RESEARCH ARTICLE

EVALUATION OF CANDIDA SPECIES FROM CLINICAL SPECIMENS BY USING CHROMAGAR.

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Manuscript Info	*Abstract*
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Candida species especially Non albicans Candida are increasingly being isolated from clinical specimens. Candida spp. are the most common cause of fungal infections and are the fourth leading cause of health care associated infections. The main aim of the study is to isolate and identify the various Candida species from clinical samples by both Conventional and chromgenic method and to check which one is better and specific method. Samples such as blood, urine and pus received in laboratory from patients of all age group and both sexes with suspected Candida infection and the positive isolates were identified by conventional as well as chromogenic method. Total 80 isolates of Candida species were recovered from the clinical specimens. *C.krusei* was most commonly isolated yeast followed by *C.albicans.* CHROMagar falsely identified *C.parapsilosis* as *C.glabrata*. Species identification using CHROMagar is rapid, technically simple, and easy as compared to conventional method.

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Introduction:-
Candida species are the fellow members of the normal flora of the skin, mucous membranes and gastrointestinal tract. They are an endogenous opportunist which means that they can cause secondary infection in individuals with some underlying immunocompromised conditions [1]. The genus is composed of a heterogeneous group of organisms and more than 17 different Candida species are known to be the aetiological agents of human infections [3]. The major etiological agent is *Candida albicans*, whereas different Candida species can cause a variety of infections including *C. tropicalis, C. dubliniensis, C. parapsilosis, C. krusei, C. guillermondii, C. glabrata*, and *C. kefyer* which epitomize many clinical forms of candidiasis. Some of these species forgather as secondary infections to another species, for example; C. *parapsilosis* is secondary infection only when *C. albicans* as a cause of Candida endocarditis.[4] Among species of Candida, *C. albicans* is most often colligated with serious fungal infections. Other Candida species also have emerged as clinically important opportunistic pathogens [5].The vast majority of invasive Candida infections are caused by only four species which include *C. albicans, C.glabrata, C. parapsilosis and C.tropicalis.* The clinical manifestations of disease are extremely varied, ranging from acute, sub acute and chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, gastrointestinal tract or become systemic as in septicaemia, endocarditis and meningitis [1].Identification of yeast pathogens by traditional methods like germ tube test, growth pattern on cornmeal agar are labour intensive and requires several days and specific mycological media. Chromogenic media contain chromogenic substrates which react with enzymes secreted by target microorganisms to yield colonies of varying colours.

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Material & Methods:-
Present prospective study was conducted in tertiary care hospital from January 2016 to June 2016. Various samples received in laboratory from patients of all age group and both sexes with suspected Candida Infection. Clinical details were noted in the case record form. Patients who were on antifungal treatment were excluded. The specimens for laboratory investigation were collected undertaking strict aseptic precautions. The various clinical specimens collected were blood, urine, pus etc. Two swabs/specimens were taken from each case. One of the specimens was subjected for direct examination and the other for the culture. All the above samples were subjected to various mycological tests.
1) Direct examination by KOH Mount
2) Gram stain
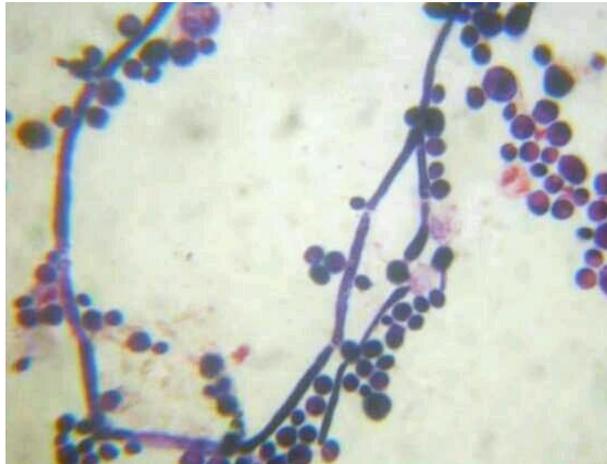
Fig A-Candida on Gram stain

3) Culture on SDA (at 25°C and 37°C)
4) Germ tube test for speciation: A small portion colony of the yeast to be tested was suspended in a test tube containing 0.5 ml human serum. The test tube was incubated at 35ºC for 2-3 hours. A drop of yeast–serum suspension was placed on a microscopic slide, overlaid with a cover slip and examined microscopically for presence of germ tubes.
 Observation: Filamentous extension from yeast cell with no constriction was considered as germ tube.
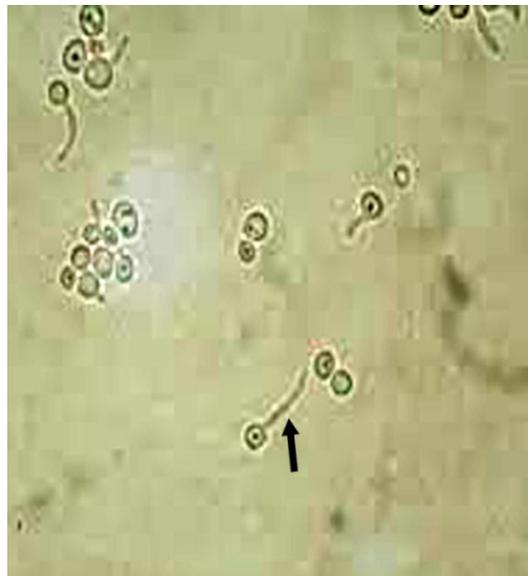
Fig B- Germ tube formation

Growth pattern on CMA for speciation:-
Isolated colonies of Candida were picked up with inoculating loop. Three parallel cuts 1 cm apart was made into the surface of Cornmeal-Tween agar, holding the inoculating loop at about a 45-degree angle. A sterile cover slip was laid on the surface of agar, covering a portion of the inoculated streaks. The inoculated plates were incubated at 25-30ºC for 24-72 hours. At the end of incubation period plates were examined microscopically (under 10x and 40x) at the edge of cover slip and the pattern of growth was observed to make a presumptive identification.

Fig C- *C.krusei* on CMA

Growth on CHROMagar:-
Isolated species were inoculated on Candida CHROMagar to improve species identification based on coloured colony morphology. These agar plates were incubated at 37^0 C for 48 hours. The species were identified by characteristic colony colour.
- *C. albicans*- Light green coloured colonies
- *C. tropicalis* - Blue to metallic blue coloured colonies
- *C. glabrata* - Cream to white smooth colonies
- *C. krusei*- Pink colonies
- *C. dubliniensis* - Dark green colonies

Fig D- Candida species on Candida CHROMagar

Observation and Results:-

In present study, 80 isolates of Candida species were recovered. Species identification was done by both conventional method and Candida CHROMagar.

Samples included in the study were 80. Out of which blood (40), urine (26) and Pus (14) respectively.
Fig1-Distribution of Various samples size-
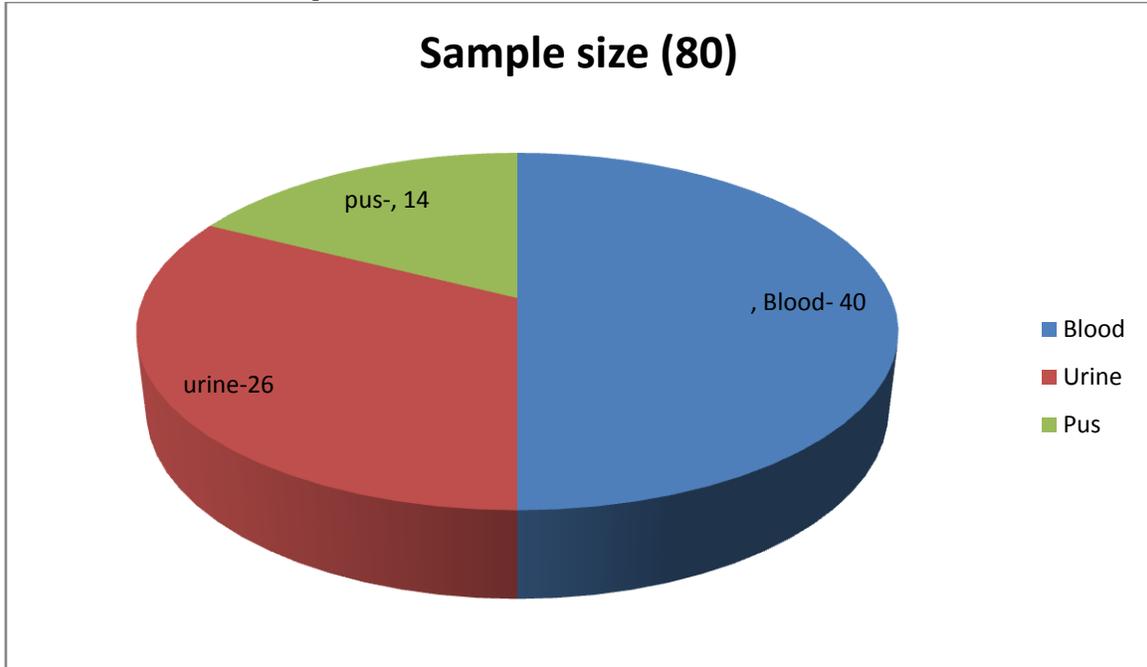
Fig1-samples size n=80 which includes blood (n=40), Urine (n=26) and Pus (n=14)

Fig 2-Showing Candida isolates in various samples such as Blood, Urine and pus.
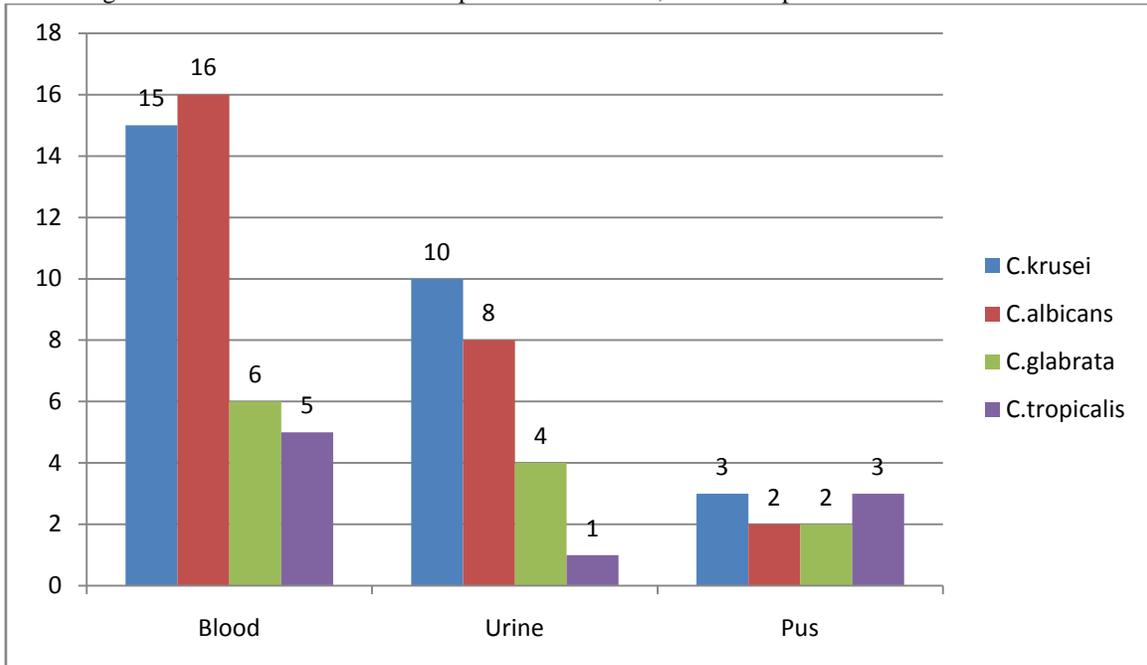
Fig 2:- Showing number of Candida isolates in various samples such as Blood, Urine and pus.

Table 1:-Distribution of different species of Candida-

Candida species	Number of isolates(n=80)	Percentage (%)
C. krusei	28	35%
C. albicans	26	32.5%
C. glabrata	12	15%
C. tropicalis	09	11.25%
C.parapsilosis	05	6.25%

Table1-Total number of Candida species isolated was 80. Out of 80 isolates, Candida krusei (35. %) was the most common species followed by C.albicans 32.5%) was most common followed by C. glabrata (15%), C.tropicalis (11.25 %.), C.parapsilosis (6.25%).

Table 2:-Identification of various species of Candida by conventional method and Candida CHROMagar

Candida species (n=80)	Conventional method	CHROMagar
C. krusei	28	28
C.albicans	26	26
C.glabrata	12	17
C.tropicalis	09	09
C.parapsilosis	05	-

Table 2-Showing Candida species isolated from Conventional method and CHROMagar.

Table 3:-Sensitivity and specificity of Candida CHROMagar for each species

Species	Sensitivity (%)	Specificity (%)
C. krusei	100%	100%
C.albicans	100%	100%
C.glabrata	100%	72.22%
C.tropicalis	100%	100%

Table 3- We obtained 100% sensitivity and specificity of Candida CHROMagar for *C. krusei*, *C. albicans*, *C.tropicalis* but sensitivity and specificity of Candida CHROMagar for *C. glabrata* was 100% and 72.22% respectively.

Discussion:-

The potential clinical importance of species-level identification has been recognized as Candida species differ in the expression of virulence factors and antifungal susceptibility. Non albicans Candida are on the rise due to increasing immunocompromised states. Non albicans Candida are more resistant to fluconazole, therefore species level identification has a direct impact on choice of empirical antifungal treatment. [6] The incidence of infections caused by Candida species has increased considerably over the past three decades, mainly due to the rise of the AIDS epidemic, an increasingly aged population, higher numbers of immune-compromised patients and the more widespread use of in dwelling medical devices. *Candida albicans* is the main cause of candidiasis; however, non-albicans Candida species such as *C. glabrata*, *C. tropicalis* and *C.parapsilosis* are now frequently identified as human pathogens. [7] Total 80 Candida species were isolated from various clinical samples. Among the various clinical isolates of Candida species we obtained *C.krusei* (28) as the most common isolate followed by *C. albicans* (26), *C. glabrata* (12), *C. tropicalis* (09), *C. parapsilosis* (Table1). Factors like increased use of antifungal drugs, use of broad spectrum antibiotics, long term use of catheters and increase in the number of immunocompromised patients contributes to the emergence of non-albicans Candida species.[8] For differentiation among different species of Candida conventionally germ tube test, growth pattern on cornmeal agar and sugar assimilation tests are being used which are technically difficult, time consuming and difficult to interpret which may take 72 hours to two weeks for species identification[9], [10]Chromogenic agar is technically simple, easy to interpret and rapid method to differentiate among different Candida species. It facilitates the detection and identification of Candida species and provides result in 24-48 hours. Among the new tests, Candida CHROMagar is rapid and cost effective as compared to other expensive systems like API systems, Vitek 2 ID system and molecular methods. [11]

In our study, for *C. glabrata* specificity of Candida CHROMagar was 72.22% as 5 species of *C.glabrata* were falsely identified by Candida CHROMagar as C. parapsilosis. Shettar SK et al [12] reported that on Candida CHROMagar, C.parapsilosis gave same cream colour as that of *C.glabrata*. This may be because of *C. glabrata*; *C. kefyr*, *C. parapsilosis* and *C.lusitaniae* appear as a variety of beige/brown/yellow colours due to the mixture of

natural Pigmentation and some alkaline phosphatase activity. [13]*C.glabrata* and *C. parapsilosis* can be easily differentiated from growth pattern on Cornmeal agar as C. glabrata doesn't produce pseudo-hyphae. Thus, the combination of Cornmeal agar Hi Candida agar can be used for early identification of *C. glabrata*. [12]

Conclusion:-
Our study showed *C. krusei* as most common Non –albicans Candida species causing candidiasis, which shows the rise of Non-albicans Candida among various clinical samples. CHROMagar is a simplistic, rapid, easy and inexpensive method with good sensitivity and specificity for identification of Candida species. CHROMagar can be reliably used for identification for *C. krusei, C.albicans, C. tropicalis* but for early identification of *C. glabrata* and C. parapsilosis both the corn meal agar and CHROMagar should be used.

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About the Author:-
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References:-
1. Shivprakasha S, Radhakrishan K, Karim PMS. Candida spp other than Candida albicans. A major cause of fungemia in a tertiary care centre. Ind J Med Microbiol 2007;25(4):405-407.
2. Bailey and Scotts, diagnostic microbiology, thirteenth edition, chapter 63 Mycology.
3. Pahwa N, Kumar R, Nirkhiwale S, Bandi A. Species distribution and drug susceptibility of Candida in clinical isolates from a tertiary care centre at Indore. Indian J Med Microbiol 2014; 32(1): 44–8.
4. Amy, R. (2000). Current issues in women's health. Controlling yeast
5. Mohandas V, Ballal M. Distribution of Candida Species in different clinical samples and their virulence: Bio-film formation, proteinase and phospholipase production: A study on hospitalized patients in Southern India. J Glob Infect Dis 2011; 3: 4–8.
6. Manjunath V, Vidya GS, Sharma A, Prakash MR, Murugesh. Speciation of Candida by Hichrom agar and Sugar assimilation test in both HIV infected and non infected patients. Int J Biol Med Res.2012;3(2):1778-1782.
7. Dharwad S, Dominic S. Species identification of Candida isolates in various clinical specimens with their anti-fungal susceptibility patterns. J Clin diagnostic Res 2011; 5(6): 1177–81
8. Deorukhkar S, Saini S. Non albicans Candida species: its isolation pattern, species distribution, virulence factors and antifungal susceptibility profile. Int J Med Sci Public Heal 2013; 2(3): 533–8.
9. Baradkar VP, Mathur M, Kumar S. Hichrom candida agar for identification of Candida species. Indian J Pathol Microbiol 2010; 53(1): 93–5.
10. Madhavan P, Jamal F, Chong PP, Ng KP. Identification of local clinical Candida isolates using CHROMagar Candida™ as a primary identification method for various Candida species. Trop Biomed 2011; 28(2): 269–74.
11. Devi LS, Maheshwari M. Speciation of Candida Species Isolated From Clinical Specimens by Using Chrom Agar and Conventional Methods. Int J Sci Res Publ 2014;4(3): 1-5.
12. Shettar SK, Patil AB, Nadagir SD, Shepur TA, Mythri BA, Gadadavar S. Evaluation of HiCrome differential agar for speciation of Candida. J Acad Med Sci 2012; 2(3): 101–4
13. Oxoid Ltd.Oxoid brilliance candida agar [pamphlet].Oxoid Ltd;UK.