

## ORIGINAL ARTICLE

# Study of Candida Species and its Antifungal Susceptibility Pattern in a Tertiary Care Hospital

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## ABSTRACT

**Introduction:** Opportunistic infections by Candida spp are becoming quite common in hospitals today with antifungal resistance, an increasing problem in many wards. Candida albicans has been the commonest species causing infection for many years but indiscriminate use of azole group of drugs has led to increase in NCA infection and resistance to antifungal drugs in Candida species

**Methodology:** Duration of the study was from 7<sup>th</sup> June- 2018 to 12<sup>th</sup> Mar 2019. Candida species isolated from various clinical specimens were subjected to speciation using standard yeast identification protocol and CHROM agar. Antifungal susceptibility testing was performed and interpreted for all the isolates of Candida using disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A document guidelines. The inoculum was prepared by suspending five colonies of growth in 5 ml of sterile saline and compared the turbidity to 0.5 McFarland Standard. A cotton swab was dipped into the inoculum suspension and evenly streaked onto Mueller–Hinton agar supplemented with 2% glucose and 5 µg/ml methylene blue against Amphotericin B and Azole group of antifungals like Fluconazole, Itraconazole, Clotrimazole and Voriconazole.

**Results:** Among the 268 culture positive isolates 152(56.7%) were C. albicans and 116 (43.2%) were non candida albicans. Among NCA, 53(19.7%) were C. tropicalis followed by other species. Susceptibility pattern showed that Azole group 73.0% sensitive among C. albicans and 67.9% sensitive among C. tropicalis while in Amphotericin B sensitivity varies from 50 % to 100% to all isolated spp. of candida.

**Conclusion:** In this study C.albicans 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.

**Key words:** Candida, antifungal, opportunistic infection, susceptibility

## INTRODUCTION

Opportunistic infections by Candida sp are becoming quite common in hospitals today with antifungal resistance, an increasing problem in many wards. With interventions and management of diseases becoming more invasive and complicated, patients' immunity can be affected due to various reasons. Apart from immunosuppression and immunodeficiencies from underlying causes such as chemotherapy, corticosteroids, surgery, malignancy, any manipulation, intervention or prolonged hospital stay increases the risk of infections due to Candida spp. and other fungal pathogens.<sup>1,2</sup>

In Hospital acquired infection Candidiasis is the one of the leading causes with mortality noted between 15-35%.<sup>3</sup> Candida albicans has been the commonest species causing infection for many years but discriminate use of azole group of drugs has lead to increase in NCA infection and resistance to antifungal drugs in candida species.<sup>4,5</sup>

The accurate species identification of Candida is important for the treatment, as not all species respond to the same antifungal drugs because of the problem of anti-fungal resistance.<sup>6</sup> 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 268 candida isolates from various Clinical specimens (blood, urine, sputum, pus, swab, ET fluid, vaginal swab etc. were taken up for the study. Duration of the study was from 7<sup>th</sup> June 2018 to 12<sup>th</sup> Mar 2019. 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 can-

didia isolates which were obtained were further speciated by the germ tube test, chlamyospore formation on cornmeal agar and inoculation on chromogenic medium.<sup>7,8</sup> 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. kefyr* produces creamish, *C. glabrata* produces pink to mauve and *C. krusei* produces purple colour colonies. The isolates were also identified on the basis of microscopic morphological features of the growth obtained on CMA (Corn Meal Agar) by doing Dalmau technique.<sup>9</sup> Also sugar assimilation test were done. In sabourad Dextrose Broth (SDB), *C. tropicalis* shows surface pellicle and bubbling while *C. albicans*, *C. Parapsilosis*, *C. glabarata* produces deposit and *C. krusei* produces side growth with surface pellicle. Antifungal susceptibility testing was performed and interpreted for all the isolates of Candida using disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI)

M44-A document guidelines.<sup>10,11</sup> The inoculum was prepared by suspending five colonies of growth in 5 ml of sterile saline and compared the turbidity to 0.5 McFarland Standard. A cotton swab was dipped into the inoculum suspension and evenly streaked onto Mueller–Hinton agar supplemented with 2% glucose and 5 µg/ml methylene blue.<sup>12,13</sup> against Amphotericin B and Azole group of antifungals like Fluconazole, Itraconazole, Clotrimazole and Voriconazole.

## RESULTS

Table -1 shows Candida spp isolated in routine clinical specimens were received during 7 th june- 2018 to 12<sup>th</sup> Mar 2019 at our department.

Table 2 shows, out of 268 candida spp., *C. albicans* was the most frequently isolated species, accounting for 56.7% followed by *C. Tropicalis* (19.7%), *C. krusei* (15.2%), *C. glabarata* (3.7%), *C. parapsilosis* (2.2%) and *C. gullermondii* (1.4%) and *C. kefyr* (0.7%) respectively. Table 2 shows all isolated candida spp. were 75% to 100% sensitivity to the Azole group except *C. tropicalis* showed 67.9% & *C. glabarata* showed 20% while NCA were 75% to 100% sensitive to the Amphotericin B except *C. glabarata* showed 60% & *C. parapsilosis* 50 %.

**Table 1: Candida spp. isolated from clinical specimens**

Candida isolate	Sputum (%)	Urine (%)	Blood (%)	Swab (%)	Pus (%)	ET (%)	V.swab (%)	Stool (%)	Total (%)
<i>C. albicans</i>	126(47)	16	4(1.4)	1(0.3)	-	1(0.3)	4(1.4)	-	152(56.7)
<i>C. tropicalis</i>	36(13.4)	8(2.9)	3(1.1)	2(0.7)	2(0.7)	1(0.3)	-	1(0.3)	53(19.7)
<i>C. krusei</i>	26(9.7)	10(3.7)	3(1.1)	-	-	2(0.7)	-	-	41(15.2)
<i>C. glabarata</i>	7(2.6)	3(1.1)	-	-	-	-	-	-	10(3.7)
<i>C. parapsilosis</i>	5(1.8)	1(0.3)	-	-	-	-	-	-	6(2.2)
<i>C. gullermondii</i>	4(1.4)	-	-	-	-	-	-	-	4(1.4)
<i>C. kefyr</i>	1(0.3)	1(0.3)	-	-	-	-	-	-	2(0.7)
Total	205(76.4)	39(14.5)	10(3.7)	3(1.1)	2(0.7)	4(1.4)	4(1.4)	1(0.3)	268

**Table 2: Isolated Candida spp. and its antifungal sensitivity pattern**

Candida species	Isolates(n=268)(%)	Sensitive to Ampho.B	Sensitive to Azole group
<i>C. albicans</i>	152(56.7%)	132(86.8%)	111(73%)
<i>C. tropicalis</i>	53(19.7%)	41(77.3%)	36(67.9%)
<i>C. krusei</i>	41(15.2%)	36(87.8%)	33(80.4%)
<i>C. glabarata</i>	10(3.7%)	6(60%)	2(20%)
<i>C. parapsilosis</i>	6(2.2%)	3(50%)	5(83.3%)
<i>C. gullermondii</i>	4(1.4%)	3(75%)	3(75%)
<i>C. kefyr</i>	2(0.75%)	2(100%)	2(100%)

## DISCUSSION

A total of 268 Candida isolates from various clinical specimens were included in our study, highest no. of candida isolates are from sputum specimen (76.4 %), followed by urine (14.5%). Studies which were done earlier Khadka et al. and Sunayana M . Jangla et al. have reported similar results.

Of the 268 candida isolates *C. albicans* was the predominant species (56.7%) followed by *C. Tropicalis* (19.7%), *C. krusei* (15.2%), *C. glabarata* (3.7%), *C. parapsilosis* (2.2%) and *C. gullermondii* (1.4%) and *C. kefyr* (0.7%) respectively. Our finding shows similar prevalence scenario of Candida species to the previous data reported by two independent groups from India which showed *C. albicans* is more preva-

lent among the *Candida* isolates.<sup>16,17</sup> Similar study conducted by Sajjan et al. also reported *C. albicans* as the major isolate. Among the NAC species, *C. tropicalis* was most prevalent followed by *C. krusei* and *C. glabrata* respectively.<sup>18</sup> Jayalakshmi et al. also showed that *C. tropicalis* (19.7%) was prevalent among the NCA species.<sup>19</sup> Similar result has been depicted in various studies conducted in different countries of Europe.<sup>20-22</sup> However, many studies have shown that NCA species have more isolation rate than *C. albicans* which suggest the emergence of non-*albicans* *Candida* species as important pathogens.<sup>23,24</sup>

Speciation of *Candida* species by CHROMagar on the basis of colour differentiation offered a rapid, convenient and reliable method for identification of clinically important *Candida* species when compared with cumbersome traditional techniques. In developing countries, CHROMagar can be taken as a simple phenotypic test alternative to molecular based assay. CHROMagar has high sensitivity as well as specificity for the identification of *Candida* species.<sup>25, 26</sup> According to various finding from our regions, these four species are more prevalent, so we chose this medium for isolation of *Candida* spp.<sup>17, 27</sup> It facilitates the detection and identification of *Candida* species from mixed culture and provides results within 24–48 h.

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. The *C. tropicalis* isolates were 77.3% sensitive to Amphotericin B and showed 67.9% sensitive to Azole group of drugs. The *C. albicans* isolates were 86.8% sensitive to Amphotericin B, & showed 73% sensitive to Azole group of drugs. The *C. krusei* isolates were 87.8% sensitive to Amphotericin B, & 80.4% to Azole group of drugs and *C. guilliermondii* isolates were 75% sensitive to Amphotericin B, & Azole group of drugs. The *C. kefyr* isolates were 100% sensitive to Amphotericin B and Azole group of drugs. The *C. parapsilosis* isolates were 50% sensitive to Amphotericin B and showed 83.3% sensitive to Azole group of drugs. The *C. glabrata* were 20 % sensitive to Azole group of drugs & 60% sensitive to Amphotericin B.

In our study we found that *C. albicans* was the predominant species responsible for various Candidal infections. the species level identification by using of chrom agar medium will helpful to mycology laboratories for rapid screening of clinically important *Candida* spp. More importantly this capability will also enable clinicians to choose appropriate antifungal agents, thus decreasing cost effect and patient's morbidity and mortality.

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