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Species Distribution and Drug Susceptibility of *Candida* Isolates from Various Clinical Specimens at a Tertiary Care Hospital in Kashmir

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Authors' contributions

This work was carried out in collaboration between all authors. Author AN designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors AN, FK and AF managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of our study was to identify the distribution of *Candida* species among clinical isolates and their sensitivity pattern for common antifungal drugs.
Study Design: Prospective observational study.
Place and Duration of Study: Department of Microbiology, Government Medical College Srinagar, Kashmir, India, from December 2014 to January 2016.
Methodology: Identification of hundred and five different *Candida* species as well as antifungal sensitivity testing was performed with Vitek2 Compact (Biomerieux France) using vitek 2 cards for identification of yeast and yeast like organisms (ID-YST cards).
Results: Among the 105 culture positive isolates, 35 (33.3%) were *C. albicans* and 70 (66.6%) were *Non Candida albicans* (NCA). Among NCA, 35 (50%) were *C. tropicalis* followed by other species. All the *Candida* isolates were sensitive to micafungin and capsofungin whereas the susceptibility pattern of amphotericin B varied from 75.6% to 100% and the highest rates of resistance were seen for fluconazole.

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Conclusions: Infections caused by *Candida* sp are on the rise. Hence accurate identification and susceptibility pattern is necessary for management of all Candida infections.

Keywords: Candida; antifungal susceptibility; Non Candida albicans; fluconazole; amphotrericin B.

1. INTRODUCTION

Candidiasis is the most common fungal disease in humans and includes infections of skin, nail, mucosa and internal organs of the body. The pathogenic species of the genus *Candida* which are commonly implicated in humans are *Candida albicans Candida tropicalis, Candida krusei, Candida glabrata, Candida guilliermondii* and *Candida parapsilosis* [1].

Candida causes acute, sub-acute, chronic and episodic infections involving skin, mucocutaneous membranes and various systems of the body. Candidal infections vary from oral thrush, glossitis, vulvovaginitis, intertrigo, paronychia, urinary tract infection, endocarditis to meningitis [1-3]. The incidence of Candidaemia of 6.9 per 1,000 intensive care unit (ICU) patients was reported in a recent study, and 7.5% of ICU patients received antifungal therapy [4-6].

In the past two decades, there has been increased incidence of infections caused by *Non Candida albicans* species [2]. This shift has been attributed to the rise in the immuno-compromised states such as HIV, cancer chemotherapy, transplantation, diabetes, burns, indiscriminate use of anti-bacterial antibiotics, steroids, pregnancy and increased use of fluconazole [7]. Antifungal resistance has become a major cause of concern in the management of candidemia. *C. krusei* and *C. glabarata* have been shown to be resistant to fluconazole and other triazoles. *C. tropicalis* and *C. parapsilosis* have been found to have variable susceptibility pattern to azoles. Few reports show *Candida* species being resistant to amphotericin B and echinocandins also [8]. Identification of yeasts isolated from clinical specimens up to species level has become increasingly important for the diagnostic laboratory as the changing epidemiology of Candida infections highlights the need for monitoring of species distribution and susceptibility of Candida in order to optimize therapy. As no relevant data on these pathogens is available from the Kashmir valley, therefore this study was undertaken to identify the spectrum of *Candida* species in clinical infections and to identify their sensitivity pattern to available antifungal agents.

2. MATERIALS AND METHODS

This prospective study was conducted from December 2014 to January 2016 with prior approval from institutional research committee. A total of 105 isolates of *Candida* obtained from different clinical specimens submitted to the Department of Microbiology, of a tertiary care hospital at Kashmir were included in the study.

Blood cultures samples were incubated in BacTAlert3D (Biomerieux, India ®) automated blood culture system .On getting a positive alarm, the blood culture broth was subjected to gram staining to detect the presence of budding yeast cells and was subsequently sub cultured onto Sabouraud dextrose agar (HiMedia, India) and blood agar plates. All other specimens i.e. pus, nail scrapings, skin scrapings, sputum, high vaginal swab were inoculated onto Sabouraud Dextrose agar plates. Suspected colonies of Candida in all cases were confirmed on Gram stain and then identified with automated Vitek 2 compact 60 system (BioMerieux India ®) using vitek 2 cards for identification of yeast and yeast like organisms Kits (ID-YST cards). The VITEK® 2 (Biomerieux) compact systems is a fully automated growth based technology that performs bacterial / yeast identification by biochemical analysis using colorimetric method. The YST reagent cards are incubated in the instrument and interpreted automatically. The YST identification card is based on established biochemical methods and newly developed substrates. There are 46 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Antifungal sensitivity was performed against Amphotericin B, 5 Flucytosine, Fluconazole, Caspofungin, Micafungin and Voriconazole on Vitek2 Compact 60 system (BioMerieux India ®). Results were interpreted according to CLSI guidelines (2012). Standard operative procedures as described by the manufacturer were followed.

3. RESULTS

A total of 105 clinical isolates of *Candida* from various clinical specimens were processed during the study period. The distribution of *Candida* isolates in various specimens is displayed in (Table 1).

Maximum number of *Candida* isolates were obtained from blood and nail specimens (26.6%each) followed by sputum (14.3%), high vaginal swab (12.3%), skin scrapings (10.4%), pus (6.6%) and urine (2.8%). *C. albicans* (33.3%) and *C. tropicalis* (33.3%) were the most common species isolated followed by C. *parapsilosis* (17.1%), *C. krusei* (13%) and *C. guilliermondii* (3.8%). *Non Candida albicans* isolation was higher (66.6%) than *C. albicans* (33.3%) with the predominant isolate being *C. tropicalis.* as shown in (Table 2).

In the present study all the isolates were susceptible to micafungin and capsofungin, 79% of *Candida* isolates were sensitive to fluconazole, 97% to amphotericin B and 91% were sensitive to flucytosine as shown in (Table 3).

4. DISCUSSION

Among various pathogenic species of fungi, *Candida* is the most prominent cause of fungal infections [9]. Although a part of normal micro biota, *Candida* is capable of causing various clinical manifestations ranging from mucocutaneous overgrowth to disseminated infections like candidemia [10]. A total of 105 *Candida* isolates from various clinical specimens were included in our study and the highest number of isolates (26.6%) were obtained from blood and nail scrapings, followed by sputum (14.3%), high vaginal swab (12.3%), skin scrapings (10.4%), pus (6.6%) and urine (2.8%). Data from surveillance and control of pathogens of epidemiological importance (SCOPE) surveillance system confirms that *Candida* species have become the fourth leading cause of blood stream infections [11].

In our study also, we noticed a very high rate of infections due to *Non Candida albicans* species (66.6%), whereas infections due to *Candida albicans* constituted only about 33.3%. Many studies have also shown an increase in the rate of *Non Candida albicans* species [12,13].

Patel et al have also reported high rate of *Non Candida albicans* infection (62.6%) and only 37.4% of infections caused by *Candida albicans* [12]. Our study also correlates with the study by Chakrabarti et al. [14] who has reported an increase in Non Candida albicans species from 52.6% in 1992 to 89.5% in 1995.

Table 1. Distribution of specimens and *Candida* isolates *(n=105)*

Specimen	Male (%)	Female (%)	Total (%)
Blood	13(46.4)	15(53.5)	28(26.6)
Nail scrapings	08(28.5)	20(71.4)	28(26.6)
Sputum	10(66.6)	05(33.3)	15(14.3)
High vaginal swab	0	13(100)	13(12.3)
Skin scrapings	06(54.5)	05(45.4)	11(10.4)
Pus	02(28.5)	05(71.4)	07(6.6)
Urine	0	03(100)	03(2.8)
Total	39(37.14)	66(62.85)	105

Table 2. Species distribution of *Candida* isolates *(n=105)*

Candida isolates	Blood (%)	Nail scrapings (%)	Sputum (%)	High vaginal Swab (%)	Skin scrapings (%)	Pus (%)	Urine (%)	Total (%)
C. albicans	2(5.7)	2(5.7)	12(34.2)	8(22.8)	4(11.4)	5(14.2)	2(5.7)	35(33.3)
C. tropicalis	18(51.4)	04(11.4)	3(8.5)	05(14.2)	2(5.7)	2(5.7)	1(2.8)	35(33.3)
C. parapsilosis	00	14(77.7)	00	00	04(22.2)	00	00	18(17.14)
C. krusei	08(61.5)	04(30.7)	00	00	01(7.6)	00	00	13(12.38)
C. guilliermondii	00	04(100)	00	00	00	00	00	04(3.8)
Total	28(26.6)	28(26.6)	15(14.3)	13(12.3)	11(10.4)	07(6.6)	03(2.8)	105

Table 3. Antifungal susceptibility pattern of *Candida* isolates

Candida Sp	*Candida albicans*	*C. tropicalis*	*C. parapsilosos*	*C. krusei*	*C. guilleirmondii*	*Total (%)*
Fluconazole						
Sensitive	30	32	17	00	04	83 (79.0)
Intermediate	02	03	01	00	0	06(5.7)
Resistance	03	0	0	13	0	16 (15.2)
Amphotericin B						
Sensitive	31	35	16	13	02	97(92.3)
Intermediate	2	0	02	0	02	06(5.7)
Resistance	2	0	0	0	0	02(1.9)
Flucytosine						
Sensitive	32	32	18	06	03	91(86.6)
Intermediate	03	03	0	01	01	08(7.6)
Resistance	00	0	0	06	0	06(5.7)
Micafungin						
Sensitive	35	35	18	13	04	105(100)
Intermediate	0	0	0	0	0	00
Resistance	0	0	0	0	0	00
Voriconazole						
Sensitive	35	34	18	13	04	105
Intermediate	0	01	0	0	0	00
Resistance	0	0	0	0	0	00
Capsofungin						
Sensitive	35	34	18	08	04	99(94.2)
Intermediate	0	01	0	0	0	01(.95)
Resistance	0	0	0	05	0	05(4.7)

Mokaddas et al. [15] also reported the Non Candida albicans incidence (60.5%) to be higher than that of *C. albicans* (39.5%). The emergence of Non Candida albicans species could be because of frequent use of antifungal agents like fluconazole for prophylaxis & therapy, which results in selection of less susceptible species. *Candida tropicalis* species has emerged as the major *Non Candida albicans* species (33.3%) in our study, followed by *C. parapsilosis* (17.4%) and *C. krusei* (12.3%). These findings correlate with the findings of Manchanda et al. [3]. In the present study, females (62.8%) were more commonly affected than males (37.1%) with a ratio of 0.59:1(M:F). In a similar study by Kandhari KC et al. [16] at AIIMS, New Delhi, the incidence in females was about 61.2% while in males it was only 38.8% with a ratio of 1:1.57(M:F) and Rizvi MW et al. [17] also reported female preponderance in their study group with a ratio of 0.85:1(M:F).

In the present study, antifungal susceptibility testing was done for the *Candida* isolates by using Vitek2 Compact (Biomerieux India) using vitek 2 cards. The *C. albicans* isolates were 100% susceptible to capsofungin and micafungin and showed 8.5% resistance to fluconazole, 5.7% resistance to amphotericin B and 8.5% showed intermediate resistance to flucytosine. In case of *Candida tropicalis*, 8.5% isolates showed intermediate resistance to fluconazole and flucytosine while as 100% isolates were sensitive to amphotericin B. The resistance rates for fluconazole, flucytosine, and capsofungin for *C. krusei* were 100%, 46.1% and 38.4 & respectively. Frequent use of fluconazole selects for the emergence of *Candida krusei* as a commonly isolated opportunistic pathogen. Furthermore, this organism is intrinsically resistant to fluconazole both in vivo and in vitro [18]. The findings of the present study correlated with those of study done by Vijaya D, et al. [19] in which it was seen *C. albicans* & *Non Candida albicans* have better sensitivity to amphotericin B than the azole group of drugs. Khotari et al. [20] from North India reported the susceptibility profile of *Candida* isolates as 92% were sensitive to amphotericin B and 36% to fluconazole. Also the finding was correlated with those of a study done by Shivanand Dharwad et al. [21] in which *C. tropicalis* was 87.5% susceptible to amphotericin B.

5. CONCLUSION

To conclude, the present study showed that prevalence of *Non Candida albicans* was higher from various clinical specimens. This study therefore emphasizes the need for rapid and precise identification of *Candida* isolates to species level for effective treatment and management strategies. There is also the need for periodic surveillance of the antifungal susceptibility pattern of the prevalent *Candida species*, as it would enlighten clinicians to choose appropriate antifungal agents, thus decreasing patient's morbidity and mortality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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