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Identification and Differentiation of Candida Species from Various Clinical Isolates through Conventional Methods in the Era of Molecular Diagnosis

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Abstract— Background: ***Candida species are commensals of the gastrointestinal tract, mucous membranes, and skin. These are the endogenous opportunists that lead to the infection cycle. The consumption of immunosuppressants, indwelling devices, and broad-spectrum antibiotics has risen over the past few decades, leading to a noteworthy rise in the prevalence of Candida infections. The purpose of this study is to use conventional methods to speciate Candida.***

Methods: ***The study included 54 isolates of Candida from various clinical samples. Gram stain, germ tube testing, along with inoculation on readily accessible CHROM agar (HiMedia India) were performed on these isolates. The Dalmau technique was used to detect the formation of chlamydospores.***

Results: ***The study comprised 54 Candida isolates obtained from various clinical samples. Most of the Candida isolates in the present investigation came from urine (50%) and were followed by sputum (26%), high vaginal swabs (14%), pus from surgical sites, and other sources (10%). The most prevalent species of candida was found to be Candida albicans 28/54 (51.85%), followed by C. tropicalis 08/54(14.81%), C. krusei 07/54(12.9%), C. dubliniensis 06/54(11.11%) and C. glabrata 5/54(9.25%).***

Conclusion: ***In order to treat the disease, it is essential to quickly and accurately identify the species of Candida that are causing it, that is, to recognize any species that may naturally defy conventional antifungal drugs. Non-albicans candida species, like C. tropicalis, C. dubliniensis, C. krusei, as well as C. glabrata, are also being removed from different clinical specimens more frequently than Candida albicans. For the quick and affordable identification of candida species, use CHROM agar.***

Index Terms: Candida, Non- albicans candida, CHROM agar, Dalmau technique, germ tube test.

I. INTRODUCTION

The widespread use of antibiotics with a wide range of undertakings, the rise in HIV infections, and other immunocompromised states have all contributed to an increase in the incidence of candidiasis during the past ten years. Around 60% to 80% of cases of candidiasis are caused by Candida albicans. Although Candida albicans is the most common species, other Candida species that may trigger both superficial and systemic mycoses are also becoming more prevalent. Among these species are C. guilliermondii, C. parapsilosis, C. glabrata, C. tropicalis, C. dubliniensis, and C. krusei. Antifungal agents can cause intrinsic resistance in certain species of Candida. Azole resistance is inherent in both C. glabrata and C. krusei. Candida species that are not albicans, such as Candida tropicalis and Candida parapsilosis, are less sensitive to azoles, especially fluconazole. The identification of isolates that may possess inherent resistance to specific antifungal agents is facilitated by species-level profiling. Therefore, timely and accurate recognition of Candida species can be crucial for both treatment and lowering death rates. It is now crucial to determine the species of Candida that has been isolated from different clinical specimens. Traditionally, tests such as the germ tube assessment, inoculation on corn meal agar, Dalmau technique, sugar assimilation, as well as fermentation are used to identify the species of Candida isolates.

II. METHODS

The study included 54 isolates of Candida from different clinical samples. Gram's stain, germ tube test, the urease test, along with inoculation on readily accessible CHROM agar (HiMedia India) were performed on these isolates. Direct samples were used for Gram's staining, and the samples were inoculated on Sabouraud dextrose agar. It was then incubated for 24 hours at 37°C. The Dalmau method was utilized to identify the growth of chlamydospores. To ensure ultimate species confirmation, a sugar assimilation test was performed on each isolate.

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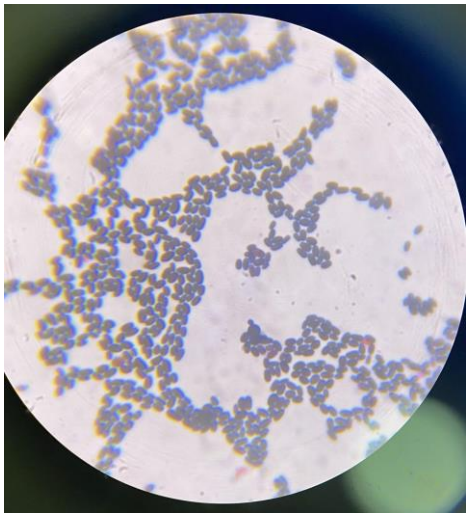

Figure 1: Demonstration of Candida in Gram staining at 100x.

Germ tube test

It is believed that this test is an easy, affordable, and an operative way to distinguish between C. albicans and other similar "species. It is predicated on the finding that C. albicans generate structures known as germ tubes that resemble tubes. Put 0.5 ml of either human or sheep serum into a tiny tube. Touch a Candida colony with a Pasteur pipette and slowly emulsify it in the serum. Incubated at 35–37°C for 2-3 hours. Place a small amount of the serum onto a slide for analysis, cover it with a coverslip, and" use low- and high-power objectives to examine the sample under a microscope. A brief filamentous projection, or hyphal, emerges "laterally from a yeast cell and is unconstrictible at the origin. The germ tube lacks a nucleus and is half as wide and three to four times longer than a yeast cell. The germ-tube test offers a quick way to identify C. albicans. C. albicans (ATCC 10231) was the positive control, and C. tropicalis (ATCC 13803) and C. glabrata (ATCC 2001)" were the negative control.

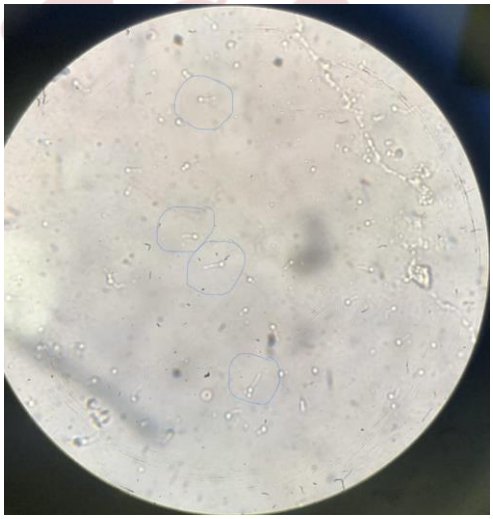

Figure 2: Demonstration of germ tubes.

Dalmau technique

On nutritionally inadequate media such as corn meal agar, specific species of Candida, such as Candida albicans, Candida dubliniensis, and a few strains of Candida tropicalis, produce chlamydospores. Chlamydospores are produced by a small number of saprophytic, non-pathogenic Candida species, among which are Candida australis and Candida clausenii. Compared to the germ tube technique, the chlamydospore formation test requires more time. Large and extremely refractile walled chlamydospores can be observed at the terminal branches or on the short lateral branches of Candida albicans using the Dalmau technique. While cornmeal and rice extract agar remain the most well-known chlamydospore-inducing media, casein agar is among the other growth media that have been recommended and shown to induce chlamydospore formation. This test is useful in differentiating amongst Candida dubliniensis and Candida albicans.

Figure 3: Dalmau technique on corn-meal agar.

Urease test

Candida species like Candida lipolytica, Candida krusei, and Candida humicola can be identified using the urease test. A positive urease test results in the color changing from yellow to pink. If there's no color change, the result is deemed negative. The Candida species is added to Christensen's urea agar slant, which is then incubated at 25ºC for more than the span of two days before being analyzed.

CHROM agar (HiMedia India)

The study included 54 isolates of Candida from different clinical samples. Gram's stain, germ tube test, the urease test,

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along with inoculation on readily accessible CHROM agar (HiMedia India) were performed on these isolates. The Candida species were cultivated on CHROM agar (Hi-media, India) and incubated at 37°C for the whole night, as per the manufacturer's directions. The species were differentiated by the color of the colonies on CHROM agar media.

Table 1: Colour of various *Candida spp*. on CHROM agar for identification.

Name	Colour on CHROM agar
C. albicans	Light green
C. tropicalis	Metallic blue
C. glabrata	White
C. krusei	Rose pink
C. parapsilosis	Pale cream
C. dubliniensis	Dark green

III. RESULTS

54 of these isolates of Candida from various clinical samples were included in the study. A majority of the detected Candida isolates in the current study (50%) came from urine, with sputum (26%) and high vaginal swabs (14%), pus from surgical sites, and other sources making up the remaining 10%. study included 54 isolates of Candida from different clinical samples. Gram's stain, germ tube test, the urease test, along with inoculation on readily accessible CHROM agar (HiMedia India) were performed on these isolates. The most prevalent Candida species was Candida albicans 28/54 (51.85%), followed by Candida tropicalis 08/54 (14.81%), Candida krusei 07/54 (12.9%), Candida dubliniensis 06/54 (11.11%), and Candida glabrata 5/54 (9.55%). Both the C. tropicalis and C. krusei isolates of Candida showed 100% specificity and sensitivity in CHROM agar. For C. albicans, the corresponding sensitivity as well as specificity were 100% and 85.7%. The degrees of sensitivity and specificity were 66.6% and 100% for C. glabarata and 20% and 100% for C. dubliniensis, respectively.

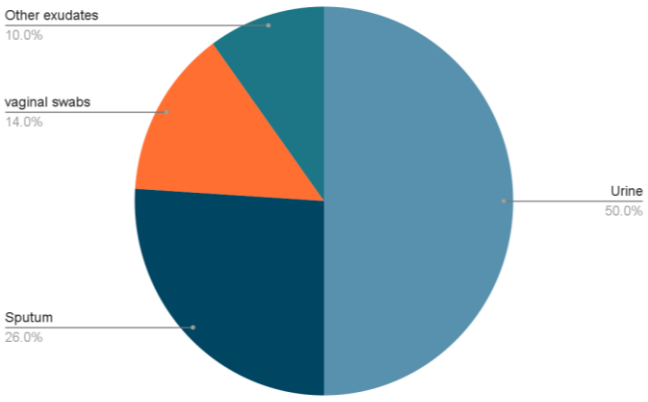

Chart 1: Distribution of Candida in clinical samples

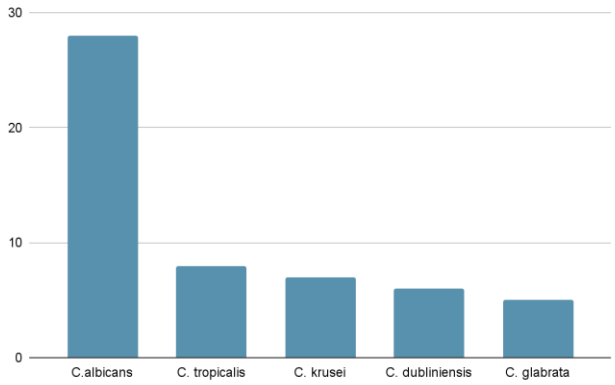

Chart 2: Number of each species of Candida isolated in various clinical samples.

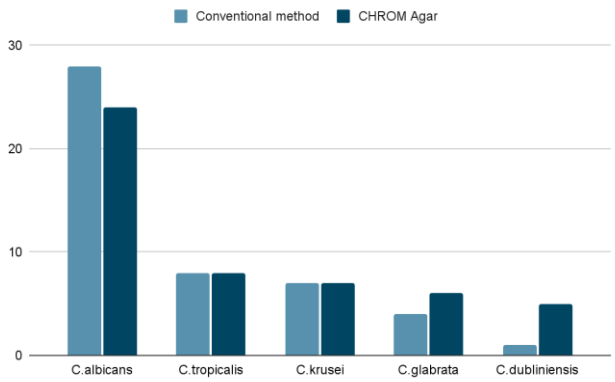

Chart 3: Sensitivity and specificity of CHROM agar for speciation of *Candida.*

IV. DISCUSSION

50% of the study's participants are Candida albicans. An investigation was carried out by Manjunath et al. also revealed the predominance of Candida albicans. Yet, several research studies have shown a greater prevalence "of non-albicans candida, ranging from 54 to 74%. According to reports, Candida tropicalis is the most common species out of the non-albicans Candida. In our investigation, Candida tropicalis was also the most common non-albicans species. The Dalmau technique, the urease test, the germ tube test, the sugar fermentation test, and the assimilation test were used in the conventional speciation of" Candida isolates. They require a lot of work and time. In just 24 to 48 hours, CHROM agar is a quick way to distinguish between the different species of Candida. In the research we conducted, the CHROM agar's sensitivity and specificity for Candida albicans were 100% for both the C. tropicalis and C. krusei isolates. For C. albicans, the corresponding sensitivity as well as the specificity were 100% and 85.7%. For C. glabarata, the corresponding degree of sensitivity as well as the specificity were 66.6% and 100%, while for C. dubliniensis, they were 20% and 100% respectively. A research endeavor by Jain N, Mathur P et al. reported a sensitivity of 80% for Candida

tropicalis and 89% for Candida albicans. Conventional methods presented difficulties in the identification of Candida dubliniensis in the present research. The results of this study on the sensitivity as well as the specificity of CHROM agar on different species of Candida were in accordance with the studies conducted by Kanna et al., Kumar et al., Swapna et al., and Easow et al. Comparing CHROM agar to traditional methods revealed that it was more practical. The current research was limited by its small sample size and the incapacity to conduct tests for antifungal susceptibility.

V. CONCLUSION

The widespread use of antibiotics with a wide range of undertakings, the rise in HIV infections, and other immunocompromised states have all contributed to an increase in the incidence of candidiasis during the past ten years. To identify species of Candida that may be naturally immune to commonly used antifungal agents, it is imperative to promptly and accurately identify the infectious species of Candida in order to initiate intervention. A quick and affordable way to identify different species of candida is to use CHROM agar. Comparing CHROM agar to traditional methods revealed that it was more practical. The current research was limited by its small sample size and the incapacity to conduct tests for antifungal susceptibility. The identification of isolates that may possess inherent resistance to specific antifungal agents is facilitated by species-level profiling. Therefore, timely and accurate recognition of Candida species can be crucial for both treatment and lowering death rates.

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