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Research Article
Candida

Speciation of Candida using CHROMagar isolated from Various Clinical Samples

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Introduction: Infections due to *Candida* especially *Non-albicans Candida* (NAC) species are increasingly being reported in recent years. Isolation and identification up to species level are of utmost importance in the early management of these infections.**Materials and methods:** All the clinical samples received in the Microbiology laboratory were inoculated on to Blood agar and MacConkey agar. The isolates which revealed Gram-positive budding yeast cells on Gram staining were further inoculated on to SDA (Sabouraud Dextrose agar) and Hicrome Candida differential agar. *Candida* was differentiated into species based on color and morphology on Hicrome agar. **Result**: A total of 126 *Candida* were isolated from various clinical samples. *C.Albicans* was the most common species isolated (49.2%) followed by *C. tropicalis* (19.8%), *C. glabrata* (19%), and *C. krusei* (12%).**Conclusion**: Hicrome agar is a simple, rapid, and cost-effective medium for the speciation of *Candida*.

Keywords: Candida albicans, Hicrome agar, Non-albicans Candida

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Introduction

Over the last few years, the incidence of mycotic infections has progressively increased. Fungi once considered as nonpathogenic or less virulent are now recognized as a primary cause of morbidity and mortality in immunocompromised and severely ill patients [1].

Candida species belong to the normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract, and vagina and are responsible for various clinical manifestations from simple mucocutaneous overgrowth to invasive infections like bloodstream infections, which is due to their great adaptability to different host environment [2].

In the 1980s *C.Albicans* accounted for more than 80% of all *Candida* isolates recovered from yeast infections. More recently *Non-albicans Candida* (NAC) species have been recovered with increasing frequency [3] Isolation and prompt identification of the infectingorganism to the species level is essential to optimize the earlyantifungal therapy as certain species like *C. krusei*are inherently resistant to azole drugs [4,5].

The longer turnaround time taken by conventional methods of identification makes them less popular among the clinicians as early diagnosis is essential for initiating appropriate therapy. In order to facilitate rapid identification, several chromogenic substrates containing culture media have been developed. Hicrome agar is a differential media that allows selective isolationof yeasts and identifies colonies of *C. Albicans*, *C. glabrata*, *C. krusei,* and *C. tropicalis*[6].

The present study was undertaken to determine the prevalence of *Candida* species among various clinical samples.

Materials and Methods

Thiscross-sectionalstudy was conducted in the Department of Microbiology of a Tertiary care hospital situated in a rural setup. Periodic sampling was done for a period of 7 months from April 2019 to October 2019.

Inclusion criteria- The study includes *Candida* isolates from various clinical samplessent routinely to the Microbiology laboratory

Exclusion criteria-Patients on any antifungal therapy 6 weeks prior to sample collection were Excluded from the study.

This present study was done after obtaining the Institutional ethical committee clearance.

All samples were collected using aseptic precautions. After receiving in the Microbiology laboratory, the samples were inoculated onto both Blood agar and MacConkey agar, and plates were incubated at 37^{0}C for 24-48 hours aerobically. Colonies that appearedsmooth, pasty, opaque, white, or beige were suspected as*Candida*colony (Figure 1)and Gram stain was done for confirmation (Figure 2).

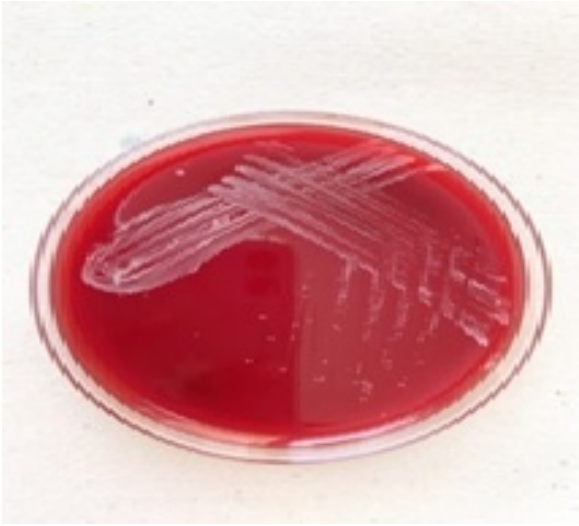

Fig-1: Candida on Blood agar.

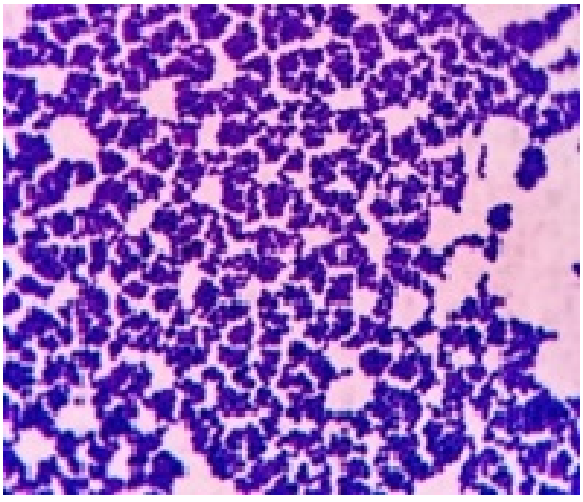

Fig-2: Gram stain showing *Candida.*

The isolate that revealed gram-positive budding yeast cells was inoculated ontoSabouraud Dextrose agar(Figure 3)and Hicrome Candida differential agar.

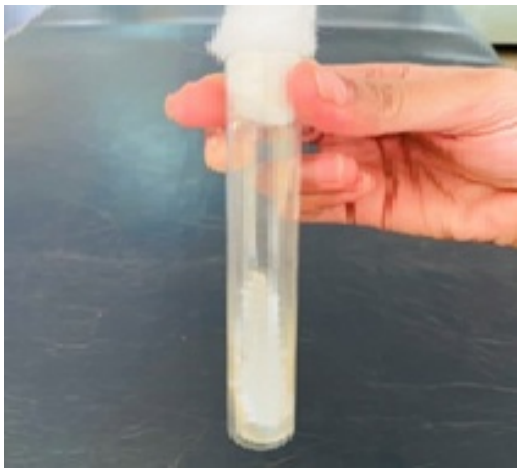

Fig-3: Candida growth on Sabouraud Dextrose agar.

The growth obtained on SDA was further subjected to Gram stain and germ tube test. Germ tube test was done to differentiate *C.Albicans* and *C.dublinenses* from other *Candida* species. An isolated colony of *Candida*was suspended in 0.5ml of serum and was incubated at 37°C for 3 hours. A drop of this suspension was placed on a microscope slide and examined for the presence of germ tubes[7]. Speciation of the Candida isolates was done by inoculating it on Hicrome Candida differential agar.Hicrome agar was prepared as per the manufacturer's instructions and incubated at 30oc for 24-48 hours aerobically. Species identification was done by the morphology and color of the colony. C.Albicansproduce light green colonies, C. tropicalismetallic blue colonies, C.kruseiproduces purple fuzzy colonies, and C. glabrata white to cream-colored colonies (Figure 4).

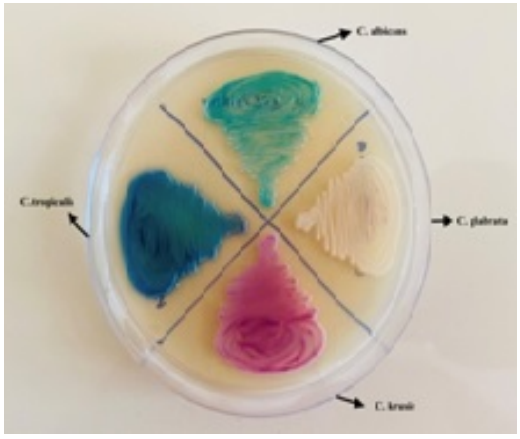

Fig-4: *Candida* species on CHROM agar.

The isolates that remained doubtful in their appearanceon Hicromewas considered as unidentified and excluded from the study. *Candida* isolates from clinical specimens like oropharyngeal, Vaginal, urinary, and bronchial secretions were considered significant only after repeated isolation. Descriptive statistics such as proportion and frequency were used to interpret the results.

Results

A total number of 126 *Candida* were isolated from various clinical samples. All isolates grew well on Hicrome Candida differential agar after 48 hours of incubation at 30°C. Most of the isolates were from High vaginal swab (n=78) followed by Urine (n=36), Sputum (n=04), Pus (n=04), Blood (n=02), Cerebrospinal fluid (n=01) and Endo Tracheal Tube (n=01) respectively (Table 1).

Table 1: Distribution of *Candida* species.

Sample	Candida species				Total
	C. albicans	C. tropicalis	C. glabrata	C. krusei	
High Vaginal swab	39	12	18	09	78 (61.9%)
Urine	16	10	06	04	36 (28.6%)
Pus	04	00	00	00	04 (3.2%)
Sputum	01	03	00	00	04 (3.2%)
Blood	01	00	00	01	02 (1.5%)
Cerebro spinal fluid	01	00	00	00	01 (0.8%)
Endotracheal tube	00	00	00	01	01 (0.8%)
Total	62 (49.2%)	25 (19.8%)	24 (19%)	15 (12%)	126(100%)

Out of 126 *Candida* isolates, *C. Albicans*was the most common species in 62 (49.2%) strains. The remaining 64 (50.8%) strains showed *Non-albicans Candida*. Out of 64 *Non-albicans Candida*isolates, 25 were *C. tropicalis*(19.8%), 24 were *C. glabrata*(19%) and 15 were *C. krusei* (12%) respectively.

Note: Out of 78 isolates from High Vaginal Swab (HVS), the most common species was *C. Albicans* followed by *C. glabrata,C. tropicalis,* and *C. krusei*. Among the 36 urine samples, the most common species was *C. Albicans* followed by *C. tropicalis*, *C. glabrata,* and *C. krusei*. Species wise distribution of *Candida* isolates from various clinical samples is shown in Table 1.

Discussion

Identification of *Candida* strains to the species level is increasingly necessary because of their variation both in their ability to cause infection and also in their susceptibility to antifungal agents. Species-level of yeast identification is mandatory for epide-

Miological purpose and laboratory diagnosis[8].Hicrome Candida differential agar medium accurately identifies the important *Candida* species namely *C. Albicans, C. tropicalis, C. glabrata, C. dubliniensis,* and *C. krusei* based on their color and morphological features [9].

Fromthe present study, the rate of isolation of NAC was 50.8% and the isolation rate of *C.Albicans* was 49.2%. *Non-albicans Candida*predominated over C

Albicans which correlates well with many other studies. In a study done by Kaup S et al[10], the *Non-albicans Candida* accounted for 50% of the isolates and the commonest species was *C. Albicans* (50%) followed by *C. tropicalis* (27.08%). *Non-albicans Candida*(52.8%) isolates were more than *C. Albicans*(47.2%) in a study by Samyuktha AA et al[11]Predominance of *Non-albicans Candida*(54%) over *C. Albicans*(46%) was also shown in a study by Madhumathi B et al[12].The present study also correlated with the study done by Marak MB et al [13] where the rate of isolation of *Non-albicans Candida* was 54.5%.

Though *Candida albicans* is considered to be the commonest species causing human diseases, there is a striking increase in the isolation rate of*Non-albicans Candida*species, primarily*Candida tropicalis*, *Candida glabrata, Candida krusei*, and *Candida parapsilosis*. This rise in *Non-albicans Candida* species has been associated with significant morbidity and mortality [14]. Hence, identification of *Candida* upto species level becomes necessary for the initiation of early and effective therapy. As NAC species significantly vary in their prevalence among different countries and health-care setups within a country, species identification plays an important role in the formulation of local therapeutic guidelines [15].

In routine diagnostic laboratories,Sabouraud dextrose agar (SDA) iswidely used for the isolation of all yeast species from a clinical specimen. Sabouraud dextrose agar is not a differential medium, and multiple yeast growth cannot be easily distinguished from each other. In the majority of the laboratories germ tube test is used to differentiate *C. Albicans* and *C. dubliniensis* from other *Candida* species. Although it is a rapid test, it may lead to false positive and false negative results [16].The other conventional methods like sugar fermentation and sugar assimilation tests used for the speciation of *Candida*are very time consuming and cumbersome. Molecular methods are very expensive And available only at reference centers.

Chromogenic agar is a rapid method to differentiate*Candida*species asit contains enzymatic substrates that are linked to chromogenic compounds. When a specific enzyme cleaves the substrate, the chromogenic substances produce color. The action of different enzymes produced by yeast species results in color variation which is useful for the presumptive identification of some

Yeasts [14]. Another important advantage of chromogenic medium is it greatly facilitates the detection of specimens containing a mixture of yeast species though there were no mixed cultures in the present study. The prompt detection of such clinical scenarios of multiple yeast etiology may be an aid for early appropriate treatment decisions [16].

In the present study, amongst the NACmost frequently isolated species was *C.tropicalis* followed by *C. glabrata* and *C. krusei*. Many other studies have also shown the preponderance of *C. tropicalis* over other NACspecies [17,18,19,20,21,22,23].

Limitations of the study

Conventional methods for the identification of *Candida* species like sugar fermentation and assimilation tests are not included in the present study. Hence, other species of *Candida* like *C. guilliermondii*, *C. parapsilosis*, *C. kefyr* could not be identified in the present study.

Conclusion

Identification of *Candida* up to species level is very important in the early management of Candidiasis. *Non-albicans Candida* species are increasingly associated with invasive Candidiasis and differ from *C. Albicans* with respect to epidemiology and antifungal susceptibility.The present study highlights the fact thatNAC has emerged as an important cause of infections even in our set up and can no longer be ignored as non-pathogens and contaminants.

What does the study add to the existing knowledge?

The current study results are also important for local monitoring of different *Candida* species which also helps in planning empirical therapies.Hicrome agar is a simple, rapid,and inexpensive method for

The identification of *Candida* species and is suitable for laboratories with limited resources.The major pathogenic species like *C. Albicans*, *C. tropicalis*, *C. glabrata,* and *C. krusei*are easily differentiated by their color and colony morphology within a short time.

Author's contribution

Dr. Shwetha D C.: Contributed for Study design, Literature search, Data collection, statistical analysis, manuscript preparation, editing,and review

Dr. Venkatesha D.: Contributed for Study design, Smanuscript preparation, editing and review

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