TITLE: OCCURRENCE OF THE *ANT(6')-IA* GENE PRESENTING DELETIONS AND MUTATIONS AMONG *ENTEROCOCCUS FAECIUM* ISOLATES SUSCEPTIBLE TO HIGH LEVELS OF STREPTOMYCIN

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ABSTRACT

Enterococci are intrinsically resistant to low levels of aminoglycosides, although in the case of serious enterococcal infections, the use of a cell wall synthesis inhibitor, such as ampicillin or vancomycin, combined with an aminoglycoside has been recommended due to a synergistic effect. High-level resistance to aminoglycosides (HLR-A) (MIC ≥ 2000 μg/ml), conferred by production of a variety of aminoglycoside modifying enzymes (AMES), has been reported worldwide and a nucleotidyltransferase (ANT) enzyme encoded by the *ant(6')-Ia* gene is associated with high-level resistance to streptomycin. However, during a study conducted by our group on whole genome sequencing (WGS) analyses of *Enterococcus faecium* isolates, we observed that 34 *E. faecium* strains identified as susceptible to high-levels of streptomycin by the disk diffusion method had the of *ant(6')-Ia* gene annotated in their genomes. The aim of the present study was to investigate this unexpected finding. Antimicrobial susceptibility to streptomycin was reassessed by phenotypic testing and the presence of the *ant(6')-Ia* gene was confirmed by PCR in all the isolates. The amino acid sequence was extracted from the annotated *ant(6')-Ia* gene of each isolate. Alignment of the *ant(6')-Ia* gene with a reference sequence revealed a deletion of the first 17 amino acids and single amino acid polymorphisms in codons 18 (Glutamine – Methionine) and 19 (Aspartic Acid – Asparagine). The protein structure was modelled using the Phyre2 platform and the results indicated alterations in the C-terminus region. Also, these alterations in the protein structure caused by the deletions led to changes in the predicted binding site. Using the Primer-BLAST tool we have observed that the pair of primers frequently used to identify the *ant(6')-Ia* gene was designed to a region located in the middle of the gene that was not affected by the deletions. Tracking antibiotic resistance is one of the major tasks of the clinical microbiology laboratory due to the direct impact in the selection of a more appropriate treatment. Molecular techniques have increasingly been used in clinical laboratory routines because they constitute fast and accurate tools to identify resistant strains. However, in some cases they may not reflect the biological variations in the expression of certain characteristics and efforts should be made to clarify the occurrence and impact of incongruences between phenotypic and genetic characteristics.

Keywords: Aminoglycoside resistance, *Enterococcus faecium*, phenotypic characterization, molecular techniques

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