Original Research Paper

Volume - 11 | Issue - 09 | September - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

# PHENOTYPIC CHARACTERIZATION OF CLINICAL ISOLATES OF *CANDIDA* SPECIES AND ITS SUSCEPTIBILITY TO VARIOUS ANTIFUNGAL AGENTS: A STUDY FROM A TERTIARY CARE HOSPITAL IN WESTERN UTTAR PRADESH

| | |
|---|---|
| **Navdeep Gambhir** | MBBS intern, Subharti Medical College, Meerut, 250005. |
| **Abhigyan Goel** | MBBS Intern, Subharti Medical College, Meerut,250005. |
| **Anita Pandey*** | Professor & Head, Post Graduate Department of Microbiology, Subharti Medical College, Meerut, 250005. *Corresponding Author |
| **Arjun Singh Bisht** | Tutor, Post Graduate Department of Microbiology , Subharti Medical College, Meerut, 250005. |
| **Sadab Khan** | Msc Medical Microbiology, Post Graduate Department of Microbiology, Subharti Medical College, Meerut, 250005. |
| **Deepali Saini** | Assistant Professor , Post Graduate Department of Microbiology, Subharti Medical College, Meerut, 250005. |

**ABSTRACT** **Background:** Indiscriminate use of antifungal agents has led to rise in infections caused by *Candida* species in recent years. Studies on characterization of *Candida* species followed by antifungal susceptibility testing can be beneficial in managing this problem.

**Objectives:** To phenotypically characterize *Candida* species isolated from various clinical samples and to determine its susceptibility to various antifungal agents.

**Methods:** A total of 119 Candida spp. isolated from various clinical samples were subjected for species identification and antifungal susceptibility testing using an automated Vitek-2 compact system.

**Results:** There was predominance of Non albicans (NAC) species (82.35%) isolated from our Hospital. Candida species were isolated predominantly from blood (68.06%) sample followed by urine (26.05%). *C. tropicalis* was the predominant NAC species isolated (27.73%) followed by *C.krusei, C.guilliermondii* (12.61% each), *C.parapsilosis* (10.08%) and *C. glabrata* (7.56%). Overall the NAC isolates were resistant to fluconazole, voriconazole, caspofungin, micafungin, amphotericin-B, and flucytosine as compared to *C. albicans*.

**Conclusion:** Predominance of NAC species and emergence of antifungal drug resistance among NAC species is a matter of concern. Thus highlighting that susceptibility should be performed in all cases to achieve good therapeutic results. Strict infection control strategies and a restrictive antifungal policy should be implemented for better clinical outcome.

**KEYWORDS :** Candida species, Non- albicans Candida, Antifungal agents.

## INTRODUCTION

Candida species is one of the most common causes of blood stream infections (BSIs) as well as many other types of infections. [1,2] Emergence of non-albicans Candida (NAC) species as one of the common cause of candidemia in the recent years shows a mycological shift .[3,4]. Prolonged antibiotic therapy, premature and /or low birth weight babies, total parental nutrition, mechanical ventilation contribute to the risk of candidal infection.[5,6,7] Prompt treatment with antifungal agent is required in these babies. Infections due to *Candida* species have been on the rise in recent decades mainly due to indiscriminate use of antifungal agents. Intrinsic and emerging resistance to azoles represent a major challenge for empirical antifungal therapy in NAC species thus contributing to significant morbidity and mortality.[8] Limited data on phenotypic characterization and antifungal susceptibility pattern of *Candida* species prompted us to undertake the present study which will help in the therapeutic management of the patients with candidiasis .

## MATERIALS AND METHOD

A prospective study was carried out for a period of six months in a tertiary care teaching hospital. A total of 119 *Candida* spp. isolated from various clinical samples received in mycology laboratory were subjected for species identification and antifungal susceptibility testing. Approval from the Institutional Ethical committee was taken before conducting the study.

Briefly, the clinical samples were first inoculated on sheep blood agar plates and Sabouraud dextrose agar slant (Hi-Media Pvt. Ltd., Mumbai, India) to obtain growth. The growth on culture media was identified as genus Candida as per standard mycological techniques.[9] Further , the species identification and antifungal susceptibility testing was performed by an automated Vitek-2 compact system (Biomerieux, France). Identification of yeast and yeast like organism and antifungal susceptibility testing was carried out using ID-YST card and AST-YS08 cards respectively purchased from Biomerieux , France. The demographic details of the patients, the clinical samples from which *Candida* spp. was isolated, the species of Candida identified and its susceptibility to various antifungal agents were analyzed.

## RESULTS

The present study showed predominance of NAC (82.35%) species in our Hospital. Candida species was isolated predominantly from blood (68.06%) followed by urine (26.05%) and pus (3.36%).[Table 1] The NAC species were isolated more from male patients in the age group of <10 years. The age and gender wise distribution of *C. albicans* and NAC species is shown in Table 2.

*C. tropicalis* (27. 73%) was the predominant NAC species isolated in our setting followed by *C.krusei* and *C.guilliermondii* (12.61% each), *C.parapsilosis* (10.08%) and *C. glabrata* (7.56%). *C. pelliculosa*, *C.ciferrii* , *C.famata* and *C.utilis* were other less common NAC species isolated from our hospital. [Table 3 & 1] Overall, *C.albicans* was the second most common Candida isolated from 17.65% samples after *C. tropicalis*.

The clinical isolates of *C.albicans* had good susceptibility towards fluconazole, voriconazole, caspofungin, micafungin, amphotericin-B (95.23%, 95.23% 100%, 100%, 100%) respectively. However, reduced susceptibility towards flucytosine was seen (76.20%) in *C. albicans* . On the other hand, the NAC species were found to be much more resistant to various antifungal agents. Overall among the antifungal agents reduced susceptibility was seen towards flucytosine (75.63%) followed by fluconazole (85.71%) and voriconzole (91.6%). However, the Candida species isolated from our hospital showed good susceptibility towards antifungal agents like caspofungin, micafungin & amphotericin-B [Table 4]

## DISCUSSION

Infection due to Candida, particularly NAC species is an emerging healthcare problem worldwide. High level of resistance to antifungal agents among Candida species in the past few years is a matter of great concern. NAC species, especially *C. tropicalis, C. krusei, C. glabrata*

and *C. parapsilosis*, tend to be less-susceptible to azoles, particularly fluconazole, than *C. albicans.C. krusei* and *C. glabrata* are inherently resistant to fluconazole,[3] thus emphasizing the need to identify Candida up to species level and determining antifungal susceptibility testing so that empirical treatment guidelines can be planned well on time.

The present study shows a changing trend of Candida species, with predominance of NAC (82.35%) . Similarly, increasing rates of NAC have been reported by various workers from different regions of India.[5,10,11,12,13]

Blood was the commonest clinical sample from which Candida species was isolated especially in the age group of <10 years of age in our setting. Similar findings of increased isolation of Candida from blood samples has been reported by previous workers.[14, 15] A shift has been observed in the frequency of each Candida spp. in the present study. *C. tropicalis* (27. 73%) being the predominant NAC species isolated from our Hospital followed by *C.krusei* and *C.guilliermondii* *C.parapsilosis* and *C. glabrata*. Similar finding has been reported. [11,12] The NAC species isolated were found to be much more resistant to various antifungal agents. [Table 4] Similar finding of increase in resistance to antifungal agents in NAC as compared to *C. albicans* has been reported. [8] Maximum resistance among NAC species was seen with flucytosine (17.65%) followed by fluconazole (10.09%) and Voriconazole (5.88%). As fluconazole is one of the common and extensively used antifungal agents for treatment of candidiasis development of resistance to fluconazole is a matter of concern. In comparison, the Candida species showed good susceptibility towards voriconazole thus highlighting that voriconazole can be used in the treatment of Candidiasis caused by fluconazole resistant strains. In our study the isolates of *C.albicans* showed good susceptibility towards various antifungal agents as compared to NAC. Similar finding has been reported by Kaur *et al.* [8]

A three year study conducted at an Indian trauma center from 2009 to 2012 found emergence of resistance against Amphotericin-B.[16] On the contrary almost all our clinical isolates of Candida species were sensitive to Amphotericin –B. Even though Amphotericin –B is effective against most strains of Candida species in vitro, it is not the first-line treatment for candidemia due to its nephrotoxicity. [17] However, the Candida species isolated from our hospital showed good susceptibility towards newer antifungal agents like caspofungin and micafungin. Similar finding has been reported. [17] Thus these agents can be useful in treatment of Candida infection caused by resistant strains.

**Table 1: Sample wise distribution of various Candida species (n=119)**

| Sample (No) | *C.albicans* | *C.tropicalis* | *C.parapsilosis* | *C.glabrata* | *C.krusei* | *C.guilliermondii* | *C.ciferrii* | *C.utilis* | *C. pelliculosa* | *C.famata* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Blood n=81 (68.06%)** | 14 (11.77%) | 20 (16.80%) | 6 (5.04%) | 7 (5.88%) | 9 (7.54%) | 14 (11.77%) | 4 (3.36%) | 2 (1.68%) | 4 (3.36%) | 2 (1.68%) |
| **Urine n=31 (26.05%)** | 6 (5.04%) | 10 (8.41%) | 4 (3.36%) | 2 (1.68%) | 5 (4.20%) | 01 (0.84%) | - | - | 2 (1.68%) | - |
| **Pus n=4 (3.36%)** | - | 03 (2.52%) | - | - | 01 (0.84%) | - | - | - | - | - |
| **Other* n=3 (2.52%)** | 01 (0.84%) | - | 2 (1.68%) | - | - | - | - | - | - | - |
| **Total (119)** | 21 (17.65%) | 33 (27. 73%) | 12 (10.08%) | 9 (7.56%) | 15 (12.61%) | 15 (12.61%) | 4 (3.36%) | 2 (1.68%) | 6 (5.04%) | 2 (1.68%) |

* other= High vaginal swab

**Table 2: Age and gender-wise distribution of Candida albicans and NAC species (n=119)**

| Age | Candida albicans | | Non- albicans Candida | | Total (%) |
|---|---|---|---|---|---|
| | Male (%) | Female (%) | Male (%) | Female (%) | |
| 0-10 | **06** | **-** | **30** | **10** | **46 (38.65%)** |
| 11-20 | **08** | **-** | **10** | **-** | **18 (15.13%)** |
| 21-30 | **03** | **01** | **10** | **04** | **18 (15.13%)** |
| 31-40 | **-** | **-** | **08** | **-** | **08 (6.72%)** |
| 41-50 | **-** | **-** | **04** | **02** | **06 (5.04%)** |
| 51-60 | **-** | **-** | **06** | **02** | **08 (6.72%)** |
| >60 | **03** | **-** | **06** | **06** | **15 (12.61%)** |
| Total | **20 (16.81 %)** | **1 (0.84 %))** | **74 (62.19 %)** | **24 (20.16 %)** | **119 (100 %)** |

**Table 3 : Distribution of various Candida species isolated from our Hospital (n=119)**

| Species | No. of isolates | Percentage |
|---|---|---|
| **NAC species :** | | |
| ***C.tropicalis*** | 33 | 27. 73% |
| ***C.krusei*** | 15 | 12.61% |
| ***C.guilliermondii*** | 15 | 12.61% |
| ***C.parapsilosis*** | 12 | 10.08% |
| ***C.glabrata*** | 9 | 7.56% |
| ***C. pelliculosa*** | 6 | 5.04% |
| ***C.ciferrii*** | 4 | 3.36% |
| ***C. C.utilis*** | 2 | 1.68% |
| ***C.famata*** | 2 | 1.68% |
| ***C.albicans*** | 21 | 17,65% |
| **Total** | 119 | 100 % |

**Table 4: Susceptibility of Candida species to antifungal drugs (n=119)**

| Name of the organisms | Antifungal drug | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fluconazole | | | Voriconazole | | | Caspofungin | | | Micafungin | | | AmphotericinB | | | Flucytosine | | |
| | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| ***C.albicans* n=21 (%)** | 20 (95.23) | 1 (4.77) | 0 (00) | 20 (95.23) | 1 (4.77) | 0 (00) | 21 (100) | 0 (00) | 0 (00) | 21 (100) | 0 (00) | 0 (00) | 21 (100) | 0 (00) | 0 (00) | 16 (76.20) | 3 (14.28) | 2 (9.52) |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***C.tropicalis* n= 33(%)** | 30 (90.91) | 1 (3.03) | 2 (6.06) | 33 (100) | 0 (00) | 0 (00) | 28 (84.85) | 3 (9.09) | 2 (6.06) | 33 (100) | 0 (00) | 0 (00) | 33 (100) | 0 (00) | 0 (00) | 27 (81.82) | 2 (6.06) | 4 (12.12) |
| ***C.parapsilosis* n= 12 (%)** | 09 (75) | 2 (16.66) | 1 (8.34) | 11 (91.67) | 1 (8.33) | 0 (00) | 10 (83.33) | 0 (00) | 2 (16.67) | 12 (100) | 0 (00) | 0 (00) | 12 (100) | 0 (00) | 0 (00) | 8 (66.67) | 1 (8.33) | 3 (25) |
| ***C.glabrata* n=9 (%)** | 7 (77.79) | 0 (00) | 2 (22.21) | 9 (100) | 0 (00) | 0 (00) | 9 (100) | 0 (00) | 0 (00) | 9 (100) | 0 (00) | 0 (00) | 9 (100) | 0 (00) | 0 (00) | 9 (100) | 0 (00) | 0 (00) |
| ***C.krusei* (n=15 (%)** | 12 (80) | 1 (6.67) | 2 (13.33) | 14 (93.33) | 0 (00) | 1 (6.67) | 15 (100) | 0 (00) | 0 (00) | 15 (100 | 0 (00) | 0 (00) | 15 (100 | 0 (00) | 0 (00) | 10 (66.67) | 2 (13.33) | 3 (20) |
| ***C.guilliermondii* n=15 (%)** | 12 (80) | 0 (00) | 3 (20) | 11 (73.33) | 1 (6.67) | 3 (20) | 15 (100) | 0 (00) | 0 (00) | 15 (100) | 0 (00) | 0 (00) | 15 (100) | 0 (00) | 0 (00) | 9 (60) | 3 (75) | 6 (40) |
| ***C.ciferrii* n=4 (%)** | 2 (50) | 0 (00) | 2 (50) | 1 (25) | 0 (00) | 3 (75) | 2 (50) | 1 (25) | 1 (25) | 2 (50) | 1 (25) | 1 (25) | 3 (75) | 0 (00) | 1 (25) | 1 (25) | 0 (00) | 3 (75) |
| ***C.utilis* n= 2 (%)** | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) |
| ***C. pelliculosa* n=6 (%)** | 6 (100) | 0 (00) | 0 (00) | 6 (100) | 0 (00) | 0 (00) | 6 (100) | 0 (00) | 0 (00) | 6 (100) | 0 (00) | 0 (00) | 6 (100) | 0 (00) | 0 (00) | 6 (100) | 0 (00) | 0 (00) |
| ***C.famata* n= 2) (%)** | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) | 2 (100) | 0 (00) | 0 (00) |
| **Total n=119 (%)** | 102 (85.71) | 05 (4.20) | 12 (10.09) | 109 (91.6) | 03 (2.52) | 07 (5.88) | 110 (92.43) | 04 (3.37) | 05 (4.20) | 117 (98. 32) | 01 (0. 84) | 01 (0. 84) | 118 (99. 16) | 0 (00) | 01 (0.84) | 90 (75.63) | 8 (6.72) | 21 (17.65) |

S- Sensitive, I-Intermediate, R-Resistant

## CONCLUSION

Predominant isolation of NAC species from various clinical samples definitely indicates a changing trend. The emergence of antifungal drug resistance among the clinical isolates is a matter of therapeutic concern. Strict infection control protocols and a restrictive antifungal policy should be implemented in the healthcare settings.

**Conflicts of interest:** None declared.
**Financial disclosure:** None

## REFERENCES

1. Chander J. Texbook of Medical Mycology, 4th edition Jaypee Brothers Medical publisher:2017..
2. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P. et al(eds.) Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6thedition. Philadelphia: Lippincott Williams & Wilkins;2006.
3. Sardana V, Pandey A, Madan M, Goel S P, Asthana AK Neonatal candidemia: A changing trend. Indian J PatholMicrobiol 2012;55:132-3
4. Goel N, RajanPk, Aggarwal R, Chaudhary U, Sanjeev N. Emergence of non albicans candida in neonatal septicaemia and antifugal susceptibility: Experience from a tertiary care center. J Lab physicians. 2009; 1: 53-5.
5. Narain S, Shastri JS, Mathur M, Mehta PR. Neonatal systemic Candidiasis in a tertiary care centre. Ind J Med Microbiol 2003; 21:56-8.
6. Juyal D, Adekhandi S, Negi V, Sharma N. An Outbreak Of Neonatal Candidemia Due To Non-Albicans Candida Species In A Resource Constrained Setting Of Uttarakhand State, India. J. Clin.Neonatal.2013 2(4):183-6 [9].
7. Singhi S, Rao DS, Chakrabarti A. Candida colonization and candidemia in a pediatric intensive care unit. PediatrCrit Care Med. 2008;9:91–5.
8. Kaur R, Jaggi S, Dhakad MS, Rawat D. An etiological and antifungal profile of candidemia in children. Int J Community Med Public Health 2019;6:3899-904.
9. Moore GS, Jaciow DM. Mycology for the Clinical Laboratory. Reston, Virginia: Reston Publishing Company Inc.; 1979. p. 323.
10. Agarwal J, Bansal S, Malik GK, Jain A. Trends in neonatal septicemia: Emergence of non-albicans Candida. Indian Pediatr 2004;41:712-5.
11. Chakrabarti A, Singh K, Das S. Changing face of nosocomial candidemia. Ind J Med Microbiol 1999;17:160-6
12. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care center of North India: 5 year study. Infection. 2007;35:256–9.
13. Chakrabarti A, Chatterjee SS, Rao KL, Zameer MM, Shivaprakash MR, Singhi S, et al. Recent experience with fungaemia: Change in species distribution and azole resistance. Scand J Infect Dis. 2009;41:275–84.
14. Rani R, Mohapatra NP, Mehta G, Randhawa VS, Changing species in neonatal septicemia in a tertiary North Indian hospital. Ind J Med Mirobiol 2002;20:424 [15].
15. Roy A, Maiti PK, Adhya S etal. Neonatal candidemia . Indian J Pediatr. 1993;60:799-801
16. Tak V, Mathur P, Varghese P, Gunjiyal J, Xess I, Misra MC. The epidemiological profile of candidemia at an Indian trauma care center. J Lab Physicians. 2014;6(2):96–101. doi:10.4103/0974-2727.141506
17. Bhhattacharjee P. Epidemiology and antifungal susceptibility of Candida species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016; 2(2): 20-27. DOI: 10.18869/acadpub.cmm.2.2.5