

**Speciation and anti-fungal susceptibility testing of candida isolates from various clinical samples in a tertiary care hospital, Hyderabad**

<sup>1</sup>Dr. Shaik Meherunnisa Begum, Assistant Professor, Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India.

<sup>2</sup>Dr. Mehjabeen Fatima, Post Graduate, Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India.

<sup>3</sup>Dr. B.M Shankar Venkatesh, Head of the Department, Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India.

**Corresponding Author:** Dr. Mehjabeen Fatima, Post Graduate, Department of Microbiology, Osmania Medical College, Hyderabad, Telangana, India.

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**Abstract**

The objective of present study is to speciate the Candida albicans and nonalbicans by using selective, differential media and methods and to determine the anti-fungal susceptibility pattern of the isolates.

Biological samples of urine, surgical biopsy (FESS), blood were processed by routine culture methods. All the Candida isolates were speciated by using differential methods like germ tube test, sugar fermentation test, inoculation on media such as Sabouraud dextrose agar, MALT extract agar, Cornmeal -Tween 80 agar and CHROMagar and anti-fungal susceptibility was performed on modified Mueller Hinton agar by Kirby Bauer disc diffusion method.

During the study period 75 Candida species were isolated. In which, 42(56%) were Germ tube test Positive (C. albicans) and 33(44%) were Negative (c. nonalbicans). The growth of candida is more favoured on

SDA than on MALT extract agar specially the non-albicans species. Among sugars, all c.albicans fermented glucose and maltose while all the c.non-albicans fermented glucose. In 33 C.nonalbicans 15 were C. tropicalis, 6 C. parapsilosis, 4 C. glabrata and 3 C. krusei, 2 C. gullermondi. In anti-fungal susceptibility testing candida albicans showed highest sensitivity to Amphotericin B(100%), Nystatin(100%) and the lowest sensitivity to fluconazole(64%) and candida non-albicans showed highest sensitivity to Nystatin(100%), Amphotericin B(91%) and Polymyxin B(91%) with lowest sensitivity to fluconazole(60%). In present study, although Candida albicans continues to be most frequently isolated Yeast but the emergence of nonalbicans has been clearly demonstrated with the emergence of species specific anti-fungal resistance.

**Keywords:** CHROMagar, Cornmeal Agar, Dalmau Plate, Sugar Fermentation Test.

## Introduction

In view of increase in mixed infections with multiple Yeast species and inherent species specific anti-fungal resistance, it is important to perform primary isolation and identification of Yeasts species and determining its sensitivity pattern for effective treatment.<sup>[1]</sup> Yeasts grow in different culture media but their growth is suppressed in mixed bacterial cultures, so several selective and differential media has been recommended for isolation of Yeast.<sup>[1]</sup> The objective of present study is to speciate the *Candida albicans* and nonalbicans by using differential methods like inoculation on media such as Sabouraud dextrose agar, MALT extract agar, Cornmeal-Tween 80 agar and CHROMagar and sugar fermentation test and determining the anti-fungal susceptibility pattern of the isolates.

## Materials and method

A prospective study is conducted from October 2023 to March 2024 in Microbiology laboratory of Osmania General Hospital. Biological samples of urine, surgical biopsy (FESS), blood were processed by routine culture methods, then the growth is subjected to Gram stain and Gram positive budding yeast cells were isolated as *Candida*. All the *Candida* isolates were inoculated on SDA with antibiotics and MALT extract agar, incubated for 48-72 hours at 37 °C. The growth on SDA is compared with MALT extract agar. Identification of species is done by Germ tube test then inoculation on Cornmeal-Tween 80 agar by Dalmau plate method and CHROMagar is performed, incubated at 37°C for 24-48hrs, the arrangement of chlamydospores is observed on Cornmeal-Tween 80 agar and pigment production on CHROMagar which were later correlated. Sugar fermentation is tested with glucose, lactose, sucrose, maltose, mannitol and xylose. Anti-fungal susceptibility was performed on modified Mueller Hinton agar with

added 2% glucose and methylene blue by Kirby Bauer disc diffusion method using Amphotericin B (AP-100U), Polymyxin B (PB-300U), Ketoconazole (KT-10µg), Itraconazole (IT-10µg), fluconazole (FL-10µg) and Nystatin (NS-100U).

## Results

During the study period 75 *Candida* species were isolated. Out of which 48(64%) were from urine, 21(28%) from surgical biopsy, 6(8%) from blood [Figure-1]. Among 75 *Candida*, 42(56%) were Germ tube test Positive (*C. albicans*) and 33(44%) were Negative (*C. nonalbicans*) [Figure-2].

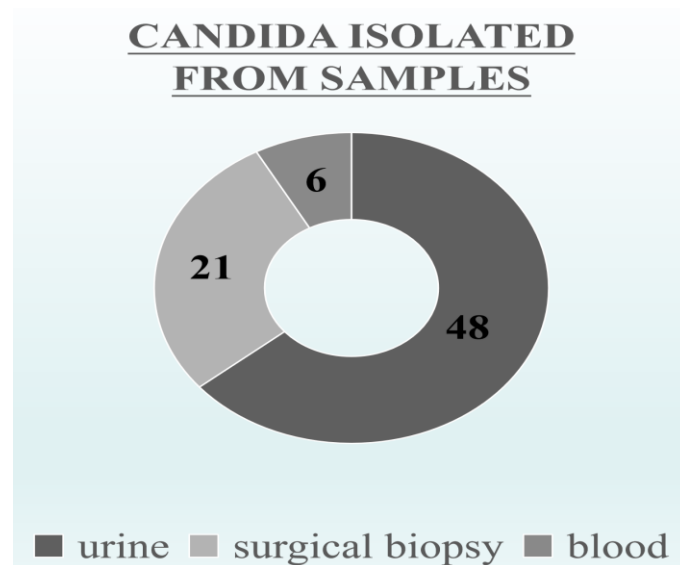


Fig. 1: No. of *Candida* isolated from samples

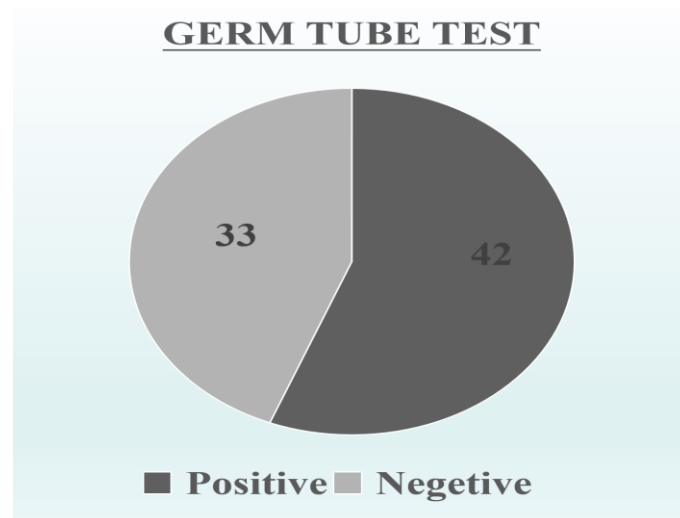


Fig. 2: Germ tube test

The growth of candida is more favoured on SDA than on MALT extract agar specially the non-albicans species. Among sugars, all *C. albicans* fermented glucose and maltose while all the *C. non-albicans* fermented glucose except *C. tropicalis* which fermented glucose, maltose and sucrose. [Figure 03]



Fig. 3: Sugar Fermentation Test

In 75 candida species, 42(56%) were *C. albicans*(light green), 15(20%) *C. tropicalis* (blue), 6(8%) *C. parapsilosis*(cream), 4(5%) were *C. glabrata* (pink to purple) and 3(4%) *C. krusei* (pink), 2(2%)*C. gullermondi*. [Table 01] [Figure 04, 05, 06].



Fig. 4: CHROMagar plate

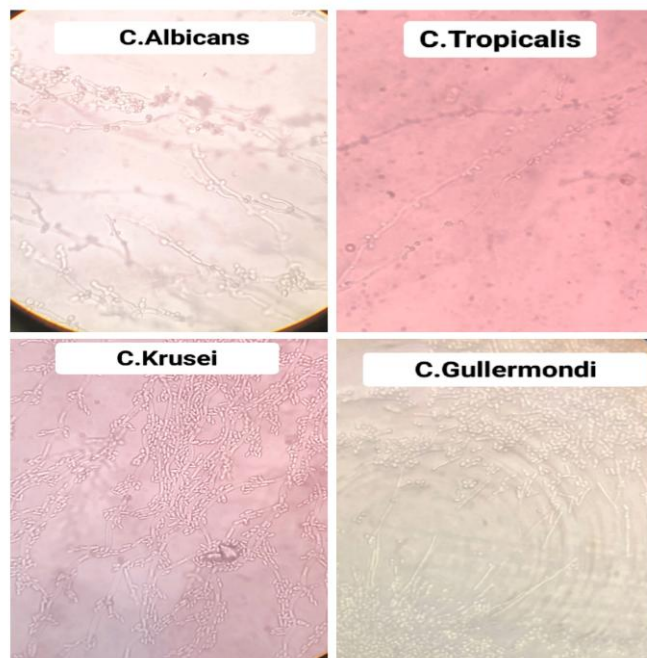


Fig. 5: Chlymadospore Arrangement in Cornmeal Agar

Species	No. of Isolates
<i>C. Albicans</i>	42(56%)
<i>C. Tropicalis</i>	15(20%)
<i>C. Parapsilosis</i>	06(8%)
<i>C. Glabrata</i>	04(5%)
<i>C. Krusei</i>	03(4%)
<i>C. Gullermondi</i>	02(2%)

Table 1: No. of isolates of various Candida species

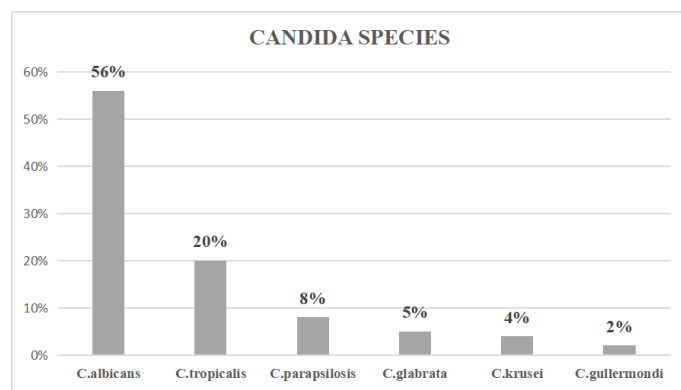


Fig. 6: Percentages of various Candida species.

In anti-fungal susceptibility testing candida albicans show highest sensitivity to Amphotericin B(100%) and Nystatin(100%) followed by Polymyxin B (90%), Itraconazole (80%), ketoconazole (79%) and the lowest

sensitivity to fluconazole(64%). Candida non-albicans show highest sensitivity to Nystatin (100%), Amphotericin B(91%), Polymyxin B(91%), followed by Itraconazole (81%), ketoconazole (81%) with lowest sensitivity to fluconazole (60%)[Table 02] [figure 07,08].



Fig. 7: Anti-fungal Susceptibility Testing

Drugs	C. Albicans	C. Nonalbicans
Amphotericin B	42(100%)	30(91%)
Nystatin	42(100%)	33(100%)
Polymyxin B	38(90%)	30(91%)
Itraconazole	33(80%)	27(81%)
Ketoconazole	33(79%)	27(81%)
Fluconazole	27(64%)	20(60%)

Table 2: Anti-fungal sensitivity percentage

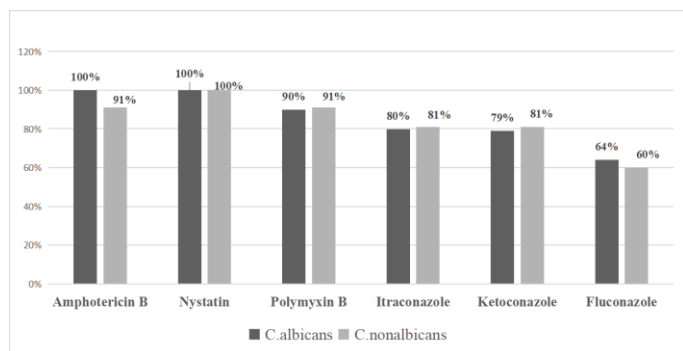


Fig. 8: Anti-fungal sensitivity percentage

## Discussion

In laboratory diagnosis, the selection of a culture medium for the initial isolation of Candida is crucial. Even though a few studies have shown that earlier differentiated media are effective, these media are not commonly used on a regular basis. Due to their high cost, the differential

culture media that have been produced in recent years have only been utilized in large laboratories.<sup>[1]</sup>

In the present study conducted on 75 biological samples, majority of isolates were *C. albicans* but a considerable number of *C. nonalbicans* was also seen. Similar trends has been shown in the studies done by C.Roopa, et.al.<sup>[2]</sup>, yucesoy M,et.al.<sup>[3]</sup> and Bilal H et.al.<sup>[12]</sup> while the other studies by Giri, Sidhartha et.al.<sup>[7]</sup> and Iyampillai T et.al.<sup>[6]</sup> Shows a contrast results of *C. tropicalis* (*C.nonalbicans*) being the most common isolates.

The most common isolated *C.nonalbicans* was *C.tropicalis* followed by *C.parapsilosis*, *C.glabrata*, *C.krusei* and the least isolated was *C.gullermondi*. Similar pattern of isolation is also seen in C.Roopa, et.al.<sup>[2]</sup> and yucesoy M, et.al.<sup>[3]</sup>

A study done by Skaar I, et.al.(1996)<sup>[8]</sup> estimates that MALT extract agar is a suitable medium for isolation of moulds and yeasts where as Bovill R et.al.<sup>[10]</sup> and Larry R et.al.<sup>[9]</sup> study show a low colony count in MALT extract agar which is in similarity to our study observation showing low growth in MALT extract agar and preferring SDA for the isolation of yeasts.

CHROMagar produces a reliable and rapid results, it is highly selective for yeast and often allows direct identification to species level.<sup>[5][6]</sup> But some species of *C.nonalbicans* give varying shades of pink coloured colonies leading to difficulties in identification, hence the efficacy of CHROMagar appears to be rather limited.<sup>[4]</sup>

So, the identification of Candida species should be done by correlating more than two conventional methods like CHROMagar pigment production with cornmeal agar (dalmau plate) chlamydospores arrangement supporting the identification with sugar fermentation test. Sugar fermentation test is a conventional method for identification of Candida isolates to species level which can support the findings of the standard methods.<sup>[7]</sup>

Anti-fungal susceptibility testing done in present study shows highest sensitivity to Nystatin, Amphotericin B, polymyxin B and lowest sensitivity to fluconazole which is supported by the study done by C.Roopa, et.al.<sup>[2]</sup> C.nonalbicans showed more resistant to fluconazole as few species like C.krusei, C.glabrata are inherently resistant as stated in a study by Noake T et.al.<sup>[11]</sup>

### Conclusion

In present study, although *Candida albicans* continues to be most frequently isolated Yeast but the emergence of nonalbicans has been clearly demonstrated. Cornmeal-Tween 80 agar is effective for appreciating production and arrangement of Chlamydospores and CHROMagar has advantage of being simple, rapid, cost effective for species identification. It has been demonstrated the emergence of species specific anti-fungal resistance with *C.nonalbicans* being more resistant to fluconazole than *C.albicans*.

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### References

1. Silva JO, Franceschini SA, Lavrador MA, Candido RC. Performance of selective and differential media in the primary isolation of yeasts from different biological samples. *Mycopathologia*. 2004 Jan; 157(1): 29-36. doi: 10.1023/ b:myco. 0000012223. 38967.7d. PMID: 15008342.
2. Roopa, C and Sunilkumar Biradar. "Isolation of *Candida* and its Speciation in Various Samples in a Tertiary Care Hospital in North Karnataka, India." (2015).
3. Yucesoy M, Ozbek OA, Marol S. Comparison of three differential media for the presumptive identification of yeasts. *Clin Microbiol Infect*. 2005 Mar;11(3):245-7. doi: 10.1111/j.1469-0691.2004.01058.x. Erratum in: *Clin Microbiol*

- Infect*. 2005 Jun;11(6):513. Oztek AO [corrected to Ozbek, OA]. PMID: 15715727.
4. Sivakumar VG, Shankar P, Nalina K, Menon T. Use of CHROMagar in the differentiation of common species of *Candida*. *Mycopathologia*. 2009 Jan;167(1):47-9. doi: 10.1007/s11046-008-9149-5. Epub 2008 Aug 21. PMID: 18716895.
5. Hospenthal DR, Murray CK, Beckius ML, Green JA, Dooley DP. Persistence of pigment production by yeast isolates grown on CHROMagar *Candida* medium. *J Clin Microbiol*. 2002 Dec;40(12):4768-70. doi: 10.1128/JCM.40.12.4768-4770.2002. PMID: 12454192; PMCID: PMC154593.
6. Iyampillai T, Michael JS, Mathai E, Mathews MS. Use of CHROMagar medium in the differentiation of *Candida* species: is it cost-effective in developing countries? *Ann Trop Med Parasitol*. 2004 Apr;98(3):279-82. doi: 10.1179/000349804225003244. PMID: 15119973.
7. Giri, Sidhartha & Kindo, Anupma. (2015). Evaluation of Five Phenotypic Tests in the Identification of *Candida* Species. *National Journal of Laboratory Medicine*. 4. 13-18. 10.7860/NJLM/2015/13492:2057.
8. Skaar I, Stenwig H. Malt-yeast extract-sucrose agar, a suitable medium for enumeration and isolation of fungi from silage. *Appl Environ Microbiol*. 1996 Oct;62(10):3614-9. doi: 10.1128/aem.62.10.3614-3619.1996. PMID: 8837416; PMCID: PMC168168.
9. Larry R. Beuchat, Selective media for detecting and enumerating food borne yeasts, *International Journal of Food Microbiology*, Volume 19, Issue 1, 1993, Pages 1-14, ISSN 0168-1605, [https://doi.org/10.1016/0168-1605\(93\)90119](https://doi.org/10.1016/0168-1605(93)90119) (<https://www.sciencedirect.com/science/article/pii/S0168160593901192>)

10. Bovill R, Bew J, Robinson S. Comparison of selective media for the recovery and enumeration of probiotic yeasts from animal feed. *Int J Food Microbiol.* 2001 Jul 20;67(1-2):55-61. doi: 10.1016/s0168-1605(01)00448-2. PMID: 11482569.
11. Noake T, Kuriyama T, White PL, Potts AJ, Lewis MA, Williams DW, Barnes RA. Antifungal susceptibility of *Candida* species using the Clinical and Laboratory Standards Institute disk diffusion and broth microdilution methods. *J Chemother.* 2007 Jun;19(3):283-7. doi: 10.1179/joc.2007.19.3.283. PMID: 17594923.
12. Bilal H, Shafiq M, Hou B, Islam R, Khan MN, Khan RU, Zeng Y. Distribution and antifungal susceptibility pattern of *Candida* species from mainland China: A systematic analysis. *Virulence.* 2022 Dec; 13(1):1573-1589. doi: 10.1080/21505594.2022.2123325. PMID: 36120738; PMCID: PMC9487756.