

ORIGINAL ARTICLE

PREVALENCE OF CANDIDA INFECTION AND ITS ANTIFUNGAL SUSCEPTIBILITY PATTERN IN TERTIARY CARE HOSPITAL, AHMEDABAD

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ABSTRACT

Introduction: In the past three decades with the use of potent antibacterial immunosuppressive and cytotoxic drugs, lethal invasive candidiasis has been described with increasing frequency. Patients admitted at tertiary care hospitals have access to very intensive management modalities. This, along with increasing number of immune-compromised patients, has led to rise in infections caused by candida especially by NCA (Non Candida Albicans).

Methodology: Duration of the study was from 1st July- 2011 to 30th June 2012. Candida species isolated from various clinical specimens were subjected to speciation using standard yeast identification protocol and CHROM agar. Antifungal susceptibility testing was done by the disc diffusion method against Amphotericin B and Azole group of antifungals like Fluconazole, Itraconazole, Clotrimazole and Voriconazole.

Results: Among the 430 culture positive isolates 161(37.4%) were *C. albicans* and 269 (62.6%) were non candida albicans. Among NCA, 176(40.9%) were *C. tropicalis* followed by other species. Susceptibility pattern showed that Azole group 25.5% sensitive among *C. albicans* and 18.7% sensitive among *C. tropicalis* while in Amphotericin B sensitivity varies from 75.6% to 100% to all isolated spp. of candida.

Conclusion: In this study *C. tropicalis* was the most common yeast isolated from all the clinical samples. The *C. albicans* and NCA showed highly susceptible to Amphotericin B, followed by Voriconazole & Clotrimazole, is the drug of choice.

Key Words: Candida speciation, Chrom Agar, Non-Candida albicans, Antifungal susceptibility testing.

INTRODUCTION

Patients admitted at tertiary care hospitals have access to very intensive management modalities. This along with increasing number of immune-compromised patients have led to rise in infections caused by candida especially by Non Candida Albicans^{1, 2, 3}. Candidemia is found mainly in individuals with some immunocompromised condition¹. Due to variable clinical presentation of candida infections, it becomes very important to identify this pathogens from all the clinical specimens received at laboratory irrespective of clinician's suspicion. Candida species differ in their antifungal susceptibility and virulence factors^{1, 3}. Thus identification of candida up to species level along with antifungal susceptibility becomes very essential. *C. krusei* and *C. glabrata* are known for their innate resistance to fluconazole¹. Recent studies show an increase in the number of cases resulting from infections with non-candida albicans (NCA) species and an increase in antifungal resistance^{2, 3}. In the past

three decades with the use of potent antibacterial immunosuppressive and cytotoxic drugs, lethal invasive candidiasis has been described with increasing frequency⁴. Predisposing factors for candida infection are: prolonged use of antimicrobial agents, immunocompromised status, chemotherapy, catheterization^{3, 7, 8}. The accurate species identification of Candida is important for the treatment, as not all species respond to the same treatment because of the problem of anti-fungal resistance⁹. The aim of the present study was to know the prevalence of candida spp. at our hospital, to isolate and speciate candida spp. from various clinical specimens, to detect their antifungal susceptibility pattern.

MATERIALS AND METHODS

A total of 430 candida isolates from various Clinical specimens (blood, urine, sputum, pus, swab, lung aspirate, catheter tip, ET fluid, IJV tip, Nail, Throat

swab, vaginal swab etc. were taken up for the study. Duration of the study was from 1st July- 2011 to 30th June 2012. The various clinical samples were collected and processed as per the standard microbiological procedures. They were screened for budding yeast like cells with the help of Gram stain, 10% KOH, and culture on Sabourad's Dextrose Agar. The candida isolates which were obtained were further speciated by the germ tube test, chlamyospore formation on corn meal agar and inoculation on chromogenic medium¹.¹⁰. The chromogenic medium, HiMedia CHROM agar, has chromogenic substances which helps in the rapid identification of the candida species, based on the reactions between the specific enzymes of the different species and the chromogenic substances. As per the colour code which is provided with the chromogenic media, *C. albicans* produces blue-green colonies, *C. tropicalis* produces dark blue-blue grey colonies, *C. guilliermondii* produces bluish pink colonies, *C. parapsilosis* produces creamish to pink, *C. kefyri* produces creamish, *C. glabrata* produces pink to mauve and *T. beigelii* produces light blue on observe and dark

blue on reverse. The isolates were also identified on the basis of microscopic morphological features of the growth obtained through slide culture on CMA (Corn Meal Agar) by doing Dalmau technique.¹¹ Also sugar assimilation test were done. In sabourad Dextrose Broth (SDB), *C. tropicalis* shows surface pellicle and bubbling while no change was observed in other species. Antifungal susceptibility test was done using the National Committee for Clinical Laboratory Standards 2011, method for antifungal disc diffusion susceptibility for yeasts with approved guideline M44-A¹². We used the following antifungal discs: Fluconazole (25mcg), Itraconazole (10mcg), Clotrimazole (10mcg), Voriconazole (1mcg), Amphotericin B (100 units) and zone size was measured as for the instruction manual (HiMedia).

RESULTS

Table -1 shows Candida spp isolated in routine clinical specimens were received during 1st July-2011 to 31st June -2012 at our department.

Table 1: Candida species isolated from clinical samples tested

Candida isolate	Blood(%)	Urine(%)	Sputum(%)	Swab(%)	Pus(%)	Fluids(%)	Others(%)	Total(%)
<i>C. tropicalis</i>	25(5.8)	80(18.6)	40(9.3)	15(3.5)	4(0.9)	–	12(2.8)	176(40.9)
<i>C. albicans</i>	11(2.6)	42(9.8)	84(19.6)	16(3.7)	4(0.9)	–	4(0.9)	161(37.4)
<i>C. guilliermondii</i>	64(14.9)	2(0.5)	–	2(0.5)	–	–	2(0.5)	70(16.3)
<i>C. parapsilosis</i>	9(2.1)	3(0.7)	–	1(0.2)	–	1(0.2)	–	14(3.3)
<i>C. Kefyr</i>	2(0.5)	1(0.2)	–	1(0.2)	1(0.2)	–	–	5(1.2)
<i>C. glabrata</i>	1(0.2)	1(0.2)	–	–	–	–	–	2(0.5)
<i>T. beigelii</i>	–	2(0.5)	–	–	–	–	–	2(0.5)
Total	112(26)	131(30.5)	124(28.9)	35(8.1)	9(2.1)	1(0.2)	18(4.2)	430

Table 2: Isolated Candida spp. and its antifungal sensitivity patterns

Candida Species	Isolates (n= 430 (%))	Sensitive against Azole group	Sensitive against Ampho. B
<i>C. tropicalis</i>	176(40.9%)	33(18.7%)	133(75.6%)
<i>C. albicans</i>	161(37.4%)	41(25.5%)	143(88.8%)
<i>C. guilliermondii</i>	70(16.3%)	63(90%)	69(99%)
<i>C. parapsilosis</i>	14(3.3%)	11(78.6%)	14(100%)
<i>C. kefyri</i>	5(1.2%)	3(60%)	5(100%)
<i>C. glabrata</i>	2(0.5%)	2(100%)	1(50%)
<i>Trichosporon beigelii</i>	2(0.5%)	2(100%)	2(100%)

Table -2 shows, 430(2.1%) candida spp. were isolated from received clinical specimens along with its antifungal sensitivity among the Azole group & Ampho. B. From that, *C. tropicalis* was the most frequently isolated species, accounting for 40.9% followed by *C. albicans* (37.4%), *C. guilliermondii* (16.3%), *C. parapsilosis* (3.3%), *C. kefyri* (1.2%) and *C. glabrata* (0.5%) and *T. beigelii* (0.5%) respectively. All isolated candida spp. were 75% to 100% sensitivity among the Amphotericin B while NCA were 75% to 100% sensitive among the Azole group except *C. tropicalis* showed 18.7% & *C. albicans* 25.5%. In our study, candidiasis was highest in 21-40 years age group,

particularly in adult(66%) while in neonates (24%) and in paediatrics (10%) to be noted.

DISCUSSION

A total of 430 Candida isolates from various clinical specimens were included in our study, of which urine showed the highest number of isolates (30.5%), followed by sputum (28.9%) and blood (26%). Studies which were done earlier by Pfaller *et al*³, have reported Candida species as the seventh most common nosocomial pathogen hospital wide and as that which caused 25% of all the urinary tract infections.

The present study had a male preponderance, with an overall male: female ratio being 2:1, observed that male sex is a risk factor for developing candidemia¹⁴. Though candidiasis can occur at all ages, studies by Dalal PJ and Kelkar SS¹⁵ at Mumbai showed the highest incidence of candidiasis to be in the age group of 21-40 years. These findings were in concurrence with those of our study.

Comparative studies on different *Candida* species in V. Manchanda³, showed that *C.tropicalis* (55.03%) was higher while it was 47.4% in our study. Another study Kashid RA et al¹⁴ showed *C. tropicalis* (46.25%), which was correlated with our study.

Studies over the years have shown that there is a considerable increase in the NCA isolates. In the present study we observed that NCA (62.6%) are frequently encountered than *Candida albicans* (37.5%), which was in agreement with the findings of the studies by Ragini Ananth Kashid, Sandhya Bellawadi, Gaytri Devi, & Indumati, et al¹⁴, who also showed the non-candida albicans incidence (70.7%) to be higher than that of *C. albicans* (29.2%). A study by V.Manchanda³ also showed non- *candida albicans* (72.4%) to have a higher incidence than *C.albicans* (27.5%). A study by Vijaya D et al¹⁶ showed NCA (54.1%) to have a higher incidence than *C. albicans* (45.9%). These findings seem to suggest that NCA are emerging as important pathogens. The in vitro susceptibility testing of antifungal agents is becoming increasingly important because of the introduction of new antifungal agents and the recovery of clinical isolates that exhibit inherent or developed resistance to Amphotericin B, the Azole group of drugs during chemotherapy. Antifungal Susceptibility Testing was done for 430 *Candida* isolates by Disc Diffusion Method. The *C.tropicalis* isolates were 75.6% sensitive to Amphotericin B and showed 18.7% sensitive to Azole group of drugs. The *C.albicans* isolates were 88.8% sensitive to Amphotericin B, & showed 25.5% sensitive to Azole group of drugs. The *C.guilliermondii* isolates were 98.6% sensitive to Amphotericin B, & showed 90% sensitive to Azole group of drugs. The *C.kefyr* isolates were 100% sensitive to Amphotericin B and showed 60% sensitive to Azole group of drugs. The *C.parapsilosis* isolates were 100% sensitive to Amphotericin B and showed 78.6% sensitive to Azole group of drugs. The *C.glabrata* were 50 % sensitive to Voriconazole & Clotrimazole while Fluconazole & Itraconazole were developed inherent resistance & 100% sensitive to Amphotericin B. *T.beigelii* shows 100% susceptible to Amphotericin B & 100% susceptible to Azole drugs. So, in our study we consider *T. beigelii* as commensals from urinary tract. The findings of the present study correlated with those of study done by Vijaya D, et al¹⁶ in which showed *C.albicans* & NCA have 100% sensitivity to Amphotericin B while Azole group of drugs were used in second choice. Also the finding was correlated with those of a study done by Shivanand Dharwad, Saldanha Dominic R.M.⁹, in which *C.tropicalis* were 87.5%

susceptible to Amphotericin B, Itraconazole & Voriconazole while showed 25% resistance to Fluconazole. In *C.glabrata* showed 100% sensitive to Amphotericin B which was compared with present study.

To conclude, the present study showed that prevalence of Non *Candida Albicans* were higher from various clinical specimens. Therefore the species level identification by using of chrom agar medium will helpful to mycology laboratories for rapid identification of clinically important *candida* spp. More importantly this capability will also enable clinicians to choose appropriate antifungal agents, thus decreasing patient's morbidity and mortality.

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