Distribution Of Biofilm Producing Different Candida Species From Various Clinical Samples At A Tertiary Health Centre In Bihar

Shailesh Kumar* ,Sudhir Kumar ,Rajeev Ranjan* ,Swati Salila*, Manoj Kumar*,Namrata Kumari*, Manish Shankar***, S.K.Shahi***

*Department Of Microbiology, **Department Of Medicine, *** Department Of Respiratory Medicine, IGIMS, Patna-800001

Abstract: Objectives: Pathogenic fungi in the genus Candida can cause both superficial and serious systemic disease, and are now recognized as major agents of hospital-acquired infection. Many Candida infections involve the formation of biofilms on implanted devices. We undertook a prospective study to speciate the candida isolates, to detect biofilm formation among the isolates and to determine the predominant candida species in various clinical samples. Methods: This prospective, analytical study was done at IGIMS, Patna over a period of 2 months. The candida isolates were identified up to species level by standard mycological techniques like wet mount, KOH preparation and culture on Saboraud's Dextrose agar (SDA). For speciation, rapid method – KB006Hi- Candida identification kit, CHROM agar and germ tube test were used. Result**:** A total of 40 non-repetitive isolates of Candida species from the clinical samples from both medical and surgical wards and ICU were included in this study. All patients on antifungal treatment were excluded. Out of the 40 cases isolated, 13 were C. albicans, 19 were C. tropicalis , 3 C. glabrata and 5 C. krusei .The maximum no. of candida isolated was from urine (16/40,40%) followed by foley's catheter tip (10/40,25%) and endotracheal tube (8/40,20%). In our study non -albicans candida were more common than Candida albicans. Among non -albicans candida, Candida tropicalis (47.5%) was commonest.. Out of 40 candida species tested 8(20%) were found to be biofilm producers. Biofilm formation was more frequent among non-albicans species accounting for 6/8(75%) whereas C.albicans for 2/8(25%).Among biofilm producing non -albicans species C.tropicalis was the only biofilm producer. Conclusions**:** The isolation of non -albicans Candida (NAC spp). From clinical specimens should not be overlooked as these organisms are considered as emerging pathogens. Speciation is needed due to variation in species pathogenicity. Biofilm positivity was observed more with isolates from Foley's catheter tip and endotracheal tube. [Shailesh K NJIRM 2016; 7(6): 106-109]

Key words: Biofilm, Candida, fungi, Pathogenic, non-albicans Candida.

Author for Correspondence: Dr. Shailesh Kumar, Department Of Microbiology, IGIMS, Patna-800001.
E-Mail: shaileshgar@yahoo.com

Introduction: Pathogenic fungi in the genus Candida can cause both superficial and serious systemic disease, and are now recognized as major agents of hospital-acquired infection. Candida species (spp.) is the most important cause of opportunistic mycotic infection worldwide .[1] The expanding population of Human Immunodeficiency virus (HIV) infected patients, increase in the use of broad spectrum antibiotics, cytotoxic chemotherapy and transplantation have increased the incidence of candidiasis. [2] Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are immuno-compromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation. Many Candida infections involve the formation of biofilms on implanted devices such as indwelling catheters or prosthetic heart valves. The biofilms are resistant to a range of antifungal agents currently in clinical use, including amphotericin B and fluconazole, and there appear to be multiple resistance mechanisms. Among Candida spp., C. albicans is the most frequent cause of infection, but non-albicans spp. infections are increasing [3]. The important factors contributing to the virulence of candida is the formation of surface attached microbial communities known as "biofilms" [4]. Biofilms are attached to a surface and encased in a matrix of exopolymeric material. A typical laboratory fungal model of biofilm formation involves three operational steps: (a) adhesion, (b) biofilm growth, and (c) maturation [5]. We undertook this study to know the prevalent species of Candida infection from various clinical samples.

Methods: This prospective and analytical study was done over a period of two months The identification of yeasts up to species level isolated from various clinical specimens though problematic has become increasingly important for diagnostic laboratory. A combination of biochemical methods like fermentation and assimilation of sugars, growth on mycological culture media, growth on chromogenic media, automated system like mini API ,Vitek 2 and molecular methods have been used for identification of yeasts . Forty non-repetitive different clinical

samples were collected from patients after consent and processed in microbiology laboratory .In our study the Candida isolates were identified up to species level by standard mycological techniques like wet mount, Gram stain and culture on SDA. For speciation, KB006Hi- Candida identification kit, CHROM agar and germ tube test were used.

Inclusion criteria: All non-repetitive isolates of Candida species from clinical samples.
Exclusion criteria: Patients on antifungal treatment. Repeat samples from same source.
Study methods: Candida isolates from different clinical samples were speciated by KB006 HiCandida Identification kit Germ tube test Colour of the colonies on CHROM agar Detection of Biofilm formation

Speciation of Candida species using KB006 HiCandida Identification kit: It is a standardized colorimetric identification system utilizing 12 conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization. On incubation for 24-48 hours, organisms undergo metabolic changes which are indicated by spontaneous colour change from orange (normal) to yellow colour.

Germ tube test: A rapid method of identifying Candida albicans based on its ability to form germ tubes within 2 hours when incubated in human serum at 37 degree C (Reynolds- Braude) phenomenon.

CHROM agar- (CHROM agar company, Hi Media)- Identification of Candida spp. on CHROM agar culture produce different colour of colonies.

Detection of Biofilm formation: Detection of biofilm was done by visual method described by Yigit et al. [6] The isolate to be tested for production of biofilm was inoculated in glass test tube containing Sabouraud's dextrose broth supplemented with glucose (final concentration 8%). The tubes were incubated at 35degree C for 48 hours. After incubation the broth from tubes were gently aspirated using Pasteur pipette. The tubes were twice washed with distilled water to remove non -adherent cells. The tubes were stained with 2% safranin for 10 min. Excess of stain was removed by rinsing with distilled water and the tubes were examined for the presence of adherent layer. Biofilm formation was considered positive when a visible stained film lines the wall and bottom of the tube.[7] The formation of ring at the liquid interface was not considered as an indication of biofilm production.

Ethical considerations: Informed consent was obtained from all the participants. Institutional Ethics Committee (IEC) approval was obtained. (Ref.No. IGIMS/2014/104/Acad)

Result: The isolates were collected over a period of two months at tertiary care hospital. The results were analyzed and presented in the following tables.

Table 1 : Distribution of Candida spp. Among clinical specimens

Specimen(n=40)	**Total no. of isolates**	**C. albicans(**n=13)	**C. tropicalis**(n=19)	**C. glabrata**(n=3)	**C. krusei**(n=5)
Urine	16(40%)	7(53.85%)	9(47.37%)	-	-
Foley's catheter tip	10(25%)	4(36.77%)	4(21.05%)	1(33.33%)	1(20%)
Endotracheal tube(ICU)	8(20%)	1(7.7%)	3(15.79%)	2(66.66%)	2(40%)
Sputum	5(12.5%)	1(7.7%)	2(10.53%)	-	2(40%)
Blood	1(2.5%)	-	1(5.26%)	-	-
Total	40	13(32.5%)	19(47.5%)	3(7.5%)	5(12.5%)

Table 2: Conventional biochemical test for various Candida species

No.	**Test**	**C.albicans(n=13)**	**C. tropicalis(n=19)**	**C.glabrata(n=3)**	**C.krusei(n=5)**
1	**Urease**	-	-	-	+
2	**Melibiose**	-	-	-	-
3	**Lactose**	-	-	-	-
4.	**Maltose**	+	+	-	-
5	**Sucrose**	-	+	-	-
6	**Galactose**	+	+/-	-	-

7	**Cellobiose**	-	+	-	-
8	**Inositol**	-	-	-	-
9	**Xylose**	+	+	-	-
10	**Dulcitol**	-	-	-	-
11	**Raffinose**	-	-	-	-
12	**Trehalose**	+	+	+	-
13	**Germ tube test**	+	-	-	-

Table3: Colony colour of various Candida species on CHROM agar

Candida species	Colour on CHROM agar
C. albicans	Light green
C. tropicalis	Fuzzy blue
C. glabrata	Creamy white
C. krusei	Light pink fuzzy

Table 4 : Candida species and biofilm formation

(n= number of biofilm producing Candida species in different clinical samples)

Specimen	C.albicans	C. tropicalis	C. glabrata	C. krusei
Urine	-	-	-	-
Foley's catheter tip	1	3	-	-
Endotracheal tube	1	2	-	-
Sputum	-	-	-	-
Blood	-	1	-	-
Total (n=8)	2	6	-	-

A total of forty non-repetitive isolates of Candida species from both medical and surgical wards and intensive care units (ICU) were included in this study. All patients on antifungal treatment were excluded. Out of the forty cases isolated, 13 were C. albicans, 19 were C. tropicalis , 3 were C. glabrata and 5 were C. krusei (Table 1). The commonest clinical specimen from which Candida was isolated was urine, followed by endotracheal tube (ET), Foley's catheter tip, sputum and blood in descending order (Table 1). The biochemical tests done are shown in (Table2).

Discussion: Forty Candida isolates were stock cultured and following ethical committee approval speciation was done. The distribution of candida isolates from different samples was evaluated. The maximum no. of candida isolated was from urine (16/40, 40%) followed by foley's catheter tip (10/40, 25%) and endotracheal tube (8/40,20%). In our study non -albicans candida were more common than Candida albicans. The shift towards non- albicans Candida is probably driven by azole use , due to selection pressure [8.]Among non -albicans candida, Candida tropicalis (47.5%) was commonest followed by C. krusei (12.5%) and then C. glabrata (7.5%) and maximum no. of C.tropicalis was isolated from urine(47.36%). In our study Candida albicans were 13 (32.5%) and mostly isolated from urine 7 (53.85%) (fig1). Chromogenic media are frequently used in direct and rapid identification of yeasts because different Candida species produce unique colour on these media (fig2). It is a reliable method for presumptive identification of most commonly isolated Candida species .(Table 3). Biofilm formation by various candida species was evaluated.(fig3) Out of 40 candida species tested 8(20%) were found to be biofilm producers. Biofilm formation was more frequent among non- albicans species accounting for 6/8 (75%) whereas C. albicans for 2/8(25%) similar to study conducted by Girish Kumar CP etal. and Muni S etal. [9,10]Biofilm is a community of microorganisms and their extra cellular polymers that are attached to a surface. [11] Adherence of Candida to medical devices often leads to the formation of biofilms. The ability to form biofilm is associated with the pathogenicity and as such should be considered as an important virulence determinant during candidiasis. Among biofilm producing non -albicans species C.tropicalis was the only biofilm producer. In a study conducted by Sahar Ali Mohamed etal**.** [12] the biofilm positivity occurred most frequently among

isolates of C. parapsilosis (100%) followed by C. tropicalis (73.1%) Out of 13 Candida albicans isolated from different clinical samples in our study, two were biofilm producers ,one from foley's catheter tube and one from endotracheal tube. Most of the biofilm producing candida species were recovered from foley's catheter (One C. albicans and three C. tropicalis) followed by endotracheal tube., (One C. albicans and two C. tropicalis) respectively (Table 4). In 2009, a Spanish group demonstrated a significant linear association between increasing values of the "Candida score" and the rate of invasive Candida infections. [13] Limitations of our study were that Cornmeal agar for chlamydospore formation could not be done due to lack of resources. and small sample size as it was a ICMR student project over a period of 2 months .Further studies pertaining to demographic study of patients with candidiasis, study of biofilm producers candida antifungal susceptibility and other virulence factors like phospholipase activity and "Candida score" need to be done.

Conclusion: The isolation of non -albicans candida (NAC spp). from clinical specimens should not be overlooked as these organisms are considered as emerging pathogens. Speciation is needed due to variation in species pathogenicity. Biofilm positivity was observed more with Foley's catheter tip and endotracheal tube.

References:

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistant public health problem. Clin Microbiol Rev. 2007;20:133-163.
2. Enoch DA, Ludlam HA, Brown NM. Invasive fungal infections: a review of epidemiology and management options. J Med Microbiol. 2006;55:809-818.
3. Mohandas V, Ballal M. Distribution of Candida Species in Different Clinical Samples and Their Virulence: Biofilm Formation, Proteinase and Phospholipase Production: A Study on Hospitalized Patients in Southern India. J. Glob. Infect. Dis. 2011;3(1):4–8.
4. Seneviratne CJ, Jin L, Samaranayeke LP. Biofilm lifestyle of Candida: A mini review. Oral. Dis. 2008;14:582-590.
5. Aparna MS, Yadav S .Biofilms: Microbes and Disease. The BrazJ. Infect. Dis. 2008;12:526-530.
6. Yigit N,Aktas E, Digistan Setal. Investing biofilm production, coagulase and hemolytic activity in Candida species isolated from denture stomatitis patients. The Eurasean Journal of Medicine. 2011;43:27-32.
7. Christensen G.D., Simpson W.A., Bisno A.L., Beachey E.H. "Adherence of slime – producing strains of Staphylococcus epidermidis to smooth surfaces." Infect Immun 1982; 37(1): 318-26
8. Purva Mathur. Intensive care units. In Hospital acquired infections Prevention and control 1st .ed. New Delhi: Wolters Kluwer 2010 : 237
9. 9. Girish Kumar CP, Menon T. Biofilm production by clinical isolates of Candidaspecies. Med. Mycol. 2006;44:99-101.
10. Muni S, Menon S, Chande C, Gohil A, Chowdhary A, Joshi A. Candida biofilm. Bombay. Hosp. J. 2012;54(1).
11. Pfaller MA, Houston A, Coffmann S. Application of CHROMagar Candida for rapid screening of clinical specimens for Candida albicans, Candida tropicalis, Candida krusei, and Candida (Torulopsis) glabrata. J. Clin. Microbiol. 1996;34:58-61.
12. Sahar Ali Mohamed etal.Biofilm formation and Antifungal susceptibility of Candida isolates from various clinical specimens. British Microbiology research Journal, 3(4):590-601, 2013.
13. León C, Ruiz-Santana S, Saavedra P etal., Cava Study Group: Usefulness of the "Candida score" for discriminating between Candida colonization and invasive candidiasis in non-neutropenic critically ill patients: a prospective multicenter study. Crit Care Med 2009, 37:1624-1633.Leroy et al. Annals of Intensive Care 2011, 1:50;6 .

Conflict of interest: None
Funding: None
Cite this Article as: Shailesh K, Sudhir K,Rajeev R, Swati S, Manoj K,Namrata K, Manish S S.K.Shahi. Distribution Of Biofilm Producing Different Candida Species From Various Clinical Samples At A Tertiary Health Centre In Bihar. Natl J Integr Res Med 2016; 7(6): Page no: 106-109