TITLE: BIOFILM FORMATION BY *Candida haemulonii* SPECIES COMPLEX

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ABSTRACT: *Candida haemulonii* species complex (*Candida haemulonii* - *Ch*, *Candida duobushaemulonii* - *Cd* and *Candida haemulonii* var. *vulnera* - C*hv*) have emerged as multidrug-resistant yeasts able to cause fungemia worldwide. However, very little is known about the virulence factors produced by them. Biofilms are microbial communities attached to a surface covered by an extracellular matrix, which confers resistance to antimicrobial agents and immune system defenses. In this sense, we aimed to investigate the *in vitro* biofilm production on polystyrene surface of 12 Brazilian clinical isolates comprising the *C. haemulonii* complex (five *Ch*, four *Cd* and three *Chv*). Biofilm biomasses, metabolic activities and extracellular matrix production were assessed by crystal violet staining, XTT reduction and safranin staining, respectively. Additionally, biofilms were analyzed by light microscopy and confocal laser scanning microscopy (CLSM). Finally, the main extracellular matrix (ECM) components, such as proteins, polysaccharides, sterols and DNA were also measured. All the fungal isolates formed biofilms, but with different degrees, exhibiting better results after 48 h of incubation on polystyrene. *Ch* biofilms exhibited significant higher metabolic activity than *Cd*. No significant differences were observed between biofilm biomass and extracellular matrix production by the three species. Individually, the isolate LIPCh4 (*Ch*) exhibited prominent biofilm formation when compared to the others. Light microscopy and CLSM corroborated these results. Biofilm thickness varied from 22.6 to 49.5 μm, but no significant differences were observed in the average between *C. haemulonii* species complex (overall mean = 30.2 μm). Regarding ECM, proteins (overall mean = 6.8 μg/mL) and polysaccharides (3.6 μg/mL) were the main components, followed by DNA (0.06 μg/mL) and sterols (0.016 μg/mL). Strain-specific differences were observed, but no significant differences were detected when comparing the three species. Collectively, our results demonstrated the ability of the species comprising the *C. haemulonii* complex to form biofilm on inert substrate, which is an incontestable virulence attribute associated with catheter-related candidemia in hospitalized individuals, representing a serious problem especially when dealing with multidrug-resistant pathogens such as *C. haemulonii* species complex.

Keywords: *Candida haemulonii* complex, biofilm, clinical isolates

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