TITLE: PHENOTYPIC AND GENOTYPIC ASPECTS INVOLVED IN BIOFILM FORMATION IN PENICILLIN- SUSCEPTIBLE AND PENICILLIN-RESISTANT *Enterococcus faecalis*

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ABSTRACT:

Enterococcus faecalis is usually found as a commensal bacterium in the human intestinal microbiota, but it is also an important opportunistic pathogen associated with nosocomial infections. Enterococci show intrinsic and acquired resistance to some antimicrobials, as well as other virulence factors, as the capacity of biofilm formation. This study aimed to evaluate the *in vitro* biofilm-forming ability and also to detect genes involved in biofilm formation in penicillin-susceptible (PSEF) and penicillin-resistant *Enterococcus faecalis* (PREF) strains isolated from clinical specimens in Uberaba, Minas Gerais. A total of 12 *E. faecalis* strains, six resistant and six susceptible to penicillin, were evaluated. The biofilm formation was evaluated in polystyrene plates, using Crystal violet. The isolates were cultured in BHI medium, in aerobiosis, at 37 °C, in the absence and presence of subinhibitory doses of penicillin. The presence of the genes *efaA* (endocarditis antigen), *ace* (collagen adhesion protein), *gel* (gelatinase), *esp* (enterococcal surface protein) and *asa1* (aggregation substance), related to the production of biofilms, were evaluated by PCR. According to the crystal violet assay the isolates were classified into moderate (58.3%) and weak (41.7%) biofilm-producing strains. PSEF isolates 228 and 277 showed moderate biofilm production and the PREF isolates were moderate producers (except the isolate 20, a weak producer). Upon the addition of penicillin concentrations equivalent to half of the minimum inhibitory concentration, PSEF isolate 277 and the PREF isolates 157, 269 and 313 were weak biofilm-formers, while the other isolates could not form any detectable biofilm. The *efaA* gene was found in all isolates regardless of the penicillin-resistance profile. The *ace* gene was detected in all PSEF isolates, while *gelE* and *esp* genes were observed in all PREF. The *asa1* gene was observed in 83.3% of the isolates. PSEF isolate 277 and the PREF isolates 20, 157, 250, 291 and 313 showed all the virulence genes evaluated. The biofilm-forming ability of enterococcal isolates sampled in this study varied between moderate and weak. Penicillin could reduce biofilm formation when used at concentrations below their MICs. Although PREF isolates have shown more genes involved in biofilm formation when compared to PSEF, additional experiments regarding the expression of these genes should be performed to confirm the higher capacity of biofilm formation of these isolates.

Keywords: *Enterococcus*, biofilm, penicillin.

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