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EVALUATION OF DIFFERENT CULTURE METHODS FOR SPECIES IDENTIFICATION FROM VARIOUS CANDIDA ISOLATES

Microbiology

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ABSTRACT

Introduction: Candida albicans is the most common species causing opportunistic mycosis .Recent epidemiological trends reveal that there is a shift from C .albicans to non albicans species that are intrinsically resistant to some azoles . That leads to importance of identification of Candida at the species level. The present study was done to evaluate the performance of conventional identification methods (phenotypic & biochemical) and commercially available chromogenic Candida differential agar (Hi Crome Candida differential agar) for identification of medically important Candida species in a routine microbiology laboratory . **Material & Methods :** A total of 90 candida isolates from various clinical specimens received in the Dept . of microbiology were taken for the study over a period of one year from July 2019 to July 2020 . The Candida isolates were speciated by conventional methods like germ tube test , Sugar fermentation test , Sugar Assimilation test & compared against Chromogenic Agar Medium . **Results :**The isolation of non albicans Candida (60) , and albicans (30) , Non albicans Candida species isolated were C .tropicalis (30) ,followed by C .krusei(10), C. glabrata (10) C .kefyr (5), C .parapsilosis (5) each . **Conclusion :** Accurate speciation of candida is very important because some of the Candida species are very resistant to antifungal treatment .Hi Crome candida differential agar is a rapid method of identification in a resource poor settings .

KEYWORDS

Candida Albicans, Candida Non Albicans, Candida Differential Agar, Rapid Identification, Conventional Method .

INTRODUCTION:

Candida is an asexual, yeast like fungus causing opportunistic mycosis called candidiasis . Commonest source being endogenous as it is a normal commensal in throat , GIT , Vagina & skin. Candidiasis has emerged due to increased number of aged immunocompromised patients receiving prolonged antimicrobials , cancer chemotherapy , undergoing organ transplantation procedures , invasive surgical procedures . Amongst the Candida species albicans is generally considered as most common species but non albicans candida species are gradually increasing in trends .Non albicans candida like *C .tropicalis ,C .glabrata , C.kruesi , C .parapsilosis* are less susceptible to azoles .So correct identification of Candida species from various clinical samples is of therapeutic & prognostic significance .

Successful identification of Candida species is also important as this may avoid prescription of certain antifungal drugs that may not work against specific species like Candida krusei which is intrinsically resistant to Fluconazole . Generally yeast identification conventionally start with germ tube test to differentiate between *Candida albicans & dubliniensis* from other candida species .Although this is a rapid test may lead to high degree of false positive & false negative results . Further identification done by slide culture on corn meal agar , carbohydrate assimilation test. .Although detection of chlamydospore in corn meal agar by slide culture technique takes 24 – 72 hours & sugar assimilation takes 72 hrs to 2 weeks, but these procedures are labour intensive & takes longer time to determine the diagnosis to judge proper antifungal agents.

For rapid identification several chromogenic media have developed . These special media contain different chromogenic substrates that reacts with enzymes secreted by the microorganisms to produce different coloured colonies .These enzymes are species specific allowing organisms to be identified at species level .

Hi – crome candida differential agar one such chromogenic medium introduced by Hi Media laboratory (Mumbai) to differentiate Candida Species namely *C. albicans , C.kruesi , C .tropicalis ,C .glabrata* based on colour .

The present study was undertaken for species identification of candida isolates from various clinical specimens by different methods

MATERIAL & METHODS :

The present study was carried out in the Dept . of Microbiology , Calcutta National Medical College & Hospital ,Kolkata . A total of 90 Candida isolated from various clinical samples received in the Dept . of Microbiology were taken up for study over a period of one year i.e. July 2019to July 2020 .

All clinical samples were screened for budding yeast cells by direct microscopy with Gram's stain , KOH preparation with 10% KOH & 40% KOH (in nail & hair samples) , followed by inoculation in Sabouraud's Dextrose Agar with chloramphenicol & incubated at 37^0C for 48 hrs .

These Candida isolates were further speciated by standard protocol like germ tube test , observation of blastospore & chlamydospore formation in corn meal agar .

Simultaneously all Candida species were inoculated in Hi Crome Candida Differential agar (Hi – media , Mumbai - India , M1297A) incubated at 37^0C for 24 to 48 hours and the species were identified by the colour of colonies on Hi Crome Candida Differential agar as per manufacturer's instruction .

Colony morphology of *Candida* species on HiCrome Candida differential agar

i. *Candida albicans*: light green coloured smooth colonies
ii. *Candida tropicalis*: blue to purple coloured raised colonies
iii. *Candida glabrata*: cream to white smooth colonies
iv. *Candida krusei*: purple fuzzy colonies
v) *Candida kefyr* : cream to white with purple centre
vi) *Candida dubliniensis* : dark green
vii) *Candida parapsilosis* : white to pink

We used ATCC strains of *Candida albicans ATCC 10231*, *Candida glabrata ATCC 15126, Candida krusei ATCC 14243* and *Candida tropicalis ATCC 750* as control

RESULTS :

Total 90 Candida strains from various clinical specimens like (urine 40 isolates , blood culture 20 , sputum 10 from immunocompromised patients , 10 oral swabs ,10 vaginal swabs) isolated .

Out of these 30 strains showed positive germ tube test (FIG 9) were further identified by slide culture on corn meal agar & subculture in Hi CROME differential agar .

Of these GERM TUBE positive strains 25 strains correctly identified as *Candida albicans* as they produced light green smooth colonies(FIG1) & Chlamydospore formation in corn meal agar (FIG 3).

Table 1 : showing distribution of albicans & non albicans species

But rest 5 germ tube positive strains identified as *Candida dubliniensis* in corn meal agar by plenty of thick walled chlamydospore in bunch or pairs was not properly identified in Hi Chrome Candida Agar(FIG 4) .

Another 60 strains were Non – albicans with absence of germ tube formation . Of these 30 isolates showed characteristics blastospore singly or in small groups & pseudohyphae as fir tree appearance(FIG 5) identified as *Candida tropicalis* .All 30 strains correctly identified in Hi CROME produced blue to metallic blue coloured raised colonies identified *as Candida tropicalis* .(FIG 1&2)

10 strains on Chrom agar showed slight creamy colonies & in corn meal agar blastospores

singly or in clusters with absence of pseudohyphae was identified as *Candida glabrata* .(FIG 8)

10 strains on chrom agar showed pinkish purple colonies & in cornmeal agar characteristics elongated pseudohyphae having crossed match stick appearance identified as *Candida krusei* (FIG1).

5 Strains showed creamy colonies on chrom agar with a lacy pattern but in corn meal agar only small blastospores singly or in small clusters seen along the pseudo hyphae identified as *Candida parapsilosis* .(FIG 6)

Amongst the remaining 5 strains there were logs in a stream morphological appearance in corn meal agar as *Candida keyfr* but could not be identified properly in Hi Crome Agar (FIG 7).

Table-2: Identification *of Candida* species by Conventional method and Hicrome Candida differential agar

Table-3: Colony colour of *Candida* isolates on Hicrome Candida differential agar

TABLES :

Table-1: Distribution of Candida albicans and Non albicans Candida isolates

Candida isolates	Number of isolates	Percentage
Candida albicans & Candida dubliniensis	30	33.3%
Non albicans Candida	60	66.6%
Total	90	100%

Table-2: Identification of Candida species by Conventional method and Hicrome Candida differential agar

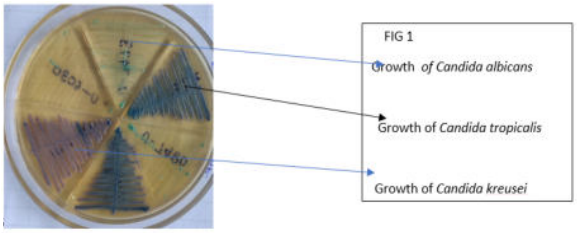
Candida species	Conventional method	Hichrome Candida differential agar
Candida albicans	25	25
Candida tropicalis	30	30
Candida dubliniensis	5	-
Candida krusei	10	10
Candida glabrata	10	10
Candida kefyr	5	-
Candida parapsilosis	5	5

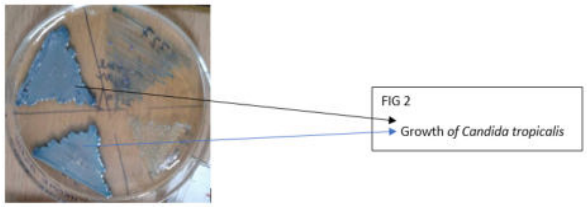
Table-3: Colony colour of Candida isolates on Hicrome Candida differential agar

	Candida species	Colons colour on Hicrome Candida differential agar	No. of Candida isolates. (n=90)
1	Candida albicans	light green	25
2	Candida tropicalis	Blue	30
3	Candida dubliniensis	dark green	-
4	Candida krusei	Purple fuzzy	10
5	Candida glabrata	White to cream	10
6	Candida kefyr	Light pink with purple centre	-
7	Candida parapsilosis	Light pink	5

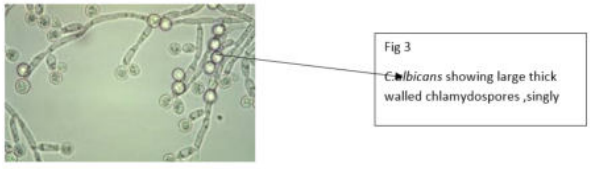
FIGURES & IMAGES :

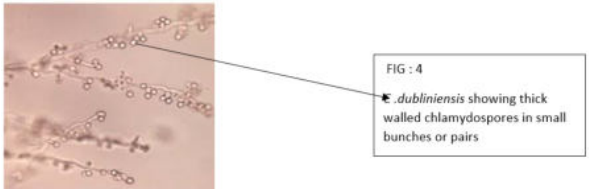
CHROM AGAR CULTURES :

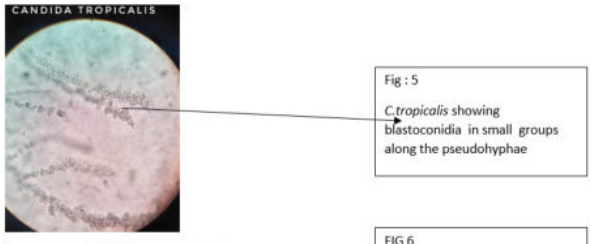


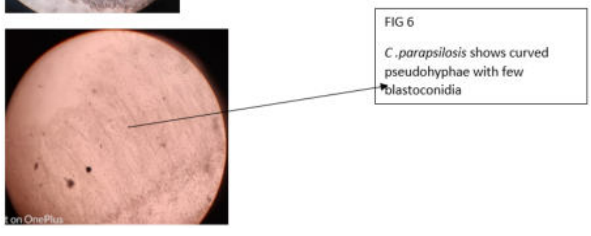


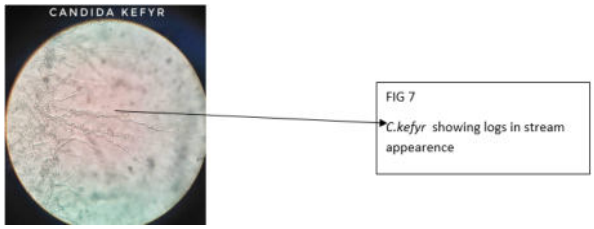
CORN MEAL AGAR CULTURES :











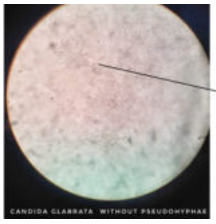


FIG 8
C.glabrata with blastospores absence of pseudohyphae

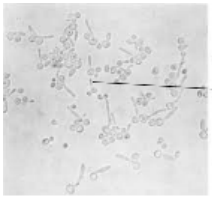


FIG 9
Germ tube formation by candida albicans

DISCUSSION :

In a resource limited countries lack of proper training , reagent &equipment supplies makes presumptive identification of yeast species quite difficult. The burden of the cost of test force most of the labs to stop at the germ tube test .Other conventional test like inoculation in corn meal agar , sugar fermentation & sugar assimilation test are not used due to lack of expertise & time consumption.

As a result direct selection of empirical antifungal prophylaxis without knowing the sensitivity profile will not only increase the burden to society but also invite emergence of resistant Candida spp.

Hi Crome Candida differential agar is found to be a suitable alternative along with conventional methods as it will reduce the cost & turn over time for species identification .

As reported by Nadeem et al, Sivakumar et al , Murray et al *C.albicans , C. tropicalis , C .krusei* was correctly identified by chrome agar as corroborated with the results of our study .

This medium easily facilitates detection of two species in a single clinical specimens by giving two different coloured colonies in a single plate .

The specificity & sensitivity of CROME AGAR in identifying pale green coloured colonies as Candida albicans was calculated to be 100% , for C. tropicalis 100%(blue to metallic blue) , C.krusei as 100%(purple) ,only C .dubliniensis & C.kefyr showed little difficulty in identificatiom

The Hi Crome candida differential agar media can be used as a culture medium for primary isolation & presumptive species identification of Candida in cases where early diagnosis is required without doing a PCR . Chrome Agar can also support the growth of moulds , so in a single specimen with a mixture of yeast & mould chrome agar can be easily utilised to detect the growth . This reduces cost and time lag for species diagnosis .

CONCLUSION :

The use of Hi Crome Candida differential agar is a reliable method for presumptive identification of *C .albicans , C. glabrata , C ,tropicalis* with sufficient sensitivity . This can well replace the conventional culture as it is less time consuming . In clinical microbiology we can save time & cost of diagnosis of fungi particularly in fungemia , in which early identification of species may require a change in antifungal agents as *C. glabrata & C.krusei* are inherently resistant to azoles . In most cases this may result in saving the patient's life .

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