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Original Article

Microbiology Section

Identifying *Candida* and Other Yeast-Like Fungi: Utility of an Identification Algorithm in Resource Limited Setting

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ABSTRACT

Aim: The increasing recovery rates of unusual yeasts with innate drug resistance make accurate identification crucial for successful therapy and infection control measures. The current study was undertaken to study the utility of CHROMagar *Candida (CC)* and evaluate an identification algorithm, using germ tube test (GT), CC and a commercial identification kit, API ID 32C.

Settings and Design: The prospective study was carried out at a private laboratory in Mumbai, India.

Materials and Methods: Identification of 533 yeast and yeast like isolates was carried out using an identification algorithm, comprising of the GT, CC and API tests.

Results: CC was useful to detect mixed cultures. We were able to identify 393/533, i.e. 73.7 % of isolates using GT and CC Tests only. This was because *C. albicans* and *C. tropicalis,* which can be reliably identified using CC, constituted 75.2 % of the isolates. We were unable to identify 140 isolates, i.e. 26.3 %, using GT and CC tests only and performed additional testing using API ID 32C. CC was not found to be reliable in identifying *C. krusei.*

Conclusion: The diverse identification profile obtained in our study substantiates the need for all diagnostic microbiology laboratories to be better prepared for identifying unusual yeasts. Though GT or CC testing cannot alone suffice for identification of all clinically encountered *Candida* and yeast-like fungi, use of GT, CC and automated identification systems in a step-wise algorithm can enable the same in a more cost effective manner.

Keywords: Algorithm, *Candida*, CHROMagar™ *Candida*, ID32C, Yeast

INTRODUCTION

Infections due to *Candida* spp. and other yeasts are on the increase, their emergence being favoured by immunosuppressive states, widespread use of chemotherapeutics and invasive devices or procedures [1-3].Though *Candida albicans* remains the most frequently isolated yeast, and there is an alarming increase in the isolation rates of other species from clinical specimens. Some of these emerging pathogens are known to show innate drug resistance.

Therefore, it is appropriate for physicians to have information on the type of *Candida* species before prescribing antifungal drugs to these patients. The ability to accurately identify yeasts, to a great extent, can determine the correct treatment and minimize treatment failure or recurrent infections [2,4,5]. Conventionally, laboratories begin the yeast identification process with the Germ tube test and often perform subcultures on Corn meal agar or rice starch agar for distinctive chlamydospore production & other morphological features. Reliable yeast identification can be achieved by using biochemical criteria i.e. ability of yeasts to assimilate certain carbon and nitrogen compounds (Assimilation reactions) and to ferment sugars (fermentation tests). Manual assimilation/fermentation tests are labour intensive and difficult to adapt in day-to day-practice[4-6]. Hence, commercially available identification kits are preferred and widely used. Several commercial chromogenic culture media are also available for the presumptive identification of yeasts. Extensive studies have documented the usefulness of these media in identifying *C. albicans, C. krusei* and *C. tropicalis.* However, these media have limited ability in differentiating unusual yeasts. Molecular methods have shown promising results in yeast identification, but are technically and monetarily difficult to introduce in a majority of diagnostic microbiology laboratories in the country. The current study was thus undertaken to study the identification profile of *Candida* & other yeast like isolates in various clinical specimens and assess the utility of CHROMagar™ *Candida*. The usefulness of a Yeast Identification Algorithm ,using a combination of germ tube test (GT), chromogenic media CHROMagar™ *Candida* (BBL™) (CC) and a commercial identification kit, API 32C (BioMérieux, France) (API) was also evaluated.

MATERIALS AND METHODS

Study design: Prospective study at a private microbiology laboratory in Mumbai, India.

Study group & period: Yeast isolates, recovered from clinical specimens, during a period of 25 months, were included in the study. Clinical relevance was not separately ascertained after isolation, as all specimens had been received for fungal culture, from patients with suspected fungal disease. Main aim of our study was to evaluate identification methodologies, and not establish clinical relevance. Isolates from repeat specimens of same patients were not included.

Materials

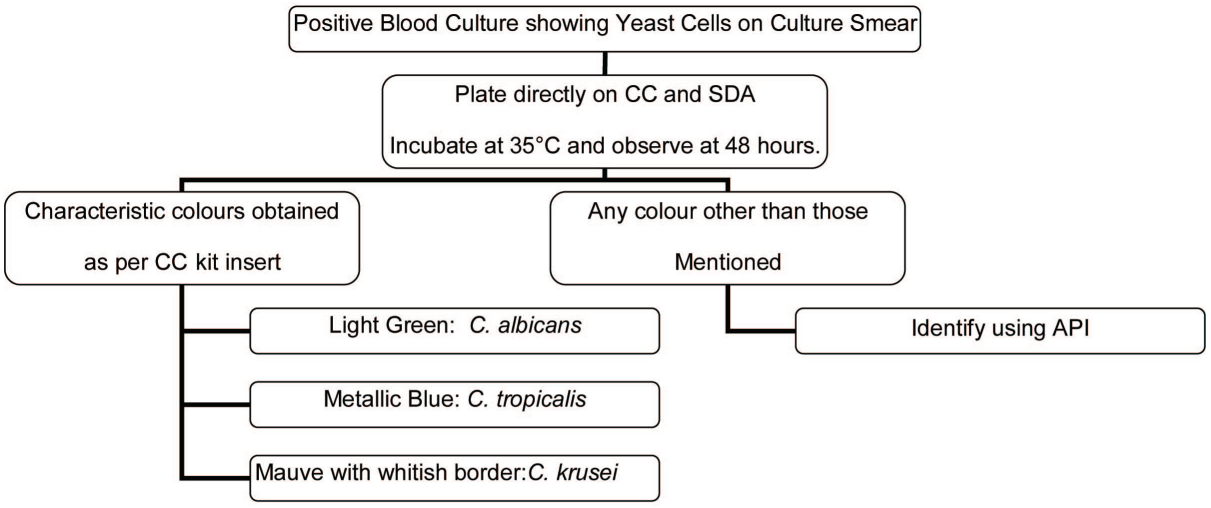
1) Sabaraud Dextrose agar (SDA) and Brain Heart Infusion agar (BHI): prepared in-house using commercial dehydrated media.
2) CHROMagar™ *Candida* (CC) (M/S BD BBL™ ,Becton Dickinson & co. Sparks, MD, USA).
3) API ID 32C Identification test (API) (Biomerieux, Marcy-l'Etoile, France).

Methods

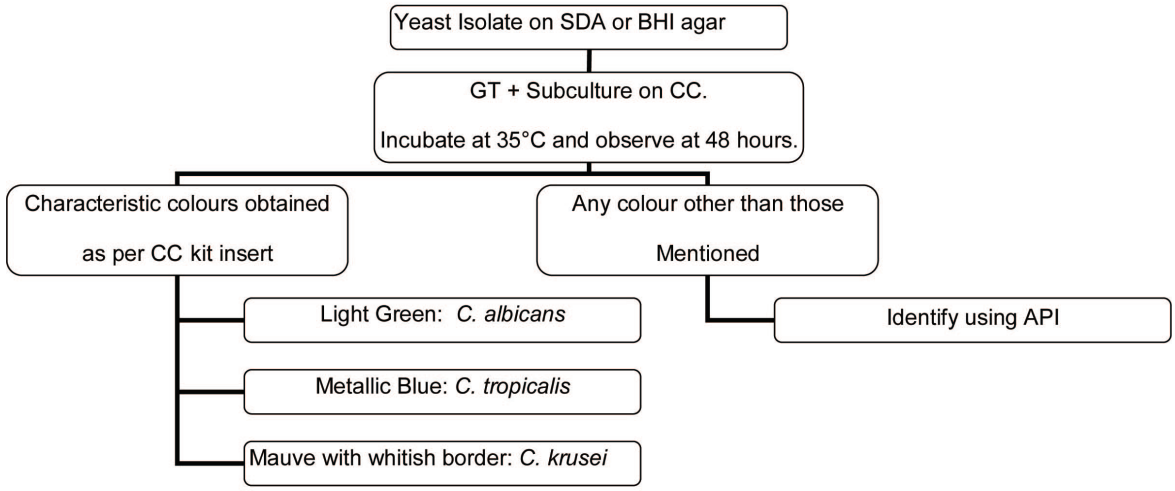
The identification algorithms shown in [Table/Fig-1&2] were used to identify the isolates.

Quality Control(QC):

1. SDA and BHI: New lot QC for its sterility and ability to support growth.



[Table/Fig-1]: Identification algorithm for blood culture isolates



[Table/Fig-2]: Identification algorithm for isolates recovered on solid media

Strain	Expected Colour Reaction
Candida albicans ATCC. 10231	Light green
Candida tropicalis ATCC 1369	Metallic blue
Candida krusei ATCC 6258	Light mauve to mauve, flat with whitish border

[Table/Fig-3]: Chromagar™ *candida* quality control

2. GT: Daily QC with positive and negative controls (ATCC strains) to check for sera reactivity.
3. CC: Prior to initiating the study, manufacturer defined colour reactions were verified for accuracy and reproducibility, using ATCC strains. Subsequently, new lot QC was done on a routine basis, as mentioned in [Table/Fig-3].
4. API: New lot QC

RESULTS

Of 6375 clinical specimens screened during the study period, 526 clinical specimens yielded yeasts on culture. Positive specimen types included Sputum(161) ,BAL(92),Urine(83),Blood(76),Tracheal secretion(31),Endotracheal secretions(17), CSF(16), Tissue(15), Pus(10), Fluids(7),Stool(7),Body fluids(6), Ear swabs(3) &Bone marrow(2) .

Seven (4 sputa and 3 urine) of the 526 specimens showed mixed cultures, with growth of 2 types of yeasts. Hence, a total of 533 isolates were obtained. The identification profile of the isolates has been shown in [Table/Fig-4].

Isolates	Number
C. albicans	285
C. tropicalis	117
C. parapsilosis	24
C. glabrata	20
C. krusei	19
Trichosporon spp.	18
C. sake	14
C.neoformans	10
C. lipolytica	5
Rhodotorula spp.	4
C. rugosa	3
C. kefyr(pseudotropicalis)	3
C. pelliculosa	3
C. dublinensis	3
Geotricum spp.	2
C. intermedia	1
C.utilis	1
C. inconspicua	1
Total	533

[Table/Fig-4]: Identification profile (N= 533)

Isolate (n=533)	Colour on CHROMagar™ *Candida*										
	Green		Blue		Mauve				Cream		Yellow orange
	Light	Dark	Metallic Blue	Light Blue Rough	Glossy	Rough with white border	Mauve	Pink Brown glossy	Glossy	Rough	
C. albicans	285	-	-	-	-	-	-	-	-	-	-
C. tropicalis	-	-	107	10	-	-	-	-	-	-	-
C. parapsilosis	-	-	-	-	-	-	-	-	24	-	-
C. glabrata	-	-	-	-	14	-	4	2	-	-	-
C. krusei	-	-	-	-	-	2	16	-	-	1	-
T. asahii	-	-	-	12	-	-	-	-	-	4	-
T. mucoides	-	-	-	-	-	1	-	1	-	-	-
C. sake	-	-	-	-	-	-	-	14	-	-	-
C.neoformans	-	-	-	-	-	-	-	-	10	-	-
C. lipolytica	-	-	-	-	-	-	-	-	5	-	-
Rhodotorula spp.	-	-	-	-	-	-	-	-	-	-	4
C. rugosa	-	-	-	3	-	-	-	-	-	-	-
C. pelliculosa	-	-	-	-	2	-	1	-	-	-	-
C. dublinensis	-	3	-	-	-	-	-	-	-	-	-
C. kefyr	-	-	-	-	-	-	-	1	1	1	-
G. candidum	-	-	-	-	-	-	-	-	2	-	-
C. inconspicua	-	-	-	-	-	-	-	-	1	-	-
C. utilis	-	-	-	-	1	-	-	-	-	-	-
C. intermedia	-	-	-	-	-	-	-	-	1	-	-

[Table/Fig-5]: Association of colour on CC with isolate identification

Light Dark		No.	Identification established by			
			%	GT+CC	GT+CC +API	%
Germ tube positive isolates(n=287)	*C. albicans*	284	284	100	0	0
	C dublinensis	3	0	0	3	100
Germ tube negative isolates(n=246	*C. albicans*	1	0	0	1	100
	C. tropicalis	117	107	91.45	10	8.55
	C. parapsilosis	24	0	0	24	100
	C. glabrata	20	0	0	20	100
	C. krusei	19	2	10.53	17	89.47
	Trichosporon spp.	18	0	0	18	100
	C. sake	14	0	0	14	100
	C. neoformans	10	0	0	10	100
	C. lipolytica	5	0	0	5	100
	Rhodotorula spp.	4	0	0	4	100
	C. pelliculosa	3	0	0	3	100
	C. kefyr	3	0	0	3	100
	C. rugosa	3	0	0	3	100
	Geotricum spp	2	0	0	2	100
	C. intermedia	1	0	0	1	100
	C. inconspicua	1	0	0	1	100
	C. utilis	1	0	0	1	100
Total		533	393	73.73	140	26.27

[Table/Fig-6]: Usefulness on the yeast identification algorithm

DISCUSSION

Identification profile of *Candida* & other yeast isolates

C. albicans, C. tropicalis, C parapsilosis and *C. glabrata* were the most commonly isolated yeasts in the study. Interestingly, a significant proportion of unusual isolates were recovered, whose identification can alter patient management. It is known that mere isolation of yeasts from clinical specimens does not establish their role in disease. Patient histories as well as repeated isolation from same site are needed to establish clinical relevance.

The diverse identification profile obtained in the present study substantiates the need for all diagnostic microbiology laboratories to be better prepared for identifying unusual yeasts. Correct identification itself may also aid in determining the significance. Larger clinical studies are also recommended to establish the clinical relevance of these unusual yeasts in the Indian context.

Utility of CHROMagar™ *Candida* (CC)

The use of CC allowed identification of 7 specimens containing mixed yeast species, an advantage shared by other investigators also [7,8]. None of these mixed cultures were evident on the primary culture media and were detected, only after subculturing on CC. In fact, some researchers have found CC to be as good as SDA for primary isolation of yeasts and superior to SDA in terms of suppressing the bacterial growth and time to positivity [7,8].

All 285 isolates of *C. albicans* gave a distinctive light green colour on CC [Table/Fig-5] The colour production was unique and was not shared by any other species. A number of studies have already substantiated this finding, reporting sensitivity & specificity of 98-100 & 100 % respectively [6,9-14]. *C. dublinensis* was found to give characteristic dark green colonies, distinctive from those of *C. albicans*. This finding has also been shared in a study by Mary-Ann et al., [15] and has been attributed to the reformulation of the CC by the manufacturer. Another study suggests that the dark green colour is found to be more pronounced, if plates are incubated beyond 48 h [10]. Majority of *C. tropicalis* isolates, i.e. 90.7 % (107/118) showed typical metallic blue colonies.10 isolates were identified using API, as the colours were not comparable to that produced by the *C. tropicalis* ATCC strain. Similar to present study, others have reported sensitivity and specificity rates for *C. tropicalis*, between 66.7-99% and 93.8-100% respectively [11,14].

The high reliability of CC to identify *C. albicans and C. tropicalis* provided rapid identification, thereby enabling faster reporting and timely patient management. CC was especially helpful in identifying blood isolates, as blood from yeast-positive Blood Culture bottles was directly plated on CC. This finding has been shared by Ainscough et al., [12].

Though CC is standardized for identification of *C. krusei*, only 2 out of 19 isolates gave characteristic mauve coloured colonies with whitish border, those matching the ATCC *C. krusei strain*. 16 isolates gave mauve coloured colonies without whitish border and were subsequently identified using API. Review of literature, also shows conflicting reports about the sensitivity of *C. krusei* detection on CC. Some studies have claimed a 100% sensitivity and specificity in their study [6,9,13]. However, there could be a bias towards CC in some studies, as the authors have used API confirmed isolates for evaluating CC, and not looked at CC as the first line of identification. Also, in studies where CC has been used as primary identification medium, identities of the "*C. krusei"* flat mauve colonies have not been subsequently confirmed using confirmatory identification [5].

Certain reports suggest CC's utility in reliably identifying *C. glabrata*, while others have discussed issues with colour intensity of C. *glabrata* strains [10,11]. Findings in present study corroborate with the latter group, as characteristic mauve glossy colonies were observed only in 14 of the 20 C. glabrata isolates. Also, 2 strains of *C. pelliculosa* and 1 strain of *C. utilis* produced similar colonies. Odds et al., [14] have also reported pink to purple coloured colonies of *C. pelliculosa* on CC.

12 out of 16 strains of *T. asahii* and 3 *C. rugosa* isolates demonstrated light blue coloured rough colonies on CC. , a finding also described by Paritpokee et al., [9]. All 24 isolates of *C. parapsilosis* in the present study showed cream coloured colonies, a finding shared by Vijaya D et al., [8]. However, cream coloured colonies were noted in a number of species and were not specific to *C. parapsilosis*.

Thus, certain characteristic colony morphologies on CC were found to be associated with *C. glabrata, T. asahii and C. sake* .Though present study data indicates that a presumptive identification of these species can be deduced from the typical morphology, these characteristics are not enough to conclude on the isolate identity. It is worthwhile to mention, that morphology on corn meal agar, if performed in addition to plating on CC, might help in reliable identification of certain species, especially *C. glabrata and T. asahii.*

Usefulness on the Yeast Identification Algorithm

Reliable identification of 393/533, i.e. 73.7% of isolates was possible using GT and CC Tests only [Table/Fig-6]. This was because C. *albicans and C. tropicalis,* which can be very reliably, identified using chromogenic media, constituted 75.2 % of the clinical isolates in our study.

Identity of 140 isolates, i.e. 26.3 %, could not be confirmed on the basis of GT and CC tests only. Identification of these isolates was performed using API ID 32C. It is thus evident that GT or CC testing cannot suffice, for accurate identification of all yeast species, and must be accompanied by more thorough identification protocols.

The cost of a single CC plate is approximately 1/10th that of a single API 32 C strip. Hence, if CC and API are used in a step-wise identification algorithm, yeast identification can be performed in a more cost effective manner.

CONCLUSION

The diverse identification profile obtained in the present study substantiates the need for all diagnostic microbiology laboratories to be better prepared for identifying unusual yeasts and thus facilitate correct treatment to minimize treatment failure or recurrent infections .Larger clinical studies are recommended to establish the clinical relevance of unusual yeasts in the Indian context.

CC is a valuable and cost effective tool for 1) identifying *C. albicans and C. tropicalis* isolates, 2) detecting mixed cultures and 3) rapid identification of blood culture isolates. GT or CC testing cannot suffice for identification of all clinically encountered yeasts. However, use of GT, CC and automated identification systems in a step-wise algorithm can enable the same in a more cost effective manner.

REFERENCES

[1] Agarwal J, Bansal S, Malik GK, Jain A. Trends in Neonatal Septicemia: Emergence of Non-albicans *Candida*. *Indian Paediatrics*. 2004;41:712-15.

[2] Verma A, Prasad K, Singh M, Dixit A, Ayyagari A. Candidaemia in patients of a tertiary health care hospital from north India. *Indian J Med Res.* 2003;117:122-28.

[3] Hazen K. New and Emerging Yeast Pathogens. *Clinical Microbiology Reviews.* 1995;8:462–78.

[4] Madhavan P, Jamal F, Chong P. Laboratory Isolation and Identification of *Candida* Species. *J Applied Sciences.* 2011;11:2870-77.

[5] Agarwal S, Manchanda V, Verma N, Bhalla P. Yeast identification in routine clinical microbiology laboratory and its clinical relevance. I*ndian Journal of Medical Microbiology.* 2011;29(2):172-77.

[6] Yücesoy M, Marol S. Performance of CHROMAGAR *Candida* and BIGGY agar for identification of yeast species. *Annals of Clinical Microbiology and Antimicrobials*. 2003;2:8.

[1] Nayak S, Kavitha B, Sriram G2 Saraswathi TR, Sivapathasundharam B, et al. Comparative study of *Candida* by conventional and CHROMagar method in non-denture and denture wearers by oral rinse technique. *IJDR*. 2012; 23(4):490-97.

[8] Vijaya D, Harsha T, Nagaratnamma T. *Candida* Speciation Using Chrom Agar. *JCDR*. 2011;5(4):755-57.

[9] Paritpokee S, Hall G, Procop G .Rapid Identification of Yeast Isolates using BD BBL™ CHROMagar™ *Candida*. *105th General Meeting of the American Society for Microbiology* 2005.

[10] P faller MA , Houston A ,Coffman S. Application of CHROMagar *Candida* for Rapid Screening of Clinical Specimens for *Candida* albicans, *Candida* tropicalis,*Candida* krusei, and *Candida* (Torulopsis) glabrata. *JCM.* 1996;34(1):58–61.

[11] Houang ETS, Chu KC, Koehler AP , Cheng AFB . Use of CHROM agar *Candida* for genital specimens in the diagnostic laboratory. *J Clin Path.* 1997;50;563-65.

[12] Ainscough S, Kibbler C. An evaluation of the effectiveness of using CHROMagar for yeast identification in a routine microbiology laboratory. *J Med Microbiol.* 1998;47:623-28.

[13] Willinger B, Manafi M. Evaluation of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida* spp. *Mycoses.* 1999;42:61-65.

[14] Odds F, Bernaert R. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* spp. *JCM.* 1994;38(8):1923-29.

[15] Mary Ann J, Brenner T, Romagnoli M, Baqui A, Merz W, Falkler W, et al. Evaluation of a Reformulated CHROMagar *Candida*. *JCM.* 2001;39(5):2015–6.

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