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Speciation of *Candida* species isolated from cutaneous skin lesions using CHROMagar and conventional methods

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ABSTRACT

Background: *Candida* species are members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. They are endogenous opportunists which cause secondary infection in individuals with some underlying immunocompromised conditions. Candidiasis is a common fungal disease found in humans affecting mucosa, skin, nails, and internal organs of the body. *Candida albicans* is generally considered the major pathogen among the *Candida* species. An increase in the prevalence of non-albicans species has been noted during the last decades. **Aims and Objectives:** The study was done to evaluate the performance of the conventional identification method and commercially available chromogenic *Candida* speciation media (CHROMagar) for the identification of medically important yeast and yeast-like organisms in a routine clinical microbiology laboratory. **Materials and Methods**: This study was conducted in the Department of Microbiology, Gandhi Medical College, Hamidia, and Associated Hospitals, Bhopal (M.P.), from January 2020 to October 2021. A total of 180 samples were collected from patients suspected of superficial mycoses which were then subjected to direct KOH examination and culture on plain Sabouraud's dextrose agar (SDA), on SDA with antibiotics slants (cycloheximide and chloramphenicol). The growths were examined macroscopically as well as microscopically. Germ tube tests and inoculations on CHROMagar *Candida* medium were performed on all growths identified as yeasts and confirmed by morphology on CMA. **Results:** In our study, the majority of patients, belonged to the age group of 21–30 years (25%) with a male-to-female ratio of 2:1. *Candida tropicalis* (44.68%) was the most common *Candida* species, followed by *C. albicans* (38.30%), *Candida krusei* (12.77%), and *Candida glabrata* (4.25%). **Conclusion:** Along with *C. albicans*, non-albicans *Candida* spp such as *C. tropicalis*, *C. krusei,* and *C. glabrata* are increasingly being isolated from clinical specimens. Identification of *Candida* species by colony color on Hicrome correlated with the morphology on CMA. Thus, the incorporation of CMA in routine yeast identification is more judicious than Hicrome as it increases the accuracy in the identification of *Candida* species within the same time as that of Hicrome.

Key words: Non-albicans *Candida*; Germ tube test; CHROMagar

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INTRODUCTION

Superficial cutaneous mycoses are fungal infections involving the outermost skin's covering as well as its appendages such as nails and hair. They are prevalent worldwide and are believed to affect 20–25% of the world's population and their incidence is on a continuous rise. They are classified into two broad groups; the "surface infections" which constitute pityriasis versicolor, tinea nigra, and piedra. And the "cutaneous infections" including dermatophytoses and candidiasis.[1]

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Candida species are members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. They are endogenous opportunists which cause secondary infection in individuals with some underlying immunocompromised conditions. Candidiasis is a common fungal disease found in humans affecting mucosa, skin, nails, and internal organs of the body. *Candida albicans* is generally considered the major pathogen among the *Candida* species. An increase in the prevalence of non-albicans species has been noted during the last decades.[2,3]

There is growing evidence of the increasing use of azoles causing this epidemiological shift. Characterization to species level helps to identify those strains which might be intrinsically resistant to some of the antifungal agents. Speciation of *Candida* isolates is conventionally done by germ tube tests, sugar assimilation, and sugar fermentation tests. Newer methods include CHROMagar, API systems, Vitek 2 ID systems, and molecular methods.[4]

Germ tube test is a rapid method to differentiate *C. albicans* and *C. dubliniensis* from other *Candida* spp. For further speciation chlamydospore formation test, sugar fermentation test, and sugar assimilation test can be done. However, these tests are time-consuming and labor-intensive. Among the newer tests, CHROMagar is rapid and cost-effective as compared to other expensive systems such as API systems, Vitek 2 ID systems, and molecular methods.[5,6]

In the present study, we speciated *Candida* isolates using a germ tube test and also evaluated the performance of commercially available chromogenic *Candida* speciation media, i.e., CHROMagar and confirmed by morphology on corn meal agar (CMA). CMA promotes the development of various microscopic morphological structures by yeasts that aid in their identification. A defining characteristic of *C. albicans* is its ability to produce chlamydospores, and their detection is an accepted criterion for the identification of this species.[7,8]

Aims and objectives

The aims of the study are:

1. The study was done to evaluate the performance of the conventional identification method and commercially available chromogenic Candida speciation media (CHROMagar) for the identification of medically important yeast and yeast-like organisms in a routine clinical microbiology laboratory.
2. To study the correlation between clinical features and the fungal isolates obtained.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Microbiology, Gandhi Medical College, Hamidia, and Associated Hospitals Bhopal, Madhya Pradesh. The study was approved by the Institutional Ethical Committee 614/MC/IEC/2020 date-January 04, 2020.

Samples from patients suspected of superficial cutaneous mycoses in the Department of Dermatology were collected and processed for mycological evaluation in the State Virology Laboratory, Department of Microbiology, Gandhi Medical College, Hamidia Hospital, Bhopal. A total of 180 samples received in the Department of Microbiology during the study period from January 2020 to October 2021 were recruited for the study.

History and other relevant data were collected from patients or their attenders and also from physicians and requisition forms from patients. Informed written consent was obtained from all the patients. After proper history-taking and physical examination of the patients, samples were collected adequately and precisely with all aseptic precautions. The samples were harvested in a sufficient amount and taken from the edge of the infected area, which corresponds to the active zone of the lesion. The following materials were used for the isolation of dermatophytes: Skin, nail, and hair. The collected specimens were divided into two portions. The first portion of the specimens was examined microscopically using 10–20% (hair and skin scrappings) potassium hydroxide (KOH). Nail clippings were immersed in 40% KOH overnight and examined the next morning.

The second portion was inoculated on plain Sabouraud's dextrose agar (SDA) and SDA with antibiotics slants (cycloheximide and chloramphenicol). All cultures were examined bi-weekly for growth and incubated for a maximum of 6 weeks before declaring them negative. The growths were noted for colony characteristics in the form of texture, surface, color on the obverse and reverse, and any diffusible pigment. Germ tube tests and inoculations on CHROMagar *Candida* Medium were performed on all growths identified as yeasts and the species were identified by type and color of the colonies on CHROMagar media as per the manufacturer's instructions. Then the colony was inoculated on a 1 cm×1 cm block of CMA block. The agar block was covered with a sterile coverslip and placed in a sterile Petri dish moistened with filter paper and incubated at room temperature for 48 h. After 48 h, the slide was placed on the microscopic stage, and the edge of the coverslip was observed using ×10 and ×40 objectives for chlamydospores, pseudohyphae, hyphae, blastospores, blastoconidia, etc. and the *Candida* were speciated.

Inclusion criteria

Patients having clinical diagnoses of superficial cutaneous mycoses were included in this study.

Exclusion criteria

Patients previously on treatment for superficial cutaneous mycoses were excluded from this study.

RESULTS

A total of 180 patients of suspected superficial cutaneous fungal infections were enrolled in the study comprising 120 (66.7%) males and 60 (33.3%) females, showing a male preponderance. Overall male-to-female ratio was 2:1. The majority of the patients were from the age group 21–30 years (25%), followed by 11–20 years (19.4%), and 31–40 years (19.4%) and least from the age group 61–70 years (1.7%). Students (38.9%), laborers (23.3%), and farmers (13.2%) dominated the study group. The majority of cases were found in July (23.3%), followed by August (12.8%) and November 23 (12.8%).

Most common type of clinical manifestation found was tinea corporis 49 (27.2%), followed by onychomycosis 28 (15.6%), tinea capitis 27 (15%), tinea manuum 23 (12.8%), tinea pedis 21 (11.7%), tinea cruris 9 (5%), tinea faciei 7 (3.9%), pruritus 5 (2.7%), tinea barbae 3 (1.7%), intertrigo 3 (1.7%), tinea versicolor 3 (1.7%), and eczema 2 (1.1%). Poor personal hygiene was the most common risk factor in the majority of the patients (42.2%), followed by topical steroid use (20%) and diabetes mellitus (20%).

A total of 47 *Candida* spp. were isolated from various clinical specimens. Growth of Candida species on Sabouraud's dextrose agar is shown in (Figure 1).

Candida tropicalis (44.68%) was the most common *Candida* species, followed by *C. albicans* (38.30%), *Candida krusei* (12.77%), and *Candida glabrata* (4.25%) (Table 1).

Figure 1: Growth of *Candida species* on Sabouraud's dextrose agar

Corn Meal Agar promotes the development of various microscopic morphological structures by yeasts such as chlamydospores which is a defining characteristic of *Candida albicans*. Pseudohyphae with terminal chlamydospores are shown in (Figure 2).

Candida albicans (green colored) and Candida krusei (pink colored) on CHROMagar Candida Medium are shown in (Figure 3).

Candida tropicalis (blue colored) and *Candida glabrata* (purple colored) on CHROMagar Candida Medium are shown in (Figure 4).

Table 1: Distribution of patients according to type of *Candida* isolates

Candida species	Number of patients	Percentage (100%)
Candida tropicalis	21	44.68
Candida albicans	18	38.30
Candida krusei	6	12.77
Candida glabrata	2	4.25
Total	47	100

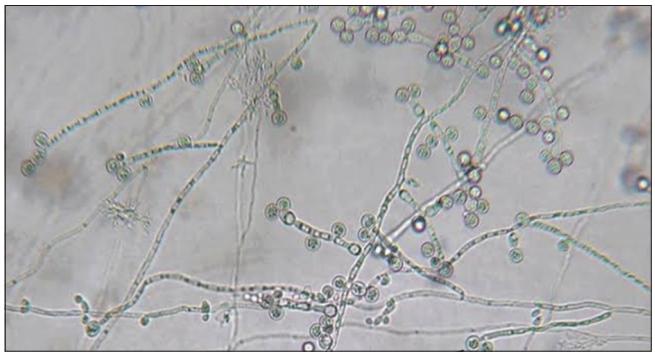

Figure 2: Pseudohyphae with terminal chlamydospores

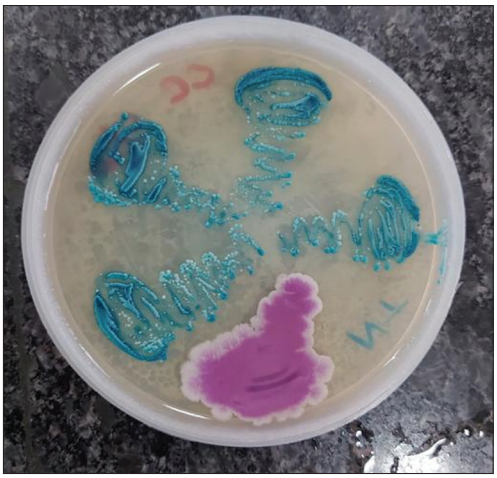

Figure 3: *Candida albicans* (green colored) and *Candida krusei* (pink colored) on CHROMagar Candida Medium

Comparison of the appearance of different *Candida* isolates on Hichrome agar as well as the morphology on corn meal agar is shown in (Table 2).

DISCUSSION

Superficial mycosis forms a large fraction of ailments in patients attending the skin outpatient department of our center. 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 the choice of empirical antifungal treatment. Furthermore, there may be geographic variation in the species isolated. In the present study, higher incidences of non-albicans *Candida* ranging from 54% to 74% have been seen in various studies.[4,9,10] Among the non-albicans species, *Candida tropicalis* is reported to be the most predominant species as discussed elsewhere. In our study also *C. tropicalis* was the most common non-albicans species.[2]

Figure 4: *Candida tropicalis* (blue colored) and *Candida glabrata* (purple colored) on CHROMagar Candida medium

Table 2: Appearance of different *Candida* isolates on Hichrome, corn meal agar

Species	Colony colour on Hichrome	Morphology on corn meal agar
Candida albicans	Light green colored smooth colonies.	Pseudohyphae with terminal chlamydospores; clusters of blastoconidia at septa.
Candida tropicalis	Blue to purple colored raised colonies.	Blastoconidia anywhere along pseudohyphae.
Candida krusei	Pink large rough colonies with a pale edge.	Pseudohyphae with cross-match sticks or tree-like blastoconidia.
Candida glabrata	Pink to purple colonies.	No pseudohyphae, cells small; terminal budding.

CHROMagar is a rapid method to differentiate between different *Candida* species. It facilitates the detection and identification of *Candida* species from mixed culture and provides results in 24–48 h. CHROMagar has the advantage of being technically simple, rapid, and cost-effective as compared to the conventional methods. Being a rural hospital and medical college, our study had its own limitations of small sample size, and inability to perform antifungal susceptibility tests. However, CHROMagar has proved to be a valuable method for the identification of *Candida* species even in resource-poor settings. Identification of Hicrome agar poses a problem as it is based on color, features such as fuzzy, hue, etc., and variations in the intensity of color with the passage of time. The interpretation becomes subjective, besides the media only recommends the identification of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, and *Candida parapsilosis*.[3-5]

The turnaround time taken for identification by morphology on CMA is 48 h which is similar to that on Hicrome as per the manufacturer's instructions. However, identification by morphological study on CMA demands skill from the laboratory personnel, which can be mastered. Koehler *et al.*[11] opine that careful observation of the yeast morphology on CMA, adds confidence in the identification of *Candida* species, which will also alert the microbiologist about the presence of unusual isolates.[11,12]

Limitations of the study

As this study had a relatively smaller sample size, the results may vary with a large study group. However, long-term studies with a larger sample size are needed to comment on the current scenario of superficial mycosis. Also no antifungal susceptibility testing of any of the isolate was performed.

CONCLUSION

Along with *Candida albicans*, non-albicans *Candida* spp such as *C. tropicalis, C. krusei,* and *C. glabrata* are increasingly being isolated from clinical specimens. CHROMagar is a simple, rapid, and inexpensive method with good sensitivity and specificity for the identification of such species. In view of accurate identification, the limitations of Hicrome in yeast identification are not to be ignored by a clinical microbiology laboratory. The incorporation of CMA in. Routine yeast identification prevents misidentification, adds confidence, and improves the mycology skills among the laboratory personnel without compromising cost, or time factors.

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Authors Contribution:
NR- Designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. **JL**- Managed the analyses of the study, concept coordination, and preparation of the manuscript. **PS**- Revision of manuscript. **MM**- Managed the literature searches. All authors read and approved the final manuscript.

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