CHROMagar as a primary isolation medium for rapid identification of Candida and its role in mixed Candida infection in sputum samples

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Abstract

Introduction: *Candida* species occurs as commensal organisms but in immunocompromised patients and those on long term antibiotics, these unrecognized opportunistic fungi may become fatal. Although *C. albicans* is the most frequently isolated pathogen, the incidence of infections due to non-albicans *Candida* are on the increase especially with fluconazole resistant strains. Identification of *Candida* to species level therefore becomes important for selecting the appropriate antifungal agents. The need for rapid identification and the difficulty in detecting mixed cultures on the traditional Sabouraud's dextrose agar (SDA) have led to the development of chromogenic media.

Aim: This study was done to evaluate the usefulness of CHROMagar *Candida* for rapid identification of *Candida* species and to identify mixed *Candida* infection from sputum samples.

Materials and methods: A total of 126 sputum samples were inoculated on CHROMagar *Candida*. The plates were incubated at 37°C for 48 hrs and the growth identified based on the colour of the colony.

Results: Single yeast infection was observed in 113 samples (89.7%) and 11 samples showed mixed infection (8.7%). In our study, *C.albicans* (61.5%) was the predominant species and among the non-albicans *Candida*, *C. tropicalis* (18.5%) was the commonest species.

Conclusion: Certainly CHROMagar *Candida* will be useful as a primary culture medium for identifying the yeast infections directly from the clinical specimens and also for the detection of mixed yeast infections which cannot be done on conventional SDA. Hence it helps us in selecting the appropriate antifungal agents for therapy and prophylaxis.

Keywords: *Candida albicans*, Non-albicans *Candida*, CHROMagar, Mixed yeast infections, Antifungal agents.

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Introduction

Candida species occurs as commensal organisms on the mucosal surfaces but increasingly becoming one of the potentially pathogenic organisms in patients with immunosuppression. In immunocompromised patients and those on long term antibiotics, these unrecognized opportunistic fungi may become fatal[1,2]. Although *C. albicans* is the most frequently isolated pathogen[3], the incidence of infections due to non-albicans *Candida* like *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis* are on the increase[4]. *C. glabrata* is emerging as the second most common species in invasive infection after *C. albicans*[5]. In recent times, there has been an increase in incidence of drug resistant *Candida* infection especially with fluconazole resistant non-albicans *Candida*[6,7]. Identification of *Candida* to species level therefore becomes important for selecting the appropriate antifungal agents to reduce the emergence of resistance[8]. Demonstration of yeast cells along with pseudohyphae in direct microscopy is seen in different species and do not have any significant correlation in species identification[8]. Most laboratories use the germ tube test and other biochemical tests for species identification which takes about 72 hrs.[9]. All these conventional methods used for identification of Candida species is time consuming and because there is close relation between the species and fluconazole sensitivity, early speciation is necessary for effective antifungal therapy[10]. Similarly early identification of mixed infection with more than one yeast species in clinical samples is also important for effective treatment of the patients. The need for rapid identification and the difficulty in detecting mixed cultures on the traditional Sabouraud's dextrose agar (SDA) have led to the development of chromogenic media[11]. CHROMagar *Candida* is a selective and differential medium which allows simultaneous isolation and identification of yeast based on the colour and colony morphology[12]. It also facilitates the identification of mixed yeast infection from clinical samples[13,14] and provides results 24-48 hrs. earlier than the standard identification methods[15,16]. Hence CHRO Magar *Candida* appears to be a very useful medium for early identification of yeast to the species level directly from clinical samples which aids us in treatment options.

Aim and Objectives

The present study was done:

1. To evaluate the usefulness of CHROMagar *Candida* for rapid identification and speciation of *Candida* species directly from sputum samples and
2. To identify mixed *Candida* infection from sputum samples using CHROMagar *Candida*.

Materials and Methods

This prospective study was done from July 2014-April 2015 in Department of Microbiology, Vinayaka Mission's Kirupananda Variyar medical college, Salem after obtaining institutional ethical clearance. The sputum samples received in the laboratory were subjected to direct gram staining. Those samples which showed pus cells and yeast cells with and without pseudohyphae (*C.glabrata* do not form pseudohyphae) were directly plated onto CHROMagar *Candida*[11]. The plates were incubated at 37°C for 48 hrs. and the growth identified based on the colour of the colony. Light green colonies- *Candida albicans*, blue colonies with pink halo - *Candida tropicalis*, purple colonies- *Candida glabrata*, pink colonies – *Candida krusei*, cream colonies- *Candida parapsilosis.*

Results

A total of 126 sputum samples were inoculated on CHROMagar *Candida*. Single yeast infection was observed in 113 samples (89.7%). 11 samples showed mixed infection with two yeast species (8.7%). Two samples did not show any growth (1.6%). A total of 135 yeasts were isolated from 126 samples.

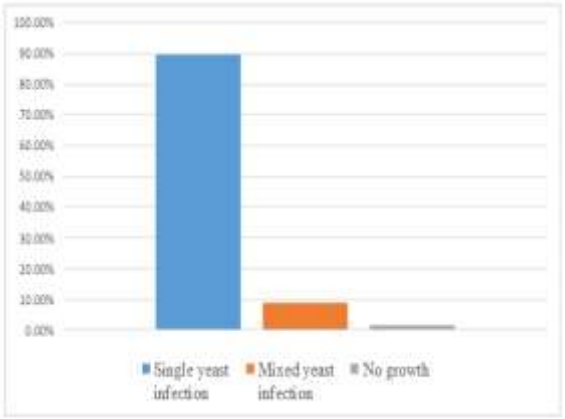

Fig. 1: Percentage of single yeast and mixed yeast infection among pulmonary candidiasis patients

Table 1: Candida species isolated from sputum sample

S. No	Fungal species	No of isolates
1	Candida albicans	83 (61.5%)
2	Candida tropicalis	25 (18.5%)
3	Candida krusei	14 (10.4%)
4	Candida parapsilosis	9 (6.7%)
5	Candida glabrata	4 (3.0%)
	Total	**135**

C.albicans was the predominant species isolated in our study. Among non-albicans *Candida*, *C.tropicalis* was the commonest.

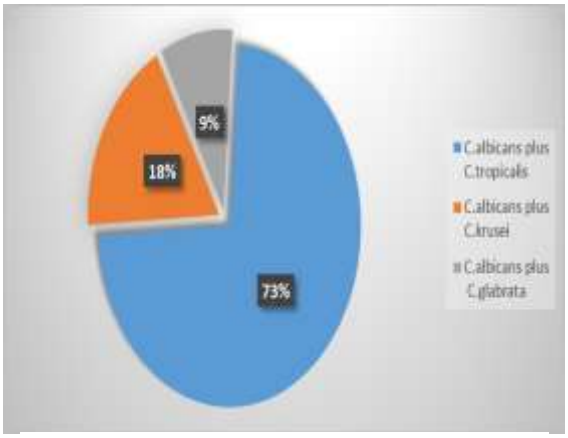

Fig. 2: Candida species among mixed infection

Discussion

The isolation of *Candida* from sputum may only represent colonization of the respiratory mucosa. The definite diagnosis of pulmonary candidiasis is based on demonstration of the yeast in the lung tissue[17] but lung biopsies cannot be routinely used for the diagnosis of suspected *Candida* infection[18]. Hence pulmonary candidiadsis was considered when sputum Gram stain showed WBCs and yeast cells with pseudohyphae (exception *C.glabrata*) and when *Candida* species were isolated in culture[1]. The above criteria was followed for diagnosis of pulmonary candidiasis in our study.

CHROMagar *Candida* is a selective medium which facilitates the identification and differentiation of several C*andida* species based on the colony colour[19]. The colour is due to the reaction of specific enzymes produced by the yeast with the chromogenic substance present in the medium. Colonies of *C. albicans* and *C. dubliniensis* appear light and dark green, *C.tropicalis* colonies appear dark blue to metallic blue and *C. krusei* colonies appear light pink and dry with a light border. *C.parapsilosis* appear cream colour and *C.glabrata* appear purple colour.

In our study, *C.albicans* was the predominant species isolated from sputum (Table 1). Out of 135 isolates, 83 isolates (61.5%) were *C.albicans*. This correlates with the study done by Pfaller[6] in which

C.albicans was the predominant isolate. This is also in accordance with the study done by Kali[20] which also demonstrates *C.albicans* to be the commonest species comprising 50% of the total *Candida* isolates.

Though *C.albicans* seems to be the predominant yeast causing invasive respiratory infections, the incidence of non-albicans *Candida* is on the increase. In our study, non-albicans *Candida* accounted for 38.5% of total isolates. This is similar to many other studies done by Pfaller[6], Moyer and Melissa[16,19] and Latha[21] in which there was an increased incidence of non-albicans *Candida*.

Among the non-albicans *Candida*, *C. tropicalis* was predominanat in our study accounting for 18.5% of the isolates. This may be because *C. tropicalis* has a greater capacity to invade the tissues in immunocompromised patients[22]. This was followed by *C.krusei* (10.4%), *C.parapsilosis* (6.7%) and *C.glabrata* (3%) (Table 1).

As indicated in other studies, CHROMagar Candida was very useful in identifying all the above species of *Candida* without any difficulty[6,23,24]. Hence CHROMagar *Candida* can be used as a primary isolation medium which isolates and identifies the species much earlier than other conventional methods[6].

Another major advantage of CHROMagar Candida was its ability to identify mixed yeast infection which cannot be done on conventional SDA[12,15,16]. It also facilitates the identification of *Candida* species within the mixed growth much earlier without the need for additional tests which aids us in treatment and prevention of drug resistance[6]. In our study, mixed infection was observed in 8.9% of the samples (Fig. 2). This could not be identified on the conventional SDA. Simultaneously, the species in the mixed growth was also identified based on the colour without any additional tests. *C.albicans* and *C.tropicalis* were the most common species among mixed infections (Fig. 2).

Conclusion

Early identification of yeast infections is very useful to initiate early therapy and reduces the cost of therapy and associated morbidity and mortality. As many laboratories do only germ tube test, the use of CHROMagar would certainly be advantageous for yeast identification. Certainly CHROMagar will be useful as a primary culture medium for identifying the yeast infections directly from the clinical specimens and also for the detection of mixed yeast infections. Hence it helps us in the selection of appropriate antifungal agents for therapy and prophylaxis.

Limitations

Although CHROMagar can accurately identify the most common *Candida* species, it cannot be considered as a substitute for standard identification protocols because of its inability to identify certain species like *C.pelliculosa*, *C.utilis*, *C.rugosa* and *C.hemulonii*. All these produce pink to purple colonies which could not be successfully identified. Similarly, mere species identification alone may not obviate the need for in vitro assessment of antifungal susceptibility in certain clinical situations.

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