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Research Article

EMERGENCE OF NON-ALBICANS *CANDIDA* AND USE OF CHROMAGAR FOR SPECIATION OF CANDIDA IN A RESOURCE-LIMITED SETTING

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ABSTRACT

Objective: *Candida* infections, especially those caused by non-albicans *Candida* species, have become increasingly common in recent years. These species' early isolation and identification are essential for their effective management. This study aimed to evaluate the efficacy of CHROMagar for the speciation of *Candida*.

Methods: All clinical samples received in the microbiology laboratory from April 2023 to March 2024 were processed as per microbiological criteria. The isolates that revealed Gram-positive budding yeast cells on Gram staining were further identified and speciated using CHROM agar and conventional methods.

Results: A total of 749 *Candida* were isolated from various clinical samples. *Candida albicans* was the most common species isolated (46.41%), followed by *Candida glabrata* (23.63%), *Candida krusei* (14.15%), *Candida parapsilosis* (9.2%), and *Candida tropicalis* (6.5%) while sensitivity and specificity of CHROM agar were 100% for *C. glabrata* and *C. krusei* and 98.3% and 100% for *C. albicans* while 100% sensitivity and 94.2%, 96% specificity for *C. parapsilosis* and *C. tropicalis,* respectively, when compared to conventional methods.

Conclusion: CHROM agar's performance was nearly identical to that of conventional methods. Compared to time-consuming, technically complex, and costly conventional methods, using this medium is quick, easy to use, and economical.

Keywords: Non-albicans *Candida*, CHROMagar, *Candida albicans*.

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INTRODUCTION

It has been observed over some time that mycotic infections have steadily grown [1]. Once considered a non-pathogenic or less virulent fungus, now *Candida* is regarded as a significant source of mycotic infections in immunocompromised people [1,2]. The impact of these infections on health in terms of morbidity and mortality is substantial, making them a major public health concern. Predisposing risk factors include increased use of broad-spectrum antibiotics, intravascular catheters, cytotoxic chemotherapies, invasive surgical procedures, and prolonged hospital stays [3].

Candida species are the most common cause of fungal infections, they are capable of initiating infections in both immunocompetent and immunocompromised hosts, but infection rates are higher in immunocompromised individuals; Therefore, candidiasis is correctly called "the disease of diseased" [4].

They constitute normal microbiota of the mucosal oral cavity, gastrointestinal tract, and vagina [4], however, can lead to superficial fungal diseases such as onychomycosis to non-life-threatening mucocutaneous candidiasis such as genital candidiasis, vulvovaginal candidiasis, and oropharyngeal candidiasis as well as life-threatening invasive candidemia [5]. All these lead to increased mortality, patient hospitalization, and health-care costs thus emerging as a public health problem [6].

In the 1980s, *Candida albicans* made up more than 80% of all candidal isolates recovered from nosocomial yeast infection [7] and *Candida* species are the third most prevalent nosocomial pathogen causing bloodstream infections [8]. Several virulence factors that contribute to its pathogenicity include epithelial and endothelial cell adherence, proteinase production, pseudohyphae formation, phenotypic switching, phospholipase production, and antigenic modulation caused by pseudohyphae formation [9].

However, during the past few decades, several studies have documented a steady shift away from the majority of *C. albicans* toward non-albicans *Candida* species (NAC), including *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, and *Candida krusei* [10]. It is crucial to identify *Candida* to species level, as many non-albicans *Candida* have reduced susceptibility to antifungal drugs [8].

For a very long time, azole drugs have been widely used to treat various types of *Candida* infections. NAC species have greater resistance to the azole than *C. albicans* [11]. The emergence of non-albicans *Candida* species could represent a selection of less susceptible species such as *C. glabrata* and naturally resistant to fluconazole like *C. krusei*. *C. tropicalis* has the highest adherence rate to inanimate materials such as urinary and vascular catheters, and it is frequently involved in the formation of antifungal-resistant biofilms. Azole resistance in *C. tropicalis* and *C. albicans* is also increasingly reported [2].

Clinicians are no longer able to base their treatment decisions on the broad classification of fungi such as yeast and mold due to changes in the *Candida* epidemiology and the availability of newer antifungal medications with distinct antifungal spectra [12]. The conventional methods for speciating *Candida* isolates include sugar assimilation and fermentation tests [13]. However, it takes 72 h to 2 weeks to complete sugar assimilation tests thus, are labor-intensive procedures that take longer time to determine proper dialogue as well as antifungal agents [8]. To effectively treat fungal infections, early diagnosis and prompt initiation of therapy are required [14].

To expedite the process of identification, multiple culture media containing chromogenic substrates have been developed as CHROMagar. These unique media produce different colored colonies of different species of Candida as a result of their enzymatic activity reacting with the chromogenic substrates present in these media [8]. It is simple, quick, and economical to use chromogenic media in clinical microbiology labs for the isolation and presumptive identification of significant *Candida* species [7].

This study aims to isolate and identify *Candida* species from various clinical samples collected at a tertiary care hospital, and characterize the isolated *Candida* species for faster reporting to start treatment.

METHODS

A study was conducted in the Department of Microbiology at Government Medical College, Amritsar from April 2023 to March 2024.

Inclusion criteria

This study includes *Candida* isolates from various clinical samples sent routinely to the Microbiology department.

Exclusion criteria

Patients on any antifungal therapy 6 weeks before sample collection.

Samples

Various clinical samples, including blood, vaginal swabs, sputum, pus, and urine.

All the samples were collected using aseptic precautions and after receiving in the microbiology department, processed as per microbiological criteria [15], isolates that were showing Gram-positive budding yeast cells on gram staining were then processed, and inoculated, and identified as follows:

1. Culture on Sabouraud's dextrose agar (SDA)

These isolates were inoculated on two SDA slants supplemented with chloramphenicol and gentamicin, one inoculated at 37°C aerobically and the other at 22°C for 24–48 h. The growth is suspected as *Candida* if it is smooth, cream-colored, white pasty colonies on SDA and confirmation is done by gram staining. On gram staining, Gram-positive budding oval-shaped yeast cells with pseudohyphae were seen (Figs. 1 and 3).

2. Germ tube

To speciate *Candida* isolates, a Germ tube test was done. It will differentiate *C. albicans* and *Candida dublienenses* from other *Candida* species. An isolated colony of *Candida* was passed in 0.5 mL of serum and incubated at 37°C for 2 h. A drop of this suspension was placed on clean microscopic slide, covered with coverslip, and examined under the microscope for the presence of Germ tube (Fig. 2).

3. Sugar assimilation test

The test was done using the Carbohydrate Assimilation Method for a large number of *Candida* isolates and *Candida* isolates were speciated as per the sugars assimilated by them. The method was done per Auxanographic Carbohydrate Assimilation Method for Large Scale Yeast Identification [16]. The carbohydrate assimilation assay was performed by preparing the basal medium with 6.75% yeast nitrogen base (YNB) and 2% agar. In addition, working solution was distributed in sterile bottles with 2 mL of YNB and 18 mL of agar. 2 mL of 10% carbohydrate solution; Glu, Suc, Mal, Lac, Cel, Raff, Tre, Xyl, Gal, Dul were supplied into sterile vials containing stock medium and sterilized by moist heat. It was aseptically poured 4 mm thick into 90 mm Petri dishes and allowed to solidify and checked for sterility. Yeast for testing was inoculated on these plates for 24 h at 35°C by making quadrants on respective carbohydrate-containing petri dishes and inoculated with 10 µL of yeast inoculum with the help of pipette, consisting of 12×10^8 cells, calibrated with McFarland standard No. 4, in respective quadrants a drop is placed, incubated at 30°C for 24 and 48 h, and growth was monitored as shown in (Fig. 4 and Table 1).

Fig. 1: *Candida* growth on Sabouraud Dextrose agar.

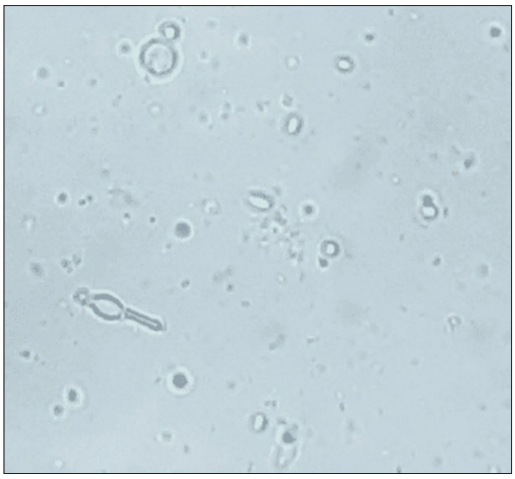

Fig. 2: Wet mount preparation of serum showing germ tube formation by Candida species

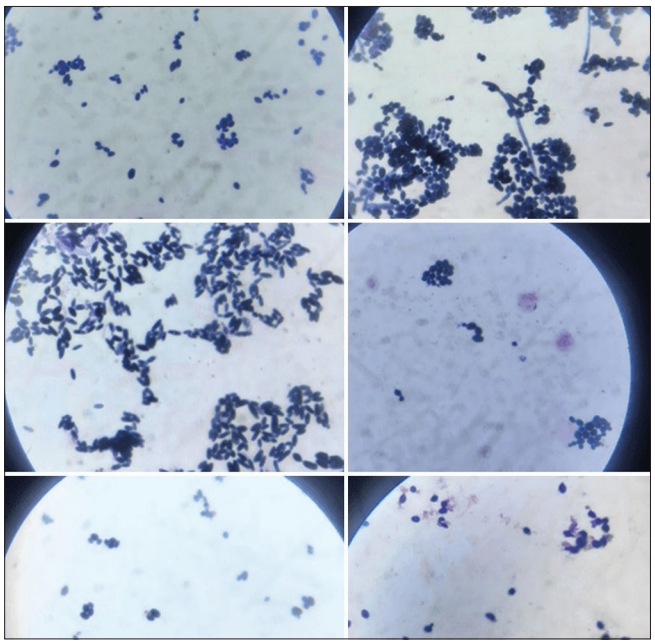

Fig. 3: Gram-stained smear showing budding yeast cells under an oil immersion field (×100)

4. Cornmeal agar (CMA)
A 1 cm by 1 cm block of CMA block was used to inoculate the colony. The agar block was put in a sterile Petri dish that had been soaked with filter paper, covered with a sterile coverslip, and incubated for 48 h at room temperature. After 48 h, the slide was put on the microscopic stage, and the *Candida* were speciated by looking for chlamydospores, pseudohyphae, hyphae, blastospores, and blastoconidia using ×10 and ×40 lenses [17] (Fig. 5 and Table 2).

5. CHROMagar
Speciation of *Candida* isolates can also be done by inoculating these isolates on CHROMagar, differential agar which was prepared as per manufacturer's instructions. After inoculating, it is incubated at 37°C aerobically for 24–48 h, followed by interpretation of morphology and color of colony as per manufacturer's specification to speciate these isolates. *C. albicans* produces light green, *C. dublienses* produces dark green, *C. tropicalis* metallic blue colonies, *C. krusei* produces purple fuzzy colonies, *C. glabrata* and *C. parapsilosis* produce cream to white colonies (Fig. 6 and Table 3).

RESULTS

A total of 749 isolates from various samples that are urine, sputum, pus, blood, and vaginal swabs are studied (Fig. 7). Isolation rate of non-albicans *Candida* spp. was higher (53.48%) than *C. albicans* (46.41%). *C. albicans* (46.41%) was the most common species to be isolated followed by *C. glabrata* (23.63%), *C. krusei* (14.15%), *C. parapsilosis* (9.2%), and *C. tropicalis* (6.5%) (Table 4).

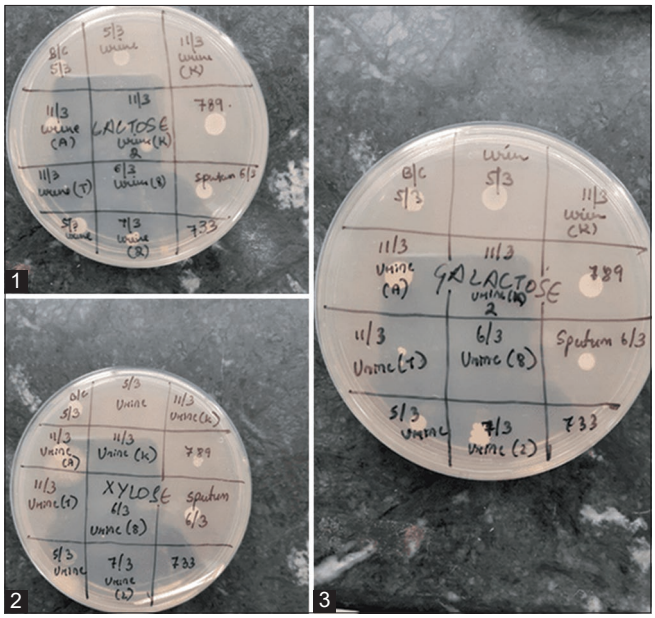

Fig. 4: Auxanographic carbohydrate assimilation plate following incubation at 30°C for 48 h for selected carbohydrates (1) Lactose, (2) Xylose, and (3) Galactose

SDA shows smooth, creamy-colored pasty colonies of approximately 2–3 mm in diameter, at both 37°C and 25°C after incubation of 18–24 h. The distribution of *Candida* species isolated is shown in Table 4.

Of CHROMagar's 749 isolates, 340C*. albicans* isolates were light green. At the same time, eight were bluish green, all 177 isolates of *C. glabrata* were initially smooth white and later became pink and all isolates of *C. krusei* were large fuzzy purple-colored, and 49 isolates of *C. tropicalis* produced metallic blue colonies. In contrast, all isolates of *C. parapsilosis* produced colorless cream-colored colonies, of approximately size 2–3 mm in diameter, at 37°C after incubation of 24 h with colors being more prominent after 48 h of incubation.

Gram staining was performed from these different colored colonies on CHROMagar and staining differentiation is as follows:

C. albicans: 5–6 µm, purple-stained round-to-oval cells arranged together

C. parapsilosis: 8–9 µm, purple-stained round cells placed individually

C. krusei: 2–3 µm, purple-stained oval elongated cells with narrow base budding placed individually

C. tropicalis: 3–4 µm, purple-stained oval cells placed together.

Species distribution of different *Candida* species among different samples is shown in Table 5.

The sensitivity and specificity of *Candida* species on CHROMagar (*Candida* differential agar) HiCrome agar are shown in Table 6.

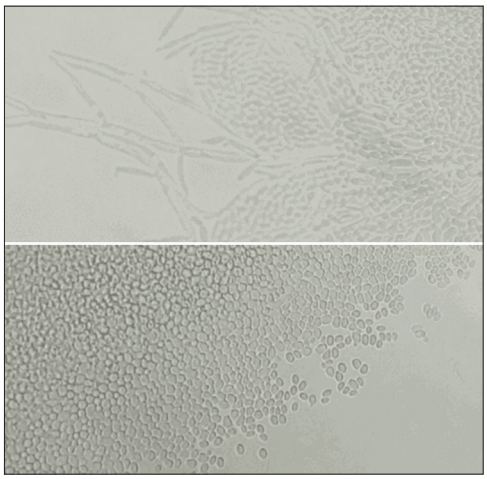

Fig. 5: Microscopic appearence of different species of *Candida* on cornmeal agar (above *Candida parapsilosis*, below *Candida glabrata*)

Table 1: Interpretation of carbohydrate assimilation test for various Candida species: Glucose (Glu), Sucrose (Suc), Maltose (Mal), Lactose (Lac), Cellobiose (Cel), Raffinose (Raff), Trehalose (Tre), Xylose (Xyl), and Galactose (Gal) [16]

Test Isolates (Wild/ATCC Strains)	Test carbohydrates								
	Glu	Lac	Suc	Mal	Tre	Xyl	Raff	Cel	Gal
Candida albicans	+	-	+	+	+	+	-	-	+
Candida tropicalis	+	-	+	+	+	+	-	+	+
Candida krusei	+	-	-	-	-	-	-	-	-
Candida parapsilosis	+	-	+	+	+	+	-	-	+
Candida glabrata	+	-	-	-	+	-	-	-	-
Candida kefyr	+	+	+	-	-	+	+	+	+

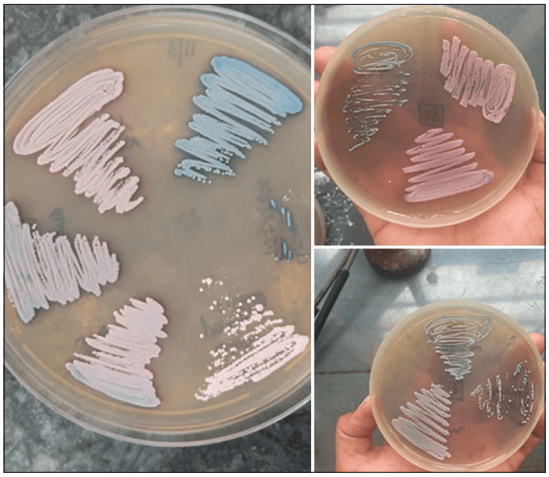

Fig. 6: CHORMagar showing different colored colony growth depicting different species of *Candida* (*Candida tropicalis, Candida glabrata, Candida parasilosis, Candida albicans*)

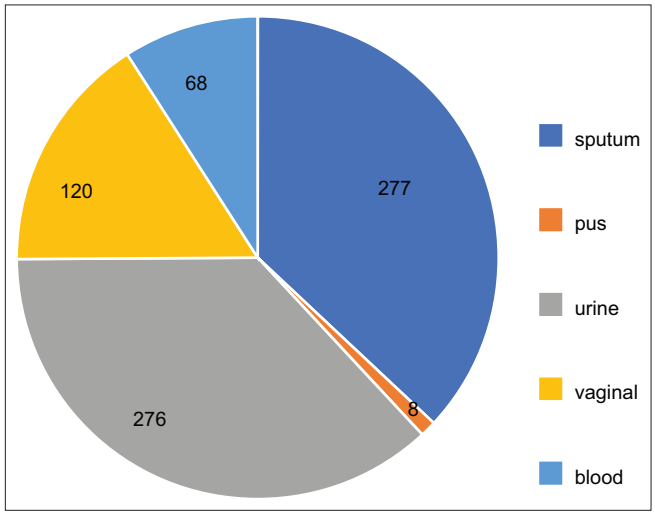

Fig. 7: Distribution of clinical samples showing growth of *Candida* species

DISCUSSION

Mucosal surfaces of all humans get colonized by *Candida* species soon after birth. [18] Various risk factors and immunosuppression favor the development of fungal infections and cause a change in the relative prevalence of different *Candida* species [13]. Systemic yeast infections and resistance to antifungal drugs are rising in Indian hospitals [19]. Increasing resistance to azoles and amphotericin B has been reported in India and other countries [8]. The identification of *Candida* at the species level is justified because the frequency of NAC infections is increasing [20]. Antifungal sensitivity to fluconazole varied between *Candida* species, with NAC being more resistant [21]. Therefore, rapid and accurate species-level identification of *Candida* species is essential for appropriate antifungal drug selection and patient management [5]. In countries with limited resources, lack of training and appropriate reagents, supplies, and equipment make rapid presumptive identification of yeast species difficult. To ease the financial burden on poor patients, these laboratories do not go beyond the germ tube test and limit their diagnosis to *C. albicans* or NAC. Biochemical assimilation and fermentation tests are not used in these laboratories due to a lack of resources and expertise and the time required for these tests, which increases the cost of mycological cultures [22]. CHROMagar is a differential medium widely used for the isolation of *Candida* species. These chromogenic media produce colonies of different colors for the chromogenic substances that react with the enzymes secreted by the organisms [23].

Table 2: Type and arrangement of blastoconidia & chlamydospore formation of different species of *Candida*

Name	Type and arrangement of blastoconidia & chlamydospore formation
Candida albicans	Large, thick-walled chlamydospore, usually terminal and present singly or in small clusters along with clusters of round blastoconidia.
Candida tropicalis	Oval blastoconidia singly or in small groups all along, long pseudohyphae.
Candida parapsilosis	Short, pencil-like pseudohyphae with blastoconidia arranged singly along pseudohyphae
Candida krusei	Yeast cells only
Candida glabrata	Pseudohyphae with blastoconidia forming cross-matchstick appearance.

Table 3: Different colored colonies on CHROMagar (*Candida* differential agar) after incubation of 48-72 hrs at 37°C depicting various species of *Candida*

Candida Species	Colony color
Candida albicans	Light green
Candida dubliniensis	Pale green
Candida parapsilosis	White to cream
Candida tropicalis	Blue with pink halo
Candida krusei	Purple, fuzzy
Candida glabrata	Cream to white

Table 4: Distribution of different species of *Candida* isolated from various clinical samples

Candida species	Number of isolates	Percentage
Candida albicans	348	46.41
Candida glabrata	177	23.63
Candida krusei	106	14.15
Candida parapsilosis	69	9.2
Candida tropicalis	49	6.5
Total	749	100

In the present study, *C. albicans* were the most commonly isolated species (46.41%), followed by non-albicans *Candida*, *C. glabrata* (23.63%), *C. krusei* (14.15%), *C. parapsilosis* (9.2%), and *C. tropicalis* (6.5%). As compared to *C. albicans* (46.41%), the isolation of non-albicans *Candida* species was higher (53.48%). These findings are in line with that of Nazir and Kanth whore ported 44.89% of *C. albicans* and 55.11% of NAC species [8]. Istalingam *et al.* [13] and Kaur *et al.* [2] also reported similar findings in their study, 56% and 61.1% of non-albicans *Candida* species while 44% and 38.9% of *C. albicans,* respectively. Various studies have shown a higher incidence of isolation of NAC ranging from 53% to 75% [3, 24, 25].

We found among non-albicans *Candida*, *C. glabrata* was the predominant isolate (23.63%), followed by *C. krusei*, *C. parapsilosis, and C. tropicalis* (23.63%, 14.15%, 9.2%, and 6.5%, respectively). These findings of *C. glabrata* as the most common NAC species to be isolated are in line with Mulu *et al.* [26] and ElFeky *et al.* [27] which reported *C. glabrata* as 2nd most common *Candida* species to be isolated after *C. albicans*, 22.5% and 12.7%, respectively.

In our study, most *Candida* was isolated from sputum (36.9%) and urine (36.8%) samples followed by vaginal swabs, blood, and pus samples. However, Jayalakshmi *et al.* [28] isolated more *Candida* from the sputum sample (31%) followed by the urine sample (28%). A study by Mohan *et al.* [29] states that the majority of isolates were from urine, followed by sputum samples.

Table 5: Distribution of *Candida* species among various clinical samples

Clinical sample	*Candida albicans* (%)	*Candida glabrata* (%)	*Candida krusei* (%)	*Candida parapsilosis* (%)	*Candida tropicalis* (%)	Total (%)
Sputum	198 (71.48)	39 (14.07)	4 (1.4)	14 (5.05)	22 (7.94)	277 (36.98)
Urine	26 (9.42)	75 (27.17)	100 (36.23)	50 (18.11)	25 (9.05)	276 (36.84)
Vaginal swabs	103 (85.83)	17 (14.16)	0	0	0	120 (16.02)
Blood	18 (26.47)	44 (64.7)	2 (2.94)	4 (5.88)	0	68 (9.07)
Pus	3 (37.5)	2 (25)	0	1 (12.5)	2 (25)	8 (1.06)
Total	348	177	106	69	49	749

Table 6: Sensitivity and Specificity Comparison of CHROMagar (*Candida* Differential Agar) versus Conventional Methods

Candida species	No. of *Candida* species identified by conventional method	No. of *Candida* species identified by CHROMagar	Sensitivity of CHROMagar (%)	Specificity of CHROMagar (%)
Candida albicans	354	348	98.3	100
Candida glabrata	177	177	100	100
Candida krusei	106	106	100	100
Candida parapsilosis	65	69	100	94.2
Candida tropicalis	47	49	100	96

In the present study, *C. krusei* was the most common species to be isolated from urine samples which was 36.23% of total urine samples showing culture growth of *Candida* isolates. Most of these patients were hospitalized and had a history of urinary catheterization thus emphasizing the fact of strong biofilm production by *C. krusei* and results are similar to Mohandas *et al.* showing *C. krusei* being the most commonly isolated *Candida* species (50%) [30].

In our study, the sensitivity and specificity of CHROMagar for *C. albicans* were 98.3% and 100%, while in a study by Nazir and Kanth [8], they showed 100% sensitivity and 96% specificity. The sensitivity and specificity of CHROMagar for *C. krusei* and *C. tropicalis* were 92.3% and 100%, while in our study, it was 100% for *C. krusei* but 100% and 96% for *C. tropicalis.*

Istalingam *et al.* [13] reported 100% sensitivity and specificity for *C. glabrata* and *C. parapsilosis.* Our study showed the same result for *C. glabrata* but it is 100% and 94.2%, respectively, for *C. parapsilosis.*

We found that CHROMagar readily detects multiple *Candida* species based on colony color and morphology and accurately discriminates the common *Candida* species, making detection of *Candida* species easy and rapid as it is technically simple, fast, as well as cost-effective in comparison to conventional methods which are technically demanding, time-consuming, and labor intensive.

CONCLUSION

Identifying *Candida* at the species level is critical to the initial treatment of candidiasis. Non-albicans candidiasis is increasingly associated with invasive candidiasis and is distinct from *C. albicans* in epidemiology and antifungal susceptibility. Our study shows that NAC has become an important cause of infection even in our setup, it cannot be dismissed as non-infectious, and CHROM agar is a simple, rapid, and inexpensive method with good sensitivity and specificity for identification of these species.

AUTHORS CONTRIBUTION

All Authors contributed satisfactorily to the study.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

AUTHOR'S FUNDING

None.

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