Species Identification of Candida Isolated from Clinical Specimens in a Tertiary Care Hospital

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Abstract:

Background: Candida species are responsible for various clinical manifestations from mucocutaneous overgrowth to blood stream infections especially in immunocompromized situations. Although C. albicans is the most prevalent species, high incidence of non-albicans Candida species with antifungal resistance are emerging which is posing a serious threat to the patients care . **Objective:** This study aimed to isolate and identify different species of Candida from different clinical specimens. **Methods:** A total of 100 different clinical specimens were studied of which 35 were oral swab, 28 were high vaginal swab, 15 were urine, 14 were nail, 04 were bronchoalveolar lavage and peritoneal fluid were 04. Among 100 clinical specimens, Candida isolates were identified in 64 specimens. Isolation of Candida species was done by primary culture in SDA. Subsequent identification of species were performed by germ tube test, subculture in chromogenic agar medium and carbohydrate assimilation test with commonly used twelve sugars. **Results:** Out of 64 isolated Candida species, Candida albicans were 51.56% and the non-albicans Candida species were 48.44%. The most prevalent Candida species was C. albicans 33 (51.53%) followed by C. tropicalis 17 (26.56%). C. glabrata 4 (6.25%), C. parapsilosis 4 (6.25%), C. krusei 3 (4.68%) and C. guilliermondii 2 (3.2%). One of the isolated Candida species was unidentified. **Conclusion:** Though Candida albicans was found as the most common species, but non-albicans Candida species are appearing as emerging pathogens as well. Exposure to chemotherapy appeared to be the commonest predisposing factor for Candida infection followed by indwelling urinary catheter in situ for prolong period.

Key words: Candida albicans, non-albicans Candida spp.

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Introduction:

Candidiasis is a primary or secondary infection which involves different species of the genus, Candida. The clinical manifestations of the disease are extremely varied, ranging from acute, sub-acute and chronic, to an episodic involvement. It may be localized to the mouth, lungs or the gastrointestinal tract, or may be systemic as in septicemia, endocarditis and meningitis. These yeasts may cause systemic infection in immunocompromised situations due to their great adaptability to different host niches[1]. Prolonged use of intravenous catheter, total parenteral nutrition, invasive procedures and increasing use of broad-spectrum antibiotics, cytotoxic chemotherapeutics and transplantations are factors that contribute to the increase in these infections[2].

More than 17 different Candida species are known to be the etiological agents causing human infections. The common species encountered are C, albicans, C. dubliniensis, C. tropicalis, C. parapsilosis, C. glabrata, C. lusitaniae, C. guilliermondii, C. pseudotropicalis(C. kefyr), C. krusei, C. rugosa, C. famata, C. lipolytica and C.zeylanoides etc. However, more than 90% of invasive infections are caused by Candida albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. krusei[3]. Candida albicans is by far the most common species causing infection in human[4]. The emergence of non-albicans Candida

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species as significant pathogens has however been well recognized during the past decades[5].

Oral candidiasis is common in the patients of medical conditions like hematological malignancies, uncontrolled diabetes mellitus and laryngeal carcinoma. In general these patients receive systemic antibiotics, cancer chemotherapy, systemic corticosteroids and irradiation therapy[6]. Another common infection with Candida spp. is vulvovaginal candidiasis, one of the most frequent infections of the female genital tract with a high incidence[7]. Device associated Candida spp. infections cause mortality rates as high as 30%[8]. The urinary tract is the anatomical site most conducive to the development of infections in hospitalized patients[9]. C.albicans is the fourth and third leading cause of hospital acquired blood stream and urinary tract infection respectively[10].

Candida albicans and non-albicans spp. are closely related but differ from each other with respect to epidemiology, virulent characteristics and antifungal susceptibility[6]. Concern is rising about the high incidence of infections caused by non-albicans species and the emergence of antifungal resistance[11]. Among the non-albicans species, C. tropicalis and C. parapsilosis are both generally susceptible to azoles; however, C. tropicalis is less susceptible to fluconazole than is C. albicans. C. glabrata is intrinsically more resistant to antifungal agents, particularly to fluconazole[11]. C. krusei is intrinsically resistant to fluconazole and infections caused by this species are strongly associated with prior fluconazole prophylaxis and neutropenia[12]

Though C. albicans is the most commonly isolated fungal species, but other species are increasingly being isolated. Particularly the non-albicans with decreased susceptibility to conventional antifungal agents are also increasing, thereby causing severe morbidity and mortality in immunocompromised patients[11]. Accurate identification of Candida spp. is therefore crucial for clinical management and for facilitating hospital control measures.

Methods:

This cross sectional study was carried out in the department of Microbiology and Immunology, BSMMU from May, 2013 to April,2014 and was approved by Institutional review board (IRB) No. BSMMU/2013/7580,Date:16-06-2013. A total of 100 specimens were collected from in-patients and out-patients of different department in BSMMU who are clinically suspected or diagnosed cases of candidiasis (oral swab 35, high vaginal swab 28, catheterized urine 15, nail 14, bronchoalveolar lavage 04 and peritoneal fluid 04).

All the clinical specimens were collected with proper clinical and laboratory procedures[13,14]. The specimens were examined microscopically by 20%KOH and Gram staining, primarily cultured in Sabourad dextrose agar (SDA) media with supplement of chloramphenicol and gentamycin in screw capped test tube. The Candida species produced a creamy colored pasty round moist colonies having a distinct yeast smell. The budding yeast cells were seen by direct microscopy in wet mount preparation both from clinical specimens and culture.

Identification of Candida species from growth in SDA media was done by germ tube test, carbohydrate assimilation test and subculture in chromogenic agar media. Germ tube test was performed to identify Candida albicans and to differentiate Candida albicans from non-albicans groups. Lightly touched single yeast colony with a sterile wire loop from the culture plate was inoculated in fresh human pooled serum and incubated at 37o C for 3 hours. The wet mount preparation showed germ tube which is a hyphal projection without constriction at the point of origin from the yeast cell.

For carbohydrate assimilation test, 12 sugars are used which are listed in carbohydrate assimilation reaction profile in mycology laboratory manuals13. A lawn culture of Candida isolate was made on yeast nitrogen base agar plate with suspension of Candida species in distilled water (equivalent to McFarland No.4 standard) and sugar disks dextrose, maltose, galactose, lactose, sucrose, mellibios, cellobios, trehalose, raffinose, xylose. Inositol and dulcitol (Hi-Media, Mumbai) were plated on the media and

incubated at 25o C to 30o C for 48 to 72 hours. Most of the isolates showed increased growth around the sugars they utilized within 48 hours except for few (dulcitol, melibiose and raffinose) which required incubation up to 5 to 7 days (Fig. I). The result was noted and tabulated.

Chromogenic agar medium was prepared following the manufacturer's instructions (Hi-Media, Mumbai). Suspension of isolates was prepared in sterile distilled water (Turbidity was adjusted to 2 McFarland standard) from primary culture in SDA and was inoculated onto Chromogenic Agar medium. The plate was incubated at 37°C for 48 hours. C. albicans produced light to medium green coloured colonies, C. trpocalis produced dark blue to metallic blue-purple colonies, C. krusei produced pink with a whitish bordered rough, dry colonies and C. glabrata produced pale pink colonies. C. parapsilosis and C. giulliermondii, both species produced colonies ranging from slight dark to light pink color (Fig.II).

Results:

A total of 100 clinical specimens were studied and 64 were positive for Candida species in culture. Out of 64 culture positive clinical specimens, Candida species were identified from oral swab 26 (40.62%), high vaginal swab 15 (23.43%), urine 12(18.75%), nail 8 (12.50%), bronchoalveolar lavage 2 (3.12%) and peritoneal fluid 1 (1.56%). Distribution of different species of Candida in various clinical specimens are shown in Table I. The commonest Candida species identified in this study was C. albicans 33 (51.56%) followed by C. tropicalis 17 (26.56%). The species of C. glabrata was 4 (6.25%), C. parapsilosis 4 (6.25%), C. krusei 3 (4.68%) and C. guilliermondii 2 (3.2%); one of the isolated Candida species was unidentified.

There was increased incidence of C. albicans in the oral swab and high vaginal swab. C. tropicalis was found in higher number in catheterized urine. C. parapsilosis (2 out of 4) was isolated more in high vaginal swab and a high incidence of C. glabrata (2 out of 4) was found in catheterized urine. C. krusei were equally distributed in oral swab, high vaginal swab and catheterized urine.

In this study the most common predisposing factor of candidiasis was exposure to chemotherapy. The second common factor was indwelling urinary catheter in situ for prolong time. Other predisposing factors were taking of multiple broad spectrum antibiotics, old age, use of intrauterine devices, taking oral contraceptives and diabetes mellitus (Table II).

Table-I :

Distribution of different species of Candida in various clinical specimens (n=64).

Name of species	Clinical specimens: Oral swab No. (%)	HVS No. (%)	Urine No. (%)	Nail No. (%)	BAL No. (%)	Peritoneal fluid No. (%)	Total No. (%)
C. albicans	13(20.31)	11(17.19)	03(4.68)	03(4.68)	02(3.12)	01(1.56)	33 (51.56)
C. tropicali	07(10.93)	-	06(9.37)	04(6.25)	-	-	17 (26.56)
C.glabrata	01(1.56)	01(1.56)	02(3.12)	-	-	-	04 (6.25)
C. parapsilosis	01(1.56)	02(3.12)	-	01(1.56)	-	-	04 (6.25)
C. krusei	01(1.56)	01(1.56)	01(1.56)	-	-	-	03 (4.68)
C. guilliermondii	02(3.12)	-	-	-	-	-	02 (3.12)
Unidentified sp ecies	01(1.56)	-	-	-	-	-	01 (1.56)
Total	26	15	12	08	02	01	64 (100)

Table-II

Distribution of predisposing factors in patients with Candida isolates.

Predisposing factors	Number of patients
History of chemotherapy	25
Urinary catheterization	12
History of use of antibiotics for prolong period	11
Old age with other debilitating conditions	06
Use of intrauterine devices	04
History of taking oral contraceptives	04
Diabetes mellitus	02

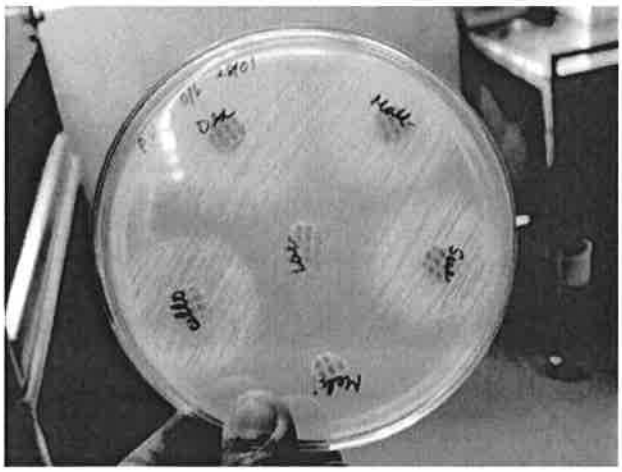

Fig-1: *Carbohydrate assimilation by Candida spp. (Dextrose, mellibiose, sucrose & cellobiose)*

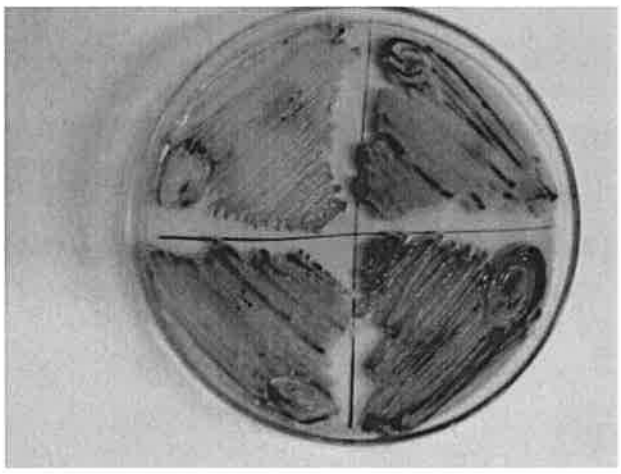

Fig -2: *C. albicans(light green),C.krusei (pinkish purple matt surface),C.tropicalis (metallic blue) in chromogenic media*

Discussion:

Present study revealed that Candida albicans were 51.56% and the non-albicans Candida species were 48.44%. Among the non-albicans Candida species, C. tropicalis were17 (26.56%), C. glabrata were 04 (6.25%), C. parapsilosis were 04 (6.25%), C. krusei were 03 (4.68%) and C. guilliermondii were 02 (3.2%). A study by Abdullah in 2006 showed, vulvovaginal candidiasis by C. albicans was the most frequent (97%) and rest 3% by non-albicans[15]. Another study was carried out in Bangladesh by Nahar et al in 1994 with the patients having symptoms of vaginitis which showed Candida albicans were 79.7% and the non-albicans were 20.3%. Among the non-albicans Candida species C. krusei were 11.9%, C. stellatoidea were 5% and C. tropicalis were 3.4%[16]. Though the above mentioned studies reveal C. albicans as the predominant Candida species with finding nearly similar to our study, but the frequency of non- albicans were higher in the present study.

Study conducted by Dharwad and Dominic in India (2011), also showed that Candida albicans was the most frequently isolated species (47%), followed by Candida tropicalis (30%)[17]. But in their study total number of isolated non-albicans Candida species were more (53%) than C. albicans (47%). In the present study C. albicans (51.56%) was found to be higher than non-albicans (48.44%). Another study with both HIV infected and non-infected patients by Manjunath et al found that C. albicans was the most frequently isolated species (52%), followed by C. tropicalis (19%)[18].

A study in Brazilian public tertiary hospitals, Nascimento et al found Candida albicans only in 33% of the total Candida isolates. C. parapsilosis was the second highest isolates (31.3%), followed by C. tropicalis (17.9%) and C. glabrata (11.8%), other species being 6.2%. In totality their study showed the non-albicans Candida predominance and C. parapsilosis was the leading cause of blood stream infection[19]. Another study by Paritpokee et al in the department of clinical pathology in Cleveland (USA) found infection by non-albicans Candida also in the rise. C. albicans was only 16.3% of the isolates and rest of the isolates were non-albicans Candida with C. parapsilosis

predominance followed by C. glabrata[20]. All these findings reveal that the non-albicans Candida are emerging as important pathogens globally, but the distribution of different species of Candida are quite different geographically.

In this study, the most commonly isolated Candida species from the specimen of oral swab was C. albicans (50%), followed by C. tropicalis (26.92%). Study by Baradkar and Kumar in 2009 in India on species identification of Candida isolates obtained from oral lesions of HIV infected patients found that C. albicans was the commonest isolates (70%), followed by C. parapsilosis (15%), C. glabrata (7.5%) and C. tropicalis (5%)[21]. A study in Ghana in 2013 with oropharyngeal candidiasis among HIV-positive patients, C. albicans was obtained as major isolates (68.5%) followed by C. tropicalis (7.4%), C. krusei (6.4%) and C. parapsilosis (3%)[22]. In all the above mentioned studies, C. albicans was found to be the major isolates, but as of the distribution of non-albicans Candida, the species showed variations in different series. The present study showed the incidence of vaginal candidiasis with C. albicans was quite high (73.33%), it was followed by C. parapsilosis (13.33%), C. glabrata (6.66%) and other species. This is quite similar to the study carried out in Iran in 2012 by Rad et al on Identification of Candida species associated with vulvovaginal candidiasis by multiplex PCR[23]. In their findings the most common species isolated was C. albicans (65.1%) followed by C. glabrata (13.1%), C. tropicalis (6.2%) and other species. Findings of present study is also similar to the study by Abdullah (2006) in which C. albicans was the most commonly identified species (97%) and rest 3% were non-albicans[15].

In this study, predominant species of Candida identified from urine specimens was C. tropicalis (50%), followed by C. albicans (25%). Nascimento et al also identified C. albicans (34%) and C. tropicalis (33%) in urinary tract infection which is also almost similar to this study[19]. In India a study only on non-albicans Candida species was carried out by Derukhkar and Saini in 2013, C. tropicalis was major isolate in their study[24]. Another study by Mahmoudabadi et al in Pakistan in 2012, identified Candida species in urine with C. albicans predominance, followed by C. glabrata and C. tropicalis respectively which is not similar to the present study[25].

The present study showed the association of predisposing factors in the patients from whom the Candida species were isolated. The most common predisposing factor was history of chemotherapy for hematological malignancies or other malignancies. Similar observation was found in the study of Shaheen and Taha in Egypt[6] and Ortaga et al in Brazil[2].

Besides cytotoxic chemotherapy, Ortega et al found, use of broad-spectrum antibiotics also a major cause of candidiasis in patients with intact immune status. Similar observation was found in present study as well. Random use of broad-spectrum antibiotics for pelvic inflammatory diseases, UTI, were one of the major causes of vulvovaginal candidiasis. Other causes of vulvovaginal candidiasis were the use of intrauterine devices and oral contraceptives. Similar findings were observed in the study of Saporiti et al [26] and Sobel et al [7].

Though diabetes mellitus (DM) is one of the major causes of candidiasis, in this study only two cases had DM, one of them was suffering from oral candidiasis chronically, another patient was having indewelling Foley's catheter for long time due to prostatic carcinoma. As the sampling technique was purposive so this finding is quite plausible. In the present study, 80% of urine samples were positive for Candida and the urine samples were collected from patients who had urinary catheters in situ. Nett et al observed that the majority of Candida causing urinary tract infections are associated with bladder catheters[27]. Deorukhkar and Saini also found in their study that long term use of catheters is one of the cause of urinary tract infection by Candida[24].

An increase in the predisposing conditions in recent years has resulted in an increasing incidence of Candida infections. Identification of Candida to the species level is important to provide a database for a given area of study. The choice of antifungals is also dependent on the species of Candida. Therefore, the species level identification of the Candida isolates can greatly influence the treatment options for the clinicians and therefore crucial for facili-

tating hospital control measures.

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