

TITLE: DETECTION OF VANCOMYCIN-RESISTANT ENTEROCOCCI CARRYING *vanA* GENE IN WASTEWATER TREATMENT PLANTS

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ABSTRACT:

Enterococcus spp. are opportunistic pathogens that have emerged as important agents responsible for nosocomial infections. The spread of vancomycin-resistant enterococci (VRE) containing several multidrug-resistant determinants is a relevant public health concern, mainly in strains harboring *vanA* gene. Wastewater treatment plants (WWTPs) might play an important role in antimicrobial resistance spreading by carrying various emerging environmental contaminants such as antibiotic resistance genes (ARGs). In the present study, we performed a genomic investigation of *vanA*-carrying enterococci from WWTPs in Brazil to assess the genotypic antimicrobial resistance profile of these microorganisms. For this purpose, 16 samples from five WWTPs supplied with sewage from different source were evaluated. Samples were concentrated by filtration in a nitrocellulose filter (0.22 µm) and inoculated in Broth Heart Infusion with 4 mg/mL of vancomycin. Bacterial identification and susceptibility to antimicrobials was analyzed using VITEK 2 Compact system. The detection of *vanA* gene was carried out by Polymerase Chain Reaction (PCR) and the Whole Genome Sequencing of *vanA*-carrying isolates were achieved by MiSeq (Illumina). From the analyzed samples, 46 *Enterococcus* spp. were recovered, most of which were identified as 63% of *Enterococcus faecium* (29/46). The *vanA* gene presence was confirmed in 12 isolates, being 66.8% of *E. faecium* (8/12) and other isolates were: one *E. faecalis*, one *E. avium*, one *E. gallinarum* and one *Enterococcus* spp.. Noteworthy, 83.3% (10/12) of these strains showed multidrug resistant profile and 58.3% (7/12) were obtained from treated wastewater. The genomic analysis pointed to the presence of multiple ARGs, some of these genes may confer resistance to antimicrobials widely used in clinical medicine, such as aminoglycosides (*aph(3')-IIIa*, *ant(6')-Ia*), macrolides (*ermB*, *msrC*) and tetracyclines (*tetM*, *tetL*). In addition, Multilocus Sequence Typing analysis revealed that six *E. faecium* belonged to CC17, which is often associated with hospital outbreaks. Therefore, the data of present study point to the environmental spread of VRE harboring several ARGs in WWTPs, indicating the relevance of studies on dissemination of antimicrobial resistance in wastewater for the purpose of epidemiological surveillance, since treated effluents are released into receiving water bodies.

Keywords: *Enterococcus faecium*, vancomycin resistance, whole genome sequencing, wastewater.

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