecium* isolates from Paraguay, by determining *E. faecium* genotypes and VanA Tn*1546* transposon diversity, and to compare the results with isolates from both South America and the rest of the world.

Two hundred and twenty-one VanA vancomycin-resistant *E. faecium* isolates were collected between January 2005 and March 2007 from five collaborating hospitals in Asunción Paraguay. These five hospitals, the Instituto de Previsión Social (IPS), Hospital de Clinicas (HCL), Hospital Nacional (HNA), Centro Medico de Bautista (CMB) and Instituto de Medicina Tropical (IMT), are representative of the national healthcare system in Paraguay. A subset of 40 isolates was chosen for further analysis, based on their site of isolation and association with disease. These infection-associated isolates were cultured from abscess material, blood, tissue and needle aspirates, representing 7% (7/97), 20% (1/5) and 27% (32/117) of the isolates obtained from HCL, HNA, and IPS, respectively. No infection-associated isolates were obtained from CMB (0/1) and IMT (0/1) hospitals.

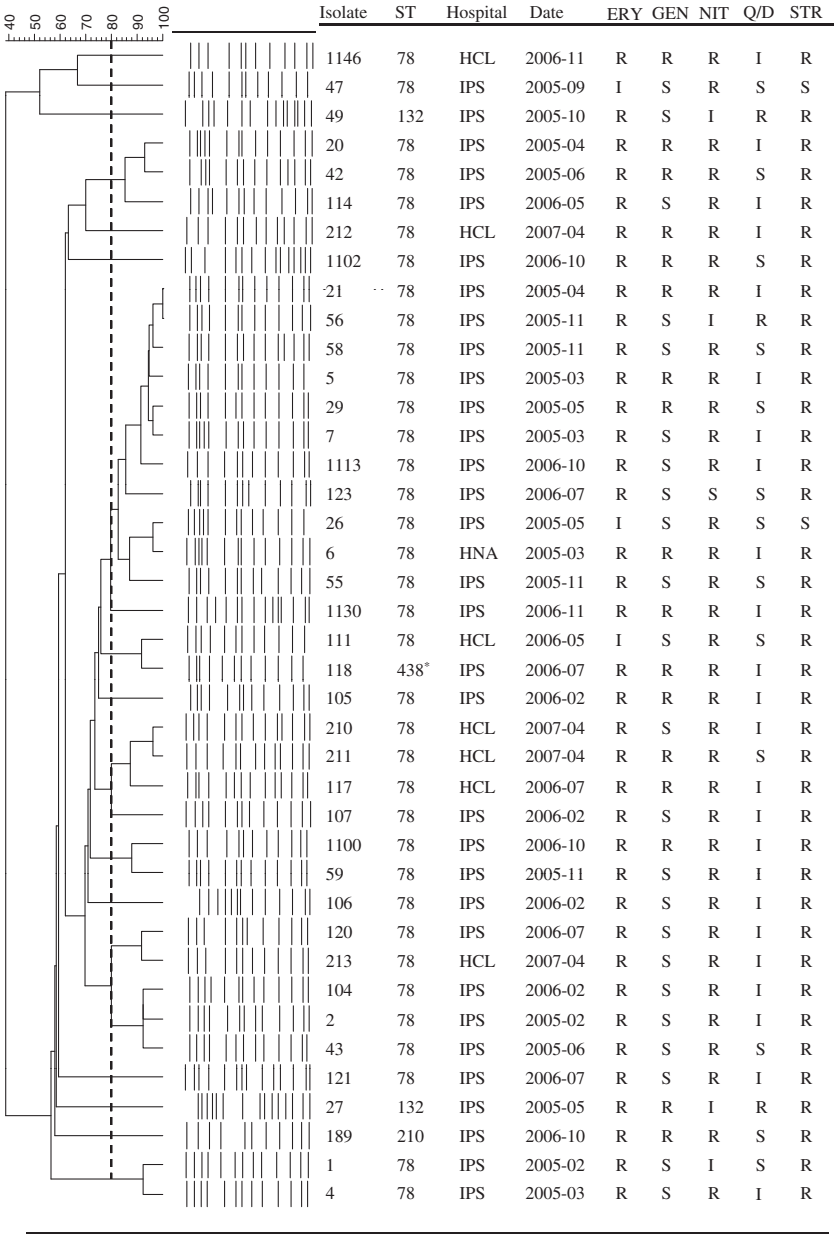

FIG. 1. Pulsed-field gel electrophoresis (PFGE) patterns obtained from 40 infection-associated vancomycin-resistant *Enterococcus faecium* isolates obtained from Paraguay between 2005 and 2007. Cluster analysis was performed using the method of DICE with UPGMA with band tolerance set to 1.5%.

Antibiotic resistance profiling was performed using the VITEK 2 system (BioMérieux, Marcy l'Etoile, France) and *E*-test (amoxicillin/clavulanic acid), according to CLSI guidelines. All 40 infection-associated isolates tested in this study were resistant to ampicillin, amoxicillin/clavulanic acid, ciprofloxacin, erythromycin, imipenem, penicillin, teicoplanin and vancomycin. All isolates were sensitive to linezolid and tetracycline. Forty-three per cent (17/40) of the isolates were resistant to gentamicin (Fig. 1).

The enterococcal surface protein gene (*esp*) was detected by PCR screening [7], revealing that all isolates were *esp* positive with the exception of one isolate (ST132). The prevalence of *esp* in these isolates is higher than previously reported for European CC17 isolates [8].

Pulsed-field gel electrophoresis (PFGE) and multi locus sequence typing (MLST) genotyping were performed as previously described [9]. PFGE results revealed 17 cluster types with no identifiable yearly pattern per cluster. Cluster analysis was performed using the method of DICE with UPGMA, with band tolerance set at 1.5% and the threshold cut-off value at 80% [10,11]. The results of MLST genotyping revealed three known (ST-78, ST-132 and ST-210) and one novel (ST-438) ST type, with 93% (36/40) of isolates comprising ST-78 genotypes. These ST-78 isolates comprised 100% of all