PHENOTYPIC IDENTIFICATION AND BIOFILM FORMATION OF *CANDIDA* SPECIES ISOLATED FROM PATIENTS WITH SURGICAL SITE INFECTION IN KARACHI PAKISTAN

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ABSTRACT: Surgical site infections (SSIs) are among major complications following surgery. The aim of this study was to determine the pattern of *Candida* species in SSIs and to evaluate their virulence potential in association with SSIs. Pus swabs (n=300) were collected from patients with SSIs. *Candida* species were identified by phenotypic characters. Biofilm forming ability was determined by visual detection method and micro titre plate assay. *Candida* species recovered 60 (20%) were from tested samples. These were identified as *Candida albicans* 26(44%), followed by *C. glabrata* 15(25%), *C. parapsilosis* 8(13%), *C. krusei* 7(11%) and *C. tropicalis* 4(7%) respectively. Biofilm forming ability of *C. albicans* was lower (46%) than other Candida non-*albicans* species (72%) assessed by both methods. This study revealed that prevalence of *Candida* species was high in SSIs. Most of *Candida* species were able to produce biofilms which may enhance the pathogenic potentials of these isolates. The rapid and accurate identification of *Candida* species should be adopted in routine culture to minimize the risk of SSIs.

Keywords: Surgical site infections, Biofilm, *Candida albicans*, microtitre plate assay.

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INTRODUCTION

Surgical Site Infections (SSIs) are among major complications following surgery accounting for approximately one fourth of all hospital acquired infections (Azer *el al.,* 2011). Patients who develop SSIs suffer significant morbidity and mortality leading to increased economic burden by extending the duration of hospital stay (Kirkland *et al.*, 1999, Coello *et al.*, 2005). Infections caused by fungi, particularly *Candida* species increased during the past ten years (Garbino and Adam, 2006, Mathews and Ashbee, 2010). Some *Candida* species are found as commensal in many healthy individuals (Shao *et al.*, 2007). The normal microflora of patients may be altered by antibacterial therapies, which may enhance the growth of *Candida* species (Jones, 1990). *Candida* species can cause life-threatening infections in immuno compromised hosts. As the incidence of *Candida* infection is increasing, rapid and accurate identification of C*andida* isolates has become a vital part in the treatment of infections (Jarvis, 1995). *Candida* species is not routinely identified by many laboratories even though it can provide a diagnostic clue to the source of infection in immuno suppressed patients (Jones, 1990). Many phenotypic methods detect *Candida* species on the basis of colonial morphology on different agars, sugar assimilation and by using API strips (Del-Castillo *et al.,* 1997). Several virulence factors contribute to pathogenicity of *Candida* species such as adherence, hydrolytic enzymes production and biofilm formation (Silva *et al.*, 2012). Micro-colonies, hyphae and pseudohyphae of yeast arrange in a complex manner forming biofilms (Kuhn *et al.*, 2002 and Chandra *et al.*, 2001). Biofilm formation is one of the important factors associated with *Candida* infections. Biofilm can be detected on the surface of implanted devices (Adair *et al.*, 1999, Crump and Collignon, 2000). However, direct evidence of *Candida* biofilms on epithelial surfaces is not clear (Douglas, 2003). The aim of this study was to identify *Candida* species isolated from SSIs by phenotypic methods and evaluate their virulence potential in order to highlight factors responsible for surgical site infections.

MATERIALS AND METHODS

Study sites and sample collection: The present study was carried out in the Department of Microbiology, University of Karachi, Karachi. Pus samples (n=300) in duplicate were collected using sterile swabs from patients who developed any signs and symptoms associated with surgical site infections, admitted at three tertiary hospitals including Abbasi Shaheed Hospital (A.S.H), Jinnah Postgraduate Medical Centre (J.P.M.C) and Civil Hospital, Karachi All patients were informed and their consent was taken on a consent form prior to sample collection.

PHENOTYPIC IDENTIFICATION OF CANDIDA ISOLATES

Isolation on Sabouraud's Dextrose Agar (SDA): All samples were cultured on Sabrouad's Dextrose Agar (SDA) (Oxoid, Basingstoke, UK) with chloramphenicol (50 mg/L) (Hungerford *et al.*, 1998). The plates were incubated at 30ºC for 48 hours. Each culture was subsequently processed for phenotypic identification.

Germ tube test: Small inoculum of Yeast cellswas inoculated into 0.5 mL of human serum and incubated at 37°C for no longer than 3h. After incubation, a drop of suspension was placed on a clean glass slide and covered with a cover-slip and examined under low-power (40 X) magnification for the presence of germ tubes (Baker, 1967).

Chlamydospore Formation: For the identification of *C. albicans*a single colony of *Candida* was picked from the a pure culture medium and inoculated on a plate of Cornmeal agar containing 1%Tween 80 and rice agar containing 0.8% Tween 80 by making 3 parallel cuts about half an inch apart at 45 degree angle to culture medium then a cover slip was added and incubated at 30°C for 2 days. After incubation the cuts into agar were investigated for the presence of chlamydospores (Kurtzman and Fell, 1998)

Chromogenic Agar: For presumptive identification of *Candida* species, Biggy agar (Oxoid) was used. A loop of culture from SDA was streaked on Biggy agar and incubated at 30°C for 48 h. Different shades of brown color with and without mycelial fringes was observed to detect *Candida* species on Biggy agar.

Carbohydrate Assimilation Test: Culturegrown in Yeast Peptone Dextrose (Difco) for 24 h at 30ºC was Subsequently centrifuged at 3000xg for 5 min. After the 3 times of successive washes with sterile saline, the suspension was adjusted to 5 McFarland Standard. Molten sterile bacteriological agar in Falcon tubes (30 mL) was allowed to cool to 45 to 50ºC, seeded with 1.5 mL of *Candida* species suspension and 300 µL of Yeast Nitrogen Base (YNB, Difco), poured into plates and allowed to solidify. After solidified, 2% carbohydrate impregnated filter paper disc (glucose, maltose, lactose, sucrose and xylose) was placed on the surface of the agar and plate was incubated at 30°C for 96 h. Since all the species of *Candida* assimilate glucose, it was used as positive control. Halo of growth around each disc indicate carbohydrate was assimilated by this specie.

Growth at 45°C: For the differentiation of *C. dubliniensis* from *C. albicans.* All species which were positive for germ tube and chlamydospore production were cultured on SDA and kept at 45°C for 48-72 h.

Opacity-test in Tween 80-$CaCl_2$ Agar: Isolates which were indicative of *C. albicans* or *C. dubliniensis*, were transferred to Tween 80-$CaCl_2$agar (pH 6.8) by sterile swab. The inoculated agar plates were incubated at 30°C and observed daily for 2- 5 days. Lypolytic activity was detected as the presence of a halo around the site of inoculation.

DETECTION OF BIOFILM FORMATION

Visual Detection Method: Biofilm formation was determined by the visual detection method (Branchini *et al.*, 1994). A loop of culture from SDA plate was inoculated into a polystyrene tube, screw capped containing 10ml Sabouraud's dextrose broth (SDB) supplemented with glucose (final concentration of 8%). The tubes were incubated at 37°C for 24 h after which the broth was aspirated out and tubes were washed with distilled water twice. The walls of the tubes were stained with safranin. Slime production was scored as negative (0), weak positive (1+), moderate positive (2+) or strong positive (3+).

Microtiter Plate Method: Well-established protocols described by Tumbarello *et al.*(2007) and Ronsania *et al.*(2011) were used with some modifications to determine biofilm formation. *Candida* species were grown on Sabouraud's dextrose agar plates at 37ºC for 24 h. Saline washed suspension of *Candida* isolates were adjusted as $3x10^7$ CFU/mL with Sabouraud's dextrose broth (SDB) containing 8% glucose and incubated at 37°C for 24 h. In each well of polystyrene microtiter plate, 20 µL suspension of each isolate was added and diluted by adding 180 µl of SDB and incubated for 24 h. Planktonic cells were removed by washing the wells twice with phosphate-buffered saline (pH 7.2) and the cells were then fixed in 99% methanol for 15 min. After evaporation of the methanol via air dry, 200 µL of 1% crystal violet (CV) was added to each well, followed by incubation for 15 min. The wells were washed three more times with sterile distilled water to remove excessive CV. 1 mL of 33% acetic acid was added in each well and incubated for 10 min to release the incorporated CV and the absorbance was determined by Microplate Reader (Spectra Max M2) at A570. To eliminate background interference the readings of blank wells were subtracted from the readings of treated wells and arithmetical mean of 3 readings was used in the analysis for each strain. *Candida* species showed strong biofilm formation in visual detection method, used as positive control and sterile SDB broth was used as the negative control.

Statistical Analysis: All Statistical analysis was performed using statistical packages for social science SPSS 19.0 software (SPSS Inc., Chicago, IL). Mean and standard deviation was estimated for quantitative observation while frequency and percentage were computed for categorical variables and analyzed by chi-

square test and fisher exact test. P≤0.05 was considered as significant.

RESULTS

A total of 300 pus samples were cultured. In 240 (80%) samples, no growth of yeast was observed, while 60 (20%) samples yielded yeast fungi. These strains were identified as *C. albicans* 26(44%) followed by *C. glabrata* 15(25%), *C. parapsilosis* 8(13%), *C. krusei* 7(11%) and *C. tropicalis* 4(7%) respectively (Fig.1).

24 (40%) isolates showed as germ tube positive, suggestive of *C. albicans* and the remaining 36 (60%) isolates did not produce germ tube. Isolates which produced chlamydospores on rice and corn meal agar were 28 (47%) in which two were not identified as *C. albicans* by other phenotypic tests. Out of 26 *C. albicans*, 24 (92.30%) were germ tube positive. On Biggy agar, growth was observed in 46 (76.66%) of the 60 *Candida* isolates. Out of which 23 (50%) showed brown and dark brown colonies with slight mycelial fringes indicative of *C. albicans* or *C. tropicalis*, 4 (8.69%) species showed large silvery brown black rough colonies with yellow halo, suggestive of *C. krusei*. 8 (17.39%) species produced cream/grey light brown colonies and 11 (23.9%) brown colonies with yellow halo on Biggy agar. Both results were indicative of *C. parapsilosis* and *C. glabrata* respectively (Fig. 2). The sensitivity of Biggy agar of each species is shown (Fig. 3)

To confirm these tests, carbohydrate assimilation test was used in all 60 strains. Sucrose, maltose and xylose were assimilated by both *C. albicans* and *C. tropicalis* whereas *C. krusei* did not assimilated any carbohydrate tested except glucose which was used as a positive control (Table 1).The results obtained using growth at 45ºC test showed that all the *C. albicans* strains gave positive results. For the differentiation of strains of *C. albicans* from the strains of *C. dubliniensis*, Tween 80 agar test was performed on all the 26 suggested strains of *C. albicans*. The opacity halo indicated that all the tested strains were *C. albicans*.

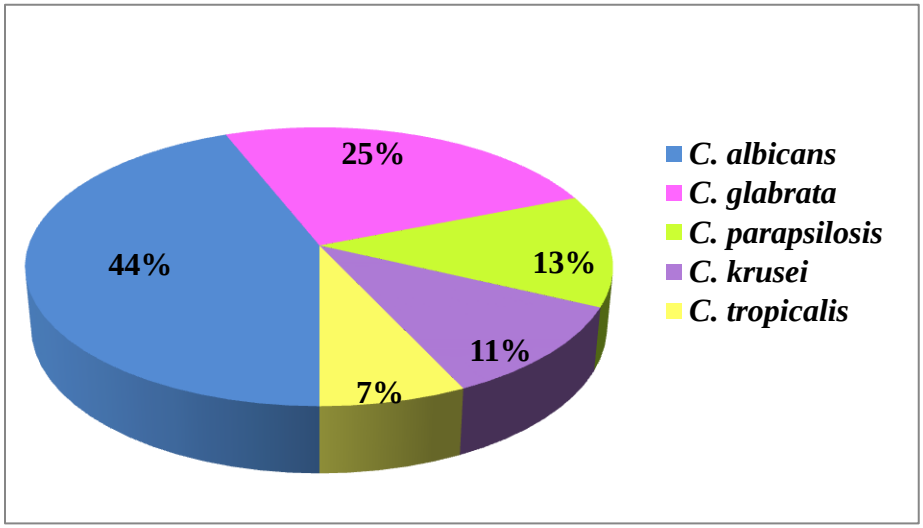

Fig- 1: Showing Pattern of *Candida* Species Isolated from Surgical Site Infections

Fig- 2: Showing different colony colors of *Candida* species on BIGGY agar

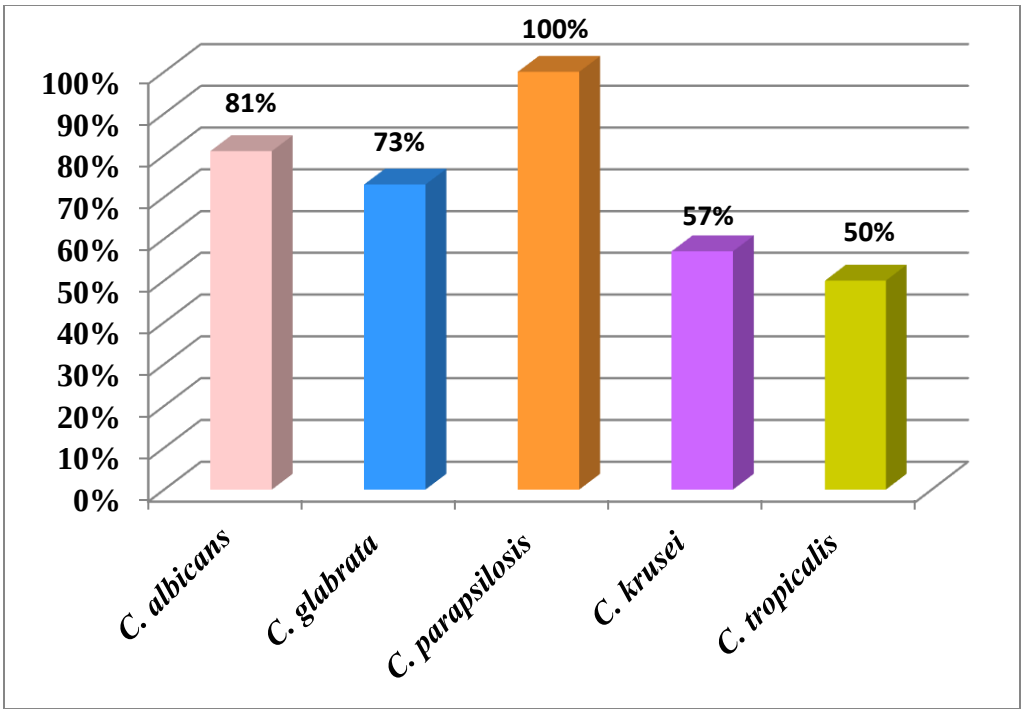

Fig- 3: Percentage of *Candida* Species Identification on Biggy Agar

Table-1: Identification of *Candida* Species by Phenotypic Methods

Candida spp.	GT	Chlamydospores formation	Growth at 45°C	Tween 80 agar test	Carbohydrate assimilation test				
					Glu	Suc	Mal	Lac	Xy
C. albicans	+	+	+	+	+	+	+	-	+
C. krusei	-	-	N/A	N/A	+	-	-	-	-
C. prapsilosis	-	-	N/A	N/A	+	+	+	-	+
C.tropicalis	-	-	N/A	N/A	+	+	+	-	+
C. glabrata	-	-	N/A	N/A	+	-	+	-	-

GT = Germ Tube Test, Glu = Glucose, Suc = Sucrose, Mal =Maltose, Lac =Lactose, Xy = Xylose ,
+ = Positive, - = Negative, N/A = Not applied

Biofilm forming ability of *Candida* species was checked by visual detection and microtitre plate method. Out of 60 tested isolates 36(60%) showed biofilm production, and fewer *C. albicans* isolates produced biofilm (46%) than all other *Candida* non-*albicans* species (72%) assessed by both methods. Biofilm production by both the visual detection method and microtitre plate method detected was similar. Specie *C. tropicalis* 4 of 4 (100%) produced the highest level of biofilm. *C. albicans* was the lowest biofilm producer (50%), as determined by the visual detection method while strain *C. glabrata* was the lowest (60%) by the microtitre plate method (Table 2).

Table-2: Biofilim formation of *Candida* species

Isolates	Biofilm production*	Biofilm production**			
		+++	++	+	**0**
C. albicans(n=26)	11(42)	4	4	5	13
C. Krusei(n=7)	6 (85)	3	2	1	1
C. prapsilosis(n=8)	6 (75)	4	1	1	2
C.tropicalis(n=4)	4 (100)	4	0	0	0
C. glabrata (n=15)	9 (60)	2	3	2	8

+++_ Strong; ++_ Moderate; +_ Weak; 0_no biofilm production; * biofilm formation is checked by microtiter plate method;
**_biofilm formation is checked by visual detection method; numbers in parenthesis are percentages

DISCUSSION

Surgical site infections are major complications following surgery (Azer *el al.*, 2011). *Candida* species, commensal of healthy individuals can cause life-threatening infections in immuno compromised hosts (Jarvis, 1995). In the present study *Candida* species accounted for 20% of the isolates which is a very high as compared to a study conducted in Nigeria (Isibor *et al.*, 2008) where 9.3% incidence of *Candida* observed in SSIs. Wroblewska *et al.* (2002)worked on 851 strains of *C. albicans* isolates from patients in internal medicine wards, surgical wards and surgical intensive care units and reported that *C. albicans* isolation rate was higher (55.5%) from patients in surgical wards as compared to that from patients in internal medicine wards (44.5%). This significant proportion of *C. albicans* isolates from surgical ward showed the growing importance of fungi in postoperative wound infections and was an alarming bell for health care providers. In the present study, *C. albicans* followed by *C. glabrata* were the most commonly isolated *Candida* species. These findings of the present study are similar to the findings of Li and An (2010) in patients of surgical intensive care units. To reduce morbidity and mortality in patients with SSIs, accurate identification of *Candida* isolates is needed. In the present workgerm-tube production was observed in 24/60 (40%) of *Candida* spp. and the remaining strains 36 (60%) failed to produce germ-tube, being identified as non-*Candidaalbicans*. These findings are in accordance with the findings of Alhussaini *et al.*(2013) where they found germ-tube production in 54% of *Candida* strains. Out of 26 *C. albicans* 24 (92.30 %) were germ-tube positive which is much similar to findings of Kangogo *et al.*(2011) who found 96.1% positivity of germ-tube formation in *C. albicans*. On the other hand, a surprising result was obtained, where 36 strains failed to produce germ-tube and out of these, 2 were positive for chlamydospores coupled with results of carbohydrate assimilation and chromogenic agar, these were identified as *C. albicans.* In the present study it was observed that all the *C. albicans* were found to be positive for chlamydospores and showed a distinct growth at 45ºC temperature. Similar findings were also reported by Kangogo *et al.* (2011). The Biggy agar is used for presumptive identification of yeasts. Most of the *Candida* spp. produced brown or dark brown colonies on Biggy agar and subsequently identified as *C. albican* and *C. tropicalis*. Due to the production of quite similar colors it was hard to differentiate the two species without other supporting tests of identification. *C. krusei* showed large rough silvery brown colonies surrounded with yellow halo and *C. parapsilosis* produced cream/grey light brown colonies, and both species were easily identified on this typical appearance. The same results were also reported by Yücesoy and Marol (2003). In comparison, after 48h of incubation *C. glabrata* showed very weak growth on Biggy agar. These findings suggest other identification procedures should be coupled with Biggy agar for accurate species identification. Odds and Bernaerts (1994) stated the same that Biggy agar alone could not provide sufficient information required for correct identification of C*andida* species. The pattern of carbohydrate assimilation is an important tests for identification of Candida spp. as of clinical interest (Zaini *et al.*, 2006). Result inTable-1 are in accordance with the study conducted in India by Manjunath *et al.*(2012) where, they found the same pattern of carbohydrate assimilation by *Candida* species. Biofilm formation isa major virulence factor which may contribute to enhance the pathogenicity of *Candida* spp. (Ozkan *et al.*, 2005). In our study the biofilm forming ability was detected in 60% of *Candida* spp. these findings are quite similar with Kumar and Sharma (2013) where they found biofilm production in 68% of *Candida* species. In the present finding biofilm production was less frequent in *C. albicans* as compared to *Candida* non-*albicans* species. Similar findings were reported by other investigators (Shin *et al.*, 2002) that *C. tropicalis* and *C. parapsilosis* are two of the *Candida* non-*albicans* species which formed strong biofilms in SDB medium supplemented with 8% glucose. In our study the highest biofilm positivity (100%) was found in *C. tropicalis* while low biofilm production was observed in *C. albicans* (42%).Similar results were observed by Kumar *et al.*(2011)who found *C. tropicalis* being the highest biofilm producer by visual detection method. Other researcher (Tumbarello *et al.*, 2007) not only found the same species as high biofilm producer by microtitre plate method but their results were also concordant with our results as for lowest biofilm producers.

Conclusion: This study revealed that *Candida* species isolates from surgical site infections have the property of biofilm production which may enhance the pathogenic potentials of these isolates. The rapid and accurate identification of *Candida* species should be adopted in routine culture to reduce the morbidity and mortality associated with surgical site infections.

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REFERENCE

Adair, C.G., S.P. Gorman, B.M. Feron, L.M. Byers, D.S. Jones, C.E. Goldsmith, J.E. Moore, J.R. Kerr, M.D. Curran, G. Hogg, C.H. Webb, G.J. McCarthy and K.R. Milligan. Implications of endotracheal tube biofilm for ventilator-

associated pneumonia. Intensive Care Med. 25(10): 1072-1076 (1999).

Alhussaini, M.S., N.F. El-Tahtawi and A.M. Moharram. Phenotypic and molecular characterization of *Candida* species in urine samples from renal failure patients. Science Journal of Clinical Medicine, 2(1): 14-25 (2013).

Azer, S.Z., S.M. Alaa Eldeen, M. Abd-Elwahb and A.M. Ahmed. Impact of Educational Program among Open Heart Surgery Patients on Minimizing the Incidence of Post Operative Infections. Journal of American Science, 7(6): 820-834 (2011).

Baker, F. Handbook of Bacteriological Technique. Pp. 415-421. 2nd ed. Butterwarth and Co. Ltd., London (1967).

Branchini, M.L., M.A. Pfaller, J. Rhine- chalk berg, T. Frempong and H.D. Isenberg. Genotype variation and slime production among blood and catheter isolates of C Parapsilosis. J. Clin. Microbiol., 32(2): 452–456 (1994).

Chandra, J., D.M. Kuhn, P.K. Mukherjee, L.L. Hoyer, T. McCormick and M.A. Ghannoum. Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J. Bacteriol., 183(18): 5385–5394 (2001).

Coello, R., A. Charlett, J. Wilson, V. Ward and B.P. Pearson. Adverse impact of surgical site infections in English hospitals. J. Hosp. Infect., 60(2): 93-103 (2005).

Crump, J.A. and P.J. Collignon. Intravascular catheter associated infections. Eur. J. Clin. Microbiol. Infect. Dis., 19(1): 1-8 (2000).

Del-Castillo, L., J. Bikandi, A. Nieto, G. Quindos, R. Sentandreu and J. Ponton. Comparison of morphotypic and genotypic methods for strain delineation in Candida. Mycoses, 40(11-12): 445-450 (1997).

Douglas, L.J. Candida biofilms and their role in infection. Trends Microbiol., 11(1): 30-36 (2003).

Garbino, J. and A. Adam. Use of highdose liposomal amphotericin B: efficacy and tolerance. Acta. Biomed., 77(4): 19-22 (2006).

Hungerford, L.L., C.L. Campbell, and A.R. Smith. Veterinary mycology laboratory manual. . Pp 27. 1st ed. Iowa State University Press, Ames, IA (1998.)

Isibor, J.O., A. Oseni, A. Eyaufe, R. Osagie and A. Turay. Incidence of aerobic bacteria and *Candida albicans* in post-operative wound infections. Afr. J. Microbiol. Res., 2(11): 288-291(2008).

Jarvis, W.R. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. Clin. Infect. Dis., 20(6): 1526–1530 (1995).

Jones, J.M. Laboratory Diagnosis of Invasive Candidiasis. Clin. Microbiol. Rev., 3(1): 32-45 (1990).

Kangogo, M.C., M.W. Wanyoike, G. Revathi and C.C. Bii. Phenotypic characterization of *Candida albicans* from clinical sources in Nairobi, Kenya. Afr. J. Health Sci., 19(1): 19-23 (2011).

Kirkland, K.B., J.P. Briggs, S.L. Trivette, W.E. Wilkinson and D.J. Sexton. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra cost. Infect. Control Hosp. Epidemiol., 20(11): 725-730 (1999).

Kuhn, D.M., J. Chandra, P.K. Mukherjee and M.A. Ghannoum. Comparison of biofilms formed by Candida albicans and Candida parapsilosis on bioprosthetic surfaces. Infect. Immun. 70(2): 878–888 (2002).

Kumar, A. and P.C. Sharma. *In Vitro* Studies on Selective Virulence Traits of Fluconazole Resistant *Candida* Species Isolated from Clinical Cases. International Journal of Biological & Pharmaceutical Research, 4(10): 685-696 (2013).

Kumar, A., V.C. Thakur, S. Thakur, A. Kumar and S. Patil. Phenotypic Characterization and In Vitro Examination of Potential Virulence Factors of candida species Isolated from Blood Stream Infection. World Journal of Science and Technology, 1(10): 38-42 (2011).

Kurtzman , C.P. and J.W. Fell. The yeasts , ataxonomic study . Pp 891-894. 4th ed. Elsevir, New york (1998).

Li, S. and Y.Z. An. Retrospective analysis of invasive fungal infection in surgical intensive care unit. Zhonghua yi xue za zhi., 90(6): 382-385 (2010).

Manjunath, V., G.S. Vidya, A. Sharma, M.R.P. Murugesh. Speciation of *Candida* by Hicrome agar and sugar assimilation test in both HIV infected and non infected patients. Int. J. Biol. Med. Res. 3(2): 1778-1782 (2012).

Mathews, M.S. and H.R. Ashbee. Pathogenic yeasts, emerging systemic yeast pathogens. p_p 231-251. The Yeast Handbook, Springer Berlin Heidelberg, London (2010).

Odds, F.C. and R. Bernaerts. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol.,32(8): 1923-1929 (1994).

Ozkan, S., F. Kaynak, A. Kalkanci, U. Abbasoglu and S. Kustimur. 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., 100(3): 319–323 (2005).

Ronsania, M.M., A.U.M. Rymovicza, T.M. Meiraa, A.M.T. Grégioc, O.G. Filhoa, O.M. Tanakaa and E.A.R. Rosab. Virulence modulation of *Candida* albicans biofilms by metal ions commonly released from orthodontic devices . Microb. Pathog.,51(6): 421–425 (2011).

Shao, L.C., C.Q. Sheng and W.N. Zhang. Recent advances in the study of antifungal lead compounds with new chemical scaffolds. Yao Xue Xue Bao, 42(11): 1129–1136 (2007).

Shin, J.H., S.J. Kee, M.G. Shin, S.H. Kim, D.H. Shin, S.K. Lee, S.P. Suh and D.W. Ryang. Biofilm production by isolates of *Candida* species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. J. Clin. Microbiol., 40(4): 1244–1248 (2002).

Silva, S., M. Negri, M. Henriques, R. Oliveira, D.W. Williams and J. Azeredo. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol. Rev., 36(2): 288–305 (2012).

Tumbarello, M., B. Posteraro, E.M. Trecarichi, B. Fiori, M. Rossi, R. Porta, K.de-G. Donati, M.L. Sorda, T. Spanu, G. Fadda, R. Cauda and M. Sanguinetti. Biofilm Production by *Candida* Species and Inadequate Antifungal Therapy as Predictors of Mortality for Patients with Candidemia. J. Clin. Microbiol.,45(6): 1843–1850 (2007).

Wroblewska, M.M., E. Swoboda-Kopec, A. Rokosz, E. Krawczyk, H. Marchel and M. Luczak. Epidemiology of clinical isolates of *Candida albicans* and their susceptibility to triazoles. Int. J. Antimicrob. Agents., 20(6): 472-475 (2002).

Yücesoy, M. and S. Marol. Performance of CHROMAGAR candida and BIGGY agar for identification of yeast species. Ann. Clin. Microbiol. Antimicrob., 2(8): 1-7 (2003).

Zaini, F., M.G. Shoar, P. Kordbacheh, E. Khedmati, M. Safara and N. Gharaeian. Performance of five phenotypical methods for identification of *Candida* isolates from clinical materials. Iranian J. Publ. Health, 35(1): 25-32 (2006).