TITLE: Distribution of virulence genes and biofilm formation in clinical *Enterococcus* Van^R strain.

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ABSTRACT:
Members of the genus *Enterococcus* have emerged as the most important nosocomial pathogens worldwide. The ability to pathogenicity is attributed to several factors of virulence. Among the factors of virulence, we have the substance of aggregate that is a glycoprotein that facilitates the contact with the cells; adhesins, which promote the link of the microorganism to the collagen and extracellular matrix of the host; gelatinase that presents an important role also in the formation of biofilm. The objective of the present study was to evaluate the occurrence of virulence genes and biofilm formation in clinical *Enterococcus* Van^R strain. We analyzed 17 clinical isolates for the occurrence the virulence genes for aggregation substances (*agg)*, collagen binding protein (*ace*) and gelatinase (*gel*E) by the use of PCR. The genomic AND was extracted by boiling and then its amplification for conventional PCR. The amplification reaction followed the usual programme with the annealing temperature 56 °C. PCR products were subjected to electrophoresis in agarose gel and the molecular weight standard used was 100 pb. The biofilm was evaluated in 96-well polystyrene microplate. A 200 µL aliquot containing 10^8 CFU / mL in BHI was used and the incubation was performed at 37 ° C for 24 hours. Then the microplates were washed with NaCl (0,85 %), fixed with methanol, stained with violet crystal, rinsed again with NaCl and added 200 µl of acetic acid. The total biofilm density (OD 540nm) was performed in spectrophotometer. All isolates were positive for at least one virulence gene evaluated, with 24% of the isolates positive for *gel*E and 6% for *ace*; none isolated showed the *agg* gene. Eighty-three percent of the isolates formed polystyrene biofilm. This study emphasizes the importance of enterococci as a reservoir of virulence genes and biofilm builders in a hospital environment.

Keywords: gelatinase, adhesins, PCR, hospital.

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