VOLUME - 12, ISSUE - 12, DECEMBER - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Clinical Microbiology

ISOLATION, SPECIATION OF CANDIDA SPECIES BY CANDIDA DIFFERENTIAL AGAR AND ANTIFUNGAL SUSCEPTIBILITY FROM VARIOUS CLINICAL SAMPLES IN TERTIARY CARE HOSPITAL, WARANGAL.

Golla Eshwara Chandra — Post Graduate, Department of Microbiology, Kakatiya Medical College, Hanumakonda.

Goteti Venkata Padmaja* — Professor and HOD, Department of Microbiology, Kakatiya Medical College, Hanumakonda. *Corresponding Author

Chilakalapalli Ramya Harshita — Post Graduate, Department of Obstetrics and Gynaecology, DY Patil Medical College, Pune.

ABSTRACT

Introduction: Candida species are fungal pathogens which are Yeast with Pseudo hyphae, they cause candidiasis (Cutaneous & Mucosal) & Systemic infections and they are one of major cause hospital acquired infection in Catheterized and Canulated patients. They are most common opportunistic fungal infection in Immunocompromised patients. **Materials and Methods:** It was a prospective study conducted in Kakatiya Medical college,. Different samples such as Urine, Blood, Oral swab, High vaginal swab and catheter tip Samples were collected and subjected to direct Gram staining, Germ tube test. Blood samples were inoculated on Brain heart infusion broth and cultured on conventional media further inoculated onto HiChrome candida differential agar to speciate candida. Antifungal drug susceptibility testing of candida species isolated is done by Kirby bauer disk diffusion method. **Results:** A total of 2124 clinical specimens were collected, among them, 195 samples tested positive for candida species. These samples included urine 98(50.2%), blood 46(23.5%), high vaginal swab 26(13.3%), oral thrush swab 18(9.2%), and catheter tip 7(3.5%). Among the candida species, Candida albicans 86(44.1%), Candida tropicalis 58(29.7%), Candida glabarata 27(13.8%), Candida krusei 18(9.2%), and Candida dubliniensis 6(3%) were isolated on culture. Candida species were most sensitive to Itraconazole158(81%) followed by Fluconazole 154(78.9%), Miconazole 151(77.4%), Ketoconazole 26(13.3%). **Conclusion:** In our study we found that candida albicans was major cause of candida infections and ketoconazole is most resistant antifungal drug. We also found that HiChrome Candida Differential agar is an effective screening tool for candida isolates speciation. We conclude that identifying candida species from various clinical specimens has become important and to know the susceptibility of candida species to antifungal drugs and guide the clinicians.

KEYWORDS : Candida species , HiChrome Candida Differential agar , Antifungal drug susceptibility.

INTRODUCTION:

Candida fungi can cause various infections, including candidiasis, both on the skin and in the mucous membranes, as well as systemic infections. These fungi are a common cause of infections in hospitalized patients with catheters or canulas, as well as in those with weakened immune systems. Candida is a type of fungus that can take on different forms, with a gram-positive, oval, budding yeast cell that produces pseudohyphae in culture and tissues [1]. Candida albicans is responsible for 60-80% of candidiasis cases, but there has been a rise in non-albicans (NAC)species that may show resistance to antifungal drugs [2]. Systemic Candida infections have a high mortality rate of 38% and can prolong hospital stays. While C. albicans still causes most hospital-acquired infections, there has been a shift towards non-albicans species such as Candida glabrata, Candida parapsilosis, Candida tropicalis, and Candida krusei. In recent years, there has also been an increase in candidiasis caused by other Candida species like Candida dubliniensis, Candida glabrata, Candida krusei, Candida tropicalis, and Candida parapsilosis [3]. For our study, we identified different Candida species by using HiCrome candida differential agar, a rapid and cost-effective method.

MATERIALS AND METHODS:

It was a prospective study conducted in Kakatiya Medical college, Warangal for a period of 6 months. Different samples such as Urine, Blood, Oral swab, High vaginal swab and catheter tip Samples were collected and subjected to Direct Gram staining, Germ tube test, Blood samples inoculated on Brain heart infusion broth and cultured on Blood agar and MacConkey agar, then they were inoculated on Sabouraud Dextrose agar and further inoculated onto HiCrome candida deferential agar to speciate candida. Antifungal drug susceptibility testing of candida species isolated is done by Kirby bauer disk diffusion and Five colonies of growth were suspended in 5ml of sterile saline and compared to 0.5 McFarland Standard for turbidity measurement [4]and further inoculated on Muller Hilton agar with 2%Glucose and 0.5 microgram Methylene blue [5] [6].

A) Macroscopic Examination:

To identify different Candida species, we picked one colony from each SDA plate and streaked it on a HiChrome Candida differential agar plate. The plates were then incubated at 37°C for 24- 48 hours. Based on the color of the colonies, we interpreted light green colonies as C. albicans, blue colonies as C. tropicalis, purple colonies as C.krusei and cream colonies as C.glabarata ,Pale green as C.dubliniensis [7] [8].

B) Microscopic Examination:

To analyze the samples, a colony was taken from each SDA plate and placed on a glass slide and smear was made. The slide was then stained with Gram stain and viewed under a microscope using an oil immersion lens for gram positive budding yeast cells.

C)Germ tube test (GTT):

To conduct the Germ Tube test, a colony was selected from each SDA plate and placed in an Eppendorf tube with 0.5 ml of human serum. The Eppendorf tubes were then incubated at 37°C for 2-3 hours. After incubation, a small amount was taken from each Eppendorf and examined under a microscope [9]. Observing the Raynaud's phenomenon involved inoculating colonies with 0.5ml of human serum and incubating at 37°C for 1-2 hours. [10].

D)Anti-fungal susceptibility testing:

Once Candida species was identified, it was inoculated onto Muller Hilton agar with 2% Glucose and 0.5 micrograms of

Methylene blue.

Then Ketoconazole 10 mcg, Fluconazole 10mcg, Miconazole 30 mcg and Clotrimazole 10 mcg were placed onto inoculated agar. They were further incubated for 24-48 hrs. An antifungal susceptibility pattern was interpreted based on zone diameter Kirby-Bauer disk diffusion method using CLSI M44(A) guidelines [4].

RESULTS:

A total of 2124 clinical specimens were collected, and out of them, 195 samples tested positive for candida species. These samples included urine 98(50.2%), blood 46(23.5%), high vaginal swab 26(13.3%), oral thrush swab 18(9.2%), and catheter tip 7(3.2%). Among the candida species, Candida albicans 86(44.1%), Candida tropicalis 58(29.7%), Candida glabarata 27(13.8%), Candida krusei 18(9.2%), and Candida dubliniensis 6(3%) were isolated on culture as shown in figure 1and table 1 .Antifungal drug susceptibility testing with following drugs has been done isolated species. In C.albicans, itraconazole(80.2%) was most sensitive and ketoconazole(81.3%) was most resistant. In C.tropicalis fluconazole(86.2%) was most sensitive and ketoconazole (96.5%) was most resistant. In C.glabarata, miconazole(74%) was most sensitive and ketoconazole (100%)was most resistant. In C. krusei miconazole (94.4%) was most sensitive to and ketoconazole(77.7%) was most resistant. C. dubliniensis was most sensitive to miconazole(100%) and ketoconazole(33.3%) and itraconazole(33.3%) was most resistant as shown in table 2 and figure 2.

Table 1. Isolation rate of Candida species from various samples

Samples	Candida species					Total Isolates from various clinical samples
	Candida albicans	Candida tropicalis	Candida glabarata	Candida krusei	Candida dublinisensis	
Urine	42(48.8%)	26(44.8%)	17(62.9%)	10(55.5%)	3(50%)	98(50.2%)
Blood	24(27.9%)	20(34.4%)	2(7.4%)	0(0%)	0(0%)	46(23.5%)
High vaginal swab	9(10.4%)	8(13.7%)	6(22.2%)	1(5.5%)	2(33.3%)	26(13.3%)
Oral thrush swab	8 (9.3%)	3 (5.1%)	2(7.4%)	4(22.2%)	1(16.6%)	18(9.2%)
Catheter tip	3 (3.4%)	1 (1.7%)	0(0%)	3(16.6%)	0 (0%)	7(3.5%)
Total Candida isolates	86(44.1%)	58(29.7%)	27(13.8%)	18(9.2%)	6(3%)	195(100%)

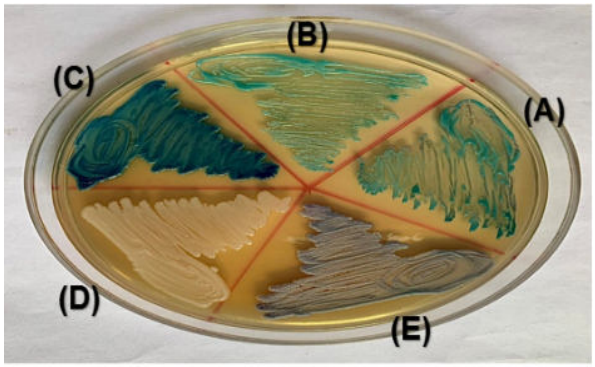

Figure 1. Growth of candida species as seen on Hicrome candida differential agar

Figure 1. A. Candida albicans(light green) ,**B.**Candida dubliniensis(pale green), **C.**Candida tropicalis(Blue), **D.**Candida glabarata(cream-white), **E.**Candida krusei (Purple).

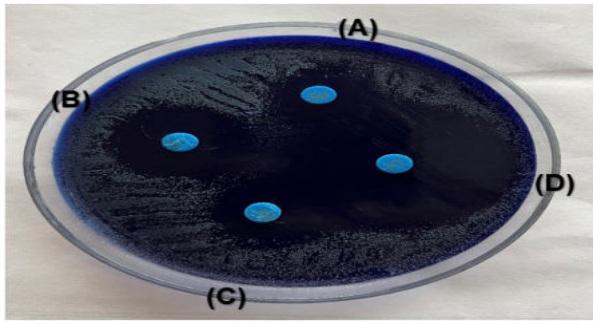

Figure2. Anti-fungal susceptibility testing by disk diffusion method of Candida albicans

Figure 2. A. Ketoconazole 10mcg **B.**Itraconazole 10mcg, **C.**Fluconazole 10mcg **D.**Miconazole 30mcg.

Table 2. Candida Species And Its Antifungal Susceptibility Pattern

Candida Species	Anti-fungal agents Ketoconazole		Itraconazole		Fluconazole		Miconazole	
	S	R	S	R	S	R	S	R
C.albicans	16(18.6%)	70(81.3%)	69(80.2%)	17(19.7%)	64(74.4%)	22(25.5%)	60(69.7%)	26(30.2%)
C.dubliniensis	4(66.6%)	2(33.3%)	4(66.6%)	2(33.3%)	5(83.3%)	1(16.6%)	6(100%)	0(0%)
C.tropicalis	2(3.4%)	56(96.5%)	49(84.4%)	9(15.5%)	50(86.2%)	8(13.7%)	47(81%)	11(18.9%)
C.glabarata	0 (0%)	27(100%)	20(74%)	7(25.9%)	19(70.3%)	8(29.6%)	21(77.7%)	6(22.2%)
C. krusei	4(22.2%)	14(77.7%)	16(88.8%)	2(11.1%)	16(88.8%)	2(11.1%)	17(94.4%)	1(5.5%)
Total	26(13.3%)	169(86.6%)	158(81%)	37(18.9%)	154(78.9%)	41(21%)	151(77.4%)	44(22.5%)

DISCUSSION:

A total of 2124 clinical specimens were collected, and out of them, 195 samples tested positive for candida species over a period of 6 months period. Among them majority of candida species isolated were from Urine and blood causing Urinary tract infection and systemic infections Speciation of Candida species by Hicrome candida differential agar on the basis of colour differentiation offered a rapid, convenient and reliable method for identification of clinically important Candida species when compared with traditional techniques [11]. In developing countries, Hicrome candida differential agar can be taken as a simple phenotypic test alternative to molecular based assay. The use of CHROMagar is highly effective in identifying Candida species with both sensitivity and specificity and antifungal susceptibility testing by conventional method as seen by other studies [12].

Among candida species which were isolated most common species was Candida albicans 86(44.1%) followed by Candida tropicalis 58(29.7%), Candida glabarata 27(13.8%), Candida krusei 18(9.2%), and Candida dubliniensis 6(3%) respectively. Our research findings reveal a comparable prevalence of Candida species and susceptibility patterns to previous data reported by independent study groups [13] [14]. They found that C. albicans is the most common Candida isolate. Another study by Sajjan et al. also reported C. albicans as the primary isolate [15]. Among non-albicans Candida species (NAC), C. tropicalis was the most prevalent, followed by C. glabarata and C. krusei. Jayalakshmi et al. also showed that C. tropicalis (26.6%) was prevalent among the non albicans candida species [16]. However, many studies have shown that non candida albicans species have more

isolation rate than C. albicans which suggest the emergence of non-albicans Candida species as major and important pathogens. Candida species are a prevalent yeast pathogen causing 8-10% of all hospital-acquired bloodstream infections In this study we found that Candida albicans 24(27.9%) and Candida tropicalis 20(34.4%) are commonly linked to bloodstream infections. This aligns with previous research indicating that C. glabrata, C. parapsilosis, C. tropicalis, and other non-albicans Candida (NAC) species are being detected more frequently in clinical samples[17] [18].In this present study candida species were found more susceptible Itraconazole 158(81%) followed by Fluconazole 154(78.9%%), Miconazole 151(77.4%), Ketoconazole 26(13.3%%). A similar study was conducted to perform an antifungal test for miconazole as of mycological interest [19] [20]. In our study candida species were found most resistant to ketoconazole 169(86.6%) followed by miconazole 44 (22.5%), fluconazole 41(21%) and itraconazole 37(18.9%). In contrast, a study conducted by Mondal et al revealed an overall resistance rate of 11.7% to ketoconazole, with the highest rates observed in Candida krusei (20%), followed by C. glabrata (17.6%), C. tropicalis (15.2%), and the lowest in C. albicans (7.8%) [12]. The highest rate of resistance to ketoconazole was observed in candida albicans 71(81.3%) followed by candida tropicalis and 56(96.5%) and candida glabarata 27(100%). Our results match with the result of khadka et al which showed ketoconazole resistance to C. albicans (89.3%), C. glabrata (85.8%), and followed by C. tropicalis (80%) [14]. According to research conducted by Zomorodian et al., fluconazole was found to be effective for 96.6% of Candida isolates. [21]. In this study fluconazole was most sensitive in candida albicans 64(74.4%) followed by candida tropicalis 50(86.2%) and candida glabarata 19(70.3%).

CONCLUSION:

we conclude that identifying candida from various clinical specimens and its speciation has become increasingly important. The changing epidemiology of candida infections calls for monitoring of species distribution and susceptibility of candida to manage such cases successfully. In our study we found that candida albicans was major cause of candida infections and ketoconazole as most resistant antifungal drug We also found that HiChrome Candida Differential agar is an effective screening tool for candida isolates speciation. Further Antifungal susceptibility testing has to be performed to know susceptibility pattern of candida isolates and guide the clinicians.

Abbreviations:

CLSI: Clinical and Laboratory Standards Institute, SDA: sabouraud dextrose agar, AFS;Anti-fungal susceptibility testing NAC spp.: non-albicans Candida species, S: susceptible, R: resistant, C. albicans: Candida albicans, GTT:Germ tube test.

Ethical approval and consent to participate:

The ethical approval for this study was taken from Kakatiya Institutional Ethics Committee, No. **ECR/840/Inst/TG/2016/RR/20/36**, on 01/08/2023, Kakatiya medical college before sample collection and processing. Consent was obtained from participants.

Authors' contributions:

1. Golla Eshwara Chandra: Concepts, Design, Literature search, Sample collection, Data acquisition, Statistical analysis, Manuscript preparation.

2. Goteti V Padmaja: Concepts, Design, Statistical analysis, Manuscript preparation Manuscript editing and review, Data analysis.

3.Chilakalapalli Ramya Harshita: Definition of intellectual content, Literature search, Data analysis, Manuscript editing and review, Clinical studies.

Conflict of interest: Nil

REFERENCES:

1. Ogaba OM, Abia-Bassy LN, Epoke J, Mandor BI, Iwatt GD (2013) Characterization of Candida species isolated from cases of lower respiratory tract infection among HIV/AIDS patients in Calabar, Nigeria. World J AIDS 3:201–206
2. Oberoi, J.K., Wattal, C., Goel, N., Raveendran, R., Datta, S., Prasad, K. (2012). Non-albicans Candida species in blood stream infections in a tertiary care hospital at New Delhi, India. Indian J Med Res, 136(6):997 1003
3. Abi-said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. The epidemiology of hematogenous candidiasis caused by different Candida species. Clin infect Dis 1997; 24:1112-28.
4. CLS Institute. Method for antifungal disk difusion susceptibility testing of yeasts: approved standard M44-A. Clinical and Laboratory Standards Institute: Wayne; 2006
5. Lee SC, Fung CP, Lee N, See LC, Huang JS, Tsai CJ, Chen KS, Shieh WB. Fluconazole disk diffusion test with methylene blue- and glucose-enriched Mueller–Hinton agar for determining susceptibility of Candida species. J Clin Microbiol. 2001; 39:1615–7.
6. Pfaller MA, Boyken L, Messer SA, Hollis RJ, Diekema DJ. Stability of Mueller–Hinton agar supplemented with glucose and methylene blue for disk diffusion testing of fluconazole and voriconazole. J Clin Microbiol. 2004; 42:1288–9.
7. Yucesoy M, Esen N, Yulung N. Use of chromogenic agar for the identifcation of Candida albicans strains. Kobe J Med Sci. 2001; 47:161–7.
8. Murray CK, Beckius ML, Green JA, Hospenthal DR. Use of chromogenic medium for the isolation of yeasts from clinical specimens. J Med Microbiol. 2005; 54:981–5.
9. Souza VM (1998) Técnicas laboratoriais utilizadas em micologia médica. In: Zaitz C, Campbell I, Marques AS, Ruiz LR, Souza VM (eds) Compêndio de micologia médica. Medsi, Rio de Janeiro, pp 51–63
10. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology.12th edition. Part V, Laboratory Methods in Basic Mycology. St. Louis: Mosby; 2007. p 629-716.
11. Nadeem SG, Hakim ST, Kazmi SU. Use of CHROMagar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited setting. Libyan J Med. 2010; 5:1–6.
12. Mondal S, Mondal A, Pal N, Banerjee P, Kumar S, Bhargava D. Species distribution and in vitro antifungal susceptibility patterns of Candida. J Inst Med. 2013; 35:45–9.
13. Odds, F.C., Bernaerts, R. 1994. CHROM agar Candida, a new differential isolation medium for presumptive identification of clinically important Candida spp. J. Clin. Microbiol., 32(8): 1923–9.
14. Khadka S, Sherchand JB, Pokhrel BM, Parajuli K, Mishra SK, Sharma S, Shah N, Kattel HP, Dhital S, Khatiwada S, Parajuli N. Isolation, speciation and antifungal susceptibility testing of Candida isolates from various clinical specimens at a tertiary care hospital, Nepal. BMC research notes. 2017 Dec; 10(1):1-5.
15. Sajjan AC, Mahalakshmi VV, Hajare V. Prevalence and antifungal susceptibility of Candida species isolated from patients attending tertiary care hospital. IOSR J Dent Med Sci. 2014; 13:44–9.
16. Jayalakshmi L, RatnaKumari G, Samson SH. Isolation, speciation and antifungal susceptibility testing of candida from clinical specimens at a tertiary care hospital. Sch J App Med Sci. 2014; 2:3193–8.
17. Krcmery, V., & Barnes, A. J. (2002). Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance. Journal of hospital infection, 50(4), 243-260.
18. Eliopoulos, G. M., Perea, S., & Patterson, T. F. (2002). Antifungal resistance in pathogenic fungi. Clinical Infectious Diseases, 35(9), 1073- 1080.
19. Hamza OJ, Matee MI, Moshi MJ, Simon EN, Mugusi F. Species distribution and in vitro antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiol. 2008; 8:135.
20. l-mamari A, Al-buryhi M, Al-heggami MA, Al-hag S. Identify and sensitivity to antifungal drugs of Candida species causing vaginitis isolated from vulvovaginal infected patients in Sana'a city. Der Pharm Chem. 2014; 6:336–42
21. Zomorodian K, Rahimi MJ, Pakshir K, Motamedi M, Ghiasi MR, Rezashah H. Determination of antifungal susceptibility patterns among the clinical isolates of Candida species. J Glob Infect Dis. s2011; 3:357–60