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Original Research Article

CANDIDA ALBICANS AND NON ALBICANS SPECIES: A STUDY OF BIOFILM PRODUCTION AND PUTATIVE VIRULENCE PROPERTIES

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Abstract: Background: The incidence of fungal infections has increased significantly, which contributes to morbidity and mortality. This is caused by an increase in antimicrobial resistance and the restricted number of antifungal drugs, which retain many side effects. *Candida* species are major human fungal pathogens that cause both mucosal and deep tissue infections due to the presence of various virulence factors such as exo-enzymes secretion, capacity of adherence and morphogenesis. The production of exo-enzymes such as proteinase and phospholipase are factors which have been classically understood. Others such as haemolytic activity and the resistance to hydrogen peroxide are still little studied especially in urine isolates. Biofilm is the structured microbial communities that are attached and encases in a matrix of exo-polymetric material and are important for the development of clinical infection. **Materials & Methods:** On this background, the current study was done to analyze the virulence factors of *Candida* species from the urine samples (n=150) collected from unmarried girls of Vivekanandha College, Tiruchengode. **Results:** Forty samples were found to be positive for *Candida* spp., among them *C.tropicalis* is the prime one in its prevalence rate followed by *C.glabrata* 25%, *C.albicans* 17.5% and *C.krusei* 12.5%. Among the 40 positive *Candida* species virulence factors like biofilm formation, haemolytic activity, exo-enzyme production (proteinase and phospholipase) were studied. Biofilm formation was prominently seen in *C.tropicalis* whereas in exo-enzyme (proteinase and phospholipase) production *C.albicans* ranks the first than any other *Candida* species. However proteinase, phospholipase and hemolytic activity were highly expressed suggesting that these virulence factors are important for the pathogenicity. **Conclusions:** Generally the different isolates expressed similar virulence potential of varying range and it reinforcing the necessity to analyze the key factor of virulence in depth and also the other factors related to the yeasts are involved in the development of the disease.

Keywords: *Candida* speciation; Hichrome agar; prevalence; antifungal susceptibility; virulence factors

Introduction

Yeasts infections of the vagina, commonly known as candidiasis, are a female infection primarily common during the fecund period. They cause diseases with severity ranging from benign to potentially life-threatening infections, with the most common yeasts being the *Candida* species. *Candida albicans* remains the

predominant species causing over half of all the yeast infection cases in the world (Pfaller & Diekema, 2007). Despite the availability of an expanded antifungal armamentarium, the mortality associated with invasive *Candida* infections remains high, ranging between 19 and 49% (Alonso-Valle *et al*., 2003; Blot *et al*., 2002; Gudlaugsson *et al*., 2003; Morgan, 2005). The incidence and associated mortality due to candidiasis can be influenced by several factors including characteristics of the population at risk, standard of the healthcare facilities available, distribution of *Candida* species and prevalence of resistance (Eggimann *et al*., 2003; Hobso 2003). Hence the *Candida* species differ in their antifungal susceptibility and virulence factors. The genus is composed of a heterogenous group of organisms and more than 17 different *Candida* species are known to be aetiological agents of human infection; however, more than 90% of invasive infections are caused by non-albicans *Candida* species such as *C.glabrata*, *C.tropicalis*, *C.krusei*, *C.dubliniensis*, *C.parapsilosis* and *C.lusitaniae* and a gradual shift in the antifungal susceptibility profile especially against azole antifungal agents have underlined the need to monitor laboratory data and to select the most appropriate antifungal agent for therapy.

The diversity and spectrum of *Candida* species of clinical significance means there is needed to develop fast and cost effective methods of identification. HICHROME agar *Candida* technique has been used and has been useful in discriminating different *Candida* species as well as mixed infestations. It is a reliable and sensitive method for presumptive identification of more commonly isolated yeast species of the genus *Candida* (Ambler *et al*., 2001, Houang *et al*., 1997 Yocesoy 2003). The virulence factors expressed by *Candida* species, to cause infections may vary depending on the type of infection, the site and stage of infection and the nature of the host response. The main virulence factors are biofilm formation, production of acid proteinase, phospholipase, etc., (Mohandas and Ballal, 2011). Many putative virulence factors have been suggested in the enhancement of *Candida* pathogenesis. The production of exoenzymes such as proteinase and phospholipase are factors which have been classically understood. Others such as haemolytic activity and the resistance to hydrogen peroxide are still little studied especially in vaginal isolates (Calderone 2007). Haemolysin is another putative virulence factor thought to contribute to candidal pathogenesis. In particular, the secretion of haemolysin, followed by iron acquisition, facilitates hyphal invasion in disseminated candidiasis (Odds 1998). This study aimed to identify the *Candida* species isolated from the urine samples at species level and to determine their virulence properties responsible for its pathogenicity and antibiotic resistance.

Materials and methods

The study was conducted among the unmarried girls of Vivekanandha College of arts and sciences for women, belonging to the age group of 18-25 from January to February, 2012 at the microbiology laboratory. The purpose of the study was explained to them. Samples of those who objected were not included in the study. Non-repeated 150 urine samples were collected and analyzed.

Analysis of urine samples

Fresh mid-stream urine samples were collected from the subjects by providing them with the clean dry sterile urine culture bottles for the samples. The study subjects were told to allow first portion of urine to flow away, and then to collect the “mid stream” of the urine directly into the bottle, replace the lid firmly on the bottle and then return the specimen as soon as possible to the laboratory. The urine samples were labeled with the patient’s number, age and sex. At the laboratory, the urine sample was transferred into clean, dry sterile test tube and then centrifuged at a speed of 3000rpm for 5 minutes. After centrifugation, the supernatant was decanted and the sediment was streaked onto sabouraud dextrose agar using a loop calibrated (0.01ml) to deliver a constant amount of urine. A wet film was then prepared and another one was dried and stained by Grams method, and then observed for yeast cells.

Identification of Candida species

The *Candida* yeast cells from sabouraud dextrose agar (SDA) were cultured on *Candida* HICHROME differential agar medium and also germ tube test were done for the species level identification. The isolates were stored as suspension in 20% glycerol broth at -4°C.

Antimicrobial susceptibility testing

Kirby-Bauer disc diffusion method is commonly employed for antibiotic sensitivity

testing (Bauer *et al*., 1996). Antifungal susceptibility testing was performed by NCCLS M44-A disc diffusion method (NCCLS, 2004). Briefly, antibiotic discs containing Itraconazole (10 mcg), Ketoconazole (10 mcg), Clotrimazole (10 mcg), Fluconazole (25 mcg), Amphotericin-B (20 mcg) and Nystatin (100 units) were tested. The zones measured only that is showing complete inhibition and the diameters of the zones recorded to the nearest millimeter.

Analysis of virulence factors

Biofilm production

Biofilm formation was determined for all the *Candida* species by the proposed method (Branchini *et al*., 1994). A loopful of organisms from the SDA plate was inoculated into a tube containing 10 ml of Sabouraud's dextrose broth supplemented with glucose (final conc of 8 %). The tube was incubated at 37^{o}C for 24 hrs after which the broth was aspirated out and the wall of the tubes were stained with safranin. Positive result was indicated by black adherence at the walls of the tube with a dry crystalline consistency. Weak slime producers usually remained pink, though occasional darkening was observed. A darkening with the absence of a dry crystalline indicated an indeterminate result. The experiment was performed in triplicates. Biofilm formation was denoted as negative (0+), weekly positive (1+), moderate positive (2+), or strongly positive (3+).

Proteinase production

Candida proteinase was detected by the slightly modified method as described (Staib *et al*., 1965). Using Bovine serum albumin medium (dextrose 2 %, KH_2 PO_4 0.1%, Mg So_4 0.05%, agar 2% mixed after cooling 50^{o}C with 1% bovine serum solution). Proteinase activity was detected by inoculating 10 µl aliquots of the yeast suspension (approximately 10^8 yeast cells / ml) into the wells punched onto the surface of the medium. The plates were incubated at 37^{o}C for 2 days. After incubation, the plates were fixed with 20% trichloraacetic acid and stained with 1.25% amido black. Decolourisation was performed with 15% acetic acid. Opaqueness of the agar, corresponding to a zone proteolysis around the wells that could not be stained with amido black indicated degradation of the protein. The diameter of unstained zone around the well was considered as a measure of proteinase production. The proteinase activity (Pz) was determined in terms of the ratio of the diameter of the well to the diameter of the proteolytic unstained zone. When Pz=1, no proteinase activity was detected in the strain. Thus, low Pz means high production of the enzyme. The experiment was performed in triplicates.

Phospholipase production

Slightly modified method (Samaranayake *et al*., 1984) was used to identify the phospholipase production. The egg yolk medium consisted of 13.0g Sabouraud Dextrose Agar (SDA), 11.7g NaCl, 0.111g $CaCl_2$, and 10% sterile egg yolks were used. The egg yolk was centrifuged at 500g for 10 min at room temperature and 20 ml of the supernatant was added to the sterile medium. Extracellular phospholipase activity was detected by inoculating 10 µl aliquots of the yeast suspension (approximately 10^8 yeast cells / ml) into the wells punched onto the surface of the egg yolk medium. The diameter of the precipitation zone around the well was measured after incubation at 37^{o}C for 48 hrs. Phospholipase activity (Pz value) was determined. When Pz=1, no phospholipase activity was detected in the strain. Thus, low Pz means high production of the enzyme. The experiment was performed in triplicates.

Haemolytic activity

The hemolytic activity was determined by plate assay method (Luo *et al*., 2001). The medium was prepared by adding 7ml of fresh sheep blood (cpp1-Brazil) to100ml of SDA supplemented with 3% glucose (sigma, USA) providing a final concentration of 7%. The final pH of the medium so prepared was 5.6± 0.2. Spot of the inoculum have been made on a sugar-enriched sheep blood agar medium. The plates were incubated at 37^{o}C in 5% Co2 for 48 hrs. The presence of a distinct translucent halo around the inoculum site, viewed with transmitted light, indicated positive hemolytic activity. The diameters of the zones of lysis and the colony were measured, and this ratio (equal to or larger than 1) was used as a hemolytic production of *Candida* species. The assay was conducted in duplicate on three separate occasions.

Results

A descriptive study was conducted and the data of information was analyzed widely to elucidate the "the Virulence Factors of *Candida* spp., isolated from urine samples" and to explore the results with various study variables. The

prevalence of *Candida* species among the college going unmarried girls of Vivekanandha College of arts and sciences for women, Namakkal was assessed and recorded in table 2. Totally 150 urine samples were collected and among them 40 samples showed positive for different species of *Candida* such as *C.tropicalis* (n=18), *C.albicans* (n=7), *C.krusei* (n=5) and *C.glabrata* (n=10) (table 3).

Table 1. Examples of commonly caused candidal diseases and its virulence and pathogenicity

FUNGAL DISEASE	CAUSATIVE AGENT	TYPE OF VIRULENCE FACTORS	ROLE OF PATHOGENICITY	SITE OF INFECTION	TRANSMISSION
Candidiasis	*C. albicans*	Adhesin (Als family, HWP1)	Adherence to epithelial cells, fibronectin, biofilm establishment	Intestinal tract, vaginal tract, skin, fingers, oral cavity	Endogenous flora, contact with secretions from infected person
		Dimorphism (phr1, hyr1, chs2, chs3, rbf1)	Hyphal phase required for invasion and adherence, yeast phase for dissemination		
		Phenotypic switching (efg1)	Conversion to more virulent forms showing increased ASP and adhesion production, evasion from host response		
		Secreted aspartyl proteinases (SAPs 1–10)	Nutrient uptake, tissue invasion, adherence and dissemination		
		Phosphlipases A,B,C,D (plb1,2,3)	Tissue invasion and adherence		
		Farnesol	Quorum sensing, Biofilm formation		
		Catalase, Superoxide Dismutase	Prevention from oxidative damages		
		SUN41, GCN4, MKc1p	Biofilm establishment		
	C. tropicalis, *C. glabrata*, *C. dubliniensis*, *C. krusei*	-	-	Intestinal tract, vaginal tract, skin, fingers, oral cavity	Endogenous flora, contact with secretions from infected person

Table 2. Prevalence of *Candida* species in clinical sample

Results	Prevalence of *Candida* spp.,	Total
Positive	40(26.66%)	40
Negative	110(73.33%)	110
Total	150	150

Table 3. Prevalence of *Candida albicans* and Non-albicans

S.No	Source of isolates	Name of *Candida* spp.,	Prevalence of *Candida* spp., in %
1	Urine (n=150)	*C.albicans*	7(17.5)
2		*C.tropicalis*	5(45)
3		*C.krusei*	18(12.5)
4		*C.glabrata*	10(25)

These isolates were identified and confirmed based on phenotypic diagnostic methods gram staining, germ tube formation (fig.1), the colour of colonies on HICHROME agar (fig.2), Chlamydospores production on Corn meal agar medium and lack of pellicle formation on SDA broth.

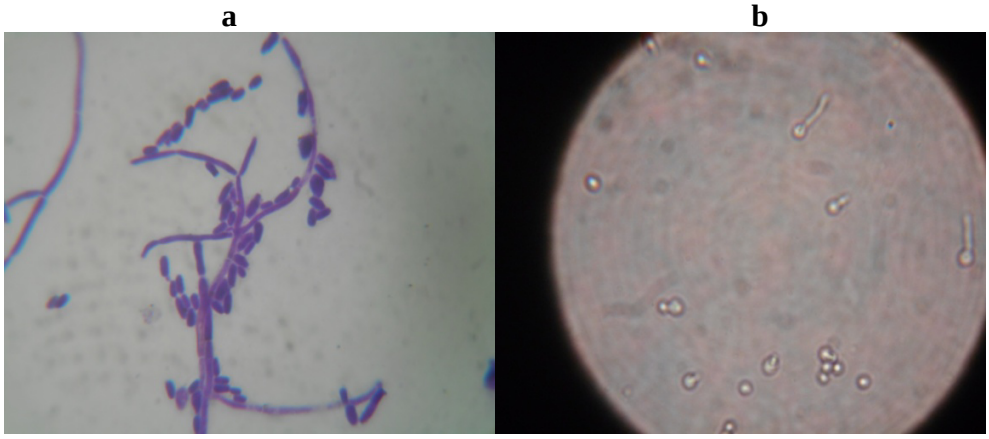


Fig. 1. a) The gram positive ovoid cells with pseudohyphae; b) germ tube formation

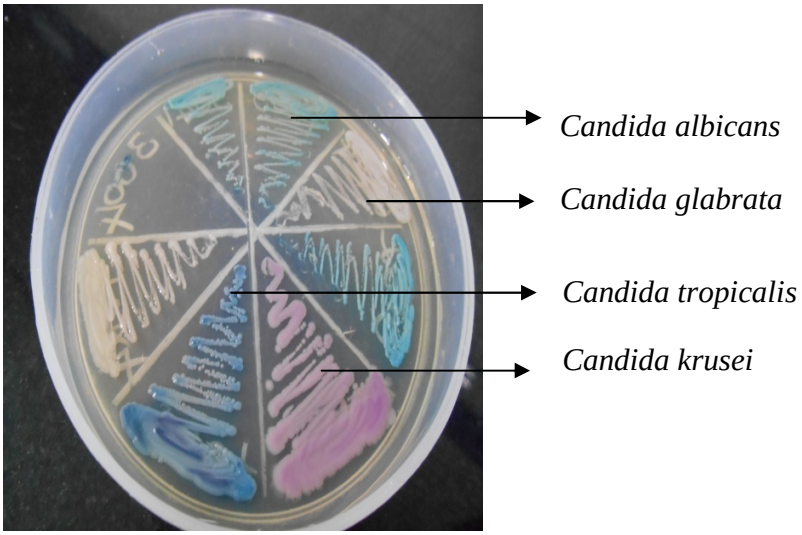


Fig. 2. Colony morphology of *Candida* spp.,

Table 4 also shows the phenotypic characteristics of *Candida tropicalis*, *Candida albicans*, *Candida krusei* and *Candida glabrata* studied during the study period. Antibiotic sensitivity test was done for all the *Candida* species isolated and the antifungal test results indicate that the yeast isolates were susceptible to Amphotericin-B 23(57.5%), Ketoconazole 9(22.5%), Fluconazole 5(12.5%) and Nystatin 3(7.5%). Various resistant levels were detected against other antifungal drugs but to Itraconazole and Clotrimazole all the isolates of *Candida* species were found to be 100% resistant. Among the 40 positive *Candida* isolates, 39 had the ability to produce biofilm at varying range of adherence (table 5).

Table 4. Morphological characteristics of *Candida* species

S.no	Name of the *Candida* spp.,	Tests			
		Gram staining	HI-chrome media	Germ tube	Incubation temperature
1	*C.albicans*	Gram +ve oval cells	Green	Positive	42^0C
2	*C.tropicalis*	Gram +ve oval cells	Blue	Later produced	37^0C
3	*C.krusei*	Gram +ve oval cells	Pink	Negative	37^0C
4	*C.glabrata*	Gram +ve oval cells	Creamy white	Negative	37^0C

Table 5.Distribution of *Candida* species according to biofilm formation

S. No	Name of organisms	Biofilm formation in %			
		3+	2+	1+	-ve
1	*C.albicans*	2(28.5)	2(28.5)	3(42.8)	0(0)
2	*C.krusei*	1(20)	4(50)	0(0)	0(0)
3	*C.tropicalis*	7(38.8)	9(50)	2(11.1)	0(0)
4	*C.glabrata*	8(80)	0(0)	(1)10%	(1)10

In the 39 species of *Candida*, strains *C.glabrata* 8(80.0%) was the strong producer of biofilm followed by *C.tropicalis* 7(38.8%) strains (fig.3). Table 6 shows the 34(85.0%) species of *Candida* producing the exo-enzyme proteinase on Bovine Serum Albumin medium **(**fig. 4**)** which is one of the virulence factors. Presently 6 strains of *C. tropicalis* and *C.albicans* were the predominant producers of proteinase enzyme when compared to other species *Candida*. Phospolipase activity of *Candida* species were identified using SDA along with sterile egg yolk medium (table 7 & fig.5). Surprisingly all the 40 positive *Candida* isolates showed phospholipase activity. All the *C.albicans* (100%), *C.krusei* (100%), *C.glabrata* (100%) and among 18 *C.tropicalis*, 16 strains (88.8%) produced phospolipase very strongly (3+) and 2(11.1%) were weak in phospholipase production. All the *Candida albicans* and non-albicans strains in the present study showed 100% β-haemolytic activity (table 8 & fig 6).

Table 6. Proteinase activity exhibited by *Candida* species isolated from urine

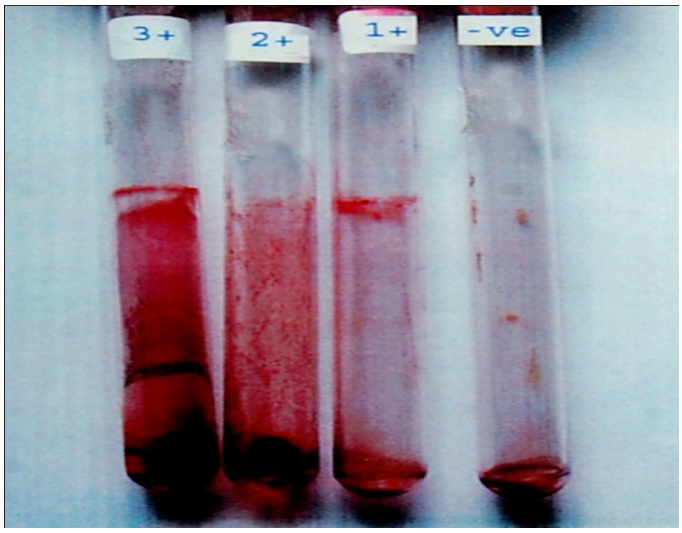
S.No	Name of organisms	Proteinase production in %			
		3+	2+	1+	-ve
1	*C.albicans*	6(85.7)	1(14.2)	0(0)	0(0)
2	*C.krusei*	1(20)	0(0)	3(60)	1(20)
3	*C.tropicalis*	6(33.3)	3(16.6)	7(38.8)	2(11.1)
4	*C.glabrata*	1(10)	3(30)	3(30)	3(30)

Table 7. Phospholipase production exhibited by *Candida* species isolated from urine

S.No	Name of organisms	Phospholipase production in %			
		3+	2+	1+	-ve
1	*C.albicans*	7(100)	0(0)	0(0)	0(0)
2	*C.krusei*	5(100)	0(0)	0(0)	0(0)
3	*C.tropicalis*	16(88.8)	2(11.1)	0(0)	0(0)
4	*C.glabrata*	10(100)	0(0)	0(0)	0(0)

Table 8. Percentage production of haemolysin by *Candida* species

S.No	Name of organisms	Haemolytic activity in %		
		A	β	γ
1	*C.albicans*	-	7(100)	-
2	*C.krusei*	-	5(100)	-
3	*C.tropicalis*	-	18(100)	-
4	*C.glabrata*	-	10(100)	-



(3+ = Strongly positive, 2+ = Moderately positive, 1+ = Weakly positive,-VE=Negative)

Fig. 3. Different range of biofilm production by *Candida* species

Fig 4 Proteinase production by *Candida* species in BSA medium

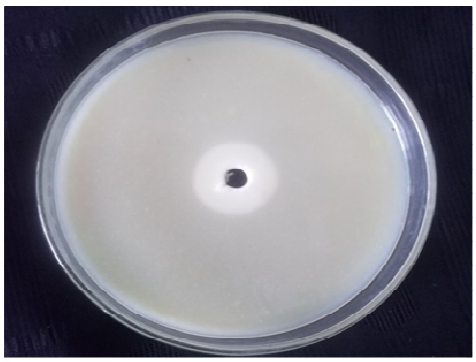


Fig 5 Phospholipase activity of *Candida* species in SDA with egg yolk containing medium

Fig 6 Beta haemolytic activity of *Candida* species on blood agar

Discussion

Candida infection of the vagina is a common problem that causes significant morbidity and affects the well-being of women. *Candida* species belong to the normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina (Shao *et al*., 2007) and are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections (Eggimann *et al*., 2003). These yeasts are commensal in healthy humans and may cause systemic infection in immune-compromised situations due to their great adaptability to different host niches. For the adherence to epithelial cells and medical biomaterials, secretion of hydrolytic enzymes (extracellular proteinases and phospholipases) and production of haemolytic factors are the principle virulence factors of *Candida* strains contributing to their pathogenicity (Ghannoum 2000). Biofilm serves as reservoirs for the cells to continually seed infection. Expression of virulence factors may be associated with specific characteristics of *Candida* isolates such as geographical origin or type of infection. The investigation of the genetic relatedness between clinical *Candida* strains may be of great importance in clinical diagnosis, epidemiology, treatment and prevention of Candidiasis (Noumi *et al*., 2011). Therefore the present study is focused on the virulence properties of *Candida* isolates.

Of the *Candida* strains isolated in our study 45% identified as *C.tropicalis*, 25% as *C.glabrata*, 17.5% as *C.albicans* and 12.5% as *C.krusei*. These results are compatible with the other research results. *C. albicans* almost always ranks first, but while the frequency of non-albicans species is increasing due to the prevailing resistance because of extensive use of antimycotic drugs particularly azoles for prolonged periods (Usharani *et al*., 2011). In many studies performed in the recent years, it has been expressed that there has been an evident change in agents of candidiasis and that the rate of candidiasis depending on non albicans *Candida* strains has reached approximately 50%. A study from Bangladesh recorded the prevalence of *Candida* spp. in that 72.7% of *C.albicans*, 16.9% of *C.glabrata*, 7.5% of *C.tropicalis* and 2.9% of *C.krusei* were noted. They concluded that *C.albicans* was the most common species isolated from vaginitis (Yusuf *et al*., 2007). The prevalence of *Candida albicans* was highly noted when compared to the non albicans species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. stellatoidea* (Germain *et al*., 1998; Jha *et al*., 2006; Zaini *et al*., 2006). But in the current study a contraindicative results was obtained stating that *C. tropicalis* was the dominant species with the prevalence rate of 45% which is non albicans species.

Biofilms are defined as structured microbial communities that are attached to a surface and encased in a matrix of exo-polymeric material. This is of particular significance since it is now estimated that a significant proportion of all

human microbial infections involve biofilm formation (Donlan 2001 & Douglas 2002). A study reported that 64% of the *Candida* strains tested were found to be biofilm producers (Girish Kumar and Menon, 2006; Vinitha and Ballal, 2007). Their result shows that *Candida* isolates form urine (75%) found to be biofilm producers. Comparatively in progress revise, 40 positive *Candida* isolates were screened for biofilm activity. Totally 39(97.5%) species of *Candida* had the ability to produce biofilm but its grade of adherences varied. Biofilm production was found to occur most frequently among non albicans *Candida* (78.9%) than *Candida albicans* (54.8%). Likely in our study, out of 40 *Candida* isolates 32(80.0%) of non *C.albicans* were dominant producer of the biofilm. Relatively in another study 135 biofilm forming *Candida* spp. isolated from ICU patients, 75(55.5%) belonged to non albicans candida (NAC) species (Deorukhkar and Saini, 2013). In contrast to the current study a report says that less biofilm production was notes in NAC sp., like *C. glabrata* and *C. parapsilosis* (Nerurkar *et al*., 2012). But in this revise, 18(12.5%) *C.tropicalis*, 10(25.0%) *C.glabrata*, 7(17.5%), *C.albicans* and 5(45.0%) *C.krusei* was dominant to produce the biofilm. Similar to the current study, biofilm production was most frequently observed for isolates of *C. tropicalis* (71.4% [20 of 28]), followed by *C. glabrata* (23.1% [6 of 26]), *C. albicans* (22.6% [38 of 168]), and *C. parapsilosis* (21.8% [14 of 64]) (Mario *et al*., 2007). Similar finding has been observed that biofilm formation was most frequent for isolates of *C. tropicalis* (80%), followed by *C. parapsilosis* (73%), *C. glabrata* (28%), and *C. albicans* (8%) (Shin *et al*., 2002). These finding were consistent and similar with the current and previous studies conducted (Mohandas *et al*., 2011; Singhai *et al*., 2012). The shift in etiology of candidiasis from *C. albicans* to NAC spp. has been documented by other researchers (Dan *et al*., 2002; Pfaller and Diekema, 2002). In the prevailing study, 34(85.0%) *Candida* species had the ability to produce proteinase as a virulence factor. Relatively in another study, the positivity for proteinase production was found in 65(59.1%) of *Candida* isolates (Sachin *et al*., 2012) and also they stated that, maximum proteinase production was seen in *C.albicans* (82.1%) followed by *C.tropicalis* (80%), which is similarly observed (Ruchel *et al*., 1983). High rates of proteinase production in *C.albicans* have been reported by other researchers too (Koelsch *et al*., 2000; Thangam *et al*., 1989; Tsang *et al*., 2007).Comparatively in present-day study, *C.albicans* 7(100%) shows the positive for proteinase activity followed by *C.tropicalis* (88.8%). The high mortality and rapidity of the spread of the disease would argue the contrary; only few studies have been conducted on virulence and its action mechanisms.

Phospholipase hydrolyze the phospholipid of the host cell membrane leading to lysis of cell and alternation of surface characteristics. These events facilitate the establishment of infection (Sachin *et al*., 2012). In previous report (Deorukhkar and Saini, 2013) 85(62.9%) isolates showed better Phospholipase production. In their cram, total of 49(81.6%) isolates of *C. albicans* showed phospholipase activity. Among NAC spp. maximum phospholipase activity was seen in *C. tropicalis* and *C. glabrata*. In our revise, phospolipase activity of *Candida* species was identified using SDA along with sterile egg yolk medium. Surprisingly all the *Candida albicans* and non albicans species showed 100% positive for phospholipase activity. Reasonably in a study, out of 110 *Candida* species studied, phospholipase activity was seen in 67 (60.9%) isolates (Sachin *et al*., 2012). The same positivity rate of phospholipase activity, in samples from patients with invasive *Candida* infection was also noted (Kantarcioglu and Yucel, 2002). Currently in this study, all the *C.albicans*, *C.krusei*, *C.glabrata* and *C.tropicalis* (100%) showed phospholipase activity. Comparably in one more research established that 92.3% of *C.albicans* produced phospholipase, among the NAC species *C.tropicalis* followed by *C.dubliniensis* showed maximum phospholipase production (Sachin *et al*., 2012). An earlier study too reported that, maximum phospholipase activity was seen in *C. albicans* (81.6%) (Deorukhkar and Saini, 2013). Among NAC spp. maximum phospholipase

activity was noted in *C. tropicalis* (65.5%). In contrast to the prevailing report no phospholipase activity was observe in *C. tropicalis* (Samaranayake *et al*., 1984). Studies on the activity of haemolysin in *Candida* species are limited. A previous study, distinguished that haemolysin activity was higher in *C.albicans* (94.8%) and *C. dubliniensis* (60%) (Sachin *et al*., 2012). All the *C. albicans, C. tropicalis, C. glabrata, C. krusei* isolates investigated in the study demonstrated 100% haemolysin activity with detectable β haemolysin activity. On the other hand, contraindicatively 80 *Candida* isolates from clinical sources in different geographical locales showed alpha haemolysis but no beta haemolysis in experiments with glucose-free sheep blood agar (Luo *et al*., 2001). Haemolysis is the condition under which *Candida* species can display haemolytic activity, but found that haemolysis is non-existent when no glucose is available in the culture medium (Manns *et al*., 1994).

Conclusion

Fungal pathogens are becoming increasingly important cause of both community acquired and nosocomial infections and yeast species of the genus *Candid*a are the most pathogenic fungi. Pathogenic fungi in the genus *Candida* can cause both superficial and serious systemic diseases, and are now recognized as one of the major agents of hospital-acquired infections. In the present-day study, based on the results, the virulence factors such as biofilm formation, proteinase, phospholipase, haemolysis activity were greater for non albicans than for *Candida albicans*. Our findings provide an alarming threat towards the virulence factors of *C.albicans* and Non albicans candida species because *Candida* from the healthy girls of our study had showed the virulence capacity which may become an opportunistic pathogen. We conclude that, prevailing study confirms the elevated incidence of candidiasis caused by non albicans candida when compare to the *C.albicans*. Fungal pathogenesis is a multifactorial phenomenon; therefore, the nature of fungal pathogens, their virulence factors, and their interaction with host defense mechanisms need to be explored for the development of more effective antifungal therapy. Although phenomenal progress has been made on molecular characterization of various virulence factors and host–fungi interactions; this issue needs further investigation in order to know the exact contribution of each virulence factor under different disease conditions.

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Conflict of Interest statement

We declare that we have no conflict of interest.

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