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Candidemia in a Tertiary Care Hospital in Jammu (J&K) - A Comparison of Conventional Methods and CHROMagar Technique for Speciation

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Abstract: ***Candida species are one of the most common causes of fungal infections leading both to non-life threatening and even life threatening invasive diseases. Candida species are the third most common cause of central line health care associated bloodstream infections. Recently, non albicans Candida species are increasingly being isolated from blood samples. In view of both inherent and acquired resistance of some of the Candida species to anti fungals , early speciation is desired for the timely initiation of appropriate anti-fungal therapy. The conventional methods of identification are time consuming and very difficult to perform. The prospective study was done to evaluate the performance of conventional identification methods (phenotypic) and commercially available Chromogenic Candida agar (CHROMagar) for the identification of Candida. A total of 52 yeasts were included in our study. The conventional methods used for speciation of yeast isolates were germ tube test, chlamydospores formation on corn meal agar and were compared against chromogenic agar medium (CHROM agar). Candida krusei (44.23 %) was the most common Candida species, followed by C. albicans (34.61 %), C. glabrata (15.38. %) and C.tropicalis (5.76%). Non albicans Candida species are gaining clinical significance. CHROM agar is an easy, technically simple, convenient and rapid method of speciation.***

Keywords: Candidemia, Non-albicans Candida, *Candida albicans*, Chromogenic Candida agar (CHROMagar)

1. Introduction

The incidence of mycotic infections has progressively increased over the last few years. Fungi once considered as non-pathogenic or less virulent are now recognized as a primary cause of morbidity and mortality not only in immuno-compromised and severely ill patients but also in immunocompetent hosts [1]. Candida species are the most common cause of fungal infections worldwide, the fourth leading cause of health care associated infections and the third most common cause of central line-associated bloodstream infections [2]. Candidemia is not confined to haematological patients, intensive care units or abdominal surgery wards, but it is remarkably frequent in the internal medicine settings. The pathogenic species of the genus Candida are *Candida albicans, Candida tropicalis, Candida krusei, Candida glabrata, Candida guillermondii, Candida parapsilosis, Candida lusitaniae, Candida kefyr, Candida dubliniensis* and are known to cause various clinical manifestations [3]. The epidemiology of Candida species has been shifting towards non-albicans Candida species which have emerged as clinically important opportunistic pathogens[4], [5], [6].The vast majority of invasive Candida infections are caused by four main non albicans Candida species which include *C.glabrata, C. krusei, C.tropicalis* and *C.parapsilosis*. The clinical manifestations of disease are extremely varied, ranging from acute, sub-acute and chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, gastrointestinal tract or become systemic as in septicaemia, endocarditis and meningitis [2]. The potential clinical importance of species-level identification has been recognized as Candida species differ in the expression of virulence factors and antifungal susceptibility. Widespread use of fluconazole has been associated with the development of infections due to non-albicans species that are intrinsically resistant to fluconazole or have developed resistance during treatment [7].

Identification of Candida by conventional methods like germ tube test, growth pattern on cornmeal agar are labour intensive and require several days and specific mycological media. Chromogenic media contain chromogenic substrates which react with enzymes secreted by target microorganisms to yield colonies of varying colours. In the present study, we speciated Candida isolates using germ tube test, chlamydospores formation test, and also evaluated the performance of commercially available chromogenic Candida speciation media i.e. CHROM agar.

2. Material & Methods

The present study was carried out between January 2016 to June 2016 in the Department of Microbiology, Government Medical College and Hospital, Jammu. Blood samples obtained from NICU, ICU and in-door patients were collected aseptically, inoculated on Blood culture broth and incubated at 37^0C for 24 hours followed by inoculation on Blood Agar and MacConkey agar. Blood agar plates which showed no growth were further incubated for 48 hours. Isolated yeast colonies on Blood agar were examined by Gram-staining and then inoculated on two tubes of Sabouraud dextrose agar slants, incubated at 25^0C and 37^0C for 24 hrs. For initial speciation Germ tube test was done. A

light inoculum was suspended in a test- tube containing 0.5ml human serum and incubated at 37^0 C for 2-4 hours. The suspension was placed on a microscopic slide and examined for the presence of germ-tubes. Candida species were further identified by Chlamydospores formation on Corn meal agar. Isolated colonies of Candida were picked up with inoculating wire. Three parallel cuts 1 cm apart were made into the surface of Corn Meal Agar, holding the inoculating wire at about 45 degree angle. A sterile cover-slip was laid on the surface of agar, covering portion of the inoculated streaks. Plates were incubated at 25^0 C for 24-72 hours. Plates were examined microscopically under 10 x and 40x. Simultaneously the Candida spp. were inoculated on CHROM agar and incubated at 37 ^{0}C for 24 hrs and the species were identified by colour of the colonies on CHROM agar media as per manufacturer‟s instructions (TM Media), showing *C.albicans* as light-green, *C.krusei* as rosy pink, C.glabrata, C.tropicalis as white and metallic blue respectively.

3. Results

In the present study, 1160 samples for blood culture were processed, out of which 52 (4.48%) Candida isolates were recovered. Out of 52 isolates 7(13.46%) Candida isolates were recovered after 48-72 hours of incubation.

Non albicans Candida species (65.38%) were isolated at a higher rate as compared to *Candida albicans* (34.*61%*). *Candida krusei* (44.23%) was the most common isolated species followed by *C. albicans* (34.61%), *C. glabrata* (15.38%) and *C.tropicalis* (5.76%) (Table 1). These 52 Candida isolates were also subjected to identification using CHROM agar. The results along with sensitivity and specificity of CHROMagar for various species are given in Table 1. Only one strain which was identified as C. glabrata by the conventional method was identified falsely as C. albicans by CHROMagar.

Out of 7 slow growing *Candida species*, *C.albicans* (3) *C.glabrata* (3) and *C. tropicalis* (1) were 5.76% 5.76% and 1.92% respectively. Species identification was done by both conventional method and Chromogenic media i.e. CHROMagar.

4. Discussion

The incidence of Candida infection has increased considerably over the past few decades, due to the AIDS epidemic, increased life expectancy, immuno-compromised states and widespread use of indwelling medical devices. In the past, C.*albicans* was mainly responsible for bloodstream infections, whereas in recent years growing proportion of episodes of candidemia have been caused by non-albicans Candida species [6], [7]. As non albicans Candida are more resistant to fluconazole, therefore species level identification has a direct impact on choice of empirical antifungal treatment [8]. In the present study a total of 52 Candida isolates were isolated from blood samples; non albicans Candida species (65.38%) were isolated at higher rate as compared to *C. albicans* (34.61%). In our study there was predominance of non albicans Candida (65.38%) which is in agreement with studies conducted by Kashid et al and Vijaya D et al in which they observed higher incidence of non albicans Candida species i.e., 70.7% and 54.1% respectively[9], [10]. These findings reported progressive shift in the species of Candida. A study conducted by Mohammed S. Alhussaini et.al is also in agreement with our findings showing C.krusei as the most isolated species among non albicans Candida [11]. We obtained 100% sensitivity of CHROMagar for *C.krusei, C.albicans* and *C.tropicalis* but sensitivity for C.glabrata was 87.5%.and specificity for C.albicans was 94.7%. The finding of our study is similar to the study conducted by Sumitra Devi et.al which showed specificity for *C.albicans* was 96%[12]. A study conducted by Daef E et al. which also showed specificity of CHROM agar for *Candida albicans* as 98.9% and for *C. glabrata* as 99% respectively[13].

For differentiation among different species of Candida conventionally germ tube test, growth pattern on cornmeal agar and sugar assimilation tests are being used which are technically difficult, time consuming and difficult to interpret which may take 72 hours to two weeks for species identification [14], [15]. Chromogenic agar is technically simple, easy to interpret and rapid method to differentiate among different Candida species. It facilitates the detection and identification of Candida species and provides result in 24-48 hours. Among the newer tests, chromogenic agar is rapid and cost effective as compared to other expensive systems like API system, Vitek ID system and molecular methods [12].

5. Conclusion

Our study showed significant rise of non-albicans Candida in blood samples. *C. krusei* was the most common non albicans Candida species isolated from blood. CHROMagar is a simple, rapid, easy and inexpensive method with good sensitivity and specificity for identification of Candida species. CHROMagar can be reliably used for identification for *C. krusei, C.albicans, C. tropicalis, C. glabrata, C.dubliensis etc.*

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Table 1: Sensitivity and specificity of CHROM agar for identification of various species of Candida

Candida spp.	*Isolates (52)*	*No .of Candida spp. identified by conventional methods*	*No. of Candida spp. identified using CHROM agar*	*Sensitivity of CHROM agar*	*Specificity of CHROM agar*
C. krusei	23 (44.23%)	23	23	100%	100%
C.albicans	18(34.61%)	18	19	100%	94.7%
C.glabrata	8 (15.38%)	8	7	87.5%	100%
C.tropicalis	3(5.76%)	3	3	100%	100%

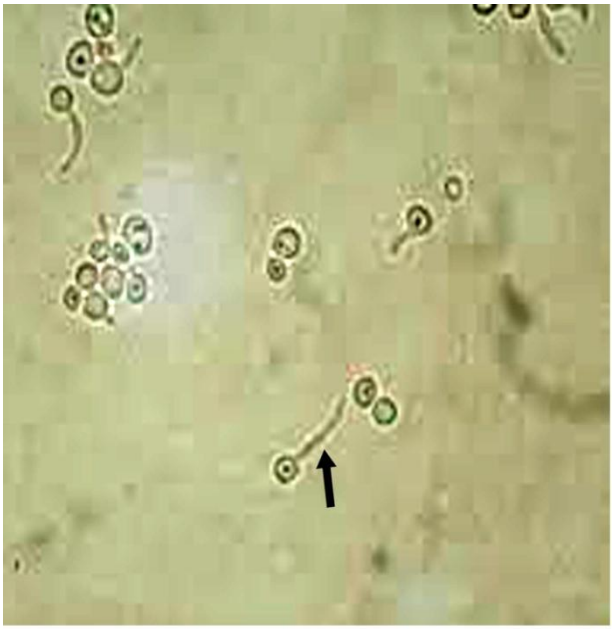

Figure 1: *C.albicans* showing Germ-tube formation.

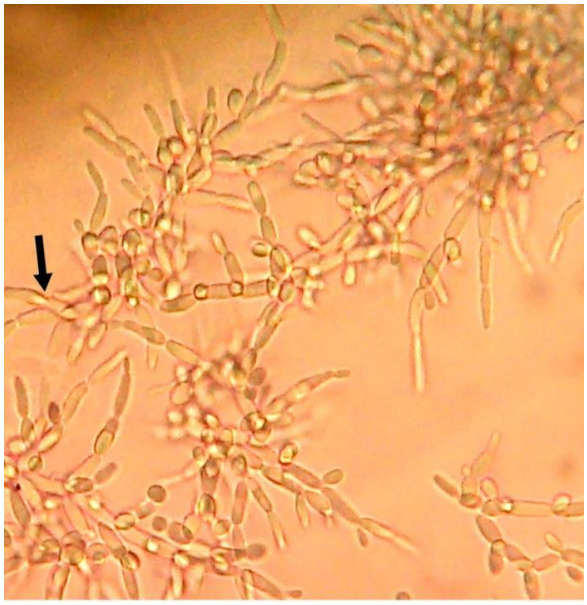

Figure 2: Growth pattern of *C.krusei* on Corn meal agar (Extensive branched pseudomycelium with chains of elongate cells giving cross matchstick appearance.)

Figure 3: *C.krusei* showing rosy pink colour on CHROMagar

Author Profile

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