Biofilm-forming ability *Candida* species isolated from oral infections

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BACKGROUND

• *Candida* pathogenicity on oral soft tissues has been attributed to several virulence factors[1-3] including adhesion to medical devices or host cells, secretion of hydrolytic enzymes [proteases, phospholipases and haemolysins][3] and biofilm formation ability[1-2].

•The ability to form biofilms of *Candida* species is recognized as a major virulence attribute of this yeast[2-3]. However among clinical *Candida* strains, biofilm formation is variable and depends on the *Candida* spp [1-3].

OBJECTIVES

• To evaluate the biofilm forming capacity among clinical isolates *Candida* species from oral candidiasis patients.

METHODS

• The biofilm forming ability of oral *Candida* spp. isolates were avaluated at Fungal Culture Collection of Oral Microbiology and Pathology Testing Service Laboratory at Dentistry Department of Faculdade da Serra Gaúcha.

• Reference *Candida* strains (*C. albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019) were used as controls in each experiment.

Clinical samples and biofilm-forming capacity

• A total of 42 yeast isolates from 40 Brazilian patients with oral candidiasis were evaluated.

• Of the clinical isolates of *Candida* spp. were examined using two methods, such as:

• **Crystal Violet (CV)**
• **Tetrazolium (XTT) reduction**

• For boths assays was used a microtiter plate reader (*SpectraMax Plus 384 Microplate Reader, Molecular Device*). For CV assay was used a wavw lenght of 620 nm[1]. For XTT reduction assay, was used to measured the absorbance at 450 nm and 620 nm[2].

• Biolfim forming ability were categorized as low metabolic activity, moderate metabolic activity, and high metabolic activity according to their cut-offs by XTT ($0.05 \leq OD \geq 0.25$)[2]. The OD value for forming biofilms were considered as weak biofilm-forming (LBF), moderate biofilm-forming (MBF), and high biofilm-forming (HBF) according to their cut-offs by crystal violet (<0.12, 0.120-0.320 and 0.320)[1].

• Data were analyzed using Kolmogorov-Smirnov's Test indicated normal distribution for biofilm data, as well one-way analysis of variance (ANOVA) for multiple strains comparisons. $P = 0.05$ was used for definition of statistical significance.

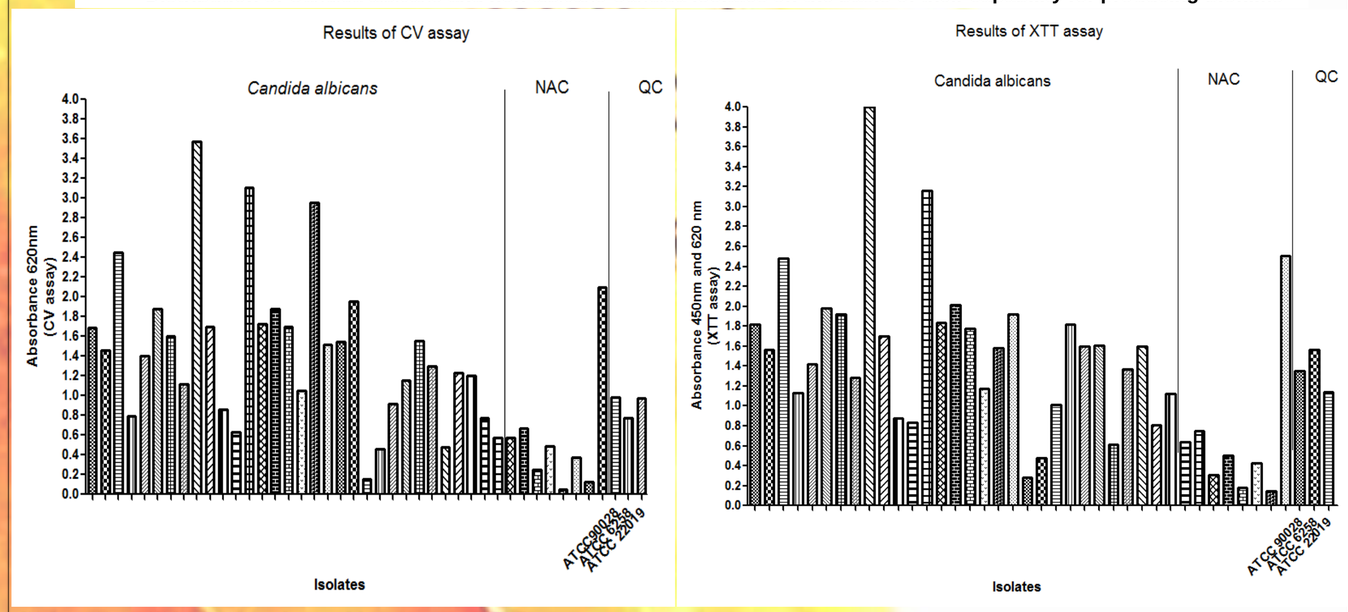
RESULTS

• Comparison of the biofilm forming ability, CV staining and XTT reduction assay of 42 clinical *Candida* isolates from patients with active oral infections were performed.

• In our study, the mean absorbance value of biofilm on *C. albicans* isolates analysed by CV staining was 1.44 (average Abs = 1.44±0.78).

• In contrast, the average absorbance value of biofilm in non-*albicans Candida* isolates was lower (mean Abs= 0.57±0,64). So interesting, the CV assay showed that the biofilm of non-*albicans Candida* species gave lower biomass than biofilm of *C. albicans* ($p < 0.001$). Within the non-*albicans Candida* species group (NAC), *C. tropicalis* yielded greater total biomass than the other species such as *C. parapsilosis*.

Distribution of *Candida albicans* and non-*albicans Candida* isolates on the basis of their capability for producing biofilms



CV: Crystal Violet; NAC: non-*albicans Candida*; QC: quality control; XTT: Tetrazoliun reduction.

• We also determined biofilm forming ability of these 42 oral *Candida* isolates with XTT reduction assay. The our results demonstrated that 40/42 (96%) of total clinical isolates were biofilm formers. The frequency of biofilm formers on *C. albicans* group was higher when was compared with non-*albicans Candida* group ($p < 0.001$). Moreover, for all clinical isolates investigated in our study, the correlation analysis between biofilm biomass (CV staining) and metabolic activity of biofilm (XTT assay) revealed a positive correlation ($r = 0.81$, $p = 0,007$).

CONCLUSION

• Our study demonstrated that *C. albicans* was responsible for aproximately 81% of oral candidiasis.

•The biofilm quantification with crystal violet staining technique was lower than total biomass of biofilm identified by XTT activity method.

• Oral clinical isolates identified as *C. albicans* have greater biofilm-forming capacity for both methods when were compared with the clinical isolates of non-*albicans Candida* species group.

References

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