Title: PHENOTYPIC ANALYSIS OF VIRULENCE TRAITS OF *Enterococcus* STRAINS ISOLATED FROM HOSPITALIZED PATIENTS WITH BLOODSTREAM INFECTION IN BELO HORIZONTE, MINAS GERAIS

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Abstract:

Enterococci have been emerged as nosocomial pathogens of growing relevance due to several properties such as persistence in hospital environment, easy dissemination, and resistance to several antimicrobial agents. In the last years, increasing frequency of bloodstream infections (BSI) has been observed and those associated with *Enterococcus* spp. seem to display higher mortality rates. Thus, data generated from the analysis of virulence abilities, antimicrobial susceptibility profile, and genetic diversity may support the designing of strategies aiming to control the microorganism dissemination. In this investigation, we addressed the identification of *Enterococcus* isolated in hemoculture from hospitalized patients with BSI at species level and the phenotypic evaluation of virulence traits. The identification of the 35 *Enterococcus* strains included in the study was carried out by VITEK® 2 (bioMérieux) microbial identification system. To evaluate the ability to produce gelatinase the microorganisms were inoculated onto the surface of nutrient agar (agar + 3% gelatin) and incubated at 37 ^{o}C for 18 h. Then, Petri dishes were incubated at 4 ^{o}C for 5 h and analyzed. Gelatin hydrolysis was indicated by clear zones around gelatinase-positive colonies. To investigate hemolysin production bacterial strains were cultivated onto blood agar (tryptic soy agar + 5% horse blood). After 24 h at 37 ^{o}C the presence of hemolysis areas was evaluated and then characterized as alpha (partial) or beta (total) hemolysis. Biofilm production was assessed by a simple microtiter plate assay using TSBg (tryptic soy broth + 2% glucose). *Enterococcus* isolates were identified as *E. faecalis* (28/80.0%), *E. faecium* (4/11.4%), and *E. casseliflavus* (3/8.6%). The production of gelatinase and hemolysin was detected for 14/40% of the study group. Among hemolytic strains 12 and two were classified as alpha- and beta-hemolytic, respectively. Only five isolates of *E. faecalis* were able to sinthesize both enzymes. Biofilm formation by 33 strains differentiated as weak (10), moderate (21), and high (2) producers was observed. Our results showed the higher prevalence of *E. faecalis* in *Enterococcus*-associated BSI in Belo Horizonte and demonstrated that they exhibited at least one of the virulence factors evaluated.

Keywords: *Enterococcus*, virulence, biofilm, hemolysin, gelatinase

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