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

Asifa Nazir*, Farhath Kanth and Anjum Farhana

1Department of Microbiology, Government Medical College, Srinagar, Jammu and Kashmir, India.

**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.

**Article Information**

DOI: 10.9734/BMRJ/2016/24548

Editor(s): (1) Raul Rodriguez-Herrera, Autonomous University of Coahuila, México.

Reviews: (1) Anonymous, Ege University, Turkey.

(2) Nathanej Luplertlop, Mahidol University, Thailand.

(3) Anonymous, University of Canterbury, New Zealand.

Complete Peer review History: [http://sciencedomain.org/review-history/13810](http://sciencedomain.org/review-history/13810)

Received 25th January 2016

Accepted 9th March 2016

Published 22nd March 2016

**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.

*Corresponding author: E-mail: asifanazir@gmail.com;*
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; amphotericin 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 Fluocytosine, 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%), 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 microbiota, 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 constituted only about 33.3%. Many studies have also shown an increase in the rate of 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. parapsilosis | C. krusei | C. guilleimondii | 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) |
| Fluocytosine | 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.

REFERENCES

1. Chander J. Textbook of medical mycology. Candidiasis. 3rd ed. New Delhi: Mehta Publishers. 2009;266-90.
2. Pfaller MA, Diekema J. Epidemiology of invasive candidiasis: A persistent public health problem. Clinical Microbiology Reviews. 2007;20(1):133-163.
3. Manchanda V, Agarwal S, Verma N. Yeast identification in routine clinical microbiology laboratory and its clinical relevance. Indian Journal of Medical Microbiology. 2011;29(2):172.
4. Kett DH, Azoulay E, Echeverria PM, Vincent JL. Extended prevalence of infection in ICU study (EPIC II) Group of investigators. Candida bloodstream infections in intensive care units: Analysis of the extended prevalence of infection in intensive care unit study. Crit Care Med. 2011;39:665-70.
5. Azoulay E, Dupont H, Tabah A, Lortholary O, Stahl JP, Francois A, et al. Systemic antifungal therapy in critically ill patients without invasive fungal infection. Crit Care Med. 2012;40:813-22.
6. Rani R, Mohapatra NP, Mehta G, Randhawa VS. Changing trends of Candida species in neonatal septicaemia in a tertiary north Indian hospital. Indian J Med Microbiol. 2002;20:42-4.
7. Concila E, Azzini AM, Conti M. Epidemiology, incidence and risk factors for invasive candidiasis in high-risk patients. Drugs. 2009;69:5-14.
8. Giri S, Kindo AJ, Kalyani J. Candidemia in intensive care unit patients: A one year study from a tertiary care centre in South. J Postgrad Med. 2013;59:190-5.
9. Sullivan D, Coleman D. Candida dubliniensis: Characteristics and identification. J Clin Microbiol. 1998;36:329-334.
10. Eggimann P, Gorbach J, Pittet D. Epidemiology of Candida species infections in critically ill non immune-suppressed patients. Lancet Infect Dis. 2003;3:685-702.
11. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. National surveillance of nosocomial blood stream infection due to species of Candida other than Candida albicans: Frequency of occurrence and antifungal susceptibility in the SCOPE Program. SCOPE Participant Group. Surveillance and Control of Pathogens of Epidemiologic. Diagn Microbiol Infect Dis. 1998;30(2):121-9.
12. Patel LR, Pethani JD, Bhatia P, Rathod SD, Shah PD. Prevalence of Candida infection and its antifungal susceptibility pattern in tertiary care hospital, Ahmedabad. Natl J Med Res. 2012;2(4):439-441.
13. Kashid RA, Belawadi S, Devi G, Indumati. Characterization and antifungal susceptibility testing for Candida species in a tertiary care hospital. Journal of Health Sciences & Research. 2011;2(2):1-7.
14. Chakrabarti A, Ghosh A, Batra R, Kaushal A, Roy P, Singh H. Antifungal susceptibility pattern of non-albicans Candida species & distribution of species isolated from Candidaemia cases over a 5 year period. Indian J Med Res. 1996;104:171-6.
15. Mokaddas EM, Al-Sweih NA, Khan ZU. The species distribution and the antifungal susceptibility of Candida bloodstream isolates in Kuwait: A 10 year study. J Med Microbiol. 2007;56:259-9.
16. Khandari KC, Rama KM Rao. Clinical and laboratory studies on cutaneous candidiasis. md J Der Ven Lep. 1969;35(2):102-107.
17. Rizvi MW, Malik A, Shahid M, Singhal S. Candida albicans infections in a north Indian tertiary care hospital: Antifungal resistance pattern and role of SDS-PAGE for characterization. Biology and Medicine 2011;3(2),Special Issue:176-181.
18. Orozco AS, Higginbotham LM, Hitchcock CA, Parkinson T, Falconer D, Ibrahim AS, et al. Mechanism of fluconazole resistance in *Candida krusei*. Antimicrobial Agents and Chemotherapy. 1998;42(10):2645-2649.

19. Vijaya D, Harsha TR, Nagaratnamma T, *Candida* speciation using chrom agar. Journal of Clinical and Diagnostic Research. 2011;5(4):755-7.

20. Kothari A, Sagar V. Epidemiology of candida bloodstream infections in a tertiary care institute in India. Indian J Med Microbiol. 2008;27:171-2.

21. Shivanand Dharwad, Saldanha Dominic RM. Species identification of *Candida* isolates in various clinical specimens with their anti-fungal susceptibility patterns. Journal of Clinical & Diagnostic Research. 2011;5(6),(suppl-1):1177-1181.

© 2016 Nazir et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13810