Original Article

"TO STUDY THE SPECTRUM OF CANDIDA SPECIES IN CLINICAL SPECIMENS AT A TERTIARY CARE CENTER IN KANPUR"

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ABSTRACT:

Candida species causes opportunistic endogenous infections mostly in hospitalised patients. This study aimed to isolate different candida species from clinical sample. **Method:** This prospective study was conducted in Microbiology Department over a period of 1 year(2016 Jan to 2016 Dec). Total 75 candida sp. were isolated from 400 clinical specimens on routine culture medium Blood agar and Mac-conkey agar, isolated colonies on were further subcultured on hichrome candida differential media for identificationof candida Germ tube test and corn meal media were also used for identification.Antifungal susceptibility were tested by disk diffusion method. Previous history and clinical finding was also recorded. **Result:**75 candida isolates from 400 clinical samples, the prevalence rate is 18.75%.Of the 75 Candida isolates,32%(24 out of 75) were C. albicans result 68%(51 out of 75) were non candida albicans species. Non candida albicans includes C. tropicalis 26.6%(20 out of 75) followed by C. dublinensis 21.3%(16 out of 75).C. krusei 16% (12 out of 75) and C.glabrata 4%(3 out of 75).Antifungal susceptibility testing revealed that 62% candida albicans were resitant to fluconazole. None of the isolate showed resistance towards Amphoterecin B. & Ketoconazol).Non – candida albicans also showed resistance towards fluconazole. **Conclusion**: This study concluded that non candida albicans mainly C.dubliensis and C.tropicalis was the may cause of candidiasis. The intrinsic resistance of non candida albicans cause problem and complication in patients care and treatment. Hence, regular monitoring of Antifungal susceptibility is a must in a given area to decrease resistance.

Keywords: Candida Species, Antifungal susceptibility.

INTRODUCTION

C*andida* spp. are the normal flora of human skin and mucosa, but have been reported more frequently as pathogen due to risk factors suc asexcessiveconsumption of a broad spectrum of antibiotics, underlying malignant diseases, HIV infection, organ transplantation, prolonged hospital stay, and exposure to invasive procedures [1, 2]. *Candida* species are the most common cause of fungal infections worldwide. The genus *Candida* comprises about 200 species, of which close to 20 can cause serious infections in humans and are now recognised as major agent of hospital acquired (nosocomial) infections i.e. 8–10% of all nosocomial infections.[3,4] *Candida albicans* is generally considered the major pathogen among the *Candida* species. An increase in the prevalence of non-*C.albicans* species has been emerged during the lastdecade. Besides *C. albicans*, the most prevalent non-*C. albicans* species are *C. tropicalis,C. parapsilosis, C. glabrata* and *C. krusei*[5,6]. The extensive use of antimycotic drugs for prolonged therapeutic courses has led to changes in the relative prevalence of various *Candida* species, with a decrease in the proportion of *C. albicans* as the etiological agent of candidiasis and an increase in the proportion of non-*C. albicans* species[5].

In recent years, there has been marked increase in the incidence of treatment failures in candidiasis patients receiving long-term antifungal therapy, which has posed a serious problem in its successful use in chemotherapy. Candida cells acquire multi drug resistance (MDR) during the course of treatment. These infections may be confined superficially to the mucosa, but in immunocompromised hosts they can develop systemic infections and ultimately lead to life-threatening situations[6]. Although antifungal therapies are available and recent epidemiological studies reveal a stable or a slightly decreasing trend of systemic infections, Candidiasis is still a serious medical problem[7,8]. Therefore, efforts have been made during recent years on accurate diagnosis and antifungal susceptibility to improve the clinical outcome. This present study was conducted in a tertiary carehospital to determine the prevalence of *Candida* speciesand its antifungal profile from various clinical samples received in the Department of Microbiology.

MATERIALS & METHODS

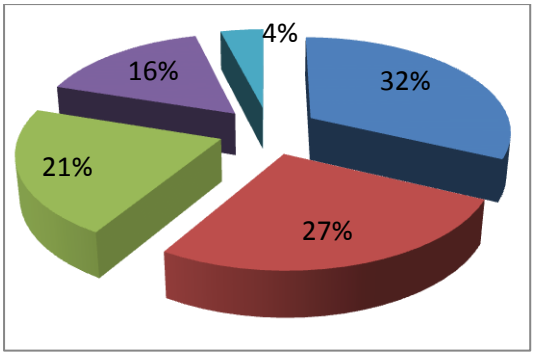
This prospective study was conducted in Microbiology Department over a period of 1 year(2016 Jan to 2016 Dec). A total of 75 clinical specimens from 400 patients suffering from mucocutaneous and systemic candidiasis, were collected i.e. sputum, sterile body fluids, pus, urine, blood, corneal

scrapping, nail clipping, vaginal swabs and plastic devices (endotracheal tube, catheter tip, suction tip) etc. from various fungal infections. Laboratory diagnosis of candidiasis was performed by (i) direct examination of clinical specimen to demonstrate fungal presence and (ii) isolation of the *Candida* inculture, its identification and antifungal susceptibility. Direct microscopic examination was done by 10% KOHmount and gram staining, which reveals the presence ofbudding yeasts, pseudohyphae, and/or hyphae. *Candida*isolation and identification were done by standard conventionalmethods i.e. inoculation of samples on SDAmedia and incubating for 48–72 h, on which most clinicallyrelevant yeast species grow within 48 hrs.Speciation of *Candida* isolates were done by germtube test to identify the albicans group and throughcorn meal agar morphology (CMA) by doing Dalmautechnique[7,8.] Further speciation of *Candida* species weredone on CHROM agar (Hi Media Pvt. Ltd, Mumbai) andplates were incubated at 37°C for 24–48 hours[7.] Colour of the colonies was noted down and the presumptive identification of the species was done as per colour code provided by the manufacturer and by referring to different literatures (Fig. 3). Biochemical tests like sugar assimilation and fermentation tests were also done[9].Antifungal susceptibility testing of all the yeast isolates was assessed using the agar disc diffusion method as per CLSI guidelines[10] The antifungal discs and Mueller-Hintonagar were procured from Hi-Media, Mumbai. Mueller-Hinton agar used for sensitivity testing was supplementedwith 2% glucose and 0.5 µg/ml methylene blue[10].

In the present study, the antifungal agents used for disc diffusion method were Amphotericin B(100 IU), Fluconazole (25 µg), Ketoconazole (15 µg),Clotrimazole (10 µg), Voriconazole (1 µg) and Nystatin(50 µg). To determine whether the isolates were susceptible,intermediate resistance or resistance against theabove antifungals; zone diameters were compared withthe standard zone interpretive break points published byCLSI (M44-A2) guidelines[9,10,11,12].

RESULTS

75 candida isolates from 400 clinical samples, the prevalence rate is 18.75%.Of candida species 32%(24 out of 75) were C. albicans result 68%(51 out of 75) were non candida albicans. Non candida albicans includes C. dubliensis 26.6%(20 out of 75) followed by C. tropicalis 21.3%(16 out of 75).C. krusei 16% (12 out of 75) and C.glabrata 4%(3 out of 75)**[FIG-1]**. Antifungal susceptibility testing revealed that 62% candida albicans were resitant to fluconazole**[FIG-2]**. None of the isolate showed resistance towards Amphoterecin B. & Ketoconazole). Non–candida albicans also showed resistance towards fluconazole.



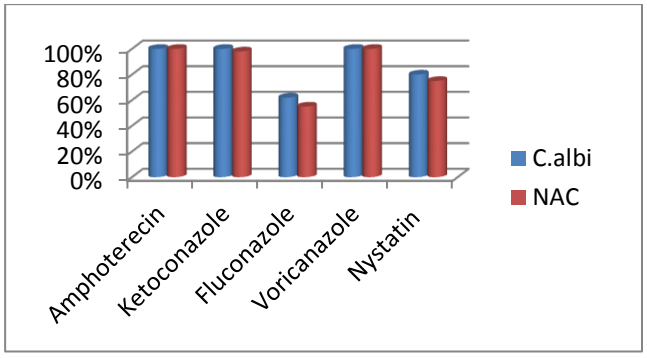


Fig 1: Distribution of candida species **Fig 2: Antifungal susceptibility of C.albicans and non albicans Candida**

DISCUSSION

Significant increased incidence of fungal infections contributes to morbidity and mortality. In the present study it was found that Candidiasis occurred at all ages and in both sexes, and females are more commonly affected than males with incidence of 60% and 40%, respectively. In a similar study by Khandari et al at AIIMS New Delhi, the incidencein females was about 61.2% while in males it was only38.8%[13]. The possible reason being females are infected with the genital candidiasis during the reproductiveperiod. During pregnancy, levels of both progesterone and estrogen hormones are elevated. Progesterone hassuppressive effects on the anti-*Candida* activity of neutrophils, while estrogen has been found to reduce the ability of vaginal epithelial cells, inhibit the growth of *Candida* species and also decrease immunoglobulins in vaginal secretion resulting in increased vulnerability of pregnant women to vaginal candidiasis [14]. The majority of the patients were in the age group of 20–40 years. According to study conducted by Dalal & Kelkar and Puri et al., maximum cases were in the age group of 21–40[15,16,17]. A total of 75 *Candida* isolates from various clinical samples, sputum showed the highest number of isolates(37%), followed by body fluid (33%), urine(27%) and blood (3%). *Candida* is a normal inhabitant

of the mouth and can be recovered from sputumin 20% to 55% of normal subjects[16]. The prevalence and prognosis of pulmonary *Candidal* infections has been difficult to evaluate since diagnosis were seldom confirmed, *Candida* isolated from sputum samples is mostly a coloniser of respiratory tract. The role of *Candida* in pulmonary Candidiasis and its diagnosis is still controversial. Possibility of colonisation cannot be ruled out because sometimes they are associated with other pathogens. To confirm *Candida* infection of respiratory tract at least more than one sputum sample was takenand no other established pathogen was detected.

Studies over the years have shown that there is a consider able increase in the non-*C. albicans* (NAC) species. In the present study we found out that NAC (68%) were frequently encountered than *C. albicans* (32%),which was in agreement with the findings by Shivanand& Saldanha, Ragini et al., Manchanda et al. and Vijayaet al., who also showed the non-*C. albicans* incidence to behigher than

that of *C. albicans[*17–21]. The predominant NACisolated in our tertiary care centre was *C. tropicalis*. Thiswas in agreement with the studies conducted by Ragini et al., Chakrabarthi et al. and Agarwal et al.[18,21,22]. These findings are suggestive of non-*C. albicans* are emerging asimportant pathogens and major threat for future.

In the present study we identified 75 *Candida* speciesusing CHROM agar which is a selective and differentialmethod for direct identification and isolation.This medium relies on the ability of different *Candida*species to form pigmented colonies due to the breakdownof substrates by enzymes of the yeasts resultingin the change of colour. A major advantage is the abilityto detect mixed cultures of yeast in clinical specimens.Hence CHROM agar would be a useful, cost effectivetool for rapid identification of various *Candida* speciesdirectly from clinical samples [23,24]

The *in vitro* susceptibility testing of antifungal agentsis becoming increasingly important because of theintroduction of new antifungal agents and recoveryof clinical isolates that exhibit inherent or developedresistance to Amphotericin B, the Azole group of drugsduring chemotherapy. In the present study, antifungalsusceptibility testing was done for 75 *Candida* isolatesby disc diffusion method. *C. albicans* isolates were 100%sensitive to Voriconazole, Ketoconazole and Amphotericin Band Nystatin; 62% resistance to Fluconazole. The *C. tropicalis* isolates were 100% susceptible to Voriconazole,Ketoconazole and Nystatin, but 2.9% and 6% resistanceto Fluconazole respectively, while 3% resistance and 3% intermediate sensitive to AmphotericinB.

The *C. krusei* isolates were 100% susceptible to Amphotericin B, Clotrimazole, Voriconazole,Ketoconazole and Nystatin while it showed 100% resistanceto Fluconazole. The findings of the present studycorrelate with those of a study by Lee et al., in which *C.krusei* showed 100% resistance to Fluconazole[26]. Fungalinfections are increasing in western part of India [27–29]. The present study showed that along with *C. albicans*, non-*C.albicans* species are increasingly being isolated from clinicalspecimens. So it is essential to identify all yeasts isolates up to species level and their antifungal susceptibility.It will speed up specific therapy, reduces morbidity andmortality in patients infected with *Candida*. As epidemiologyof Candidaemia differs markedly, the study promotethat it is important for every setting to speciate and performthe antifungal susceptibility testing.

CONCLUSION

This study concluded that non candida albicans mainly C.dubliensis and C.tropicalis was the may cause of candidiasis. The intrinsic resistance of non candida albicans cause problem and complication in patients care and treatment. Hence, regular monitoring of Antifungal susceptibility is a must in a given area to decrease resistance.

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