Original Article

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Microbiology Section

Candida tropicalis as a Predominant Isolate from Clinical Specimens and its Antifungal Susceptibility Pattern in a Tertiary Care Hospital in Southern India

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ABSTRACT

Background: The incidence of infections caused by *Candida* species has increased considerably over the past three decades mainly due to the rise of the AIDS epidemic, an increasingly aged population, higher numbers of immunocompromised patients and the more widespread use of indwelling medical devices. *Candida tropicalis* is emerging pathogenic yeast among non-albicans species. Recently drug-resistant *C.tropicalis* was also reported in hospitals.

Aim and Objective: The present study aimed to isolate and speciate *C. tropicalis* from various clinical samples and to determine its antifungal susceptibility profile.

Materials and Methods: Clinical samples such as urine, blood, exudates and vaginal swab which were submitted to the Microbiology laboratory during the year 2013 were screened for the growth of *Candidia* species, which then identified as *C.tropicalis* by the routine microbiological procedures such as germ tube formation, assimilation and fermentation of sugars and colony color on HICHROM *Candida* agar. Antifungal susceptibility was performed by disc diffusion method with the drugs Amphotericin-B, Itraconazole, Ketaconazole and Fluconazole on *C. tropicalis* isolates.

Results: A total number of 112 *Candida* isolates were isolated during the year 2012 from various clinical specimens. Among them 61 (54.3%) were identified as *C.tropicalis*. All the *C. tropicalis* isolates were sensitive to Amphotericin-B (100%) but 23 isolates (37.7%) were resistant to Fluconazole.

Conclusion: We conclude that identification of *Candida* species is important to know the prevalent species in the clinical setup and routine antifungal susceptibility should be performed to avoid inappropriate treatment.

Keywords: Chrom agar, Drug-resistance, Prevalence

INTRODUCTION

In last three decades there has been a significant increase in the incidence of fungal infections in humans [1] ranging from superficial infections involving skin, hair and nail to systemic infections affecting major organs [2]. Various factors like widespread use of immunosuppressive drugs, use of broad-spectrum antibiotics, low-dose maintenance therapy and invasive surgical procedures are accounted for the increase incidence of fungal diseases [3,4]. Among the fungal infection in human beings, *Candidia*l infections are predominantly reported. More than 200 species of *Candida* have been described, but approximately 90% of human invasive fungal infections are caused by only five species: *Candida albicans*, *Candida tropicalis*, *Candida* parapsilosis, *Candida glabrata* and *Candida krusei* [5]. Although *Candida albicans* remains the most common *Candida* species encountered, the morbidity and mortality caused bynon-albicans *Candida* (NAC) species are increasing [6].

C. tropicalis is now considered an important emerging fungal pathogen as it is associated with high mortality rate [7]. It is one of the most common *Candida* species that causes disease in humans, especially in tropical climates and it is responsible for 3 to 66% of cases of candidemia, depending on the geographic region [8].

C.tropicalis bloodstream infection accounts for 40 to 70% mortality [9], and these rates may vary depending on various other co-morbid conditions like leukemia, neutropenia and factors like central venous catheters and parental nutrition. In India, *C.tropicalis* is the most common cause of nosocomial *Candida*emia. Epidemiological data from the Indian subcontinent showed that 67–90% of nosocomial *Candida*emia cases were due to NAC species of which *C.tropicalis* was the most dominant [10].

Superficial mucosal *C.tropicalis* infections include oral thrush and vulvovaginitis. Meningeal, articular, osteomyelitis, pneumonia, endocarditis and pericardial infections has been reported. Urinary tract infections with *C.tropicalis* occur in patients with indwelling catheters, structural genitourinary abnormalities, diabetes mellitus and antibiotic therapy. *C. tropicalis* suppurative thrombophlebitis is related to intravenous therapy [11]. The increased isolation of this species from various *Candida* infections and emergence of azole resistance [12] necessitated the study. So, this study aims at determining the prevalence and antifungal susceptibility pattern of *C. tropicalis* from various clinical specimens in our clinical setup or hospital.

MATERIALS AND METHODS

This study was done during a period of one year, from January 2013 to December 2013 in a teaching hospital, which is located at Chennai.The study was conducted after obtaining the ethical committee's clearance and the university scientific review board's approval. Samples such as urine, exudates, blood and vaginal swab were collected under aseptic techniques in a sterile container and transported immediately to laboratory. Urine samples were collected after giving proper instructions to patients and catheterized urine samples were collected using sterile syringe from the port of urine catheter.

The samples such as urine,exudates and vaginal swab were subjected to wet mount and Gram stain and inoculated on Sabouraud's Dextrose Agar with Actidione slope. Blood samples were first inoculated in Brain heart infusion broth then after 48 hours it was subcultured on Sabouraud's Dextrose Agar with Actidione slope. The inoculated media were incubated at 37ºC for 24-48

hours. The isolates were considered significant by correlation with Gram stain, growth of two consecutive cultures and the clinical presentation. The suspected *Candida* colonies were processed further for their species level identification.

Identification of *Candida* species: The isolates had been identified by gram stain were further speciated by a panel of tests like germ tube test, morphology on corn meal agar, carbohydrate fermentation and assimilation tests [13].

Carbohydrate assimilation tests: The *Candida* species to be tested was freshly grown on a carbohydrate free medium like nutrient agar. A suspension of the isolate in saline or distilled water was prepared.The surface of the agar plate was inoculated by rotating a swab soaked in suspension and the agar surface was allowed to dry. With a sterile forceps carbohydrate discs (filter paper discs impregnated with a drop of test sugar) were placed on the surface of the medium. The sugars used in this test were Glucose, Sucrose, Lactose, Maltose, Galactose, Cellibiose and Trehalose. The plate was incubated at 30ºC for 24-48 hours. After incubation, the plate was removed and observed. A positive result is indicated by the presence of growth surrounding the carbohydrate discs. The carbohydrates assimilated by the *Candida* species were noted and the species identification was done by comparing with the standard chart [14].

Carbohydrate fermentation tests: Overnight incubated isolates in nutrient agar was inoculated in the individual sugar tubes and incubated at 37ºC for 10 days. The sugars used for the study were Dextrose, Lactose, Sucrose, Galactose, Trehalose and Maltose. Individual sugars are prepared with Yeast extract, peptone and Bromothymol blue as indicator. Acid or acid and gas production was noted in each tube and accordingly speciation was done [15]. *C.tropicalis* ferments with gas production in dextrose, sucrose, maltose, galactose, and trehalose but does not ferment lactose.

HiCrome *Candida* differential agar M1456A was obtained commercially from Hi Media, Mumbai, India. As this agar is intended to differentiate the *Candida spp* by producing different pigment and colony morphology it is used in our study [16]. It identifies steel blue to blue as *C. tropicalis*, light green colonies as C. albicans, cream to white as *C. glabarata* and purple fuzzy colonies as *C. krusei*. The medium was prepared as per the instructions given by the manufacturer and the colonies from SDA were subcultured on it and observed for the colony appearance and pigment production.

Antifungal susceptibility testing was performed by disc diffusion method on Yeast Nitrogen Base Glucose Agar (YNBGA). A lawn culture was made with the medium and commercially available antifungal disks (Himedia) Amphotericin B, Ketaconazole, Itraconazole, and Fluconazole (10µl) were placed and incubated at 35ºC for 48 hours [17].

RESULTS

A total number of 238 *Candida* isolates were isolated during the year 2013 (Jan to Dec). Among them 112 non-repeat isolates of *Candida* were included in the study after confirming the growth in the second sample. A total of 61 *C.tropicalis* was isolated from various clinical specimens. [Table/Fig-1] show the clinical specimen wise distribution of *C. tropicalis*. Majority of the isolates were obtained from urine samples 39 (63.93%) followed by vaginal swabs 12 (19.67%). Among the 61 isolates 43 (70.5%) were from critically ill patients admitted in ICU and 18(29.5%) from non-ICU patients. Out of 39 *C. tropicalis* isolated from urine samples; 35 isolates were from catheterized patients and 4 from non-catheterized patients. Other isolates apart from *C.tropicalis* were speciated and the results as follows: *C.albicans* 42 (37.5%). *C. parapsilosis* 6 (5.4%) and the least common isolate was *C.glabrata* 3 (2.7%). All the *C.tropicalis* isolates formed blue colour colonies on CHROM agar medium.The antifungal susceptibility testing for *C.tropicalis* was performed by disc diffusion method with the drugs, Amphotericin-B, Itraconazole,

Samples	Urine	Vaginal Swab	Exudates	Blood	Total
No of *C.tropicalis isolates*	39(63.93%)	12(19.67%)	04(6.55%)	06(9.83%)	61

[Table/Fig-1]: Distribution of C tropicalis in various specimens

Number of *C.tropicalis* Isolates	Amphotericin -B		Itraconazole		Ketoconazole		Fluconazole	
	S	R	S	R	S	R	S	R
61	61 (100%)	–	45 (73.8%)	16 (26.2%)	46 (75.4%)	15 (24.6%)	38 (62.3%)	23 (37.7%)

[Table/Fig-2]: Sensitivity and resistance pattern of *C.tropicalis* for Antifungal drugs
*The values represented were statistically significant (p<0.05) at 95% CI

Ketaconazole and Fluconazole. All the *C. tropicalis* isolates were sensitive to Amphotericin-B (100%) but 26 isolates (37.7%) were resistant to Fluconazole. [Table/Fig-2] show the antifungal susceptibility pattern of *C.tropicalis*.

DISCUSSION

*Candidia*sis is defined as infections caused by *Candida* species. It is considered as commensals in healthy individual and its capacity to produce superficial or systemic infections depends on the host immune system and various risk factors [18].

In recent years there is remarkable increase in the opportunistic pathogen *Candida* which causes life threating infections in immunocompromised patients [19]. HIV infection, the new world pandemic provided a potent soil for *Candidial* infection. Modern medical procedures of various kinds contribute to the risk factors for developing *Candidia*sis. The early identification and antifungal susceptibility testing is necessary for decrease of mortality rate.

In the present study 112 non-repeat isolates of *Candida* were speciated. *C.tropicalis* was the predominant isolate from clinical samples 61 (54.5%); similar observation was also reported by other researchers or studies [20,21]. Out of 39 urine isolates 35 (89.7%) patients had urinary catheterization as a risk factor. Kobayashi CC et al., in their study observed that one of the principle risk factors in patients with candiduria was urinary catheterization (38.4%) [22]. Another multicentre surveillance study had documented the removal of catheter has cleared funguria in 31.3% of their patients. So, all these studies favour urinary catheterization as one of the risk factor in candiduria [23]. Our observation of higher percentage (70.5%) of *C.tropicalis* isolation in ICU could be due to previous history of treatment with broad spectrum antibiotics, or total parenteral nutrition or cytotoxic chemotherapy and other co-morbid conditions such as was diabetes mellitus and malignancy.

Next to urine, 19.67% of *C.tropicalis* was isolated from vaginal swab. Similar observation was made by another study where the isolation of *C.tropicalis* from vaginal swab was the second to urine specimen. Studies have identified pregnancy; uncontrolled diabetes and practise of low dosage azole in the treatment of various *Candida* infections were predisposing factors for *C. Tropicalis* vulvovaginitis [20].

We identified 6(9.83%) cases of *C.tropicalis* fungemia in ICU patients. *C.tropicalis*is responsible for 3 to 66% of cases of candidemia, depending on the geographic region and mortality rates of 40 to 70% have been associated with the presence of *C. tropicalis* in the bloodstream. ICU stay, total parenteral nutrition (TPN), prior exposure to fluconazole, and diabetes mellitus were the major risk factors for candidemia [7]. Pathogenesis of *Candidia*sis depends on expression of various virulence factors like adhesins, phenotypic switching, thigomotropism and hydrolytic enzymes [24]. Above all, expression of these virulence factors may vary depending on the infecting species, geographical origin, type of infection, the site and stage of infection, and host immune response [20].

Some studies showed that *C. tropicalis*is even more invasive than C. albicans in the human intestine, particularly in oncology patients and they express increased virulence factors in immunocompromised

patients [25]. Various virulence factors [26] are responsible for forming biofilm by *Candida* spp. and detection of these virulence factors remains a key indicator of possible haematogenous infection.

C.tropicalis has its ability to express variety of virulence factors [20] indicating its capacity to contribute to the pathogenesis and produce invasive infections. Furthermore, *C. tropicalis* causes invasive disease in neonatal intensive care units (ICUs) through cross-contamination and has a slight tendency to progress from colonization to infection [6].

C.tropicalis is being increasingly isolated from human disease and is associated with invasive infection it remains a major concern because of its ability to develop rapid resistance to fluconazole. Resistance to antifungal agents has represented a major challenge for the clinic and a major public health problem. In our study, all the *C. tropicalis* isolates were sensitive to amphotericin-B (100%) but 23 isolates (37.7%) were resistant to fluconazole. When compared with itraconazole and ketaconazole, the fluconazole had shown higher degree of resistance. Similar observation was made by another study in India [27] where fluconazole resistance was high (38.8%) when compared to ketoconazole (28.2%) and itraconazole (32.9%). As determined by other study [28], we also observed maximum resistance to fluconazole. Resistance to azole by *C. tropicalis* is mediated by up-regulation and mutations of ERG11 gene [6]. The increasing rate of fluconazole resistance in *C.tropicalis* remains a major concern in the management of its infection. All the *C.tropicalis* isolates were sensitive to amphotericin; its use is limited in the treatment because of various side effects including renal toxicity [27].

CONCLUSION

C. tropicalis, cannot be overlooked as contaminants or non-pathogenic commensals. It remains an important pathogen, especially in ICU patients. Studies have shown increased prevalence and high incidence of drug resistance in *C. tropicalis*, as well as increased morbidity and mortality caused by this pathogen in hospitalized individuals, identification and susceptibility tests should be performed on all yeast isolates for appropriate therapy and it will potentially reduce the risk of drug-resistant life-threatening infections and nosocomial infections.

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