TITLE: Fifteen years of *Enterococcus faecium* in hospitals of Rio de Janeiro: from antimicrobial susceptibility test to genomic insights.

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ABSTRACT

Classified by the World Health Organization as a global priority pathogen, vancomycin-resistant *Enterococcus faecium* (VREfm) was firstly documented in Rio de Janeiro in 2002. This study aimed to evaluate the circulation of *Enterococcusfaecium* in Rio de Janeiro, Brazil. For that, 1005 strains received in our laboratory, from different hospitals settings, between 2002 to 2016, were tested by the disk diffusion method. Moreover, 74 strains had their genetic diversity assessed by whole-genome sequencing (WGS) and multiple bioinformatics analyses. Rates of resistance to ampicillin, ciprofloxacin, erythromycin, levofloxacin, norfloxacin, penicillin, rifampicin, teicoplanin and vancomycin were higher than 90%. High- level resistance to aminoglycosides (HLRA) was found in 57% of the isolates. We also compared the resistance profiles of strains isolated before 2002 and observed the increase in the rates of resistance to aminoglycosides (12.1% to 57.7%), beta- lactams (27.2% to 99.7%) and quinolones (48.5% to 100%). Phylogenetic analysesindicated the occurrence of emergent waves of distinct lineages. ST78 was predominant in the period from 2002 to 2008, being replaced by ST412, which wasprevalent until 2012. Subsequently, the ST963 and ST896 lineages emerged in 2013and 2015, respectively. Some characteristics, such as the presence of the *drfF* gene (for trimethoprim resistance), *hyl* (a virulence determinant), and IS*Lgar5*, in addition to the allele 44 of the *purK* gene, were associated with the lineages that replaced ST78. Molecular clock calculations suggested that the successive waves of expansion of these clonal lineages started about ~58 years ago, time to MRCA of these lineages. Genome analyses revealed different mechanisms associated with the divergence of these lineages, including homologous recombination, variation in the R-M system and exclusive acquisition of genes involved in cell wall biogenesis andmetabolism. Overall, our results indicate that the emergence of resistance to vancomycin among *E. faecium* was associated with increasing rates of resistance toseveral other antimicrobials. Moreover, the persistence of this species in the hospitalenvironment in our State was driven by a highly dynamic evolutionary processes with successive waves of hospital-adapted lineages emerging and expanding.

Keywords: Vancomycin resistance, *Enterococcus faecium*, Whole-Genome Sequencing, Bacterial Evolution

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