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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTINGOF *CANDIDA* ISOLATES FROM VARIOUS CLINICAL SPECIMENS AT A TERTIARY CARE HOSPITAL, WESTERN RAJASTHAN

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Abstract

Background: *Candida spp* is a member of the normal flora of the skin, mucous membrane and gastrointestinal tract. Candida continues to be leading cause of morbidity and mortality in large population of immunocompromised and hospitalized patients. Invasive Candidiasis due to non-albicans candida has been on the rise in last few years. This study aims to *Spectate Candida* using chromogenic medium.The emerging pathogens are resistant to conventional antifungal therapy.

Objective: To identify the various species of candida isolated from different clinical specimens and to compare the susceptibility pattern of these isolated species towards different antifungal agents.

Methods: All Candida isolates recovered from various clinical samples during the period from September 2017 and august 2018 were studied., These isolates were subjected to gram's stain, germ tube test and inoculation on commercially available CHROM agar (HiMedia India).

Results: A total of 155 Candida species were isolated from the different clinical specimens of suspected candida infection cases. Most of the isolates obtained were from urine samples 93 (60%) followed by blood 26(16.77%). Non albicans Candida were isolated at a higher rate 101 (65.16%) than Candida albicans 54 (34.84%) . Among 101 non *C. albicans, C. tropicalis* 55 (35.48%) was the most common species followed by 19 (12.26%) *C. parapsilosis.* Among all species of Candida commonest isolate was C. tropicalis 55(35.48%) followed by C. albicans 54(34.83%). Candida species from various samples were high resistant to itraconazole (72.26%) followed by fluconazole(70.92%), voriconazole (68.39%) and ketoconazole (57.42%)while there was minimum resistance to amphotericin-B (20%).This study emphasizes the need for monitoring local epidemiologic data and antifungal susceptibility pattern of candida isolates for proper treatment.

Conclusions: Along with *Candida albicans*, non-albicans *candida spp* like *C. tropicalis*, *parapsilosis*, *C. krusei* and *C. glabrata* are increasingly being isolated from clinical samples. CHROM agar is a

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simple, rapid and inexpensive method for identification of such species. Characterization to species level helps to identify species which might be intrinsically resistant to commonly used antifungal agents.

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Introduction:-

Candida is yeast like fungus and ubiquitous human commensal. They become pathogens and cause infections when local or systemic host resistance lowered down[1].The Candida species have been recognized as the fourth commonest cause of nosocomial invasive infections[2]. Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who areaged, immune-compromised, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation[3].

Candida albicans is the most common cause of candidiasis accounting for about 60-80% of infections. An increase in prevalence of non-albicans species has been noted during last decades[4,5].Also, in recent year non-*albicans Candida* (NAC) species are considered as major pathogens causing severe infections in human beings[6].Characterization to species level helps to identify those strains which might be intrinsically resistant to some antifungal agents[5,7].

The commonly used antifungal drugs show significant variation in the susceptibility pattern among the types of *Candida* species. The drug resistance scenario has been increasing during last decades due to over growing use of random antifungal agents[8]. Several previous studies reported the emergence of drug resistance *Candida*species in global scenario[9,10]. Therefore, the change in drug susceptibility pattern of *Candida* species and introduction of newer antifungal agents has made the invitro susceptibility testing of antifungal agents more relevant for using specific and sensitive drugs[11].Thus, the early identification, speciation and susceptibility testing of *Candida* species in clinical specimens have become increasingly important to prevent the treatment failure using appropriate antifungal agent.

Aims and Objectives:-

1. To identify the various species of candida isolated from different clinical specimens.
2. To compare the susceptibility pattern of these isolated species towards different antifungal agents.

Materials And Methods:-

We enrolled 2997 clinical specimens for this study. This study was conducted between September 2017 and august 2018. The clinical specimens wereurine, pus from ear& trachea, tracheal devices, blood, CSF, sputum, throat swab, indwelling medical devices, conjunctival swab, various body fluidsand semen. These specimens were collectedfrom OPD and IPD patients of all age and sex groupsduring the period of 1 year. All specimens were investigated for fungal culture and identification of speciesafter obtaining ethical clearance from the Institutional Ethics Committee.

All clinically suspected samples were subjected to gram staining to look for presence of Gram positive yeast like budding cells with pseudohyphae indicating presence of candida species and KOH mount. The samples were inoculated on Sabouraud's dextrose agar (SDA) with chloramphenicol & Blood agar and incubated at 37^0C and 25^0C 24-72 hours.Further identification and speciation of Candida on SDA were confirmed by Gram's stain, Germ tube test, 0.1% Glucose agar test,Sugar fermentation test,Sugar assimilation test, CHROM agar according to standard microbiological techniques[12].

Germ tube test was carried out by inoculating isolated yeast cells into 0.5 ml of pooled human serum in a small tube and incubation at 37ºC for 2 hours. Germ tubes formation was observed microscopically as tubular elongation extending from the yeast cells without constriction or septa at the point of attachment to the yeast cells. 0.1% Glucose agar test were inoculated and incubated at 30ºC for 2-5 days and studied microscopically for the presence of pseudohyphae, chlamydospores andblastospores. Isolated candida species were sub-cultured onchromogenic Candida medium (HICHROME Candida agar) and incubated at 37ºC for 48 hours. Presumptive species identification was done based on specific colony colors produced by the chromogenic substrates in the medium. All isolates were further identified by carbohydrate assimilation.

All the isolates were subjected to the antifungal susceptibility test according to CLSI document M 44 – A2 by disk diffusion testing method for yeasts. Muller Hinton agar supplemented with 0.5µg/ml Methylene Blue Dye and 2% Glucose (MHMB) was used for sensitivity testing. The inoculated plates were incubated at 37^0C for 24 hours or longer[13].

Results:-

We processed total 2997 samples during the time period ofthis study, in which 155(05.17%) Candida species strains were isolated.Majority of the patients belonged to 0-10 years age group 36(23.23%)followed by 41-50 years of age group 24(15.48%).The male and female patients ratio of Candidiasis was 90:65(1.38 : 1) respectively.In this study, maximum number of Candida species strainsrecovered from Urine samples 93 (06.97%) followed by Blood 26 (12.56%), Sputum 22 (14.76%), Indwelling medical devices 05 (06.10%), Tracheal 03 (01.17%), Pus/Ear swab 2 (00.37%), Fluid sample 2 (03.57%), Conjunctival swab 1 (01.75%) andThroat swab 01 (01.04%).Out of 155 Candida species, 54 (34.84%) were *C. albicans* and101 (65.16%) were non *C. albicans*(**Fig.no. 1**). Among 101 non *C. albicans*, *C. tropicalis*55 (35.48%) was the most common species followedby 19 (12.26%) *C. parapsilosis*, 14 (09.03%) *C. krusei*, 09(05.81%) *C. glabrata* and 04 (02.58%) were *C.kefyr*(**Table no. 2**).Candida species strains were isolated 90.97% in indoor patientsand 09.03% in outdoor patients. *C.tropicalis*(37.59%) is the major isolatedspecies in IPD patients which is followed by*C.albicans* (32.62%), *C.parapsilosis* (12.77%), *C.krusei*(08.51%), *C.glabrata* (05.67%) and *C.kefyr* (02.84%) whereas inOPD patient *C. albicans* (57.14%) is the major cause ofcandidiasis.The isolated Candida species from various samples were high resistant to itraconazole (72.26%) followed by fluconazole(70.92%), voriconazole (68.39%) and ketoconazole (57.42%)while there was minimum resistance to amphotericin-B (20%)(**Table no. 3**)

Fig. no. 1:- Distribution of *Candida albicans* and *Nonalbicans Candida* isolated from different clinical specimens.

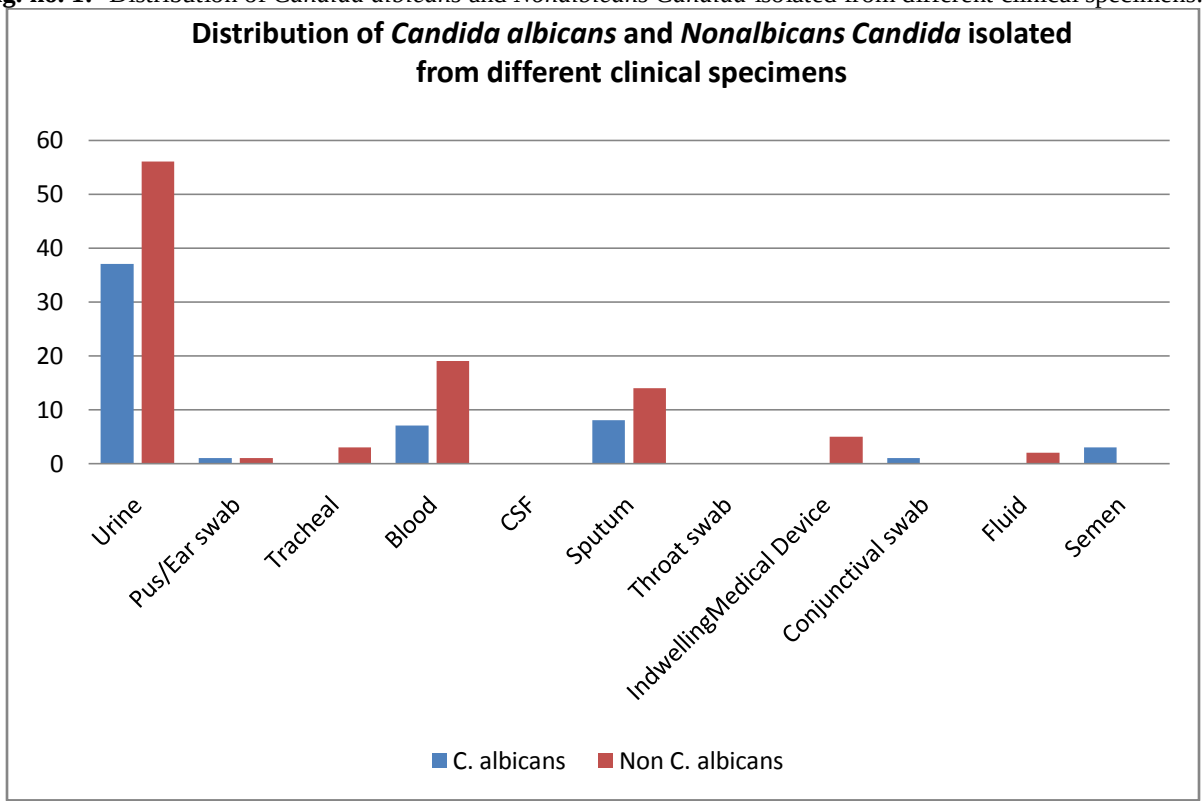
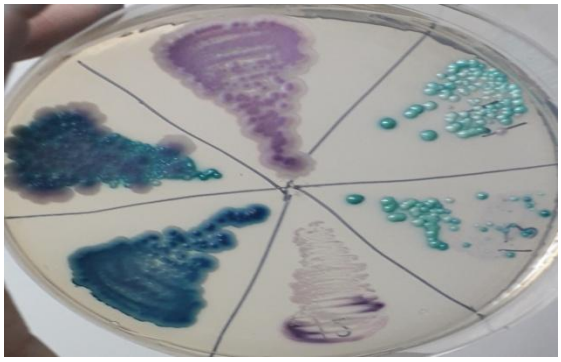


Table 2:- Candida species isolated from different clinical samples.

Types of Specimen	C. albicans (%)	C. glabrata (%)	C. krusei (%)	C.parapsilosis (%)	C.tropicalis (%)	C. kefyr (%)	Total (%)
Urine	37 (39.79%)	08 (08.60%)	06 (06.45%)	08 (08.60%)	31 (33.33%)	03 (03.23%)	93 (60.0%)

Blood	07 (26.92%)	00 (00%)	02 (07.69%)	05 (19.23%)	12 (46.16%)	00 (00%)	26 (16.77%)
Sputum	08 (36.36%)	01 (04.55%)	04 (18.18%)	02 (09.09%)	07 (31.82%)	00 (00%)	22 (14.19%)
Indwelling medical device	00 (00%)	00 (00%)	01 (20%)	02 (40%)	01 (20%)	01 (20%)	05 (03.23%)
Tracheal	00 (00%)	00 (00%)	00 (00%)	02 (66.67%)	01 (33.33%)	00 (00%)	03 (01.93%)
Pus/Ear swab	01 (50%)	00 (00%)	00 (00%)	00 (00%)	01 (50%)	00 (00%)	02 (01.29%)
Fluid	00 (00%)	00 (00%)	00 (00%)	00 (00%)	02 (100%)	00 (00%)	02 (01.29%)
Throat swab	00 (00%)	00 (00%)	01 (100%)	00 (00%)	00 (00%)	00 (00%)	01 (00.65%)
Conjunctival swab	01 (100%)	00 (00%)	00 (00%)	00 (00%)	00 (00%)	00 (00%)	01 (00.65%)
Total	54	9	14	19	55	4	155



CHROM agar

Table 3:- Antifungal susceptibility Pattern for Candida species isolatedfrom varioussamples.

CANDIDA SPECIES ISOLATED	KT		FLC		IT		AP		VRC	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
C.albicans	21 (38.8%)	33 (61.1%)	15 (27.7%)	39 (72.2%)	12 (22.2%)	42 (77.7%)	39 (72.2%)	15 (27.7%)	16 (29.6%)	38 (70.3%)
C.krusei	03 (21.4%)	11 (78.57%)	-	-	03 (21.4%)	11 (78.5%)	08 (57.1%)	06 (27.7%)	03 (21.4%)	11 (78.5%)
C.tropicalis	27 (49.1%)	28 (50.9%)	14 (25.4%)	41 (74.5%)	17 (30.9%)	38 (69.1%)	47 (85.4%)	08 (14.5%)	17 (30.9%)	38 (69.1%)
C.glabrata	05 (55.5%)	04 (44.4%)	04 (44.4%)	05 (55.5%)	04 (44.4%)	05 (55.5%)	08 (88.9%)	01 (11.1%)	04 (44.4%)	05 (55.5%)
C.kefyr	01 (25%)	03 (75%)	01 (25%)	03 (75%)	02 (50%)	02 (50%)	03 (75%)	01 (25%)	01 (25%)	03 (75%)
C.parapsilosis	09 (47.3%)	10 (52.6%)	07 (36.8%)	12 (63.2%)	05 (26.3%)	14 (73.6%)	19 (100%)	00 (0%)	08 (42.1%)	11 (57.8%)
TOTAL	99	89	41	100	43	112	124	31	49	106

	(42.5%)	(57.4%)	(29.0%)	(70.9%)	(27.7%)	(72.2%)	(124%)	(20%)	(31.6%)	(68.3%)

Discussion:-

Fungal infections, particularly those attributed to Candida species, are frequent complications for hospitalized patients contributing to increased morbidity and mortality and healthcare cost.Furthermore there is increasing prevalence of infections caused by non-albicans Candida worldwide with various degree of susceptibility to routinely use antifungal agents indicating the importance of laboratory diagnoses[14].

In this study, it was observed that candidiasis can occur at all ages and in both sexes.The highest number of isolates were obtained in the age group of0-10 years 36 (23.23%), followed by the age groups of 41-50 years 24(15.48%). Infections were more common in the 0-10 age groups in thisstudy.This can be attributed to the various co-morbid conditions and thehealth issues pertaining to the particular age-groups, as is relevant in thisstudy. However, further studies have to be carried out to justify thesignificance of the fact.In present study, Males were more affected than females with anoverall male:female ratio of (90:65)1.38. Candida tropicalis is major isolated species. In male patient Candida tropicalis 36.67% is the major isolated species whereas in female patients Candida albicans 36.92% is the major organisms.The preponderance of male patientssuffering from candidiasis in this study correlates with Singh et al[15].

In the present study, out of total 2997 specimen ,155 (05.17%) Candida species strains isolated. Candida species were isolated mainly from urine samples 93 (60%) followed by blood 26 (16.77%), sputum 22 (14.19%), indwelling medical devices samples 05 (03.23%). The study done by Shilpaet al[16] shows maximum Candida species isolates from urine samples 66 (46.5%) followed by genital discharge 39 (27.5%), blood 16 (11.3%), sputum 9 (6.3%), pus 7 (4.9%) and 5 (3.5%) from plastic devices (catheter tip, central line tip). Duttaet al[17] also reported maximum number of Candida isolates from urine samples 31 (37%) followed by sputum 18 (21%) and blood 03 (4%) which are less then present study. These prevalence ratesare different in other studies because these depend upon factors like kind and number of samples received, type of hospital and the geographical place where the studies going to be done.

Among these 155 Candida species isolated, *non-Candida albicans*species were more in number than *C. albicans*. The most common among*non C. albicans* species included *Candida tropicalis* 55 (35.48%)followed by 19 (12.26%) *C.parapsilosis*, 14 (09.03%) *C. krusei*, 09 (05.81%),*C. glabrata* and 04 (02.58%) were *C. kefyr*. *C. albicans* accounted for 54 (34.84%) of the isolates. The studies done by Duttaetal[17], Shivaprakasha et al[18], Verma et al[19] alsoindicate a trend towards an increasing prevalence of infections caused byspecies of *non-Candida albicans*.Generally, Candida causes opportunistic infection. If a person ishospitalized, having low immunity, taking prolonged antibacterialtreatment etc. these are the conditions where one can be more susceptibleto Candida infection.

The disk diffusion method, according to CLSI guidelines, was usedfor antifungal susceptibility testing. The isolates in the study showedlower resistance to amphotericin-B.

Among the Candida species, the rate of resistance exhibited by *C.albicans* is maximum in itraconazole (77.78%) followed by fluconazole(72.22%), voriconazole (70.37%), ketoconazole (61.11%) whereasamphotericin-B (27.78%) shows minimum resistance.

Candida krusei has intrinsic resistance towards fluconazole. Theother antifungal like ketoconazole, itraconazole and voriconazole shows78.57% rate of resistance whereas in amphotericin-B, it is 42.86%.The resistance to antifungals in *Candida tropicalis* is maximum tofluconazole (74.55%) followed by 69.09% in itraconazole andvoriconazole, 50.90% in ketoconazole and minimum in amphotericin-B(14.55%).

Candida glabrata shows 55.56% of resistance to fluconazole,itraconazole and voriconazole while 44.44% to ketoconazole whereasonly 11.11 % of resistance shown by amphotericin-B.*Candida kefyr* is mostly sensitive to amphotericin-B (75%)followed by itraconazole (50%) and 25% to ketoconazole, fluconazoleand voriconazole.The resistance pattern in *Candida parapsilosis* shows highestresistance towards itraconazole (73.68%) followed by fluconazole (63.16%), voriconazole (57.89%) and ketoconazole (52.63%) whileamphotericin-B shows no resistance.

Overall, the *non-Candida albicans* species showed more resistancethan *C. albicans* to applied antifungal agents except amphotericin-B.These similar findings corelates with study done by Duttaet al[17].

Conclusion:-

The present study suggests an increasing prevalence of non-Candida albicans species in the various clinical samples isolated. An emergence of resistance of these Candida species isolates to the routinely used antifungals, make them difficult to treat.

Therefore, detection of distribution of Candidathrough presumptive identification, followed by confirmation and antifungal treatment, has a efficient effect on successful treatment as it helps in optimum selection of the therapeutic agent and use of CHROMagar is a simple, rapid and inexpensive method for identification of *Candida* species especially in the laboratory with limited resources.

References:-

1. Anaissie EJ, McGinnis MR, Pfaller MA (eds) (2009) Clinical Mycology, 2nd edn. Churchill Livingstone Elsevier, Philadelphia.
2. Douglas LJ. Candida biofilms and their role in infection. Trends Microbiol. 2003 Jan; 11(1):30-6.
3. Mohandas V and Ballal M. Distribution of Candida species in different clinical samples and their virulence: biofilm formation, proteinase and phospholipase production: a study on hospitalized patients in southern India. J Glob Infect Dis. 2011; Jan-Mar; 3(1): 4–8.
4. Mokaddas EM, Al-Sweith NA, Khan ZU. Species distribution and antifungal susceptibility of *Candida* bloodstream isolates in Kuwait: a 10-years study. J Med Microbiol. 2007;56:255-9.
5. Srinivasan L, Kenneth J. Antibiotic susceptibility of *Candida* isolates in a tertiary care hospital in Southern India. Ind J Med Microbiol. 2006;24:1-8.
6. Gullo A. Invasive fungal infections: the challenge continues. Drugs. 2009;69(Suppl 1):65–73.
7. Golia S, Reddy KM, Karjigi KS, Hittinahalli V. Speciation of *Candida* using chromogenic and cornmeal agar with determination of fluconazole sensitivity. Al Ameen J Med Sci. 2013;6(2):163-6.
8. Yang YL, Cheng HH, Ho YA, Hsiao CF, Lo HJ. Fluconazole resistance rate of *Candida* species from different regions and hospital types in Taiwan. J MicrobiolImmunol Infect. 2003;36:187–91.
9. Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Tanabe K, Niimi M, Goffeau A, Monk BC. Efflux mediated antifungal drug resistance. ClinMicrobiol Rev. 2009;22:291–321.
10. White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. ClinMicrobiol Rev. 1998;11:382–402.
11. Hospenthal DR, Beckius ML, Floyd KL, Horvath LL, Murray CK. Presumptive identification of *Candida* species other than *C. albicans*, *C. krusei*, and *C. tropicalis* with the chromogenic medium CHROMagar*Candida*. Ann ClinMicrobiolAntimicrob. 2006;5:1.
12. Chander, J. (2009). Text book of Medical Mycology, Chapter 2, 5, 6, 45, 3 rd edition, Meheta publishers, 9-20,35-36, 266-290.
13. Clinical and Laboratory Standards Institute (CLSI) (2009). Method for antifungal disk diffusion susceptibility testing of yeasts; Approved guidelines –Second edition. CLSI document M44-A2.Pennsylvnia .ISBN 1-56238-703-0.
14. Alonso-Valle H, Acha O, Garcia-Palomo JD, Farinas-Alvarez C, Fernandez-Mazarrasa, C, Farinas MC. (2003). Candidemia in a tertiary care hospital: epidemiology and factors influencing mortality. *European Journal of Clinical Microbiology and Infectious Diseases, 22*(4), 254-257.
15. Singh K, Chakrabarti A, Narang A and Gopalan S. Yeastcolonization and fungaemia in preterm neonates in a tertiary carecentre. Indian J Med Res. 1999;110:169-73.
16. Arora S, Dhuria N, Jindal N, Galhotra S. Speciation, biofilmformation and antifungal susceptibility of Candida isolates. Int J ResDev Pharm L Sci. 2017; 6(2): 2517-2521.
17. Dutta V, Lyngdoh WV, Bora I, Choudhary B, Khyriem AB andBhattacharyya P. Characterization of Candida species from IntensiveCare Unit Isolates in a Tertiary Care Centre in North-East India: Aretrospective study. Int J Med Public Health. 2015; 5:312-6.
18. Shivaprakasha S, Radhakrishnan K and Karim PM. Candida spp.other than Candida albicans: A major cause of fungaemia in a tertiarycare centre. Indian J Med Microbiol. 2007; 25:405-7.
19. Verma AK, Prasad KN, Singh M, Dixit AK and Ayyagari A.Candidaemia inpatients of a tertiary health care hospital from NorthIndia. Indian J Med Res. 2003;117:122-8.