

COMPARISON OF CHROMAGAR WITH CORN MEAL AGAR FOR THE SPECIATION OF *CANDIDA* ISOLATED FROM CLINICAL SAMPLESJASLEEN KAUR*^{ORCID}, PRIYA BHAT^{ORCID}, UPASANA BHUMBALA^{ORCID}

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ABSTRACT

Objectives: The purpose of this study was to isolate and speciate *Candida* from various clinical samples and compare the colony characteristics of isolated *Candida* species on CHROMagar with the corn meal agar (CMA).

Methods: In our prospective study conducted for the duration of 6 months from February 2023 to July 2023, 81 *Candida* isolates were obtained from various clinical specimens such as high vaginal swab, blood, pus, skin scrapings, sputum, bronchoalveolar lavage (BAL), and endotracheal secretions which were subjected to KOH mount and then subcultured on Sabouraud's dextrose agar (SDA) with chloramphenicol and incubated aerobically at 37°C for 24–48 h. From the growth on SDA, further inoculation was done on CHROMagar and CMA, followed by additional identification and susceptibility through Vitek-2, and three were compared in terms of their sensitivity and specificity for isolation of various *Candida* species.

Results: Maximum *Candida* isolates were obtained from blood (18), followed by high vaginal swabs (17), pus (12), and nail clippings (10), whereas a single isolate was recovered from a patient with otorrhea. The most common risk factor was found to be prolonged antibiotic therapy. NAC was isolated at a higher rate 42 (52%) than *Candida albicans* 39 (48%). Among *non-albicans Candida* (NAC), *Candida tropicalis* was the most common species isolated 21 (50%), followed by *Candida parapsilosis* 11 (26%), *Candida glabrata* 5 (12%), *Candida krusei* 2 (4.7%), and *C. tropicalis*, which was predominant species isolated in blood 13/18 (73%) followed by pus 9/12 (75%). *C. albicans* predominated in HVS 13/17 (76%), sputum sample 4/6 (67%), BAL 6/9 (66%), and ET secretions 2/3 (67%). *Candida ciferrii* was the only species isolated from ear discharge.

Conclusion: CHROMagar showed more sensitivity in detection of *C. albicans* (100%) in comparison to CMA and Vitek-2 (97.4%) and decreased specificity (97.6%) as compared to CMA and Vitek-2 (100%) whereas for speciation of NAC particularly *C. parapsilosis* and *C. glabrata* CMA alone and also in combination with Vitek-2 exhibited much better sensitivity and specificity as compared to CHROMagar. NAC showed higher level of resistance to the azoles group of drugs and were overall less sensitive to other antifungal as compared to *C. albicans*. Hence, the amalgamation of cost-effective CMA with CHROMagar is an utmost need of an hour to give accurate yeast identification within the same time span.

Keywords: *Candida albicans*, *Non-albicans Candida*, CHROMagar, Corn meal agar.

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INTRODUCTION

Candida species constitute normal flora of the skin, mucous membranes, and gastrointestinal tract but at the same time serve as a big reservoir of endogenous opportunists capable of causing secondary infection in individuals with some underlying immunocompromised conditions [1]. They are 4th most common cause of health-care-associated bloodstream infections (BSIs) and 3rd most common cause of central line-associated BSI [2,3]. Although *Candida albicans* is the most common species responsible for both superficial and deep-seated mycoses but the focus has now shifted to *non-albicans Candida* (NAC) as the major culprit in intensive care units (ICUs), abdominal surgery wards, transplant recipients, etc. [4]. Irrational use of broad-spectrum antibiotics, indiscriminate use of azoles, placement of intravascular catheters as a nidus of biofilm formation, invasive surgeries, prolonged hospital stay, etc., are some of the risk factors implicated in the epidemiological shift in the distribution of *Candida* species [3]. Of the many pathogenic NAC known, the most frequent species associated with human infections include *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, *Candida dublinensis*, and *Candida guilliermondii*[3] that differ in their geographic variability, patient specificity, virulence factors, and their resistance pattern [2,3,5]. Therefore, characterization to species level serves as a modality for correctly identifying the strains that are intrinsically resistant to the commonly used antifungals and in addition serves as an effective aid in the initiation of proper antifungal therapy to the patient at the earliest.

In today's scenario CHROMagar *Candida*, a chromogenic species-specific, cost-effective media has gained popularity as it can be used for simultaneous isolation and identification of various *Candida* spp., based on direct detection of specific enzymatic activities by adding certain substrates of fluorochromes to the medium, in addition to the presumptive identification in poly-fungal specimens in 48–72 h [6]. Incorporating corn meal agar (CMA) for the purpose of identification based on the production of chlamydo spores, blastospores, true hyphae, and branched pseudohyphae can give an added advantage in the process. In this study, we aim to compare CHROMagar *Candida* with the CMA for the identification of *Candida* strains from different clinical samples. Moreover, identification and yeast susceptibility testing through Vitek-2 can also serve as an aid for the isolation of different *Candida* spp. and depicting the local geographical resistance pattern of the isolates, respectively.

METHODS

After taking approval from the Institutional Research Committee, and Ethics Committee for Biomedical and Health Research, Adesh University with permission from the Medical Superintendent and Head of the Department of Microbiology, Adesh Hospital, Bathinda, data collection was started. The study was conducted in the Mycology section of the Microbiology Laboratory, at Adesh Hospital, Bathinda. It was a prospective comparative study conducted for the duration of 6 months from February 2023 to July 2023.

Inclusion criteria

All the samples received in the mycology laboratory for fungal processing showing budding yeast cells on KOH mount and Gram staining with creamy pasty colonies on Sabouraud's dextrose agar (SDA) were included in the study.

Exclusion criteria

Urine samples were excluded from the study.

All clinical samples except urine received in the mycology section were processed, out of which 81 *Candida* isolates were obtained from various clinical specimens such as high vaginal swab, blood, sputum, pus, bronchoalveolar lavage, endotracheal secretions, ear swab, and nail clippings. Patient's demographic details and associated risk factors such as mechanical ventilation, central line insertion, duration of antibiotic therapy, use of corticosteroids, history of invasive surgical procedures, history of diabetes mellitus, pregnancy, use of OCP obtained from the clinical records, and test requisition form were analyzed. As per the standard operating procedures, all the clinical samples received were subjected to 10% KOH mount (40% KOH for nails), and to look for the presence of budding yeast cells. Positive samples were then subcultured on SDA with chloramphenicol and incubated aerobically at 37°C for 24–48 h. The growth of yeast was identified by smooth, cream-colored pasty colonies [7]. From the isolated colony, Gram staining was performed to observe the presence of yeast cells with or without pseudohyphae Fig. 1 represents the methodology followed for the identification of *Candida* isolates.

Collected data were compiled in the Microsoft Excel sheet and appropriate statistical analysis was done. The outcome measure was a comparison of CHROMagar and CMA for the correct isolation of *Candida* isolates with the automated identification through Vitek-2 in terms of sensitivity and specificity percentages.

RESULTS AND DISCUSSION

A total of 81 isolates were obtained from 1170 clinical samples received in the mycology laboratory. Out of 81 isolates, 34 (42%) were male

and 47 (58%) were female. 46–60 years (32%) contributed to the maximum whereas the least samples were received in the age group of below 15 years (3.6%) (Table 1). The maximum number of isolates were obtained from medical ICU (MICU) (31%), followed by obstetrics and gynecology (21%) and the least isolates were from neonatal ICU (2%), as depicted in Fig. 2.

NAC was isolated at a higher rate 42 (52%) than *C. albicans* 39 (48%) (Table 2).

Among NAC, *C. tropicalis* was predominantly isolated 21/42 (50%), followed by *C. parapsilosis* 11 (26%), *C. glabrata* 5 (12%), and *C. krusei* 2 (4.7%) (Table 3). Maximum *Candida* isolates were obtained from blood (18), followed by high vaginal swabs (17) whereas a single isolate was recovered from a patient with otorrhea.

C. albicans was predominantly isolated in 13 out of 17 HVS (71%), 6 out of 9 BAL samples (67%), 4 out of 6 sputum samples (67%), 2 out of 3 (75%) in ET secretion while NAC was predominant in other samples, 13 out of 18 (73%) in blood, 9 out of 12 (75%) in pus sample, 6 out of 10 (60%) in nail clippings, and 3 out of 5 (60%) in skin scrapings. Among NAC, *C. tropicalis* was the predominant spp. found in blood 8 out of 13 (62%), pus 5 out of 9 (56%), nail clippings 3 out of 6 (50%), and skin scrapings 2 out of 3 (67%), although *C. parapsilosis* was predominant NAC isolated in BAL 2 out of 3 (67%). *C. ciferrii* was the only species isolated from ear discharge (Table 3).

The most common risk factor was found to be prolonged antibiotic therapy, as shown in Fig. 4

CHROMagar, a chromogenic medium identified different species of *Candida* on the basis of colonial colour as depicted in Fig. 5. Further different *Candida* spp on inoculation on CMA using Dalmau plate technique Fig. 6 depicted different microscopic characteristics ie arrangement of hyphae, pseudohyphae, blastospores and chlamydoconidia formation aiding in the identification of different *Candida* isolates on CMA under high power lens as depicted in Figs. 7-12.

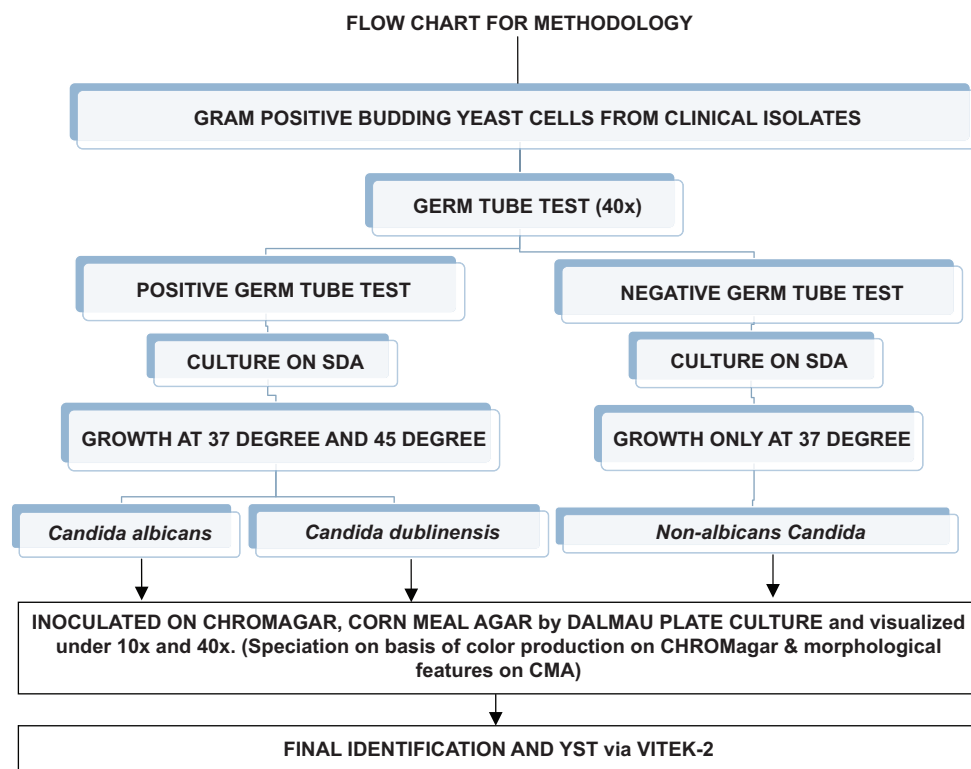


Fig. 1: Flow chart for methodology

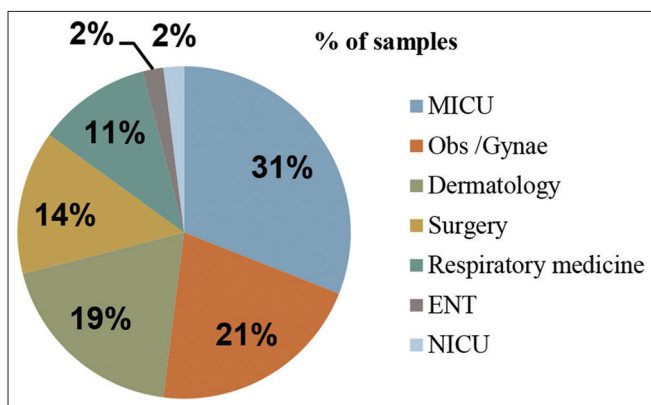


Fig. 2: Department-wise distribution of samples



Fig. 5: CHROMagar showing different Candida spp.

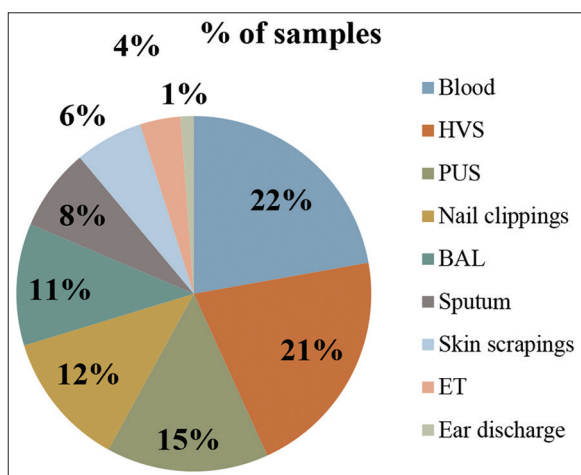


Fig. 3: Sample-wise distribution of Candida species (n=81)

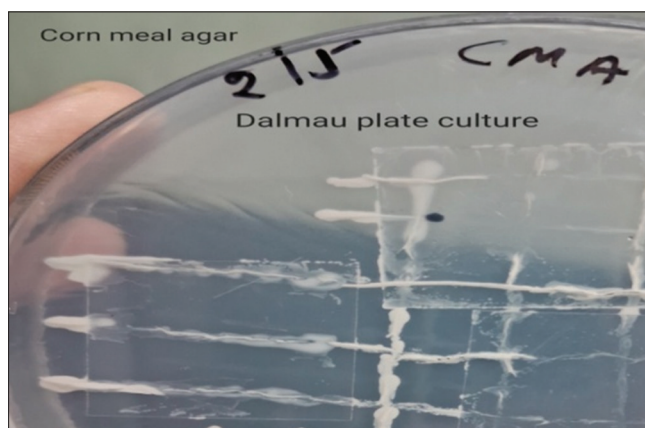


Fig. 6: Dalmau plate culture on corn meal agar

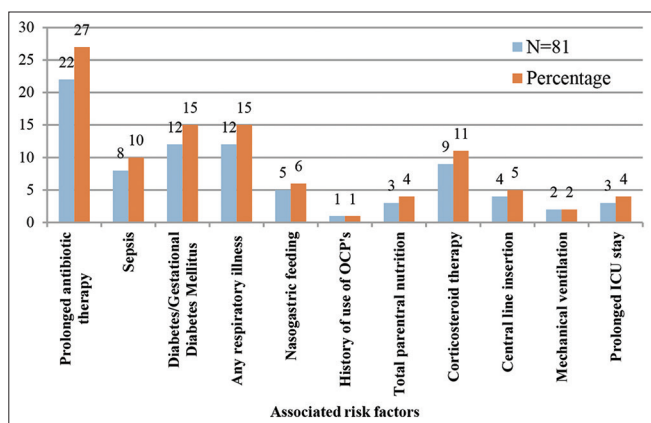


Fig. 4: Associated risk factors

Further co-relation of species identification was done via CHROMAgar, CMA and Vitek-2 as shown in Table 4.

CHROM agar showed more sensitivity (100%) in comparison to CMA and VITEK 2 (97.4%) but decreased specificity (97.6%) than CMA and Vitek-2 (100%) in detection of *Candida albicans* whereas for speciation of *Non-albicans Candida* particularly *C. parapsilosis* and *C. glabrata* CMA alone and also in combination with Vitek 2 exhibited much better sensitivity and specificity as compared to .CHROMAgar as depicted in Table 5.

NAC showed a higher level of resistance to azoles group of drugs and was overall less sensitive to other antifungals as compared to *C. albicans*.

Table 1: Age- and gender-wise distribution of Candida isolates

Age group	Male (n=34)	Male %	Female (n=47)	Female %	Total n=81 (%)
Up to 15 years	1	2.9	2	4.3	3 (3.6)
16-30 years	5	14.7	8	17.1	13 (16.1)
31-45 years	10	29.4	13	27.6	23 (28.4)
46-60 years	11	32.4	15	31.9	26 (32.1)
61-75 years	5	14.7	8	16.1	13 (16.1)
>75 years	2	5.9	1	3.0	3 (3.7)

Table 2: Distribution of Candida albicans and non-albicans Candida isolates

Candida isolates	Number of isolates (n=81)	Percentage (%)
<i>Candida albicans</i>	39	48%
<i>Non-albicans Candida</i>	42	52%
Total	81	100%

However, flucytosine and amphotericin B showed 100% sensitivity in all the *Candida* isolates (Fig. 13).

Fungal infections by *Candida* species are on a rising trend over the past decade, especially in immune-compromised patients due to the increased use of broad-spectrum antibiotics and the widespread use of indwelling medical devices. Although *C. albicans* was earlier considered

Table 3: Species-wise distribution of *Candida* isolates in different clinical specimens

Clinical specimen	<i>Candida albicans</i> (n=39)	<i>Candida tropicalis</i> (n=21)	<i>Candida dublinensis</i> (n=1)	<i>Candida parapsilosis</i> (n=11)	<i>Candida glabrata</i> (n=5)	<i>Candida guilliermondii</i> (n=1)	<i>Candida krusei</i> (n=2)	<i>Candida ciferrii</i> (n=1)
Blood	5	8	0	4	0	0	1	0
Vaginal swab	13	1	0	0	3	0	0	0
Pus	3	5	1	2	0	1	0	0
Nail clippings	4	3	0	0	2	0	1	0
BAL	6	1	0	2	0	0	0	0
Sputum	4	1	0	1	0	0	0	0
Skin scrapping	2	2	0	1	0	0	0	0
ET secretion	2	0	0	1	0	0	0	0

Table 4: Appearance of different *Candida* species on CHROMagar and Corn Meal Agar and identification through Vitek-2 system

Species	Total number of isolates	Colony characteristics on CHROMagar <i>Candida</i>	Morphological features on Corn Meal Agar	Identification with CHROMagar	Identification with CMA	Species on Vitek-2 system	Comments Identification of CHROM versus CMA versus Vitek-2
<i>Candida albicans</i>	39	Light green colonies	Terminal chlamydo spores, clusters of blastospores with abundant pseudohyphae	39 strains identified as <i>Candida albicans</i> Morphology essential	Identified 38 strains as <i>Candida albicans</i> one strain out of 39 misidentified as <i>Candida dublinensis</i>	Identified 38 strains as <i>Candida albicans</i> one strain was misidentified as <i>Candida ciferrii</i>	38 strains correlated by all three One strain out of 39 was misidentified as <i>Candida dublinensis</i> on CMA and one strain out of 39 was misidentified as <i>Candida ciferrii</i> on Vitek-2
<i>Candida tropicalis</i>	21	Purple-colored raised colonies	Blastoconidia arranged singly and in clusters along long pseudohyphae	Identified all 21 strains as <i>Candida tropicalis</i>	Identified all 21 strains as <i>Candida tropicalis</i>	Identified all 21 strains as <i>Candida tropicalis</i>	21 strains correlated by all three
<i>Candida dublinensis</i>	1	Dark green colonies	Chlamydo spores arranged in clusters at the terminal position on pseudohyphae	Identified the strain as <i>Candida dublinensis</i>	Identified the strain as <i>Candida dublinensis</i>	Identified the strain as <i>Candida dublinensis</i>	One strain correlated by all three
<i>Candida parapsilosis</i>	11	Cream-colored colonies with a mauve tinge	Blastoconidia arising at nodes along short pseudohyphae	Identified seven strains as <i>Candida parapsilosis</i> . Morphology essential Four strains were misidentified as <i>Candida glabrata</i>	Identified all 11 strains as <i>Candida parapsilosis</i>	Identified all 11 strains as <i>Candida parapsilosis</i>	Seven strains correlated by all three Four strains out of 11 were misidentified as <i>Candida glabrata</i> on CHROM
<i>Candida guilliermondii</i>	1	Cream to white colored colonies	Blastoconidia arranged in clusters at septa along short pseudohyphae	Misidentified as <i>Candida glabrata</i>	Identified the strain as <i>Candida guilliermondii</i>	Identified the strain as <i>Candida guilliermondii</i>	Strain correlated by Vitek and CMA Misidentified as <i>Candida glabrata</i> on CHROM
<i>Candida krusei</i>	2	Purple-colored colonies	Pseudohyphae lying at angles give a matchstick-like appearance.	Identified all two strains as <i>Candida krusei</i>	Identified all two strains as <i>Candida krusei</i>	Identified all two strains as <i>Candida krusei</i>	Two strains correlated by all 3
<i>Candida ciferrii</i>	1	Green-colored colonies	Lateral blastoconidia arranged on short pseudohyphae	Misidentified as <i>Candida albicans</i>	Identified the strain as <i>Candida ciferrii</i>	Identified strain as <i>Candida ciferrii</i>	1 strain correlated by Vitek and CMA Strain misidentified as <i>Candida albicans</i> on CHROMagar

the most common cause of *Candidiasis*, the emergence of NAC as an important opportunistic pathogen in recent years is a matter of concern to be emphasized [7]. NAC is associated with increased morbidity

and mortality due to increased resistance to antifungal agents either intrinsic or acquired [8]. Hence, species level identification has gained an utmost importance in the recognition of *Candida* due to

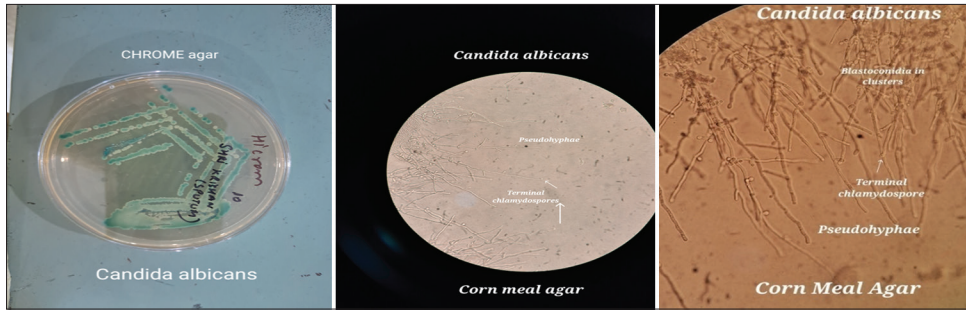


Fig. 7: *Candida albicans* on CHROMagar and corn meal agar

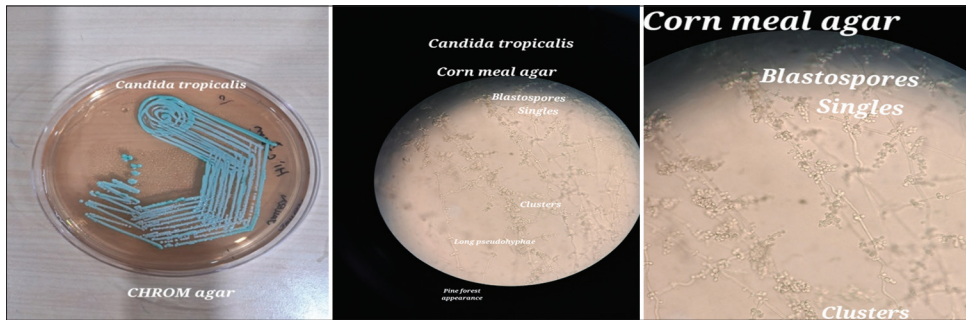


Fig. 8: *Candida tropicalis* on CHROMagar and corn meal agar



Fig. 9: *Candida parapsilosis* on CHROMagar and corn meal agar



Fig. 10: *Candida glabrata* and *Candida ciferrii* on CHROMagar and corn meal agar

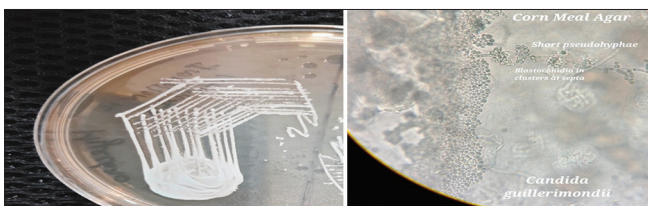


Fig. 11: *Candida guilliermondii* on CHROM agar and corn meal agar

the variability in expression of putative virulence determinants and decreased susceptibility to various antifungals [1]. Therefore, rapid identification to species level should be done on a priority basis keeping in mind the affordability, reliability, and time constraints for the test results that can directly influence the treatment option thus avoiding treatment failure [2].

A total of 81 *Candida* isolates were obtained from 1170 clinical samples received in our microbiology laboratory for fungal processing during a

Table 5: Sensitivity and specificity of CHROMagar, corn meal agar, and Vitek-2

Species isolated	No. of <i>Candida</i> spp. identified by CHROMagar	No. of <i>Candida</i> spp. Identified by CMA	No. of <i>Candida</i> spp. Identified by Vitek-2	Sensitivity % of CHROMagar	Specificity % of CHROMagar	Sensitivity % of CMA	Specificity % of CMA	Sensitivity % of Vitek-2	Specificity % of Vitek-2
<i>Candida albicans</i> (39)	39	38	38	100	97.6	97.4	100	97.4	100
<i>Candida tropicalis</i> (21)	1 (FP)	21	21	100	100	100	100	100	100
<i>Candida dublinensis</i> (1)	1	1 (TP)	1	100	100	100	98.7	100	100
<i>Candida parapsilosis</i> (11)	7 (TP)	11	11	64	97.2	100	100	100	100
<i>Candida glabrata</i> (5)	2 (FP)	5	5	60	93.8	100	100	100	100
<i>Candida krusei</i> (2)	2	2	2	100	100	100	100	100	100

CHROMagar showed more sensitivity (100%) in comparison to CMA and vitek-2 (97.4%) but decreased specificity (97.6%) than CMA and Vitek-2 (100%) in detection of *Candida albicans* whereas for speciation of non-*albicans* *Candida* particularly *C. parapsilosis* and *C. glabrata* CMA alone and also in combination with Vitek-2 exhibited much better sensitivity and specificity as compared to CHROMagar. TP: True positive, FP: False positive



Fig. 12: *Candida krusei* on CHROMagar and corn meal agar

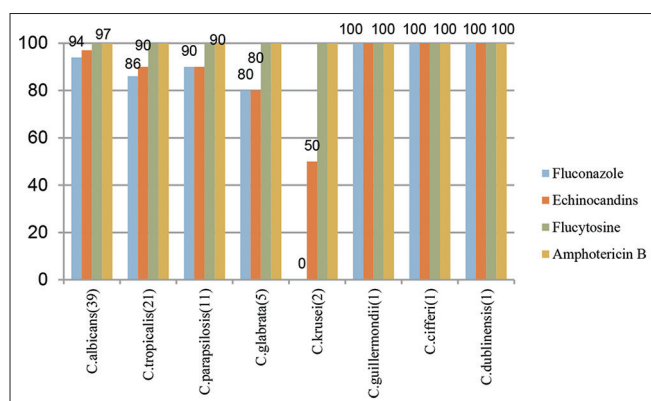


Fig. 13: Antifungal susceptibility testing of *Candida* isolates

period of 6 months. Female (58%) outnumbered as compared to males (42%) with maximum isolates covering the 46–60 year age group (32.1%) and minimal isolates from extremes of age group (3.6–3.7%). Maximum isolates were obtained from medicine ICU (31%) followed by obstetrics and gynecology (21%). This was due to the fact as maximum admissions were of females in the MICU of age group 46–60 years with comorbidities such as diabetes, chronic obstructive pulmonary disease, and prolonged antibiotic therapy from which *Candida* isolates were recovered, and moreover, the affected females were multipara with history of vaginitis coming to the obstetrics and gynecology outpatient department. Similar results were shown by Somsundaram [7] where

maximum growth was isolated from females (64%) as compared to males (36%). However, male preponderance has been reported by Patel et al. [9].

In the present study, the rate of isolation of NAC was higher (52%) than *C. albicans* (48%). *C. tropicalis* was the predominant non-*albicans* species isolated (21/81), followed by *C. parapsilosis*, (11/81), *C. glabrata* (5/81), and *C. krusei* (2/81). A single isolate of *C. guilliermondii* and *C. dublinensis* in pus sample was also recovered. The predominance of NAC correlated well with many other studies like Nirmal et al. [1] Bharathi [4]. In a study done by Nirmal et al. [1], NAC accounted for 58% of isolates and the most common species was *C. albicans* (42%) followed by *C. tropicalis* (30%). Similar results were also obtained by Shwetha and Venkatesha [10] where the rate of isolation of NAC was 50.8% and *C. albicans* was 49.2%. In a surveillance study conducted around various countries, an increase in the prevalence of *C. tropicalis* (4.6% in 1997 to 7.5% in 2003) and *C. parapsilosis* (4.2–7.3% in 2003) has been reported with isolation rates of less common species such as *C. guilliermondii* [8].

In our study, maximum isolates were recovered from blood (18/81) among which *C. tropicalis* was the predominant species (8/18) followed by high vaginal swabs (17/81) where *C. albicans* (13/17) was isolated maximally. *C. parapsilosis* was the third most common species (11/81) and most of the isolates were recovered from blood samples (4/11) from MICU. A single isolate of *C. ciferrii* was obtained from a diabetic patient who presented to ENT with complaints of ear discharge. Similar results were obtained from the study done by K Pavneet in which maximum isolates were from blood (57.4%) followed by sputum

(31.5%). This study correlates with the observation of many other authors such as Jaggi *et al.* where 33.6% of isolates were from blood, 50% from Gill *et al.*, and 57.7% by Sandhu *et al.* unlike other studies by Nirmaladevi [7] where the maximum isolates were from urine (54%) followed by high vaginal swabs (17%). In a study done by Sathi *et al.* [5], maximum species were obtained from high vaginal swabs (52%) followed by blood (39%).

The striking increase in the isolation rate of NAC primarily *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* over the last few years is a matter of concern as they are associated with increased morbidity and mortality in addition to their varied susceptibility pattern. Hence, species level identification plays a pivotal role in the formulation of local therapeutic guidelines and initiation of early and effective antifungal therapy [10].

The risk factors responsible for changing epidemiology could be attributed mostly to the prolonged antibiotic therapy in the ICUs, history of comorbidities such as diabetes, severe immunosuppressant, patient on corticosteroids, prolonged use of oral contraceptive pills by females, and any device insertion that can act as a nidus for biofilm formation, previous history of any respiratory illness, etc. In our study, prolonged antibiotic therapy in the ICUs was the most common risk factor (25%) followed by diabetes (13%). Administration of broad-spectrum antibiotics is responsible for suppressing the endogenous flora leading to fungal overgrowth. The hyperglycemic environment in diabetic patients leads to immune dysfunction thus increasing the susceptibility to fungal infections, especially by *Candida*. Similar results were obtained by Chongtam [11] where prolonged antibiotic therapy was the most common risk factor (26%), followed by diabetes (21%) and HIV (16%). Chakrabarty and Shivaprakash observed a higher rate of *Candida* infection in those patients with more than three antibiotic administrations for 7 days or more.

The conventional methods used for identification are cumbersome and time-consuming. Moreover, the germ tube test also known as the Reynolds-Braude phenomenon used in routine diagnostic laboratories as a rapid test for differentiating *C. albicans* and *C. dublinensis* from other *Candida* species can also give false-positive and false-negative results. The formation of unconstructed filaments in response to serum forms the basis of this test [12]. In our study, all *C. albicans* isolates and a single isolate of *C. dublinensis* showed germ tube test positive unlike the study conducted by Bhaskaran *et al.* [8] that showed germ tube sensitivity and specificity of 88% and 100%, respectively, for *C. albicans*. Hence, it has become mandatory to evaluate the effectiveness of alternative methods in terms of reliability and feasibility such as for HiCHROM chromogenic medium along with CMA for identification of *Candida* up to species level.

In our study, we evaluated the performance of CHROMagar with CMA in terms of sensitivity and specificity for the speciation of isolates. Eighty-one *Candida* isolates were identified through the combination of growth on SDA, Gram staining, germ tube test, Hichrom agar, and CMA with additional antifungal susceptibility pattern through Vitek-2.

In the present study, while identifying *Candida* species based on color description by manufacturer, CHROMagar showed 100% sensitivity for identification of *C. albicans*, *C. tropicalis*, and *C. krusei* whereas CMA and Vitek-2 showed 100% sensitivity for identification of *C. tropicalis*, *C. krusei*, *Candida parapsilosis*, *C. glabrata*, *Candida dubliniensis*, and 97.4% sensitivity for identifying *C. albicans*. CMA and Vitek-2 showed 100% specificity for all *Candida* isolates except *C. dubliniensis* for which CMA showed 98.7% specificity. In the present study, CHROMagar was less specific in the identification of *C. albicans*, *C. parapsilosis*, and *C. glabrata*. In our study, Hichrome falsely identified 4/11 isolates of *C. parapsilosis* as *C. glabrata* and similarly 2/5 isolates of *C. glabrata* as *C. parapsilosis*.

Similar results were also seen by Bharathi [4] where CHROMagar showed more sensitivity (100%) in comparison to CMA and Vitek-2 (97.4%) but decreased specificity (97.6%) than CMA and Vitek-2 (100%) in detection of *C. albicans* whereas for speciation of NAC particularly *C. parapsilosis* and *C. glabrata* CMA alone and also in combination with Vitek-2 exhibited much better sensitivity and specificity as compared to CHROMagar. Although the interpretation of HiCHROM based on color production is subjective, the intensity of color varies with time leading to altered sensitivity and specificity in the identification of various species, the ease and rapidity of CHROMagar along with the identification of mixed infection cannot be ignored in the era of the emergence of NAC. Moreover, the turnaround time taken for identification by CMA is almost similar to Hichrom agar; hence, it serves as an added advantage in identification by morphological characteristics by expertise and, further, leads to the correct identification of species with increased sensitivity and specificity. Therefore, CHROMagar and CMA should both go hand in hand while coming to the diagnostics.

Moreover, knowledge of the antifungal susceptibility pattern through Vitek-2 will prevent resistance to the available antifungal agents. In our study, all *Candida* isolates were sensitive to amphotericin B and flucytosine [13]. Among the azoles, *C. albicans* was more sensitive to fluconazole 37/39 (94%) as compared to *C. tropicalis* 18/21 (86%) and *C. glabrata* 4/5 (80%). Both strains of *C. krusei* were resistant to fluconazole as they exhibit intrinsic resistance to the above [10]. However, in the present study, *C. albicans* showed 38/39 (97%) sensitivity to echinocandins followed by *C. tropicalis* and *C. parapsilosis* that showed 90% sensitivity. *C. glabrata* and *C. krusei* were 4/5 (80%) and ½ (50%) sensitive, respectively. Single strains of *C. guilliermondi*, *C. ciferrii*, and *C. dublinensis* were sensitive to all the antifungal.

Limitations of the study

Urine samples were not included in the present study. Conventional methods used for identification of *Candida* species such as sugar fermentation and assimilation tests were excluded from the study.

CONCLUSION

Prompt identification of *Candida* to species level is mandatory for the early management of Candidiasis. The present study has highlighted the emergence of NAC as important pathogens in our setup; hence, these cannot be considered non-pathogens and contaminants. Accurate identification in the era of increasing NAC species requires the incorporation of CMA with CHROMagar for proper yeast identification thus preventing misidentification of species from various clinical samples with better test results without compensating cost and time constraints. Moreover, the findings of this study have served as a boon for our clinical laboratory to incorporate Hichrom agar and CMA in the routine isolation of *Candida* species for their presumptive identification.

CONFLICTS OF INTEREST

No conflicts of interest.

AUTHORS' CONTRIBUTIONS

Jasleen Kaur is the corresponding author as she has prepared, reviewed, and supervised the manuscript. Priya Bhat has prepared and reviewed the manuscript, and Upasana Bhumbra has supervised the manuscript.

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