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# Characterisation of *Candida albicans* Bilofilm Formed on Prosthetic Heart Valves

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## ABSTRACT

Microbial biofilm on prosthetic heart valves seriously complicate the care of patients, *Candida albicans* had been isolated from the biofilm matrix formed on heart valves removed from patients. Ten *Candida albicans* isolates were tested for biofilm formation at different time interval and maximum biofilm formation was achieved by one isolate at 48 h. The optical density of the maximum adhered isolates was 1.8 nm but for the other 9 isolates OD value was in the range 0.6-1.5 nm. The *Candida albicans* isolated from the heart values were tested for antibiotic sensitivity and found that only a higher dose of Amphotericin B (0.5 μg $mL^{-1}$) and gentamycin (12 μg $mL^{-1}$) inhibited 50% of the metabolic activity in *Candida albicans*.

**Key words:** *Candida albicans*, biofilm, drug susceptibility, heart valves, biofilm matrix

## INTRODUCTION

In recent years, the use of medical implants such as catheters, pacemakers, prosthetic heart valves and joint replacements have increased dramatically. These devices can become colonized by microorganisms which form a biofilm consisting of a mono-or multilayer of cells embedded within a matrix of extra cellular polymeric material. Release of microorganisms from the biofilm may initiate an acute disseminated infection. Implant associated infection are difficult to resolve because biofilm microorganisms are resistant both to host defense mechanisms and antibiotic therapy.

*Candida albicans* is an opportunistic pathogen that infects primarily immuno-compromised hosts. *Candida albicans* infections manifest as a complex biofilm consisting of yeast, hyphae and desquamated epithelial cells. Use of indwelling catheters, increases the risk of *Candida* infection. Devices, such as shunts, prostheses, endotracheal tubes, vascular catheters have been shown to support colonization and biofilm formation by *Candida* species. Biofilms of *Candida albicans* consisted of mixture of yeasts, hyphae and pseudohyphae and were resistant to the action of a variety of antifungal agents, including amphoteicin B and fluconazole. Hawser *et al.* (1998) studied *Candida* biofilms growing on the surface of small disc of catheter material and demonstrated that after 48 h, *Candida albicans* biofilm was formed of a dense network of yeasts, germ tubes, pseudohyphae and hyphae.

Chandra *et al.* (2001) reported that *Candida albicans* growing as a biofilm, exhibited resistance to Amphotericin B, Nystatin, Chlorhexidine and Fluconazole. Ramage *et al.* (2001) reported

multidrug efflux pumps in relation to fluconazole resistance in *Candida albicans* biofilms. Kuhn *et al.* (2002) reported that *Candida* biofilm show unique susceptibilities to Echinocandins and AMB lipid formations.

Kuhn *et al.* (2002) concluded that *Candida albicans* produces quantitatively larger and qualitatively more complex biofilms than other species. Kumamoto (2005) reported that Candida *albicans* an opportunistic fungal pathogen that normally resides within a mammalian host also exhibits contact dependent cellular behaviors such as invasive hyphal growth and biofilm development and concluded that *Candida albicans* uses the cell integrity pathway to mediate multiple contact-dependent responses.

Al-Fattani and Douglas (2006) demonstrated that the matrix can make a significant contribution to drug resistance in *Candida* biofilms, especially under conditions similar to those found in catheter infections *invivo* and that the composition of the matrix material is an important determinant in resistance. Hence, in the present study attempt has been made to study the Characteristics of biofilms formed by *C. albicans* on prosthetic heart valves implanted in cardiac patients.

## MATERIALS AND METHODS

Prosthetic heart valve removed for replacement from ten patient admitted at Jipmer, Medical College, Hospital, Puduchery. India was choosen for the isolation of *Candida albicans* that constitute biofilm over the implant. The heart valve samples were collected under aseptic conditions and brought to the laboratory for investigation. Using standard procedure (Pruthi *et al.*, 2003) the microbes attached to the heart valves were isolated and from these microbial consortium *Candida albicans* were isolated using Hichrom Candida agar (Hi-Media, Bombay). *C. albicans* strains isolated were maintained on slopes of Sabouraud dextrose agar (Hi-Media).

**Biofilm quantification:** *C. albicans* isolates were tested for their biofilm-forming ability at different time intervals (8, 16, 24, 32, 40, 48, 56, 64 and 72 h).The cultures were grown on yeast peptone dextrose (YEPD) broth containing 20 g $mL^{-1}$ peptone, 10 g $mL^{-1}$ Yeast extract and 20 g $L^{-1}$ dextrose and incubated at 48 h at 35°C with agitation (120 rpm).

Biofilm quantification was performed using modified 2, 3 bis (2-methoxy-4 nitro-5-sulphophenyl 1) -2H-5-tetrazolium-carboxanilidae (XTT) reduction assay on capped polypropylene tubes. The heart valve material was cut in to small discs (0.5 cm surface area) and sterilized by exposure to ultraviolet radiation for over 15 min on each side. *C. albicans* cell suspension isolated from the biofilm was added to the surface of the sterilized discs ($5\times10^8$ cfu $mL^{-1}$ of 48 h grown *C. albicans*). The plates were incubated for 48 h at 37°C, according to the study of Baillie and Douglas (1999). The discs were then washed with sterilized phosphate buffered saline (PBS) to remove loosely adherent cells. One milliliter of sterilized YEPD broth was added to the washed discs and incubated at 37°C for 24 h. The biofilm thus formed was then quantified using XTT reduction assay.

An XTT (Sigma) solutions (1 mg $mL^{-1}$ in PBS) was prepared, filters sterilized through a 0.22 μm pore size filter and stored at 70°C. Menadione solution (0.4 mM) was prepared and filter sterilized before each assay. Prior to each assay, XTT solution was thawed and mixed with the Menadione solution at a ratio of 5 to 1 by volume. The biofilms formed on discs were first washed five times with 1 mL of PBS and then 1 mL of PBS and 60 μL of the XTT-Menadione solution were added to each of the prewashed and control tubes. The tubes were then incubated in the dark for

2 h at 37°C. Following incubation the color change in solution was measured spectrophotometrically at 492 nm (Systronics, India).

Biofilm of *C. albicans* formed on YEPD was isolated and tested for antibiotic sensitivity. For antibiotic sensitivity assays tissue culture polystyrene 96 well flat bottom plates were used. Isolates from the culture medium was removed and filled in the wells of culture plate. Each well was filled with 0.2 mL aliquots of the culture and broth was used as control to check sterility and non-specific binding media.

YEPD broth (200 μL) containing different concentrations of antifungal agents were then added to each well (Amphotericin B-0.1 μg $mL^{-1}$ and flucanozole 0.65 μg $mL^{-1}$). The plates were incubated for 24 h at 37°C and washed in PBS. Biofilm quantification was done by XTT reduction assay.

## RESULTS AND DISCUSSION

Along with some bacteria *Candida albicans* may attach and develop biofilms on components of mechanical heart valves and surrounding tissues of the heart, leading to a condition known as prosthetic valve endocarditis (Donlan, 2001) Biofilm forming microorganism are reported to originate from the skin, other indwelling devices such as central venous catheters or dental work (Braunwald, 1997) *C. albicans* was one among the different microbes that form biofilm on indwelling device and cause endocarditis. Lewis (1998) reported that *C. albicans* in the biofilm was resistant to many antibiotics and relatively few patients can be cured of a biofilm infection by antibiotic therapy alone. In this study, it is imperative assess the extent of biofilm formation by *C. albicans* in heart valve implants and the sensitivity of *C. albicans* isolated from the prosthetic heart valve implants. The emergence of *Candida* species notably, *C. albicans,* an opportunistic pathogen in these infected heart valves are of serious concern. Data showed that out of the 10 *C. albicans* isolates screened for biofilm formation at different time periods (12-72h) maximum biofilm formation was achieved by isolates number 8 at 48 h revealing its strongest ability to form biofilm compared to all other isolates (Fig. 1).

Present investigation to test the *in vitro* activity of antifungal agents revealed increased drug resistance in *C. albicans*. Data showed 50% reduction in metabolic activity at a concentration of 0.5 μg $mL^{-1}$ of amphotericin B and 12 μg $mL^{-1}$ of flucanozole. The high antifungal resistance in *C. albicans* pose threat to patients having heart valve transplants. Antifungal resistance in *C. albicans* exposes the need develop strong.

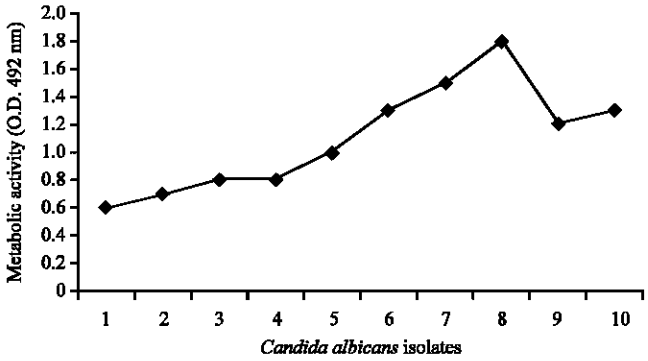


Fig. 1: Characterisaton of *C. albicans* bilofilm formed on prosthetic heart valves

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