Biofilm formation by *Candida Tropicalis*: comparison between isolates from immunocompromised and immunocompetent patients

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ABSTRACT

Introduction: *Candida tropicalis* is an important pathogen. Infections caused by *Candida spp.* are often associated with biofilm on implanted medical devices and on epithelial surfaces. **Materials and methods:** We compared clinical isolates of *C. tropicalis* from immunocompromised patients (n=46) with those from immunocompetent (n=41) patients for biofilm formation. **Results:** Biofilms were produced in 73/87 *C. tropicalis* isolates; 41 (56.2%); biofilm activity 3+ (n=2), 2+ (n=14) and 1+ (n=25) from immunocompromised patients and 32 (43.8%) biofilm activity 3+ (n=3), 2+ (n=7) and 1+ (n=22) from immunocompetent patients. **Conclusion:** Our study showed that *C. tropicalis* can produce biofilms in both immunocompetent and immunocompromised patients. Further analysis of a large number of isolates from immunocompromised patients would validate the present finding.

Keywords: *Candida tropicalis,* biofilm, immunocompromised, immunocompetent

INTRODUCTION

Candida (C) species are commonly regarded as normal commensals of mucosal surfaces in healthy humans. However these commensal yeasts can be serious opportunistic pathogens in human hosts with conditions such as Acquired Immune Deficiency Syndrome (AIDS), diabetes mellitus, organ transplantations and cancers. *Candida* spp. are now recognised as major agents of hospital acquired infection and are reportedly the fourth most common cause of blood stream infection. [1] Recent reports have also indicated a trend towards non-albicans candida (NAC) such as *C. parapsilosis* and *C. tropicalis* as causative agents of candideamia. [2] The production of biofilm enhances resistance to antifungal agents. Hence biofilm-associated infections are frequently refractory to conventional therapy. [3]

Biofilms are interdependent communities enclosed in an exopolysaccharide matrix. It is found on any surface, including medical devices such as venous catheters, voice prostheses, intrauterine devices and prosthetic joints. Biofilms are notoriously dif-

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ficult to eliminate and serve as a source of recalcitrant infections. [4] A variety of microbial infections are caused by biofilms ranging from the common, urinary tract infections, catheter infections, child middle ear infections, and dental plaque, to more serious infections such as endocarditis and infections of heart valves. [5] Immunocompromised patient populations such as those with cancers or HIV infection are most susceptible to biofilm related infections owing to their debilitated immune status and use of bio prostheses. [6] With a paucity of data on biofilm production among *C. tropicalis*, the present study was undertaken to demonstrate and compare biofilm production among clinical isolates of *C. tropicalis* from immunocompromised and immunocompetent patients.

MATERIALS AND METHODS

A total of 87 clinical isolates of *C. tropicalis* were included in this study. Of these, 46 isolates were from immunocompromised patients and 41 from immunocompetent patients. The immunocompromised patients, included HIV patients (n=30) and patients with malignancies (n=16). Samples consisted of oral swabs (n=34), blood (n=7) and urine (n=2), and one sample each of sputum, vaginal swab and endotracheal secretion. Isolates from immunocompetent patients (n=41) consisted of various clinical conditions such as fever (n=8), road accident cases (n=11), diabetes mellitus (n=5), heart disease (n=4), vaginal thrush (n=8) and stent (n=5). Samples included urine (n=30), blood (n=5), pus (n=2) and one each from sputum, vaginal swab, wound swab and bronchial wash. A standard strain of *C. tropicalis* ATCC 13803 was also included. All isolates were speciated using standard mycological procedures like colour on CHROM agar, germ tube test, sugar fermentation and assimilation tests. Biofilm production was studied by the micro titre plate method as described by Shin *et al.* [7]

Sabouraud's dextrose broth (SDB) was prepared with a final concentration of 8% glucose. Test isolates grown on SDA at 37 °C for 24 h were washed and suspended in sterile saline. The turbidity of the suspension was adjusted to $\sim 3 \acute{} 10^7$ CFU mL^{-1}. Biofilm production was performed in flat bottom micro titre plates. To 20 µl aliquots of cell suspension,180 µL of SDB was inoculated and incubated at 37 °C for 24 h without agitation. Distilled water (200 µL) was added to each well and spectrophotometer readings were taken at 405 nm with a micro titre plate reader (Lab systems Multiskan MS, Finland). Mean of the readings (duplicates) were used to calculate %T values. The %T value for each test sample was subtracted from the %T value of the blank to obtain the $\%T_{bloc'}$ i.e. the measure of the amount of light blocked when passing through the wells. $\%T_{bloc}$ was scored as negative for <5, 1+ for 5-20, 2+ for 20-35, 3+ for 35-50 and 4+ for ≥50.

Table 1: Biofilm productions among immunocompromised and immunocompetent patients.

Isolates	No. of isolates tested	Scoring of biofilm production No of isolates (%)			
		3+	2+	1+	-ve
Immunocompromised patients	46	2 (4.3)	14 (30.4)	25 (54.3)	5 (10.8)
Immunocompetent patients	41	3 (7.3)	7 (17)	22 (53.6)	9 (21.9)
Total	87	5 (5.7)	21 (24.1)	47 (54)	14 (16)

RESULTS

Biofilms were produced in 73/87 *C. tropicalis* isolates. Among the 73 biofilm producers 41 (56.2%) were from immunocompromised patients and 32 (43.8%) were from immunocompetent patients.

Among the 41 *C. tropicalis* isolates from the immunocompromised patients 25 (54.3%) isolates showed 1+ biofilm activity, 14 (30.4%) showed 2+ biofilm activity. Among the 32 *C. tropicalis* isolates from the immunocompetent patients 22 (53.6%) isolates showed 1+ biofilm activity, 7 (17.0%) showed 2+ biofilm activity (Table 1). There was no significant difference in biofilm forming ability between *C. tropicalis* isolates from immunocompromised and immunocompetent patients.

DISCUSSION

It is increasingly obvious that infections caused by *Candida* spp. are an escalating problem and of major concern is the limited arsenal of anti-fungals agents. In this study, the ability of *C. tropicalis* to adhere and form biofilm on polystyrene surfaces under static conditions was demonstrated. [7] The use of indwelling medical devices is associated with a high risk for candida infections. Most candida infections are associated with biofilm formation on implanted medical devices or on host epithelial cell surfaces. Formation of *C. tropicalis* biofilms has gained clinical significance due to their ability to resist host immune defences. [6, 9]

In this present study, 46 *C. tropicalis* isolates from immunocompromised patients were tested for biofilm production. Of these 89.1% (n=41/46) of the isolates were positive for biofilm which was similar to a study by Kumar *et al*. which reported biofilm productions in 95.8% (n=23/24) of *C. tropicalis* isolates from immunocompromised patients. [8]

Candida biofilms are intrinsically resistance to most antifungal drugs. Life threatening candida infections has increased dramatically worldwide in part due to the increase in the number of immunocompromised patients, particularly HIV patients, patients undergoing chemotherapy with resultant neutropenia, organ transplant associated immunosuppressive therapy, invasive medical procedures such as use of prosthetic devices and vascular catheters, parenteral nutrition and peritoneal dialysis haemodialysis etc.

The treatment of invasive candidiasis is not straightforward given the associated severity of illness of patients, and also the toxicity of anti-fungal agents. Production of biofilms further decreases the effect of anti-fungals, contributing to treatment failures or requirement for prolonged treatments, removal of foreign devices and use of prolonged and complex regime of anti-fungal. This may also promote development of fungal resistance.

In conclusion, our study showed that *C. tropicalis* from immunocompetent and immunocompromised patients can form biofilms and this may significantly impact in the management of *C. tropicalis* infection. However, our sample size is small and further analysis of a large number of isolates from immunocompromised patients would validate the present finding.

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