# Investigation of biofilm production by *Candida* species isolated from various clinical samples

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*Candida* species are the fourth most common cause of bloodstream infections in hospitalised patients according to data from the US National Nosocomial Infections Surveillance system,[14] and the number and severity of infections have increased dramatically over recent years.[23] Although *Candida albicans* is the major human pathogen among yeasts, the proportion of infections due to other *Candida* species is increasing.[9]

The continual increase in the use of medical devices (e.g., catheters, prosthetic heart valves and joint replacements) is associated with an important risk of infectious complications. Infections related to biomedical devices are a leading cause of mortality in patients. In addition, these infections are associated with prolonged hospital stay and higher medical costs.[6,7,13]

Various factors play a role in the pathogenesis of *Candida* infections, including the effect of toxin and enzyme production, adherence, biofilm production, dimorphism and cell surface composition.[10,14] Biofilm production has been associated with adherence to the surfaces of catheters and other biomedical devices.[21]

The aim of this study is to determine the biofilm production of different *Candida* species isolated from various clinical samples and compare the intensity of biofilm formation.

A total of 173 *Candida* species were tested for biofilm production. These were recovered from different clinical specimens and consisted of 65 *C. albicans*, 29 *C. parapsilosis*, 25 *C. glabrata*, 25 *C. tropicalis*, 18 *C. kefyr*, five *C. guilliermondii*, four *C. lipolytica* and two *C. krusei*. Of the test organisms, 65 were isolated from blood and comprised 30 *C. albicans*, 18 *C. tropicalis*, 12 *C. parapsilosis*, three *C. kefyr*, one *C. glabrata* and one *C. krusei*. The remaining strains were isolated from urine ($n$=39), respiratory specimens ($n$=25), body fluids ($n$=18), wounds ($n$=17) and other sites ($n$=9). Species distribution showed 35 *C. albicans*, 24 *C. glabrata*, 17 *C. parapsilosis*, 15 *C. kefyr*, seven *C. tropicalis*, five *C. guilliermondii*, four *C. lipolytica* and one *C. krusei*.

Primary isolation from samples was performed on Sabouraud dextrose agar (SDA) supplemented with 1% chloramphenicol. Blood specimens were inoculated into aerobic media and processed using the BACTEC blood culture system (Becton Dickinson). All blood cultures were then subcultured on SDA. The yeast isolates were identified by a germ-tube test, development of blastospores, chlamydospores and pseudohyphae and assimilation tests using the API 20C AUX system (bioMérieux, France).

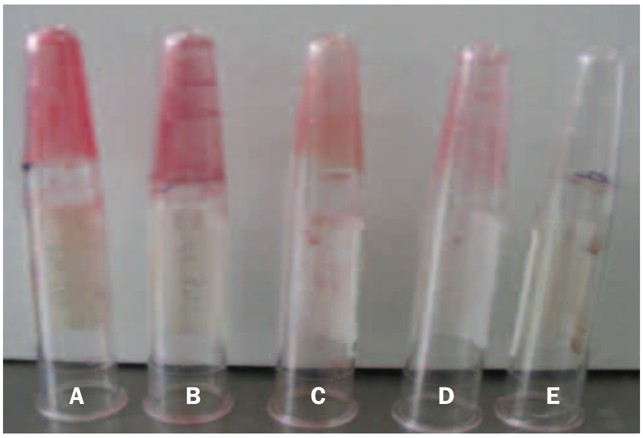


**Fig. 1.** Intensity of biofilm production by the *Candida* strains. A) Strong; B, C) Moderate; D) Weak; E) Negative.

Biofilm production was determined using a modification of the test described for coagulase-negative staphylococci by Christensen *et al*.[5] and for *Candida* by Branchini *et al*.[3] A loopful of organisms from the surface of an SDA plate was inoculated into a polystyrene conical tube containing 10 mL Sabouraud broth supplemented with glucose (final concentration: 8%). These were incubated at 35°C for 24 h. After removal of the liquid medium, the tubes were washed gently with distilled water and stained with 1% safranin. Each tube was examined visually for the presence of a biofilm layer on the internal wall. Biofilm production was scored as negative, weak positive (1+), moderate positive (2+ or 3+) or strong positive (4+), as described by Pfaller *et al*.[18] Intensity of the biofilm layer obtained with *Candida* strains is shown in Figure 1. Biofilm-positive *Staphylococcus epidermidis* ATCC 35984 was used as a positive control. Each isolate was tested at least three times and scored independently by two observers.

The differences in biofilm production by *Candida* strains between those recovered from blood and those from other sites were determined using the $\chi^2$ test. $P<0.05$ was considered to be significant.

Biofilm production was demonstrated in 114 (65.9%) of the 173 *Candida* isolates tested. Thirty-eight (58.5%) of the 65 *C. albicans* strains and 76 (70.4%) of the 108 non-albicans strains were biofilm-positive. No significant difference was found between *C. albicans* and non-albicans species in terms of biofilm activity ($P>0.05$).

Overall results obtained with the *Candida* strains are shown in Table 1. In the 65 *C. albicans* strains, biofilm production was weak in 18 (27.7%) and moderate in 20 (30.8%). Strong biofilm production was not found in the *C. albicans* strains tested. In non-albicans strains, biofilm intensity was weak, moderate and strong in 16 (14.8%), 32 (29.6%) and 28 (25.9%), respectively (Table 1).

No significant difference in biofilm production between bloodstream *C. albicans* isolates and those from other clinical samples was observed ($P>0.05$). In contrast, biofilm activity in non-albicans strains obtained from blood was significantly higher than those isolated from other sites ($P<0.05$; Table 2).

Over the past decade, the incidence of nosocomial fungal infection has increased. This is associated with parenteral nutrition, use of extended-spectrum antibiotics, duration of

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**Table 1.** Distribution of biofilm production by *Candida* species.

| Yeast (*n*) | Biofilm production | | | |
|---|---|---|---|---|
| | Strong | Moderate | Weak | Negative |
| *C. albicans* (65) | 0 (0) | 20 (30.8%) | 18 (27.7%) | 27 (41.5%) |
| Non-albicans *Candida* (108) | 28 (25.9%) | 32 (29.6%) | 16 (14.8%) | 32 (29.6%) |
| *C. parapsilosis* (29) | 5 | 6 | 7 | 11 |
| *C. galabrata* (25) | 0 | 11 | 4 | 10 |
| *C. tropicalis* (25) | 14 | 10 | 0 | 1 |
| *C. kefyr* (18) | 4 | 4 | 3 | 7 |
| *C. guilliermondii* (5) | 0 | 0 | 2 | 3 |
| *C. lipolytica* (4) | 3 | 1 | 0 | 0 |
| *C. krusei* (2) | 2 | 0 | 0 | 0 |
| Total (173) | 28 | 52 | 34 | 59 |

hospital stay, use of immunosuppressive agents following chemotherapy, mechanical ventilation and the use of medical devices.[11,13,14,17] Although *C. albicans* is the most commonly isolated fungal species, increase in infection due to other *Candida* species has been observed. Some of the non-albicans strains are often resistant to antifungal agents and infection is associated with a higher mortality rate.[19] A recent study showed that biofilm formation by a non-albicans species of *Candida* (*C. parapsilosis*) may play an important role in outbreaks of infection.[15]

Biofilm is an accumulation of microorganisms and their extracellular polymers which adhere to and grow on solid surfaces such as catheters and other biomedical devices, and contributes to their high prevalence as a source of nosocomial infections.[5,18,20]

Biofilm production is also associated with antimicrobial resistance.[1,8,16] Al-Fattani and Douglas[1] reported that drug resistance in *Candida* biofilms is a complex process. In a recent study, fluconazole and amphotericin B showed decreased activity against the biofilm of *C. tropicalis* strains tested,[2] making treatment of biofilm-associated infection difficult.[20]

Methods used in the observation of biofilm production give semiquantitative results. In a comparison of the visual tube method (VTM) and transmission electron microscopy (TEM) it has been reported that VTM is cheap, simple and reliable, and can be used to detect biofilm production.[4] In the present study, VTM was used to examine biofilm production by various *Candida* species isolated from blood and other sites.

It has been shown that *in vitro* biofilm formation depends on *Candida* species and strain type.[8,12,14,22] Kuhn *et al*.[15] showed that *C. parapsilosis* isolates from an outbreak had a significantly higher ability to form biofilms. Shin *et al*.[22] compared different species for their ability to produce biofilms on a polystyrene surface. These authors observed that biofilm positivity occurred most frequently in isolates of *C. tropicalis*, followed by *C. parapsilosis, C. glabrata* and *C. albicans*. Similarly, in this present study, *C. tropicalis* was the most frequent biofilm-producing species.

Tumbarello *et al*.[24] investigated the correlation between *Candida* species and biofilm production. It was found that biofilm production by *C. albicans* was significantly less frequent (22.6%) than by non-albicans species (33.3%). However, it was emphasised that non-albicans strains such as *C. parapsilosis*, *C. pseudotropicalis* and *C. glabrata* produced significantly less biofilm.[12,14] In the present study, biofilm activity was seen in 38 out of 65 *C. albicans* strains (58.5%) and in 76 out of 108 non-albicans strains (70.3%), but the difference was not significant.

The present study also evaluated biofilm production by *C. albicans* and non-albicans strains according to isolate origin. Shin *et al*.[22] compared biofilm production by *Candida* species obtained from the blood and those obtained from other anatomical sites. No significant difference was observed between *C. albicans* bloodstream isolates (7%) and those from other sites (8%). However, they showed that biofilm production by non-albicans species obtained from the blood (79%) was significantly higher than that by isolates from other sites (52%). In the present study, there was no significant difference between *Candida* bloodstream isolates and those isolated from other clinical samples. Biofilm activity for non-albicans species obtained from blood was significantly higher than for isolates from other sites ($P<0.05$). In the present study, the highest intensity of biofilm formation was observed in non-albicans strains by VTM.

**Table 2.** Biofilm production by *Candida* strains isolated from blood and other samples.

| Yeast (*n*) | Origin | Number positive (%) | Number negative (%) | *P* value |
|---|---|---|---|---|
| *C. albicans* | Blood | 18 (60) | 12 (40) | >0.05 |
| | Other sites | 20 (57.1) | 15 (42.9) | |
| Non-albicans *Candida* | Blood | 29 (82.9) | 6 (17.1) | <0.05 |
| | Other sites | 47 (64.4) | 26 (29.6) | |

In conclusion, biofilm production is an important virulence factor in infections caused by *Candida* species, bearing in mind that infections caused by non-albicans species have increased recently. Therefore, preventive measures such as the use of antimicrobial coating of biomaterials should be applied in order to prevent infections caused by *Candida* species.

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# Lack of isolation of *Pseudomonas aeruginosa* associated with agricultural practices: relevance to patients with cystic fibrosis


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Cystic fibrosis (CF) is the most common inherited disease in persons originating from a white and European background and has a genetic carriage rate of one in 20 persons and an incidence of one in 2500 live births.[1] It is an autosomal recessive condition whereby two alleles carrying a polymorphism in the CF transmembrane conductance regulator (*CFTR*) gene phenotypically manifest the disease state through a variety of multiorgan problems, and is associated with a pharmacological dysfunction to regulate chloride ion secretion across cell membranes.

The most common complication of CF is the recurrence of chronic chest infections usually caused by bacterial pathogens.[4] Cystic fibrosis patients continue to suffer from recurrent and chronic respiratory tract infections and most


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