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RESEARCH ARTICLE

SPECIATION AND BIOFILM FORMATION IN CANDIDA SPECIES ISOLATED FROM VARIOUS CLINICAL SAMPLES

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ABSTRACT

Background and Objective: Candida albicans a normal commensal of human mucosal surfaces and opportunistic pathogen in immune-compromised states, diabetes mellitus and iatrogenic factors like antibiotic use, indwelling devices, intravenous catheters, etc. Most infections are caused by C.*albicans*, the shift towards treatment resistant non albicans candida (NAC) species is evident in recent years. One of the important factors contributing to the virulence of Candida species is the formation of surface attached microbial communities known as "Biofilm". Thus this study is undertaken to identify, isolate, speciate and to determine Biofilm production.

Materials and Methods: A total of 150 Candida species isolated from various clinical samples were processed in the department of Microbiology, AIMS, B.G.Nagara for a period of 6 months from Oct 2014 to March 2015. Isolates were from vaginal swab (76%), urine (15.3%), exudates (6.7%) and endotracheal aspirate (2%). Samples were inoculated onto Sabouraud's dextrose agar (SDA) & chrom agar (Himedia, Bombay). Growth on SDA was speciated by standard methods using Germ tube, Corn meal agar, slide culture, sugar fermentation and sugar assimilation tests. By the colour of colonies on chrom agar, isolates were speciated. **Result:** Out of 150 Candida species studied, 63 were C.*albicans* (42%) and 87 NAC (58%). A total of 78 (52%) candida species were biofilm producers and 72 (48%) non biofilm producers.

Conclusion: The above results shows increasing isolation of NAC when compared to C.albicans and Biofilm production from candida species.

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INTRODUCTION

Over the last three decades, Candida species emerged as an important cause of health care associated and opportunistic infections. Candida albicans a normal commensal of human mucosal surfaces and opportunistic pathogen in immune-compromised states, diabetes mellitus and iatrogenic factors like antibiotic use, indwelling devices, intravenous catheters, etc. Serious infections with Candida species have increased dramatically to a point where Candida species have become a common cause of nosocomial blood stream infection, next to Escherichia coli and Pseudomonas species. Most infections are caused by C.*albicans*, the shift towards treatment resistant non albicans candida (NAC) species is evident in recent years. One of the important factors contributing to the virulence of Candida species is the formation of surface attached microbial communities known as "Biofilm". Biofilms are defined as highly structured communities of microorganisms that are either surface associated or attached to one another and are enclosed with a self-produced protective extracellular matrix. The advantages to an organism of forming a biofilm include protection from the environment, resistance to physical and chemical stress, metabolic co-operation and a community-based regulation of gene expression. In the recent years, there has been an increased appreciation of the role that fungal biofilms play in human disease as microbes growing within biofilms exhibit unique phenotypic characteristics compared to their planktonic counterpart cells, particularly increased resistance to antimicrobial agents. The mechanism by which biofilms resists the action of antifungal agents are not exactly known. Possible resistance mechanisms include drug exclusion by biofilm matrix and phenotypic changes resulting from nutrient limitation and growth rate. In addition to providing safe sanctuary for microorganisms, biofilms may also act as reservoirs for persistent sources of infection in a patient and as

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such adversely affect the health of an increasing number of individuals, including patients with HIV-infection, cancer, transplants, patients requiring surgery or intensive care and newborn infants. Ramage *et al*. (2005)

Although Candida albicans is the predominant etiology of Candidiasis, other non albicans candida species such as C. krusei, C. glabrata, etc., have emerged as drug resistant opportunistic pathogens. The present study was undertaken to speciate and biofilm-forming ability of Candida species isolated from various clinical samples. Approval was obtained from the institutional ethical committee.

MATERIALS AND METHODS

A total of 150 Candida species isolated from various clinical samples were processed in the department of Microbiology, AIMS, B.G.Nagara for a period of 6 months from Oct 2014 to March 2015. Isolates were from vaginal swab (76%), urine (15.3%), exudates (6.7%) and endotracheal aspirate (2%). Samples were inoculated onto Sabouraud's dextrose agar (SDA) & chrom agar (Himedia, Bombay).Growth on SDA was speciated by standard methods using Germ tube, Corn meal agar, slide culture, sugar fermentation and sugar assimilation tests. By the colour of colonies on chrom agar, isolates were speciated (Fig 1). Patients on antifungal therapy or with cutaneous candidiasis were excluded from the study.

Biofilm formation: Biofilm production was detected by tube adherence method described by Brachinet *et al*. (1994) A loopful of organisms from Sabouraud's dextrose agar was inoculated into Sabouraud's dextrose broth supplemented with glucose (8%) and incubated at 37°C. After 24 hours, broth was gently drained out; tubes were washed with distilled water and stained with 1% Safranin for 7 minutes. Safranin was emptied and tubes were observed for adherent biofilm layer, scored as visually negative, weak positive 1+, moderate positive 2+ and strong positive 3+(Fig 2).

RESULTS

Out of 150 Candida species studied, 63 were C.*albicans* (42%) and 87 NAC (58%). A total of 78 (52%) candida species were biofilm producers and 72 (48%) non biofilm producers. Among biofilm producers 30.77% and 69.23% were from Candida albicans and NAC respectively.

Table 1 Distribution of various species in relation to clinical sample and its biofilm production.

Table 2 Grading of biofilm production by Candida species in the study group.

Table 1. Results of biofilm production: specimen and species wise distribution.

Species	High vaginal swab		Urine		Pus		Endotracheal aspirate	
Total	Biofilm +ve	Number	Biofilm +ve	Number	Biofilm +ve	Number	Biofilm +ve	Number	Biofilm +ve
C. albicans No 63	24 (38.09%)	49	17 (34.69%)	9	5 (55.55%)	4	2 (50%)	1	0 (0%)
C. glabrata No 32	21 (65.62%)	24	14 (58.33%)	5	5 (100%)	3	2 (66.66%)	-	-
C. tropicalis No 21	14 (66.66%)	14	9 (64.28%)	3	3 (100%)	2	1 (50%)	2	1 (50%)
C. krusei No 16	13 (81.25%)	13	10 (76.92%)	2	2 (100%)	1	1 (50%)	-	-
C. kefyr No 8	4 (50%)	6	3 (50%)	2	1 (50%)	-	-	-	-
C. parapsilosis No 4	1 (25%)	3	0 (0%)	1	1 (100%)	-	-	-	-
C. dubiliensis No 4	1 (25%)	3	0 (0%)	1	1 (100%)	-	-	-	-
C. guilliermondi No 2	0 (0%)	2	0 (0%)	-	-	-	-	-	-
Total 150	**78** (52%)	**114** (76%)	**53** (46.49%)	**23** (15.34%)	**18** (78.26%)	**10** (6.66%)	**6** (60%)	**3** (2%)	**1** (33.33%)

Table 2. Grading of biofilm production of Candida species in the study group

	HVS			Urine			Pus			Endotracheal aspirate		
	1+	2+	3+	1+	2+	3+	1+	2+	3+	1+	2+	3+
C. albicans 63	06	08	10	01	01	03	01	01	-	-	-	-
C. glabrata 32	11	06	04	-	03	02	-	02	-	-	-	01
C. tropicalis 21	07	05	02	01	01	01	-	01	-	-	-	-
C. krusei 16	07	04	02	-	-	01	01	-	-	-	-	-
C. kefyr 8	03	-	01	-	-	-	-	-	-	-	-	-
C. parapsilosis 4	-	-	01	-	-	-	-	-	-	-	-	-
C. dubiliensis 4	-	-	01	-	-	-	-	-	-	-	-	-
C. guilliermondi 2	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Biofilm production among C.albicans and NAC as reported by various studies

	C.albicans %	Non albicans candida %
Present study (2015)	38.09	62.06
Golia (2012)	55.10	74.57
Mythreyi (2015)	40	62.5
Muni (2012)	54.83	78.9
Girish kumar (2006)	61.11	92.5

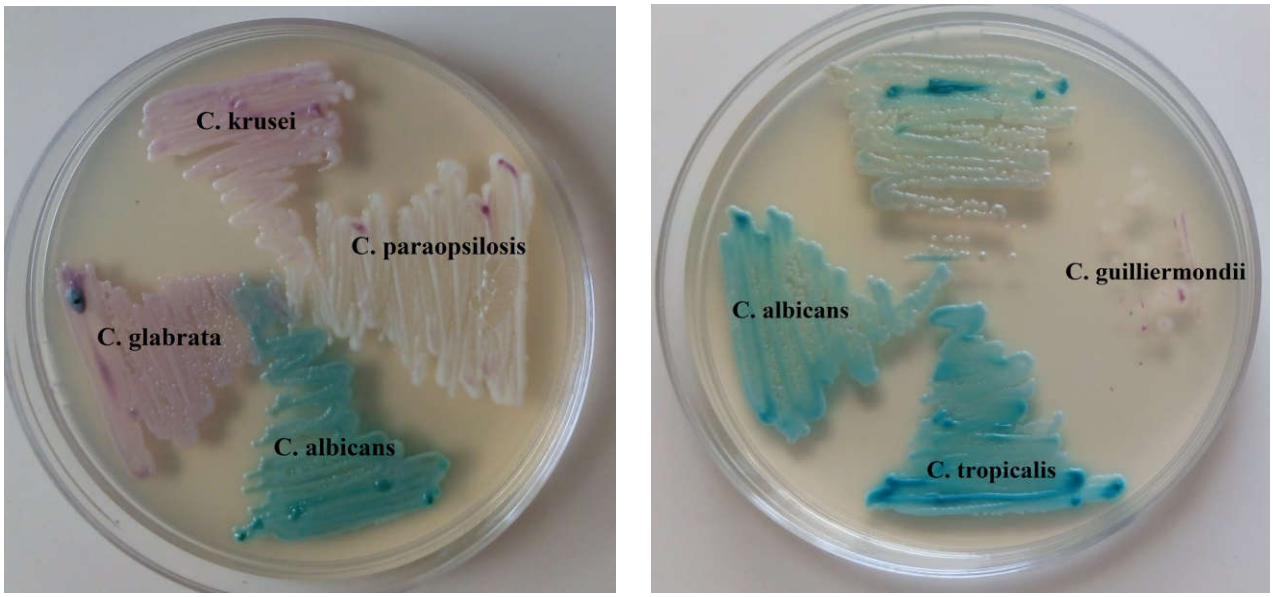

Fig. 1. Speciation of Candida by Chrom agar

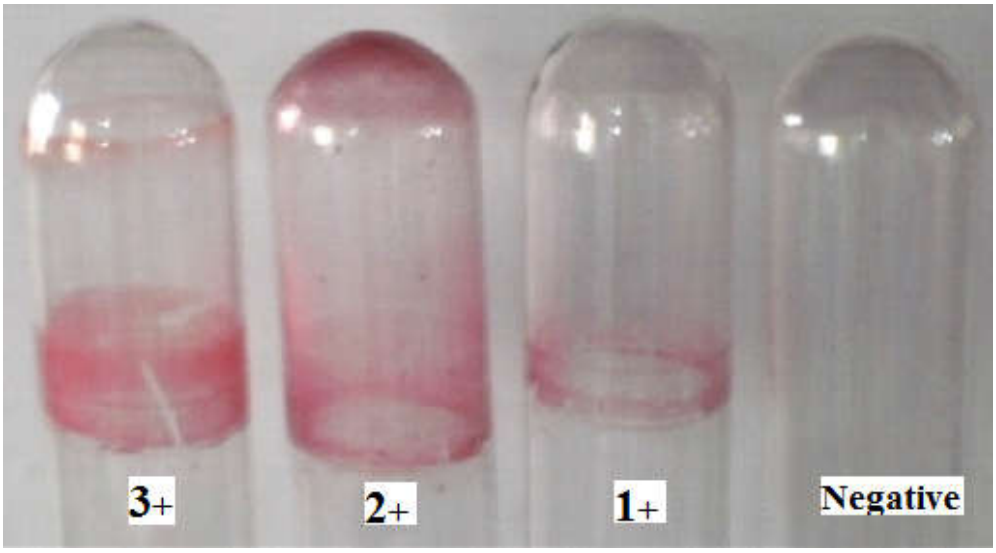

Fig. 2. Biofilm detection by safranin method

DISCUSSION

Mycosis in general and Candidiasis in particular are both widespread and increasing in frequency. C.*albicans* historically has been predominant cause of candidiasis. In 1989, C.*albicans* accounted for great than 80% of all candidial isolates recovered from nosocomial yeast infection. More recently NAC species has been appeared with increasing frequency. In the present study, maximum numbers of isolates were C. albicans (42%), followed by C. glabrata (21.3%), C. tropicalis (14%) and C. krusei (10.67%) whereas other studies showed C. albicans as common isolate followed by C. tropicalis. Higher number of Candida species were from vaginal swab (72%) and predominantly C. albicans (42.98%) followed by C. glabrata (21.1%), C. tropicalis (12.28%) and C. krusei (11.4%). In urine, most common isolate was C.albicans (39.13%) followed by C. glabrata (21.74%), C. tropicalis (13.04%) and C. krusei and C. kefyr (8.7%). In the present study, 58% were NAC correlating with Saroj *et al*. Golia *et al*. (2012) The problem of emergence of NAC species becomes more acute because different species of NAC exhibit varying degree of resistance intrinsic or acquired or both to commonly used antifungal drugs. Sachin *et al*. (2014)

In the present study, 52% of the isolates were biofilm producers which is less compared to others. Golia S*et al* (2012) The positivity was maximum in urine (78.26%), followed by pus (60%), vaginal swab (46.5%) and endotracheal aspirate (33.3%). Maximum number of biofilm formation was among urine isolates (78.26%), which may be due to catheterisation in hospitalised patients. Least biofilm producers were found in respiratory tract infection is in correlation with Muni *et al*. Muni S *et al* (2012) Among C. gulliermondi (2) isolates were negative for biofilm production, may be due to less number studied. Devices such as stents, shunts, prosthesis, implants, endotracheal tube, pace-maker and various types of catheters are associated with biofilm production which is the reason for variation in distribution of biofilm production in different samples.

Biofilm production was found more among NAC (62.06%) than C. albicans (38.09%) is in correlation with other studies. Table 3 shows Biofilm production among C.*albican*s and non albicans candida as reported by various studies. Golia *et al.* (2012), Muni *et al.* (2012), Kumar and Menon (2006), Mythreyi and Jyothi (2015) Biofilm production in urine was 100% with C. glabrata, C. tropicalis and C. krusei whereas in vaginal swab it was maximum in C. krusei (76.9%) and in pus, C. glabrata was highest with 66.67% biofilm production. Among NAC, biofilm positivity occurred more among C. krusei (81.25%), followed by C. tropicalis (66.67%), C. glabrata (65.6%), C. kefyr (50%), C. albicans (38.09%), C. parapsilosis and C. dubiliensis (25%) correlating with the other studies. Biofilm production is more among NAC than C. albicans which may correlate with higher resistance to antifungals. Strong biofilm production was maximum in C.albicans (20.6%) compared to NAC (12.6%). Chrom agar results are parallel with the conventional method in speciating the candida. Vijaya *et al*. (2011) Chrom agar has the advantages of easy to prepare, rapid, cost effective compared to technically demanding, time consuming and expensive conventional method. The present study showed biofilm formation as an important virulence factor exhibited by Candida species especially among NAC. The biofilm formation result in antifungal drug resistance and protection from host defenses, which will carry important clinical repercussion.

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