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Production of Biofilm by *Candida* and non-*Candida* spp. isolates Causing Fungemia: Comparison of Biomass Production and Metabolic Activity and Development of a Cut-off

L.J. Marcos- Zambrano[1,2], P. Escribano[1,2,3], E. Bouza[1,2,3,4], J. Guinea[1,2,3,4]

[1]Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain. [2]Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain. [3]CIBER Enfermedades Respiratorias-CIBERES (CB06/06/0058), Madrid, Spain. [4]Medicine Department, School of Medicine, Universidad Complutense de Madrid

Doctor Esquerdo, 46
28007 Madrid, Spain
Phone: +34-915867163
Fax: +34-915044906
E-mail: jguineaortega@yahoo.es

BACKGROUND

Candida species are able to form biofilm, although extracellular matrix structure and composition of the biofilm vary markedly between species. Biofilm production can be studied by quantification of biomass production using crystal violet stain (CV) and quantification of the metabolic activity of viable embedded biofilm cells based on reduction of tetrazolium salt XTT to formazan dye (XTT).

However, data on biofilm production in a large number of invasive *Candida* isolates obtained from blood cultures are lacking.

OBJECTIVES

1) Study biofilm production in a large collection of *Candida* and non-*Candida* isolates isolated from blood by XTT and CV procedures.

2) Establish tentative cut-off to classify strains according to their ability to form biofilm (low, moderate, or high biofilm-forming capacity: CV procedure) and their metabolic activity (low, moderate, or high metabolic activity: XTT procedure).

3) Compare both procedures to establish species-specific biofilm profiles.

METHODS

577 yeast isolated from blood cultures (n=512 patients) from 2007 to 2013

Molecular Identification by amplification and sequencing of the ITS1-5.8S-ITS2 region. (White T, 1990)

Biofilm formation in 96-well microtiter plates (Pierce, C.G. Nat Protoc, 2008).

Cuantification of biofilm biomass O'Toole G.A. J Vis Exp, 2011)

Cuantification of biofilm metabolic activity (Pierce, C.G. Nat Protoc, 2008)

Distribution by tertiles and development of cut-offs

Clasification of Biofilm production by CV:
LBF <0.44
MBF: 0.44 – 1.17
HBF: >1.17

Clasification of Biofilm activity by XTT:
LMA <0.097
MMA: 0.097 – 0.2
HMA: >0.2

RESULTS

Biofilm formation according to species:

Biomass production and the metabolic activity of the sessile cells for each species measured by CV and XTT procedures, respectively, are shown in Table 1.

A high strain-to-strain variability was found.

TABLE 1. Biofilm metabolic activity (XTT) and production (CV) according to different *Candida* and non-*Candida* spp

Species	n	XTT (GM ± SD)	CV (GM ± SD)
C. albicans	267	0.168 ± 0.098	1.108 ± 0.614
C. parapsilosis	162	0.155 ± 0.171	0.701 ± 0.558
C. glabrata	60	0.257 ± 0.103	0.277 ± 0.245
C. tropicalis	46	0.132 ± 0.058	1.602 ± 0.597
C. krusei	10	0.136 ± 0.081	0.204 ± 0.125
Candida spp.	19	0.148 ± 0.092	0.397 ± 0.360
Other yeasts	13	0.035 ± 0.065	0.247 ± 0.324
Overall	577	0.167 ± 0.125	0.888 ± 0.662

Figure 1a and 1b show the biofilm production (1a) and the metabolic activity of biofilm (1b) of each species.
We observed species-specific profiles of biofilm production; for example, *C. tropicalis* was associated with high amounts of biofilm, whereas *C. glabrata* was associated with high metabolic activity.

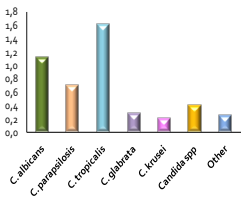

FIGURE 1a. Biofilm production measured by CV.

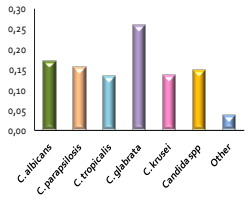

FIGURE 1b. Biofilm metabolic activity measured by XTT.

Cut-offs for classifying isolates according to biomass formation and metabolic activity.

We calculated the percentages of strains of each species according to the cut-offs for both procedures (Figures 2a and 2b). Most isolates of *C. tropicalis* were HBF (80%); a high percentage of *C. albicans* isolates were also HBF (48%) or MBF (36%), and many *C. parapsilosis* isolates were MBF (41%).
In contrast, all isolates of *C. krusei* and a high proportion of isolates of *C. glabrata* (77%), other yeasts (77%), and *Candida* spp (68%) were LBF. A species-specific pattern was also observed for metabolic activity. *C. glabrata* isolates were HMA (73%), followed by *C. albicans* and *C. parapsilosis*. Interestingly, more than 30% of isolates of *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *Candida* spp. and other yeasts were LMA.

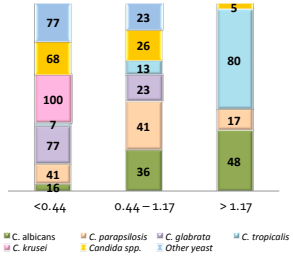

FIGURE 2a. Percentage of isolates from each species classified according to the biofilm biomass production

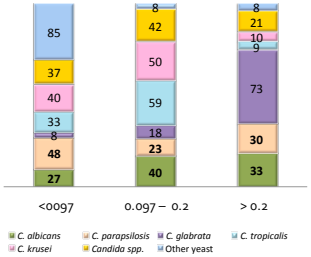

FIGURE 2b. Percentage of isolates from each species classified according to the biofilm metabolic activity

Agreement between procedures:

We compared categorical agreement between the procedures (Table 2). Full categorical agreement was defined as the % of isolates that were in the same category with both methods; partial agreement was calculated considering MBF and HBF or MMA and HMA as the same category.
The different patterns of biofilm production and metabolic activity found for each species may explain the moderate agreement observed.

TABLE 2. Agreement between the XTT reduction and CV staining procedures

% Agreement	*C. albicans*	*C. parapsilosis*	*C. glabrata*	*C. tropicalis*	*C. krusei*	*Candida* spp.	Other yeast	Overall
Full	51.5	50.6	8.3	17.4	40	26.3	84.6	43.7
Partial	81.2	71.6	31.7	65.2	40	36.8	92	70

CONCLUSIONS

- ✓ We found a high variability in the degree of biofilm formation among strains from a same species or among strains from different species.
- ✓ Some species, such as *C. glabrata* or *C. tropicalis*, showed an specific profile of biofilm formation.
- ✓ XTT and CV can serve as complementary procedures for the study of biofilm production.
- ✓ We are currently the clinical implication of these findings.

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