

Isolation and identification of *Candida* and *Non albicans Candida* species using chromogenic medium

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Abstract

Background: Identification of *Candida* to species level is required to reduce morbidity & mortality as non albicans candida species are being recovered with greater frequency nowadays. CHROM Agar has the advantage of rapid identification and speciation of *Candida* by making use of chromogenic substrates.

Material & Methods: A prospective study was carried out from February 2014 to May 2014. The material for the study constituted various clinical samples that were sent routinely to the microbiology laboratory for culture and sensitivity testing. CHROM Agar *Candida* was used to differentiate *Candida* species along with standard yeast identification methods and antifungal susceptibility testing.

Results: A total of 45 *Candida* species isolates were recovered in which Non albicans candida 35(77.78%) remained higher in comparison to *C. albicans* 10(22.22%) with *C. krusei* 15(33.3%), *C. glabrata* 13(28.9%) and *C. tropicalis* 7(15.6%). *C. albicans* was isolated mainly from respiratory medicine ward (5), *C. tropicalis* from medical intensive care unit (03), *C. glabrata* and *C. krusei* with eleven isolates each from neonatal intensive care unit. *C. krusei* (26.67%) and *C. glabrata* (26.67%) remained predominant in blood; *C. tropicalis* (13.33%) in urine and *C. albicans* (8.89%) in sputum. *C. albicans* sensitivity to Fluconazole, Voriconazole and Amphotericin B was 70%, 70% and 90% respectively. *C. krusei* remained resistant to Voriconazole (60%) but susceptible to Amphotericin B (80%) whereas *C. tropicalis* depicted sensitivity of 71% to both Fluconazole and Amphotericin B.

Conclusion: CHROM Agar *Candida* shows good potential in identification of azole and amphotericin B resistant candida species, which in combination with local antifungal susceptibility pattern shall help to optimize the initiation of antifungal therapy and further avoid treatment failures.

Keywords: CHROM agar, Non albicans candida, Azoles.

1. Introduction

Nosocomial fungal infection has been increased nowadays due to advancement in medical management, and change in patient profile. *Candida* species being ubiquitous in nature inhabit the gastrointestinal tract including the mouth, oropharynx, female genital tract and skin. Although *C. albicans* remain the most common pathogen in oropharyngeal candidiasis (OPC), non-albicans species are increasingly associated with invasive Candidiasis. Non albicans *Candida* (NAC) species are closely related to *Candida albicans*, but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility [1]. The genus *Candida* includes several species implicated in human

pathology such as *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. kefyr*, *C. guilliermondii* and *C. dubliniensis* [2]. *Candida* colonization of mucosal sites ordinarily poses no threat to the health of the host. Problems develop when the body's defenses are abridged as occurs with diabetes, human immunodeficiency virus infection, neutropenia, and immunosuppression accompanying organ transplantation or when patients undergo certain procedures, such as bladder catheterization or urologic surgery [3]. Breaches in defense allows increased colonization of mucosal surfaces and sometimes candidemia, in which case the organisms can be carried to the kidneys.

These predisposing conditions permit the survival of blood-borne or locally invasive yeast in sufficient numbers to evade the local or systemic immunity [4]. Due to variable clinical presentation of Candida infections, it is important to identify this pathogens from all the clinical specimens received at laboratory irrespective of clinician's suspicion [5]. Candida species differ in their antifungal susceptibility and virulence factors. Thus identification of Candida up to species level along with antifungal susceptibility becomes very essential. *C. krusei* and *C. glabrata* are known for their innate resistance to fluconazole [6]. Conventional yeast identification includes microscopic morphological identification and biochemical studies, which require technical expertise and may take three to four days. Several brands of chromogenic media have been developed to produce rapid yeast identification. These media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies showing various pigmentations. These enzymes are species specific, allowing organisms to be identified to the species level by their colour and colony characteristics [7]. As rapid and reliable identification of Candida to species level is now needed far more than ever for clinicians as a deciding factor for the available treatment choices, so the present study was undertaken to know the prevalence of *C.albicans* and Non-albicans Candida isolates in various clinical specimens at our tertiary care institute using CHROM agar Candida along with their antifungal susceptibility pattern.

2. Material and Method

2.1 Study design and setting

A prospective study was carried out in the department of Microbiology, BPS Govt. Medical College for Women, Khanpur Kalan from February 2014 to May 2014. The material for the study constituted all the clinical samples that were sent routinely to the microbiology laboratory for culture and sensitivity testing.

2.2 Identification

All the samples were collected using aseptic precautions as per the standard guidelines and sent to the Microbiology department for further processing. As per the standard operating procedures, all the clinical specimens were inoculated on blood agar and Mac Conkey agar except blood samples which were inoculated in biphasic brain heart

infusion agar plus broth. The culture plates were incubated aerobically at 37°C for 24 to 48 hours. The visual growth was stained and the one which revealed gram positive budding yeast cells were further subcultured on SDA and CHROM agar. CHROM Agar was prepared as per the manufacturer's instructions (HiCrome Candida Differential Agar (M1297A), HiMedia Lab Pvt. Ltd). These plates were incubated at 30°C for 24-48 hours. Species were identified on CHROM Agar by the morphology and colour of the colony (chromogenic reaction). The appearances of various Candida species on CHROM Agar as per the instruction manual of manufacturer were light green-Candida albicans, cream to white- Candida glabrata, purple fuzzy- Candida krusei and blue to purple - Candida tropicalis. The growth on SDA was further speciated by standard methods using germ tube test, chlymadospore formation on corn meal agar and sugar fermentation and assimilation test. The isolates that remained doubtful in their appearance on CHROM Agar and those which did not confirm to the accepted morphological characteristics were considered as unidentified and excluded from the study.

2.3 Susceptibility testing

The antifungal susceptibility testing of yeast isolates was carried out using the disk diffusion method as per M44-A Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. Mueller Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue was used for sensitivity testing. The isolated Candida species were cultured on SDA at 35°C for 24 hours. Inoculum was prepared by picking five distinct colonies with approximately 5mm diameter. Then the colonies were suspended in 5ml of sterile physiological saline and resulting suspension was vortexed thoroughly. The turbidity of the inoculum suspension was adjusted to 0.5 McFarland standard followed by inoculation of plates containing MHA supplemented with 2% glucose (supports growth) and 0.5µg/ml methylene blue (delineate zone size) with a sterile cotton swab moistened with inoculum suspension. The plates were allowed to dry for 3-5 minutes and then antimicrobial discs were dispensed onto the surface of inoculated agar plate. The discs tested were fluconazole (25µg), voriconazole (1µg) and Amphotericin B (100 units), with their zone diameters measured as per the instruction manual of manufacturer (HiMedia Labs Pvt. Ltd.). All the media and discs were procured from Hi Media Labs, Mumbai.

3. Results

Figure 1: Appearance of *Candida* species on CHROM Agar *Candida*; A) *C. tropicalis*, B) *C. glabrata*, C) *C. albicans* and D) *C. krusei*

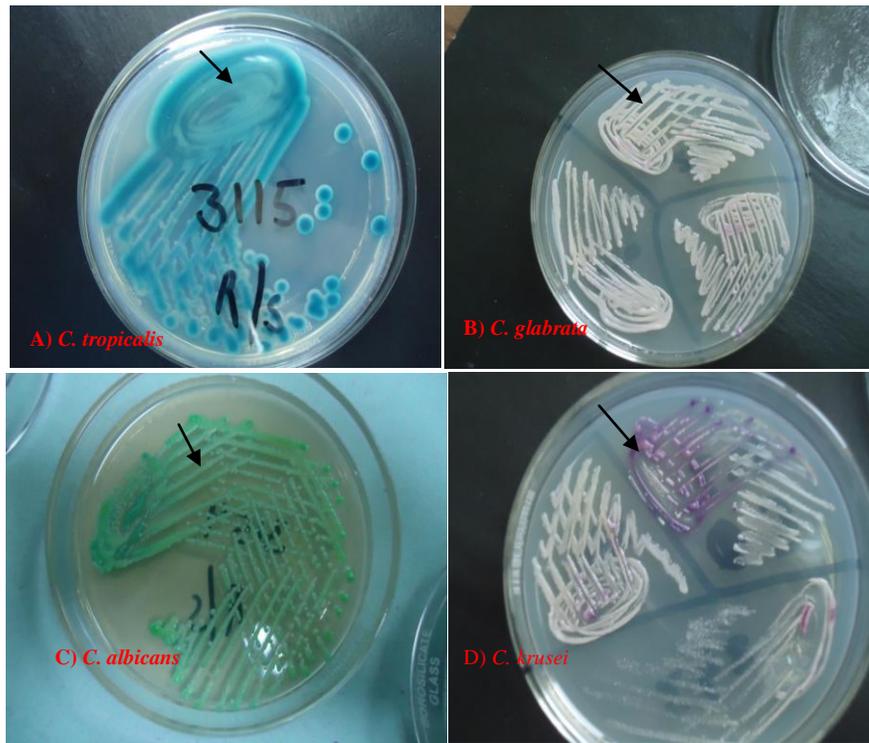


Table 1: Source of *Candida* species from different clinical samples

Candida species	Blood	Urine	Sputum	Fluids
<i>C.albicans</i> (n=10)	02(4.44%)	03(6.67%)	04(8.89%)	01(2.22%)
<i>C.krusei</i> (n=15)	12(26.67%)	03(6.67%)	-	-
<i>C.tropicalis</i> (n=7)	-	06(13.33%)	01(2.22%)	-
<i>C.glabrata</i> (n=13)	12(26.67%)	-	-	01(2.22%)
Total	26(57.78%)	12(26.67%)	5(11.11%)	2(4.44%)

Table 2: *Candida* species isolated from clinical departments

Department	<i>C.albicans</i>	<i>C.krusei</i>	<i>C.tropicalis</i>	<i>C.glabrata</i>
Neonatal ICU	-	11	-	11
Surgical ICU	02	-	01	-
Medical ICU	-	02	03	-
Pediatrics ward	-	02	01	01
Surgical ward	01	-	01	-
Gynae/obstetrics ward	02	-	-	-
Respiratory Medicine ward	05	-	01	01
Total	10	15	7	13

Table 3: *In-vitro* antifungal susceptibility pattern of *Candida* species

Candida species	Fluconazole		Voriconazole		Amphotericin B	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
<i>C.albicans</i>	7(70%)	3(30%)	7(70%)	3(30%)	9(90%)	1(10%)
<i>C.krusei</i>	-	-	6(40%)	9(60%)	12(80%)	3(20%)
<i>C.tropicalis</i>	2(28.57%)	5(71.43%)	-	-	6(85.71%)	1(14.29%)
<i>C.glabrata</i>	-	-	-	-	-	-

In present study 45 Candida species were isolated from various clinical samples which included blood (26), urine (12), body fluids (02) and sputum (05) shown in fig 1. The Non albicans Candida (NAC) species 35(77.78%) remained higher in comparison to *C. albicans* 10(22.22%). Among NAC various species recovered were *C. krusei* 15(33.3%), *C. glabrata* 13(28.9%) and *C. tropicalis* 7(15.6%) as shown in table 1. As regards isolation from various clinical units, maximum number of *C. albicans* were from respiratory medicine ward (5), *C. tropicalis* from medical intensive care unit (MICU) (03), *C. glabrata* and *C. krusei* with eleven isolates each from neonatal intensive care unit (NICU) as shown in Table 2. *C. krusei* (26.67%) and *C. glabrata* (26.67%) were predominantly isolated from blood followed by *C. tropicalis* (13.33%) from urine and *C. albicans* (8.89%) from sputum as shown in table 1. *In vitro* susceptibility testing revealed *C. albicans* sensitivity to Fluconazole, Voriconazole and amphotericin B as 70%, 70% and 90% respectively. In case of NAC, *C. krusei* remained resistant to Voriconazole (60%) but susceptible to Amphotericin B (80%) whereas *C. tropicalis* depicted sensitivity of 71% to both Fluconazole and Amphotericin B as shown in table 3.

4. Discussion

Increasingly, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and other NAC are the yeast species responsible for candidiasis. Importantly, many NAC have decreased susceptibility to antifungal agents. Specifically, *Candida krusei* and many *C. glabrata* demonstrate decreased susceptibility to fluconazole. Clinicians now depend on identification of *Candida* to the species level in order to optimize the selection of antifungal agents and to allow them to provide the best possible patient care. Several brands of chromogenic media have been developed to produce rapid yeast identification. These media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentations. These enzymes are species specific, allowing organisms to be identified to the species level by their color and colony characteristics. CHROM agar Candida has been shown to allow differentiation of candidal yeasts by color and morphology [7]. In the present study, out of 3338 clinical specimens tested during the study period, only 45 samples yielded growth of *Candida* species with prevalence rate of 1.34%. Two isolates which appeared dark green in colour on CHROM agar and with conventional tests also their morphology could not be fixed, were excluded from the study by labeling them unidentified. NAC (Non albicans *Candida*) species 35 (77.78%) outnumbered *C. albicans* 10(22.22%) which is similar to study done by Mondal S et al 2013 (NAC 57.75%; *C. albicans* 42.5%) and Vijaya D et al 2011 (NAC 55.8%; *C. albicans* 45.09%) [1, 9]. The emergence of new species of *Candida* as potential pathogens is reflection of the changing scenario in Medicine since the 1960s [10].

Distinction between species facilitates the understanding of epidemiology of *Candida* species particularly regarding the reservoir and mode of transmission which is required for the development of effective measures to prevent and control transmission of resistant pathogens [11].

The results of CHROM Agar were exactly parallel to that of conventional tests performed in all 45 isolates of *Candida* species, which is similar to observation of Vijaya D et al 2011 and Amar CS et al 2013 [9,12]. Ideally laboratories should be able to simultaneously detect and identify *C. albicans* and other major NAC species in clinical specimens. The differential agar medium, CHROM agar *Candida*, appears to meet these criteria as it facilitates the detection and identification of yeasts from mixed cultures and can provide results 24 to 48 h sooner than standard isolation and identification procedures [13].

It was found that *Candida* species remained predominant in blood stream infections (57.78%), with 53.33% of infections due to non albicans candida (NAC) species and 4.44% infections were due to *C. albicans* (4.44%). These findings are in concordance with other workers who have observed higher rates of blood stream infections with NAC [14, 15]. Among NAC, *C. krusei* (26.67%) and *C. glabrata* (26.67%) were predominantly isolated from blood stream infections. Some species like *Candida krusei* and *Candida glabrata* are emerging, possibly because they are innately less susceptible to azole drugs [16]. NAC isolation from urine specimens depicted predominance of *C. tropicalis* (13.33%), followed by *C. krusei* (6.67%) and *C. albicans* (6.67%), which is in concordance with Yashawanth R et al 2013 who also quoted *C. tropicalis* (45.45%), followed by *C. albicans* (30.3%) and *C. krusei* (15.15%) from urine samples [17]. The present study revealed Amphotericin B to be the most efficacious drug with susceptibility of 90% *C. albicans*; 85.71% *C. tropicalis* and 80% *C. krusei*, as observed from in-vitro antifungal susceptibility testing whereas among azoles, Voriconazole and fluconazole remained efficacious only for *C. albicans* strains with susceptibility of 70% for both the drugs. The resistance remained high (60%) to Voriconazole for *C. krusei* isolates whereas *C. tropicalis* exhibited 71.43% resistance to fluconazole. The in vitro susceptibility pattern depicted in present study is similar to results of studies done by Yashawanth R et al 2013 & Shivanand et al 2011 [17,18]. Azoles are amongst the most useful antifungal agents which inhibit ergosterol pathway by inhibiting 14- alpha-demethylation step. This result in the accumulation of methylated sterols which leads to disruption of fungal cell membrane structure. The resistance against azole class of antifungal agents has been increasing very rapidly due to frequent use of the drugs [19]. The number of antifungal agents available continues to increase in the setting of a shift of candidal infections to those caused by non-*albicans* species. Because of this, identification to species and

increased use of susceptibility testing has become necessary for appropriate selection of antifungal agent [20].

5. Conclusion

Use of CHROM agar *Candida* as a differential medium can serve the purpose of mycology laboratories for rapid identification of clinically important *Candida* spp. in comparison to conventional methods while potentially decreasing laboratory costs and labour. More importantly, this capability will also enable clinicians in optimizing the selection of antifungal agents and hence more rational and customized therapy which shall help in decreasing patient morbidity and mortality.

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