INCIDENCE AND VIRULENCE TRAITS OF *CANDIDA DUBLINIENSIS* ISOLATED FROM CLINICALLY SUSPECTED PATIENTS

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ABSTRACT

Objective: Fungal infections are caused by *Candida* species are increasing mainly in immunocompromised patients. Among *Candida* species, *Candida dubliniensis* has recently increasing opportunistic pathogenic yeast. The present study was aimed to assess the incidence and virulence factors of *C. dubliniensis* isolated from urine samples of various hospitalized clinically suspected patients.

Methods: Totally 1,406 urine samples were processed, among that 9 (0.64%) were identified as *C. dubliniensis* by germ tube production, biochemical test (*Candida* identification kit), CHROM agar *Candida* differential medium and growth at 45°C. Virulence factors of the *C. dubliniensis, viz.,* phospholipase, proteinase, esterase, lipase, hemolysin, and biofilm production were detected by standard procedures.

Results: Maximum number of *C. dubliniensis* have ability to produce protienase 6 (66.66%), followed by esterase 5 (55.55%), biofilm formation 4 (44.44%), hemolysin 2 (22.22%), and phospholipase and lipase 1 (11.1%), respectively.

Conclusion: The result revealed that these enzymes are potential virulence factors are the most important thing to realize pathogenesis of *C. dubliniensis*.

Keywords: *Candida dubliniensis*, Urine, Incidence, Virulence factors.

INTRODUCTION

Fungal pathogens are becoming increasingly important cause of both community-acquired and nosocomial infections; the genus *Candida* is the most pathogenic fungi [1]. Although *Candida* is consisting of more than 200 species, only a few of them has been associated with human infection [2]. Of these, *C. albicans* is the most important infectious agent and represents a serious public health challenge. In recent years, non-albicans *Candida* species are also being implicated [3]. In 1995, a new *Candida* species was identified in human immunodeficiency virus (HIV) infected patient with oropharyngeal candidiasis in Dublin, Ireland [1]. This new species have ability to forms germ tubes and chlamydospores that are almost indistinguishable from those of *Candida albicans*. Phenotypic characteristics of finding and differentiation of *Candida dubliniensis* remains problematic in routine identification. On CHROM agar *Candida* differential medium, *C. dubliniensis* produced the dark-green colonies within 48 hrs at 37°C when freshly isolated from clinical material, but this property is not retained in subculture [4]. Recently, the absence of growth of *C. dubliniensis* at 45°C has to be an easy and reliable feature for differentiation of this species from *C. albicans* [5].

C. dubliniensis has recently been added up to the rising list of potential opportunistic pathogenic yeasts, and it has been recovered from several body sites in many populations; it is most frequently recovered from the oral cavities of HIV-infected patients [6]. Extracellular hydrolytic enzymes seem to play an important role in Candidal overgrowth [7]. The enzymes produced are proteinase, lipase, esterase, and phospholipase; factors which are responsible for the invasiveness and proliferation of fungi caused by the destruction of host tissues, in which the organisms are provided with nutrients by the host tissues [8].

Hemolytic activity is another important virulence factor exhibited by the pathogens which allows growth in the host using several iron-binding proteins as a source of iron. In recent years, there has been a rapid spread of antifungal multidrug resistance, which has become a serious public health problem [9]. Biofilm production was also associated with high-level antifungal drug resistance of the associated organisms [10]. Although extensive studies on epidemiology and phylogeny of *C. dubliniensis* have been performed, little is known about virulence factors such as extracellular enzymes and hemolytic activities [6]. Therefore, the present study was aimed to determine virulence factor (production of proteinase, phospholipase, lipase, esterase, biofilm formation, and hemolytic activity) of *C. dubliniensis* isolated from urine sample.

MATERIALS AND METHODS

The study included the clinical strains of *C. dubliniensis* recovered from urine samples of various hospitalized clinically suspected patients from Microbiology Department at Doctors Diagnostic Center, Trichy, India. The collected samples were processed in the Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Namakkal, Tamil Nadu, India. All the isolates were stored with glycerol on deep freezer until further testing.

Urine sample

A total of 1,406 urine specimens were directly subjected to Gram's staining in addition specimens were routinely cultured on blood agar, MacConkey, and cystine lactose electrolyte deficient agar (Himedia, Mumbai, India), for the recovery of microbial growth (bacteria and *Candida* species). Then, the culture plates were incubated at 37°C for 24-48 hrs. After incubation, growth was observed, and suspected colonies

were inoculated on Sabouraud dextrose agar plates supplemented with chloramphenicol (Himedia, Mumbai, India) and incubated first at 37°C for 24-48 hrs to observe the growth of *Candida* spp.

Mycological investigation

Then, the isolated colonies were subsequently identified by (i) Germ tube production; (ii) conventional biochemical test using Hi *Candida* identification kit (Himedia, Mumbai, India); (iii) subculture of two or three representative colonies on CHROM agar *Candida* differential medium (Himedia, Mumbai, India) and incubation at 37°C for 24-48 hrs. The *Candida* isolates were identified by color characteristics on CHROM agar *Candida* differential medium [11], and (iv) the confirmatory test to differentiate the *C. dubliniensis* from *C. albicans* by growth at 45°C [5]. The temperature test was performed using Sabouraud's dextrose agar (SDA) with chloramphenicol (Himedia, India). Cultured plates were incubated at 45°C and growth was examined daily up to 10 days. For the tests, a standard reference strain of *C. albicans* (ATCC 90028) was used as the control.

Phospholipase activity

C. dubliniensis isolates were screened for extracellular phospholipase activity by measuring the size of the zone of precipitation after growth on egg yolk agar [12]. The egg yolk medium consisted of 13.0 g SDA, 11.7 g NaCl, 0.11 g CaCl$_2$ (Himedia, Mumbai, India), and 10% sterile egg yolk (all in 184 ml distilled water). First, these components were mixed and sterilized without the egg yolk; then, the egg yolk was centrifuged at 500 g for 10 minutes at room temperature, and 20 ml of the supernatant was added to the sterilized medium. Standard inocula of the test and control *Candida* isolates (5 ml, with 10^8 yeast cells [ml saline]$^{-1}$) were inoculated onto the egg yolk agar medium and left to dry at room temperature. Further, 5 ml of saline, but without yeast cells, was overlaid onto the plate and left to dry at room temperature. Each culture was then incubated at 37°C for 48 hrs after which the diameter of the precipitation zone (Pz) around the colony (an indicator of phospholipase activity) was determined. Reference strains of *C. albicans* (ATCC 90028) served as positive control.

Proteinase activity

Proteinase production was measured in terms of bovine serum albumin (BSA) degradation according to the technique described by Staib (1965) [13]. The suspension of 1X10^6 cells ml^{-1} was prepared from *Candida* isolate. 10 μl of suspension was inoculated on 1% BSA medium plate. The BSA medium consisted of dextrose 2%, KH$_2$PO$_4$ 0.1%, MgSO$_4$ 0.05%, agar 2%, and 1% BSA solution (Himedia, Mumbai, India). The plate was incubated for 5 days at 37°C. After incubation, the plates were fixed with 20% trichloroacetic acid and stained with 1.25% amidoblack. Decolorization was performed with acetic acid. Opaqueness of the agar, corresponding to a zone of proteolysis around the colony that could not be stained with amidoblack indicated degradation of the protein. Reference strains of *C. albicans* (ATCC 90028) served as positive control.

Lipase activity

Production of lipase was observed by inoculating the isolates of *Candida* by single diffusion method in SDA agar provided with 1% tributyrin as substrate (Himedia, Mumbai, India) [14]. Clear zone formation was found around the colony after 24-48 hrs incubation at 37°C was considered as lipase activity. Reference strains of *C. albicans* (ATCC 90028) served as positive control.

Esterase activity

Esterase activity was determined by plate method presented by Slifkin (2000) [15]. The agar medium consisted of 1 g of peptone, 0.5 g of NaCl, 0.01 g of CaCl$_2$, and 1.5 g of agar (Himedia, India). Medium was sterilized and cooled to about 50°C, then 0.5 ml of sterile Tween 80 (Himedia, India) was added. An overnight culture of each isolate was inoculated into medium by touching the center of the agar medium with a cotton swab in triplicate. The inoculated culture plates were incubated at 29°C and were examined during 10 days. The presence of a halo around a

colony was indicative for esterase production by the *Candida* isolate. Reference strains of *C. albicans* (ATCC 90028) served as positive control.

Biofilm formation

Biofilm formation was determined for all the isolates, and the standard strains using a method proposed by Branchini *et al.* (1994) [16]. A loopful of colony from the SDA plate was inoculated into 10 ml of Sabouraud's liquid medium supplemented with glucose (final concentration of 8%). The tubes were incubated at 37°C for 24 hrs, after which, the broth was aspirated out and the walls of the tubes were stained with safranin. Biofilm formation was scored as negative (0), weak positive (1+), moderate positive (2+), or strong positive (3+).

Hemolysin activity

Hemolysin activity of *Candida* spp. was detected by blood agar plate assay as described by Manns *et al.* (1994) [17]. The media was prepared by adding 7 ml of aseptically collected fresh sheep blood to 100 ml of SDA supplemented with glucose at a final concentration of 3% (w/v). 10 μl of standard inoculum [10^8 yeast cells (ml saline)$^{-1}$] prepared from both the test and the control *Candida* isolates was inoculated onto the medium. The blood agar plate was then incubated at 37°C in 5% CO$_2$ for 48 hrs. Hemolysin activity was determined by the translucent zone of hemolysis. Reference strains of *C. albicans* (ATCC 90028) served as positive control.

Enzymatic score and statistical analysis

The enzyme activities were expressed as Pz value (a=colony diameter/ b=diameter of the colony plus the precipitation zone) as described by price and co-workers [18]. According to this definition, low Pz values mean high proteinase or phospholipase and lipase production and inversely, high Pz values indicate low enzymatic production. The enzymatic activity was scored into four categories: A Pz of 1.0 indicated no enzymatic activity; a Pz between 0.999 and 0.700 indicated low enzymatic activity; Pz between 0.699 and 0.400 corresponded to moderate activity; low Pz values between 0.399 and 0.100 means high enzymatic activity. Esterase activity (Pz value) was reported as follows: A Pz of 1.0 as negative (-), 0.90-0.99 as weak (+), 0.80-0.89 as mild (+ +), 0.70-0.79 as strong (+ + +), and <0.69 as very strong (+ + + +) [19]. The data were analyzed statistically using mean and standard error of mean.

RESULTS

A total of 1,406 urine samples were included in the study, among that 9(0.64%) of the strains were identified as *C. dubliniensis* by following characteristics (i) Germ tube production was detected. (ii) as per the Hi*Candida* identification kit result interpretation, *C. dubliniensis* strains were negative in urease activity. Maltose, galactose, xylose,
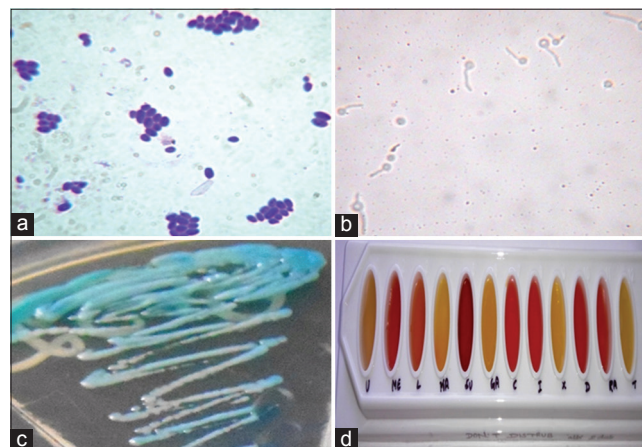

Fig. 1: Mycological investigation of *Candida dubliniensis* (a) Gram-positive budding yeast cells on Gram's staining. (b) Formation of germ tube in human serum at 37°C. (c) Dark-green colonies on CHROM agar medium. (d) Biochemical test results of *Candida dubliniensis* on Hi*Candida* identification kit

and trehalose were utilized. Remaining sugars, *viz.,* melibiose, lactose, sucrose, cellobiose, inositol, dulcitol, and raffinose were not fermented. (iii) dark green colored colonies were observed in CHROM agar *Candida* differential medium (Fig. 1). (iv) no growth was observed at 45°C.

Virulence factors of *C. dubliniensis* were demonstrated in Fig. 2. The enzymatic activities of the *C. dubliniensis* (phospholipase, proteinase, esterase, and lipase) were recorded in Table 1. Out of 9 strains, phospholipase activity was determined in only one strain (11.11%), which showed the moderate activity (Pz=0.5, mean=0.05). Proteinase production was observed in six strains (66.66%), Pz ranged from 0.5 to 0.9 (mean=0.47). Low-level production of esterase was found in five strains (55.55%), Pz ranged from 0.8 to 0.9 (mean=0.47). Lipase activity was noted as one strain (11.11%), Pz ranged at 0.9 (mean=0.10). According to the results of enzyme production, maximum number of strains has an ability to release the proteinase and esterase. Table 2 recorded the results of hemolysin and biofilm production. Beta hemolysis was observed in two strains (22.22%). Biofilm production was noted in four strains (44.44%).

DISCUSSION

C. dubliniensis is opportunistic yeast, and it appears to have a worldwide distribution [20,21]. Despite the effort that has been expended to identify *C. dubliniensis* in clinical samples, a definitive assessment of the prevalence of this species is still lacking [2]. *C. dubliniensis* have
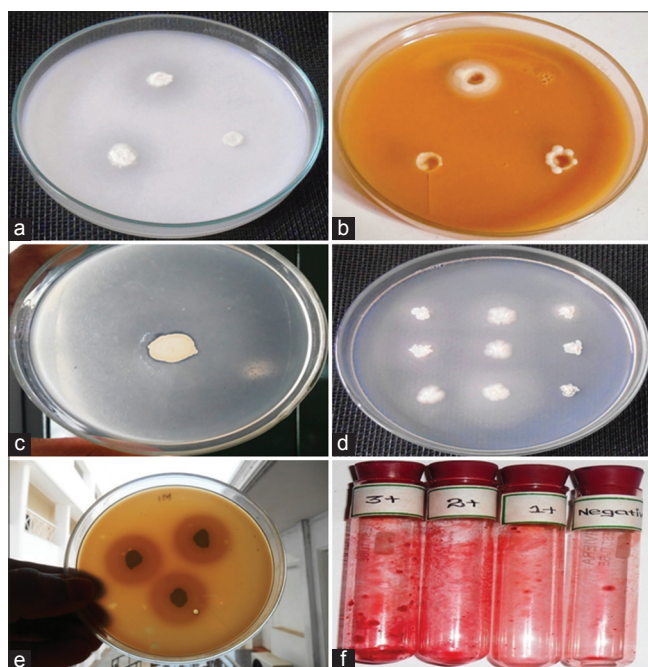

Fig. 2: Virulence factors of *Candida dubliniensis* (a) Proteinase production on bovine serum albumin medium plate, (b) Phospholipase production on egg yolk agar, (c) Lipase production on Sabouraud's dextrose agar with 1% tributyrin oil, (d) Esterase production on Tween 80 opacity medium (e) Hemolysin production on blood agar plate, and (f) Biofilm formation on Sabouraud's liquid medium with glucose

been isolated from a wide range of geographical locations including Europe, North and South America, and Australia [22]. It recovered from several body sites in many populations, but it is most frequently recovered from the oral cavities of HIV-infected patients [6], and 15-30% has been reported from oral cavities of HIV-infected and AIDS patients [23]. Due to the use of inefficient sampling and identification methods or the sampling on the wrong anatomic sites, the prevalence of *C. dubliniensis* in normal healthy individuals has been underestimated probably [2]. In a study reported that, on an Irish population of normal healthy individuals, only 3.5% of individuals were found to carry *C. dubliniensis* in the oral cavity while the prevalence of this species in the vagina was found to be even lower [24] and relatively high prevalence rates of *C. dubliniensis* have been found in oral cavities of patients suffering from diabetes [22]. As well as being associated with oral infections, *C. dubliniensis* has also been identified in a wide range of other anatomic sites [2]. Moreover, isolates of *C. dubliniensis* have been isolated from vaginal, urinary, and fecal specimens [25-27]. Likewise, a study reported that *C. dubliniensis* (31%) was isolated from urine samples of ICU patients [28]. The percentage of prevalence is very high when compared to our results. In the present study, we identified 9(0.64%) of *C. dubliniensis* from urine specimens.

Virtually nothing is known about the virulence traits of *C. dubliniensis*. However, it might be expected that this species may share the ability to produce certain putative virulence factors with *C. albicans* [29]. The virulence of *C. dubliniensis* to be low when compared to the virulence of *C. albicans*. It has been suggested that the reason for the comparatively low virulence is its lower capacity to form hyphae compared to *C. albicans* [30]. *C. dubliniensis* is, however, only *Candida* species in addition to *C. albicans* that forms true hyphae. The expression of a wide range of degradative enzymes, such as secretary aspartyl proteinases (SAPs) and phospholipases has also been recognized as important factors of *C. albicans* to cause infection [2]. *C. dubliniensis* possesses homologues of seven *C. albicans* secretory aspartyl proteinase genes (SAP) [29]. A prior study reported that proteinase activity of *C. dubliniensis* resulted in Pz range from 0.61 to 0.84 (mean = 0.75) [6], which is insignificantly low level of enzyme production when compared to the present result. In our study, proteinase production was observed at 66.66% and Pz ranged from 0.5-0.9 (mean=0.47) (Table 1). Bennett *et al.* (1998) [31] examined that *C. dubliniensis* possessed sequences homologous to *C. albicans* phospholipase C1 gene (*CAPLC1*) by southern hybridization and PCR analyses. Sachin *et al.* (2012) [32] observed 40% of phospholipase production in *C. dubliniensis*. However, in our study, only 11.11% (Pz=0.5, mean=0.05) of phospholipase activity was documented (Table 1). The production of phospholipases in *C. dubliniensis* is strain related and the amount produced shows important variations among clinical isolates and depending on the surrounding conditions [33].

Apart from the phospholipases, the term "lipolytic enzymes" comprises lipases and esterases, which hydrolyze glycerol esters of both short- and long-chain fatty acids [34]. The extracellular enzyme described here was considered an esterase, in the strict sense of the word, because of its hydrolytic activity with respect to water-soluble monoesters with a long-chain fatty acid [35]. Esterase activity has been found in a variety of pathogenic *Candida* species [35-37]. Khosravi *et al.* (2008) [38] examined esterase activity of *C. albicans* as a potential virulence factor in the animal model. The esterase activities of these yeasts were previously demonstrated in a few studies using the Tween 80 opacity test [15,35].

Table 1: Percentage production of enzymatic activities in *C. dubliniensis*

Strain	Enzyme variables	Percentage	Range (Pz)	Mean±SEM (Pz)
C. dubliniensis (n=9)	Phospholipase	11.11 (1/9)	0.5	0.05±0.05
	Proteinase	66.66 (6/9)	0.5-0.9	0.47±0.13
	Esterase	55.55 (5/9)	0.8-0.9	0.47±0.15
	Lipase	11.11 (1/9)	0.9	0.10±0.10

SEM: Standard error of mean; Pz: Precipitation zone, *C. dubliniensis: Candida dubliniensis*

Table 2: Hemolysin and biofilm production in *C. dubliniensis*

Name of the species	Strains name	Hemolysin	Biofilm
C. dubliniensis	(n1)	-	1+
	(n2)	β hemolysis	-
	(n3)	-	-
	(n4)	-	1+
	(n5)	-	-
	(n6)	β hemolysis	1+
	(n7)	-	2+
	(n8)	-	-
	(n9)	-	-

C. dubliniensis: Candida dubliniensis

Similarly, we use the Tween 80 opacity medium for the detection and 55.55% of esterase activity was determined in *C. dubliniensis* (Table 1), which is quite high when compare to 16.6% [39]. Lipase activity of *Candida* plays a key role in persistence and virulence in humans [40]. Lipase is a family of enzymes which hydrolyze the ester bonds in triacylglycerol to yield free fatty acids [41]. In the broad sense of the term, an enzyme which hydrolyzes monoacylglycerol with a long-chain fatty acid (C12 or more) is classified as a monoacylglycerol lipase [36]. *C. albicans* showed higher lipase activity in this study [42]. None of the studies recorded the lipase production in *C. dubliniensis*. In our study, we observed that 11.11% of lipase production in *C. dubliniensis* and Pz ranged at 0.9 (Table 1).

Since there is essentially no free iron in the human host, most pathogens acquire this indirectly from commonly available iron containing compounds such as hemoglobin [43]. The enzymes involved in this activity are classified as hemolysins. Studies on the activity of hemolysin in *Candida* were limited. Linares *et al.* (2007) [6] found the hemolytic activity of *C. dubliniensis*. A study observed that 60% of hemolysin activity in *C. dubliniensis* [32], which was high when compared to our results 22.22% (Table 2). Biofilm production is considered to be a potential virulence factor of *Candida* species. In our study, 44.44% of *C. dubliniensis* was found as biofilm producers (Table 2). One of the major characteristics of biofilms production is their increased resistance to antifungal drugs. Induction of ergosterol genes has also been described in *C. dubliniensis*, where incubation with fluconazole and formation of biofilm was coupled with upregulation of the Cd*ERG3* and Cd*ERG25* [44].

CONCLUSION

In conclusion, our study recommends that an incidence and virulence factor of *C. dubliniensis* from clinical specimens is very important. Although number of epidemiological studies on *Candida* species, less attention only given to the *C. dubliniensis*. The reason for this may be phenotypic isolation and identification of this species is problematic, which closely related to *C. albicans*. The majority of studies observed the *C. dubliniensis* in a specific group of populations (HIV and AIDS patients) such studies were failed to record this species from normal healthy individuals. Knowledge about the virulence factors of *C. dubliniensis* is clinically very important. Our study revealed that *C. dubliniensis* had the maximum level of proteinase, esterase activity, biofilm formation and only minimum level of phospholipase, lipase, and hemolytic activity was observed. In addition, prevalence and virulence factors such as esterase, lipase as well as hemolytic activity in *C. dubliniensis* are less studied and importance must be given.

ACKNOWLEDGEMENTS

REFERENCES

1. Sullivan D, Haynes K, Bille J, Boerlin P, Rodero L, Lloyd S, *et al*. Widespread geographic distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus infected individuals. J Clin Microbiol 1998;36:3007-12.
2. Sullivan DJ, Moran GP, Coleman DC. *Candida dubliniensis*: Ten years on. FEMS Microbiol Lett 2005;253:9-17.
3. Rizvi MW, Malik A, Shahid M, Singhal S. *Candida albicans* infections in a North Indian tertiary care hospital: Antifungal resistance pattern and role of SDS-PAGE for characterization. Biol Med 2011;3(2):176-81.
4. Schoofs A, Odds FC, Colebunders R, Ieven M, Goossens H. Use of specialised isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. Eur J Clin Microbiol Infect Dis 1997;16:296-300.
5. Pinjon E, Sullivan D, Salkin I, Shanley D, Coleman D. Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. J Clin Microbiol 1998;36:2093-5.
6. Linares CE, de Loreto ES, Silveira CP, Pozzatti P, Scheid LA, Santurio JM, *et al*. Enzymatic and hemolytic activities of *Candida dubliniensis* strains. Rev Inst Med Trop Sao Paulo 2007;49:203-6.
7. Schaller M, Borelli C, Korting HC, Hube B. Hydrolytic enzymes as virulence factors of *Candida albicans*. Mycoses 2005;48:365-77.
8. Naglik J, Albrecht A, Bader O, Hube B. *Candida albicans* proteinases and host/pathogen interactions. Cell Microbiol 2004;6:915-26.
9. Perea S, Patterson TF. Antifungal resistance in pathogenic fungi. Clin Infect Dis 2002;35:1073-80.
10. Ozkan S, Kaynak F, Kalkanci A, Abbasoglu U, Kustimur S. Slime production and proteinase activity of *Candida* species isolated from blood samples and the comparison of these activities with minimum inhibitory concentration values of antifungal agents. Mem Inst Oswaldo Cruz 2005;100(3):319-23.
11. Vijaya D, Harsha TR, Nagaratnamma T. *Candida* speciation using Chrom Agar. J Clin Diag Res 2011;5(4):755-7.
12. Samaranayake LP, Raeside JM, MacFarlane TW. Factors affecting the phospholipase activity of *Candida* species *in vitro*. Sabouraudia 1984;22:201-7.
13. Staib F. Serum-proteins as nitrogen source for yeastlike fungi. Sabouraudia 1965;4:187-93.
14. Aneeja KR. Experiments in Microbiology, Plant Pathology, Tissue Culture and Mushroom Cultivation. IInd ed. New Delhi: New Age International Publishers; 1996.
15. Slifkin M. Tween 80 opacity test responses of various *Candida* species. J Clin Microbiol 2000;38(12):4626-8.
16. Branchini ML, Pfaller MA, Rhine-Chalberg J, Frempong T, Isenberg HD. Genotypic variation and slime production among blood and catheter isolates of *Candida parapsilosis*. J Clin Microbiol 1994;32:452-6.
17. Manns JM, Mosser DM, Buckley HR. Production of a hemolytic factor by *Candida albicans*. Infect Immun 1994;62:5154-6.
18. Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity in *Candida albicans*. Sabouraudia 1982;20:7-14.
19. Ge YP, Lu GX, Shen YN, Liu WD. *In vitro* evaluation of phospholipase, proteinase, and esterase activities of *Candida parapsilosis* and *Candida metapsilosis*. Mycopathologia 2011;172(6):429-38.
20. Sullivan D, Coleman D. *Candida dubliniensis*: Characteristics and identification. J Clin Microbiol 1998;36:329-34.
21. Sullivan D, Haynes K, Bille J, Boerlin P, Rodero L, Lloyd S, *et al*. Widespread geographic distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus-infected individuals. J Clin Microbiol 1997;35(4):960-4.
22. Willis AM, Coulter WA, Sullivan DJ, Coleman DC, Hayes JR, Bell PM, *et al*. Isolation of C. Dubliniensis from insulin-using diabetes mellitus patients. J Oral Pathol Med 2000;29:86-90.
23. Pinjon E, Moran GP, Coleman DC, Sullivan DJ. Azole susceptibility and resistance in *Candida dubliniensis*. Biochem Soc Trans 2005;33(5):1210-4.
24. Pontón J, Rüchel R, Clemons KV, Coleman DC, Grillot R, Guarro J, *et al*. Emerging pathogens. Med Mycol 2000;38 Suppl 1:225-36.
25. Sullivan DJ, Westerneng TJ, Haynes KA, Bennet DE, Coleman DC. *Candida dubliniensis* sp. Nov.: Phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology 1995;141:1507-21.
26. Odds FC, Van Nuffel L, Dams G. Prevalence of *Candida dubliniensis* isolates in a yeast stock collection. J Clin Microbiol 1998;36:2869-73.
27. Quindós G, Carrillo-Muñoz AJ, Arévalo MP, Salgado J, Alonso-Vargas R, Rodrigo JM, *et al*. *In vitro* susceptibility of *Candida*

dubliniensis to current and new antifungal agents. Chemotherapy 2000;46:395-401.

28. Chaudhary U, Deep A, Chabbra N. Rapid identification and antifungal susceptibility pattern of *Candida* isolates. Infect Dis Antimicrob Agents 2009;26(2):49-53.

29. Gutiérrez J, Morales P, González MA, Quindós G. *Candida dubliniensis*, a new fungal pathogen. J Basic Microbiol 2002;42(3):207-27.

30. Stokes C, Moran GP, Spiering MJ, Cole GT, Coleman DC, Sullivan DJ. Lower filamentation rates of *Candida dubliniensis* contribute to its lower virulence in comparison with *Candida albicans*. Fungal Genet Biol 2007;44(9):920-31.

31. Bennett DE, McCreary CE, Coleman DC. Genetic characterization of a phospholipase C gene from *Candida albicans*: Presence of homologous sequences in *Candida* species other than *Candida albicans*. Microbiology 1998;144 (1):55-72.

32. Sachin CD, Ruchi K, Santosh S. *In vitro* evaluation of proteinase, phospholipase and haemolysin activities of *Candida* species isolated from clinical specimens. Int J Med Biomed Res 2012;1(2):153-7.

33. Hannula J, Saarela M, Dogan B, Paatsama J, Koukila-Kahkola P, Pirinen S, *et al*. Comparison of virulence factors of oral *Candida dubliniensis* and *Candida albicans* isolates in Healthy people and patients with chronic candidosis. Oral Microbiol Immunol 2000;15:238-44.

34. Wilhelm S, Tommassen J, Jaeger KE. A novel lipolytic enzyme located in the outer membrane of *Pseudomonas aeruginosa*. J Bacteriol 1999;181(22):6977-86.

35. Rudek W. Esterase activity in *Candida* species. J Clin Microbiol 1978;8:756-9.

36. Casal M, Linares MJ. Contribution to the study of the enzymatic profiles of yeast organisms with medical interest. Mycopathologia 1983;81:155-9.

37. Pospísil L, Kabátová A. Lipolytic activity in some *Candida* strains (author's transl). Zentralbl Bakteriol Parasitenkd Infektionskr Hyg 1976;131:692-6.

38. Khosravi AR, Riazipour M, Shokri H, Mousavi M. Intracellular esterase activity of *Candida albicans* and its correlation with pathogenicity in mice. J Med Mycol 2008;18(3):134-40.

39. Kumar CP, Menon T, Sundararajan T, Nalini S, Thirunarayan MA, Rajasekaran S, *et al*. Esterase activity of *Candida* species isolated from immunocompromised hosts. Rev Iberoam Micol 2006;23:101-3.

40 Martin S, Borelli C, Korting HC, Hube B. Hydrolytic enzymes as virulence factors of *Candida albicans*. Mycos 2005;48:365-77.

41. Bier M. Lipases. Methods Enzymol 1955;1:627-42.

42. Ramesh N, Priyadharsini M, Sumathi CS, Balasubramanian V, Hemapriya J, Kannan R. Virulence factors and antifungal sensitivity pattern of *Candida* Sp. Isolated from HIV and TB patients. Indian J Microbiol 2011;51(3):273-8.

43. Bélanger M, Bégin C, Jacques M. Lipopolysaccharides of *Actinobacillus pleuropneumoniae* bind pig hemoglobin. Infect Immun 1995;63:656-62.

44. Boreck´a-Melkusova S, Moran GP, Sullivan DJ, Kuchar´ıkov´a S, Chorv´at D Jr, Bujd´akov´a H. The expression of genes involved in the ergosterol biosynthesis pathway in *Candida albicans* and *Candida dubliniensis* biofilms exposed to fluconazole. Mycoses 2009;52(2):118-28.