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Detection and Prevalence of Candida Isolates among Patients in Amravati City of Maharashtra State, India

KEYWORDS: Prevalence, Candida albicans, non albicans Candida, Opportunistic infections, vaginitis

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ABSTRACT *This study aimed to detect and determine the prevalence of Candida isolates among patients attending hospital in Amravati. A total of two hundred patients (92 males and 108 females) of different ages and socioeconomic status attending Govt. General Hospital Amravati; District Women Hospital, Amravati; T.B. Hospital, Amravati and Private pathology laboratories in Amravati were enrolled in this study. The clinical samples from the oral thrush, blood, urine and vaginal discharge were collected and cultured. The species of Candida isolated were identified. The overall isolation rate of Candida was found to be 55%. A total of 110 Candida isolates were obtained in this study, of which 48 (43.63%) were found to be Candida albicans and 62 (56.36%) were found to be different non-albicans Candida species. The majority of isolates were obtained from patients with oral thrush (80%) followed by vaginitis/vaginal discharges (77.77%), urine (40.47%) and blood (36.84%).*

INTRODUCTION

Infectious diseases account for a larger proportion of health problems in the developing countries and the use of steroids, antibiotics and cytotoxic drugs has increased the number of fungal infections. In the past few years, a significant increase in the number of yeast infections has been reported in the medical literature. Candidiasis is the most common opportunistic fungal infection. C. albicans is the organism most often associated with serious fungal infection; other Candida species also have emerged as clinically important opportunistic pathogens (Wingard, 1995). Candidal vaginitis is the most common form of mucosal candidiasis. Candidiasis is responsible for 90% of the cases of infectious vaginitis.

Nosocomial candidiasis is gaining significance worldwide. The frequency of candidemia has increased dramatically over the past few decades accounting for 8-10% of all nosocomial blood stream infections (Pfaller et al., 2006). Among the causes of bloodstream infection, Candida ranks fourth in the United States and seventh in Europe. Candida bloodstream infections are mostly observed in hospitals, mainly from intensive care units (ICUs), oncology units, and organ transplants units etc, where most patients are subjected to heavy therapeutic protocols and are immunodeficient. Until recently, C. albicans was by far the predominant species in most countries, causing up to two thirds of all cases of invasive candidiasis. However, during recent decades, several countries around the world have witnessed a change in the epidemiology of Candida infections, characterized by a progressive shift from a predominance of Candida albicans to non-albicans Candida species (Krcmery & Barnes, 2002). There is growing evidence suggesting a role for increasing use of azole agents in this epidemiological shift.

Candiduria, presence of Candida spp. in the urine may be one of the most challenging of the Candidal infections and is associated with the use of urinary catheters and antimicrobial therapy. Approximately 10% of cases of candidemia are related to a urinary tract source (Ang et al., 1993). Mammary candidiasis, a condition that affects breastfeeding womens has also been reported (Stamatakos et al., 2008). Candida has also emerged as an important cause of neonatal infections with significant morbidity and mortality (Tiraboschi et al., 2009). Only a few studies from India have reported candidaemia rates of 6-18 per cent (Xess et al., 2007) and an increase in isolation of non-albicans Candida species from blood samples (Shivaprakasha et al., 2007). The aim of this study therefore was to determine prevalence of Candida among patients in Amravati city of Maharashtra state.

MATERIALS AND METHODS

Study area

A total of two hundred patients (92 males and 108 females) of different ages and socioeconomic status attending Government General Hospital Amravati; District Women Hospital, Amravati; District TB Centre, Amravati, and Private Pathology laboratories in Amravati were enrolled in this study. This study comprised of 60 tuberculous patients, admitted to District TB Centre, Amravati.

Specimen collection

A total of 200 different clinical samples were collected from patients being treated in hospitals in Amravati city of Maharashtra state. The samples were collected under aseptic conditions and included 60 from oral thrush, 38 from blood, 84 from urine, and 18 samples from vaginal swabs of pregnant women. Oral thrush specimen from patients admitted for the treatment of pulmonary tuberculosis was collected by swabbing affected area of mouth cavity using sterile cotton swab and immediately streaked on Sabouraud's dextrose agar (Hi-media, Mumbai) with chloramphenicol (SDAc) Medium. Sample of vaginal discharge was collected on sterile cotton wool swab with the help of a gynecologist and immediately streaked on SDAc medium. For urine sample, midstream urine was collected in sterile, wide necked, leak proof container and inoculated on growth medium within two hours. Blood was collected by a clinician by locating the vein in the arm using pressure cuff. Skin over the vein was thoroughly cleaned with ethanol ether. 5 - 7 ml of blood was collected by vein puncture using sterile syringe and before clotting blood was inoculated on growth medium.

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Wet preparation, culture isolation and identification:
Specimens were transferred to a microscope slide. A drop of 10% KOH was added and mixed. It was covered with a cover glass and examined under the microscope at 10x and 40x. In addition, the smears were gram stained and examined. The samples were inoculated on to Sabouraud's dextrose agar (Hi-media, Mumbai) with chloramphenicol as the main isolation medium. The culture plates were incubated at 370C for 24 – 48 hrs. Different Candida species and other pathogenic yeasts were identified on the basis of morphological and biochemical characteristics (Cheesbrough, 1984). The identification of the species was conducted by assessing the colony morphology, germ tube formation, capsule production, hyphal and chlamydoconidia production on cornmeal agar, pellicle formation, carbohydrate assimilation, fermentation of sugars, urease test. Yeast isolates were screened for germ tube production in serum broth. Culture on Candida agar (Hi-media, Mumbai) was used for identification of the species.

RESULT AND DISCUSSION
A total of 200 clinical samples were analyzed in present study. The samples included oral thrush (60 cases), urine (84 cases), blood (38 cases) and vaginal swab (18 cases). Among these 110 (55%) were found positive for Candida species. Of the 110 culture positive specimens studied, 48 Candida cultures were isolated from oral thrush, 34 from urine, 14 cultures from blood and 14 from vaginal swab specimens. The overall isolation rate of Candida was found to be 55% (Table 1). Of the 110 isolates, C. albicans was the most common species (48 cases; 43.63%), followed by C. tropicalis (16 cases; 14.54%), C. glabrata (12 cases; 10.90%), C. parapsilosis (12 cases; 10.90%), C. pseudotropicalis (8 cases; 7.27%), C. krusei, (6 cases; 5.45%), C. guilliermondi (4 cases; 3.63%) and C. stellatoidea (4 cases; 3.63%). Highest isolation rate of Candida was obtained with oral thrush specimen (80%), followed by vaginal swab (77.77%), urine (40.47%) and blood (36.84%). Candida species distributions in different clinical samples are shown in Table 2 and 3.

Infections caused by fungi in man and animals are common throughout the world. Fungi cause both superficial and internal mycoses. In fact fungal infections show much higher mortality rate than bacterial infections (Liu et al., 2002). Opportunistic infections caused by pathogenic yeasts are most commonly associated with AIDS and other immuno-compromised states (Dupont et al., 1992). Oropharyngeal candidiasis develops in 80 -85% of patients with AIDS.

Candida is an asexual, diploid, dimorphic fungus that is present on humans and in their environment. A relatively small number of Candida species are pathogenic for humans. These organisms are capable of causing a variety of superficial and deep-seated mycoses such as cutaneous, mucocutaneous, subcutaneous, or systemic candidiasis. As Candida organisms are commensals, general risk factors for Candida infections include immunocompromised states, diabetes mellitus, and iatrogenic factors like antibiotic use, indwelling devices, intravenous drug use, and hyperalimentation fluids. Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation. The present study showed the 55 % distribution of Candida species in different clinical samples and the predominance of non-albicans Candida, as was also shown by Mohandas & Ballal (2011).

In the present study, Candida species were found to be more frequently associated with oral thrush, vaginitis, urine and blood. C. albicans was isolated at a higher rate from oral thrush (66.66%) and vaginal swabs (57.14%). The incidence of Candida albicans was found to be higher in oral thrush and vaginal swab as compared to other Candida species. Previous studies (Rizvi & Luby, 2004) reported much higher prevalence (78%) of C. albicans among Nepalese women. Our finding is parallel with study conducted by Muvunyi & Hernandez (2009) which revealed 52.5 % prevalence of C. albicans in women with vaginal symptoms. High isolation rate (82.8%) of C. albicans was reported earlier (Ng et al., 1998) in vaginal swabs and 44.2% in blood, respiratory system, urine and skin.

Highest number of non-albicans Candida were isolated from blood (85.71%) and urine (82.35%), followed by vaginal swab (42.85%) and oral thrush (33.33%). Percent distribution of Candida albicans (CA) and Non-albicans Candida (NAC) in various clinical specimens is shown in Fig.1.
Mohandas & Ballal (2011) reported predominance of C. krusei (38.23%) in blood cultures followed by C. albicans (20.58%). In present study also C. krusei was isolated frequently from blood at the rate of 28.57% but it was not detected in urine and vaginal swabs. Hamal et al. (2001) isolated C. parapsilosis from blood samples as a major species causing candidemia with isolation rate of 50.8%. In the present study C. parapsilosis was isolated from blood at a much lower rate (14. 28%) with an overall isolation rate of 10.90%. The prevalence of Candida parapsilosis was more in urine –17.64% followed by blood and vaginal swab –14.28% and oral thrush - 4.16%.

Candiduria caused by *Candida albicans* has been widely reported in the literature. However, a case of candidiasis in the urinary bladder caused by *Candida tropicalis* is occasionally encountered. Recently Sivaneswaran et al. (2012) reported a rare case of renal dysfunction in which ball-like lesions were seen adhering to the bladder wall of urinary tract (fungus balls) caused by Candida tropicalis. In the present investigation also Candida tropicalis was found to be associated with 23.52% cases of urine infection. Chakrabarti et al. (1992) reported 15% isolation rate of C. tropicalis from urine. Thus there is a need to understand the clinical implication of *Candida tropicalis* to improve the health care for patients.

During the past decade, Candida glabrata has emerged as an important cause of fungemia. In the present study Candida glabrata was recovered at a greater rate from blood(14.28%) and was not detected in vaginal swab. Costa et al. (2000) in a two year prospective study of nosocomial fungaemia showed the recovery of Candida guilliermondii from gastrointestinal tract and C. parapsilosis from venous catheter. However in the present investigations, C. guilliermondii was recovered from vaginal swabs and urine at the rate of 14.28% and 5.88%, respectively but was not detected in oral thrush and blood.

In the present study, C. albicans (57.14%) and other Candida species (42.86%) were recovered from the vaginal swabs collected from pregnant women attending the gynecologist. The overall isolation rate of Candida from vaginal swabs was found to be 77.77%. The overall carrier rate of 57.14% observed for C. albicans is comparatively lesser than the reports of Donbraye-Emmanuel et al. (2010) who documented 26% Candida infection rate among pregnant

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women with 65.4 % C. albicans and 34.6% other Candida species.

In the present study Candida albicans and non-albicans Candida species were isolated at the rate of 43.63% and 56.36%, respectively. However, Alli et al. (2011) reported Candida albicans to be 60% and other Candida spp. to be 40% among the patients in Southwestern Nigeria. These percentages reported for Candida albican (43.63%) and non-albicans Candida species (56.36%) in this study is higher compared to what was reported by Akingbade et al. (2013), who reported overall prevalence of candida albicans among womens attending health centers in Nigeria as 24.4%. However, recent reports by Kothari & Sagar (2009) from our country indicate a trend towards an increasing prevalence of non-albicans candidemia.

The rate of opportunistic fungal infections in turberculous patients is also very high. The reasons for increased prevalence are lowering of immune system due to tuberculosis and the use of antituberculous drugs of non-specific action which promote the growth and reproduction of the fungus flora and in turn aggravate the course of underlying process in the lung tissues (Solov'eva et al., 1991). Reports of Sehar & Perween (2004) revealed 15.2% tuberculous patients co-infected with Candida species, showing high incidence rate (8.4%) of C. tropicalis as compared to C. albicans (6.8%). In present study Candida co-infection was observed in 80% of patients with pulmonary tuberculosis with a higher prevalence of C. albicans (66.66%) than C. tropicalis (8.33%). Candida species are emerging as a potentially pathogenic fungus in patients with bronchopulmonary diseases. The growth promoting association of Candida and Mycobacterium tuberculosis has raised increased concern. Such opportunistic respiratory fungal infections pose a difficult diagnostic challenge due to lack of any pathognomonic clinical syndromes. Recent studies by Kali et al. (2013) revealed Candida albicans as the predominant organism observed in 50% of the patients, followed by C. tropicalis (20%) and C. glabrata (20%) with candida co-infection rate of 40% in patients suffering from pulmonary tuberculosis. However, over the past decade there has been a distinct change in the species of Candida associated with nosocomial infections as well as rise in the prevalence of non-albicans Candida species and C. tropicalis is emerging as a predominant organism.

In conclusion, this study demonstrated the prevalence of Candida among patients attending hospitals for different causes other than candidiasis. Incidence of Candida infection was greater in the patients admitted for the treatment of tuberculosis and in the pregnant women with vaginal infection. These findings should be taken into account in further studies concerning presence of C. albicans among women. However, there is a growing emergence of non-albicans candida (NAC) and non-albicans group had not been studied as extensively as the more common albicans group. More studies should be encouraged in this direction to reduce the incidence of Candida. Treatment should be given to culture positive women in order to prevent subsequent infection of the neonate and secondary infection to the mother. There is a need for appropriate health education to reduce candidal infection.

Table 1 : Isolation rate of pathogenic yeasts in different clinical samples

Sr. No.	Specimen	No. samples collected	Cultures positive for yeast	Isolation rate
1	Oral thrush	60	48	80 %
2	Urine	84	34	40.47 %
3	Blood	38	14	36.84 %
4	Vaginal swab	18	14	77.77 %
	Total	200	110	55 %

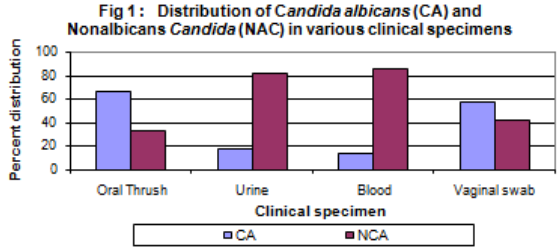
Table 2: Candida species isolated from different clinical samples (overall percent distribution of different Candida spp.)

Source of Clinical isolates	Oral thrush	Urine	Blood	Vaginal swab	Total	Overall % Distribution
C. albicans	32	06	02	08	48	43.63
C. glabrata	06	04	02	00	12	10.90
C. tropicalis	04	08	02	02	16	14.54
C. parapsilosis	02	06	02	02	12	10.90
C. pseudotropicalis	02	04	02	00	08	7.27
C. krusei	02	00	04	00	06	5.45
C. guilliermondii	00	02	00	02	04	3.63
C. stellatoidea	00	04	00	00	04	3.63

Table 3: Percent Distribution of various Candida species in different clinical samples

Organism	Clinical sample			
	Oral thrush	Urine	Blood	Vaginal swab
C. albicans	66.66 %	17.64 %	14.28 %	57.14 %
C. glabrata	12.50 %	11.76 %	14.28 %	-
C. tropicalis	8.33 %	23.52 %	14.28 %	14.28 %
C. parapsilosis	4.16 %	17.64 %	14.28 %	14.28 %
C. pseudotropicalis	4.16 %	11.76 %	14.28 %	-
C. krusei	4.16 %	-	28.57 %	-
C. guilliermondii	-	5.88 %	-	14.28 %
C. stellatoidea	-	11.76 %	-	-

Fig 1 : Distribution of *Candida albicans* (CA) and Nonalbicans *Candida* (NAC) in various clinical specimens



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