TITLE: DIVERSITY AND POPULATIONAL STRUCTURE OF *ENTEROCOCCUS FAECALIS* WITH HIGH-LEVEL RESISTANCE TO AMINOGLYCOSIDES

AUTHORS: FARIA, A.R.^{1,2}; SOUZA, S.S.R.^{1,3}; FREITAS, A.A.R.¹; MORAIS, J.M.^{1,3}, MERQUIOR, V.L.C.³; TEIXEIRA, L.M.¹.

INSTITUTIONS: ¹INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, CCS-UFRJ, RIO DE JANEIRO, RJ (AV. CARLOS CHAGAS FILHO, 373, BLOCO I, 2º ANDAR, CEP 21941-590, RIO DE JANEIRO - RJ, BRAZIL);

²FACULDADE DE MEDICINA, UFRJ, RIO DE JANEIRO, RJ (RUA BRUNO LOBO, 50, 5° ANDAR, CEP 21044-020, RIO DE JANEIRO - RJ, BRAZIL);

³FACULDADE DE CIÊNCIAS MÉDICAS, UERJ, RIO DE JANEIRO, RJ (AVENIDA PROFESSOR MANOEL DE ABREU, 444, 2° ANDAR, CEP 20550-170, RIO DE JANEIRO – RJ, BRAZIL);

ABSTRACT:

Enterococcus faecalis is an important agent of serious clinical conditions, such as bloodstream infections, that are frequently difficult to treat. In this study we investigate E. faecalis isolates showing high-level resistance (HLR) to aminoglycosides (HLR-A), in relation to antimicrobial resistance, virulence determinants and population structure. A total of 306 HLA-R E. faecalis isolates recovered from patients attended at hospital institutions located in the State of Rio de Janeiro, during the period from Jan/2005 to Jan/2013 were examined. Susceptibility to 14 antimicrobials was assessed by disk diffusion tests, and the minimum inhibitory concentrations of gentamicin and streptomycin were determined by agar dilution tests. Genes associated with HLR-A and with resistance to vancomycin, as well as genes coding for virulence traits were identified by PCR assays. Population structure analysis was carried out by PFGE and MLST techniques. Among the isolates with HLR to gentamicin (HLR-G), the aac(6')-Ie-aph(2")Ia gene was prevalent, followed by the aph(2")-lc gene. The ant6'-Ia gene predominate among isolates with HLR to streptomycin (HLR-S). The aph(3')-IIIa gene which confers resistance to other aminoglycosides was also observed in high frequency. Isolates with HLR-A showed high rates of resistance to 6 of the 14 antimicrobials tested, and also included resistance to vancomycin (13.7%) and penicillin (19.0%). Among the 42 VRE (vancomycin-resistant enterococci) isolates, 38 and 4 harbored the vanA and vanB genes, respectively. The efaA (99.7%), eep (99.7%), gelE (95.1%), ace (89.2%), asal (87.3%) and agg (79.7%) genes have been identified in larger proportions of the isolates, followed by the cyl (55.2%) and esp (42.8%) genes. PFGE analysis identified 36 clonal groups, being GP8, GP3 and GP5 the prevalent among HLR-G isolates; GP1, GP8, GP16, GP3 and GP19 among HLR-GS; and GP22, GP2 and GP24 among HLR-S. Analysis of 58 selected isolates by MLST revealed the occurrence of 17 STs besides two new STs described in this study (ST769 and ST770). The most frequent STs were ST6, ST21, ST4 and the new ST769. VRE isolates comprised ST6, ST9, ST97, ST103 and the new ST769. The clonal expansion of high-risk E. faecalis presenting HLR-A by some of these lineages that included resistance to vancomycin and penicillin and the presence of virulence determinants, indicates the emergence of more adapted and potentially pathogenic clones in the hospital environment.

Keywords: Antimicrobial resistance; Bacterial diversity; *Enterococcus faecalis*; High-level aminoglycoside resistance; Virulence determinants.

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