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Original Article

Microbiology Section

Comparison Between Virulence Factors of *Candida albicans* and Non-Albicans Species of *Candida* Isolated from Genitourinary Tract

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ABSTRACT

Background: *Candida* spp. is frequently isolated from cases of vulvovaginal candidiasis and catheter associated UTI. *C.albicans* is the most frequently isolated species but non-*albicans* species of *candida* are gaining clinical significance.

Objectives: To compare the virulence factors like biofilm production, phospholipase and hemolytic activity in *C.albicans* with that of non albicans species of *candida* isolated from urogenital tract.

Materials and Methods: Vaginal swabs and urine samples received in Microbiology laboratory during one year period were processed by standard methods. The *candida* isolates were speciated and biofilm production, phospholipase and hemolytic activity were studied in them. Statistical analysis was performed using SPSS version 11.5.

Results: Out of the 3001 urine samples processed 41 (1.3%) were positive for *candida*, out of 293 high vaginal swabs 41(14%) were positive for *candida*. *C.albicans* was the most prevalent isolate followed by *C.tropicalis*, *C.glabrata* and *C.krusei*. Out of 40 *C.albicans* strains, 22 (55%) produced biofilm, 39 (97.5%) produced hemolysin and 21 (52.5%) produced phospholipase. Out of 42 strains of non albicans species of *candida*, 19 (45%) produced biofilm, 41(97.6%) hemolysin and 5 (12%) produced phospholipase.

Conclusion: Phospholipase production was better in *C.albicans* in comparison toother species of *candida*. There was no statistically relevant difference between hemolysin and biofilm production by *C.albicans* and non albicans strains of *candida*.

Keywords: Biofilm production, *Candida*, Hemolytic activity, Phospholipase, Urogenital tract

INTRODUCTION

Candida spp. is the commensal fungi that colonize skin, gastrointestinal tract and reproductive tract. At times they cause opportunistic infections in immunocompromised individuals [1,2]. *candida albicans* are the most frequently isolated species but now non- *albicans* species are gaining clinical significance [1,2]. Most frequently observed manifestations of genitourinary candidiasis include vulvovaginal candidiasis in women, balanitis and balnoposthitics in men and candiduria in both sexes [1,2].

Vulvovaginal candidiasis is the second most common cause of abnormal discharge after bacterial vaginosis in healthy women of reproductive age. Candiduria is usually present in elderly hospitalized patients, neonates and immunocompromised patients. *Candida* is frequently isolated in nosocomial urinary tract infections [1,2]. Virulence factors of *candida* relevant to genitourinary tract are adherence, pseudohyphae formation, phenotypic switching, biofilm production, proteinase and phospholipase production [2-4]. It is a well-known fact that presence of virulence factors is a measure of the pathogenic potential of the organism.

The objective of the present study is to compare the virulence factors like biofilm production, phospholipase and hemolytic activity in *C.albicans* with that of non albicans species of *candida* isolated from urogenital tract.

MATERIALS AND METHODS

This study was conducted in the Microbiology Department of a tertiary care hospital in Southern India. All the vaginal swabs and urine samples received in the laboratory from January to December 2012 that were smear positive, were inoculated on Sabouraud's Dextrose Agar (SDA). Colonies were identified by gram's stain, germ tube test, chlamydospore formation and chrome agar [1]. The approval of the Institutional Ethics Committee of Kasturba Medical College, Mangalore, Manipal University, India has been taken for the study.

Biofilm production was determined by visual methods as given by Vinitha M et al., [3]. Biofilm production was scored as negative, moderate or strong [3].

Candida isolates were streaked onto SDA and incubated at 37°C for 18 h. Fungal suspensions equal to McFarland [2] turbidity was prepared. Ten microliters of this suspension was spotted on human blood SDA (with 3% glucose). Plates were incubated at 37°C for 48 h [4].The isolates showing a clear zone of hemolysis around the colonies were considered to be positive for hemolytic activity [4].

Detection of Phospholipase activity was done as given by Samaranayake et al., [5,6]. Fresh cultures of *candida* grown on SDA was harvested and suspended in 0.9% NaCl and adjusted to OD_{520} of 0.5 using spectrophotometer and stored in vials. Ten microliter of the suspension (approximately 10^8 yeast cells/ml) was inoculated into wells punched on the surface of the modified egg yolk agar. Plates incubated at 37^0C for 48 h. The diameter of the colonies and the diameter of the zone of opacity was measured and pz value calculated as follows [5,6]:

$$\text{Phospholipase activity P z} = \frac{\text{Diameter of colonies (mm)}}{\text{Diameter of the zone of opacity + colonies}}$$

Statistical analysis was done using SPSS version 11.5. Chi Square test or Student t-test was used wherever applicable [5,6].

RESULTS

Out of the 3001 urine samples processed 41 (1.3%) were positive for *candida*, out of 293 high vaginal swabs 41(14%) were positive

Different species of *candida* Total = 82 strains	Biofilm production Moderate/strong (%)	Hemolysin (%)	Phospholipase Production (%)
C.albicans n = 40	22 (55)	39 (97.5)	21 (52.5)
C.tropicalis n = 19	12 (63)	18 (94.7)	3 (15.8)
C.glabrata n = 14	5 (35.71)	14 (100)	0 (0)
C.krusei n = 09	2 (22.2)	9 (100)	2 (22.2)
Total n= 82	41 (50)	80 (97.6)	26 (31.7)

[Table/Fig-1]: Biofilm, hemolysin and phospholipase production by various species of *candida* isolated from the genitourinary tract

		C.albicans n = 40 (%)	Non albicans strains of *candida* n = 42(%)	p value
Biofilm production	Moderate/ strong	22 (55)	19 (45)	0.36
Hemolysin		39 (97.5)	41 (97.6)	1.00
Phospholipase		21 (52.5)	5 (12)	*<0.01

[Table/Fig-2]: Comparison between the various virulence factors expressed by *candida albicans* and non albicans strains of *candida* isolated from genitourinary tract
* *C.albicans* strains produced more phospholipase in comparison to the non albicans strains of *candida*. Whereas there was no statistically relevant difference between hemolysin and biofilm production by *C.albicans* and non albicans strains of *candida*

for *candida*. *C.albicans* 40 (48.7%) was the most prevalent isolate followed by *C.tropicalis*19 (23%),*C.glabrata*14 (17%) and *C.krusei 9* (10.9%) [Table/Fig-1].

A total of 41 (50%) out of 82 *candida* isolates produced biofilm. Of the 41 biofilm producers, 24 (58.5%) were strong biofilm producers and 17 (41.46%) were moderate biofilm producers. Twenty two (55%) out of 40 isolates of *C.albicans* produced biofilm whereas among the non albicans species, maximum numbers of *C.tropicalis* isolates produced biofilm [Table/Fig-1].

Hemolytic activity was detected in 80 (97.6%) out of 82 isolates of *candida*. One strain of *C.albicans* and one strain of *C.tropicalis* did not produce hemolytic activity whereas, all the other isolates produced hemolysis on human blood SDA [Table/Fig-1].

Phospholipase activity was detected in 26 (31.7%) isolates of *candida*. We got an average Pz value of 0.73 among the strains which produced phospholipase. Phospholipase production was better in *C.albicans* in comparison with other species of *candida* (p< 0.01) [Table/Fig-2]. Whereas there was no statistically relevant difference between biofilm production or hemolysin production of *C.albicans* and non- albicans species of *candida* [Table/Fig-2].

Biofilm production was more in *candida* isolated from urine samples in comparison to vaginal isolates. Phospholipase production was more in the *candida* isolated from vaginal samples in comparison to urine isolates. But, the difference was not statistically significant [Table/Fig-3].

		Urine samples n = 41(%)	Vaginal swabs n = 41(%)	*p-value
Biofilm production	Moderate/ strong	23 (56)	18 (44)	0.27
Hemolysin		39 (95)	41 (100)	0.15
Phospholipase		9 (22)	17 (41.5)	0.06

[Table/Fig-3]: Comparison between the various virulence factors expressed by different species of *candida* isolated from urine and vaginal samples

DISCUSSION

Previously *C.albicans* used to be isolated in large numbers and non-*C.albicans* in minority of women with vulvovaginal candidiasis [2,7]. In the present study, as in some of the previous studies *C.albicans* was found to be the predominant isolate followed by *C.tropicalis*, *C.glabrata* and *C.krusei* [7,8]. Some other studies have reported an increasing trend in occurrence of non- *C.albicans* over time [2,9].

Previous studies have shown that 60% - 70% of the isolates produced biofilm [3,10]. 50% of the isolates in the given study produced biofilm. In a study done at Manipal, India by Vinutha et al., *C.albicans* isolates recovered from blood demonstrated lower percentage of biofilm positivity [10]. In cases of *candida* vaginitis, *candida* forms a biofilm along with other members of the bacterial vaginal flora [11]. The resistance of such biofilm cells to conventional antifungal therapy might explain the frequent recurrence of vaginal thrush by *candida* [11]. *Candida* is associated with cases of nosocomial pneumonia and urinary tract infections [11,12]. Al most invariably, an implanted device such as an intravascular or urinary catheter, or endotracheal tube, is associated with these infections and a biofilm can be detected on the surface ofthe device [11,12].

A comparative study of different virulence factors of clinical isolates and commensal *candida* strains showed that the clinical isolates produced more hemolysin than the commensal *candida* but there was no statistically relevant difference in the production of other virulence factors like phospholipase or proteinase [13]. In a study all isolates of *candida* showed beta hemolysis on blood agar with 3% glucose but all isolates of *C.parapsilosis* failed to produce neither alpha nor beta hemolysis on blood agar [14]. In our study most of the isolates produced hemolysin.

In a study all isolates from urine and vaginal samples produced phospholipase [5]. Other studies have shown that *C.albicans* produce better phospholipase than the non albicans strains [6].The phospholipases in general catalyze the hydrolysis of phospholipids, which are major components of all cell membranes [15]. In our study most of the *C.albicans* strains produced phospholipase in comparison to non - albicans strains of *candida*.

CONCLUSION

Phospholipase production was better in *C.albicans* in comparison with other species of *candida*. Whereas there was no statistically relevant difference between hemolysin and biofilm production by *C.albicans* and non albicans strains of *candida*.The production of virulence factors by non albicans species shows the involvement of non albicans species of *candida* in genitourinary tract infections. More studies involving other virulence factors like phenotypic switching and proteinase production can be done in future studies.

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