

Comparison of Chromogenic Media with the Corn Meal Agar for Speciation of Candida

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In recent days, non albicans candida species are emerging, many of these species are inherently resistant to routinely used antifungals. Hence, the need for speciation of candida is important in the treatment point of view. Speciation of candida can be done by conventional methods, using chromogenic media, serological, molecular methods. Most of the laboratories use Hicrome for identification of yeast species considering its ease, rapidity. To determine the usefulness of Hicrome agar in the identification of different species of candida in comparison with corn meal agar. The candida isolated from cutaneous candidiasis were included. Species identification was done by colony color on Hicrome, and confirmed by morphology on corn meal agar for 80 candida isolates. The 10 different species of candida were identified. Emerging species of candida - *Candida kefyr*, *C.zeylanoides*, *C.lusitaniae* *C.lipolytica* were identified by morphology on corn meal agar which were misidentified as *C.glabrata* by Hicrome. Identification of *C.albicans*, *C.tropicalis*, *C.krusei* by colony colour on Hicrome correlated with the morphology on corn meal agar. So, the incorporation of corn meal agar in the routine yeast identification is more judicious than Hicrome as it increase the accuracy in the identification of candida species within in the same time span as that of Hicrome.

Keywords: Hicrome, corn meal agar, Candida.

Candida albicans remains the most common causative agent of both superficial and deep fungal infections¹. But recent reports suggest that a shift has occurred in the distribution of infections, with NAC being increasingly detected².

Due to the epidemiological alteration in the distribution of *Candida* species as well as significant increasing trend of either intrinsic or acquired resistance in some of these fungi, the precise identification of *Candida* species is necessary for effective antifungal therapy and also for prevention of nosocomial infections³. The strains of *C. lusitaniae* may show resistance to amphotericin B so, for example, the automatic prescription of amphotericin B in a patient with a septicemia due to *C. lusitaniae*⁴.

A large variety of methods have been developed with the aim of facilitating rapid, accurate yeast identification⁴. Many tests with different techniques from conventional to molecular methods are available for yeast identification.

Clinical microbiology laboratories face an important challenge to select a system for yeast identification that is accurate, cost-effective, easily interpreted and reasonably rapid⁴.

Now a days, chromogenic media are frequently used in the direct and rapid identification of yeasts because different *Candida* species produce unique colors on these media.⁵

Our study is to evaluate the usefulness of Hicrome agar for speciation of candida in comparison with the corn meal agar.

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MATERIALS AND METHODS

The samples from cutaneous candidiasis cases (nail clippings from onychomycosis, swabs from intertrigo / napkin rash) were included in the study. A total number of 80 candida were isolated following culture on the SDA slants. Candida isolates were speciated using Hicrome. Hicrome Candida agar product code number M-677 was obtained commercially. Plates were prepared as per manufacturer's instructions. 5-8 colonies from 24-48 hr old SDA slants were inoculated on Hicrome agar and incubated at 37°C for 48 hrs. Colony morphology and colour was noted and compared with HiMedia instructions⁶. As per HiMedia instructions, depending on the colour of colonies species were identified as follows

On Hicrome agar medium *C.albicans* appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to purple coloured raised colonies. *C.glabrata* colonies appear as cream to white smooth colonies, while *C.krusei*

appear as purple fuzzy colonies. *C.parapsilosis* appears as cream coloured colonies with mauve tinge⁶. Then the colony were inoculated on 1cm x 1 cm block of corn meal agar (CMA) block. The agar block was covered with sterile cover slip and placed in a sterile petridish moistened with filter paper and incubated at room temperature for 48 hours^{7,8}.

After 48 hrs, the slide was placed on the microscopic stage and the edge of the cover slip was observed using 10x and 40x objectives for chlamydospores, pseudohyphae, hyphae, blastospores, blastoconidia etc and the *Candida* were speciated^{7,8}

RESULTS

Table 1 Showing the identification of various candida species by Hicrome and cornmeal agar. In our study 10 different candida species were identified by each method. But *Candida kefyri*, *C.zeylanoides*, *C.lusitaniae*, *C.lipolytica* were not identified by Hicrome.

Table 2 Showing sensitivity and specificity of Hicrome agar against CMA 100% sensitivity of Hicrome agar was observed in identification of *C.albicans*, *C.tropicalis*, *C.krusei*, *C.glabrata*. Moderate sensitivity of 70.58% was observed in *C.parapsilosis*. 100% specificity was observed

Table 1. Showing the identification of various candida species by Hicrome and cornmeal agar

Isolates	Detected on CMA	Detected on Hicrome
<i>Candida albicans</i>	24	24
<i>Candida tropicalis</i>	18	18
<i>Candida parapsilosis</i>	17	12
<i>Candida glabrata</i>	04	16
<i>Candida guilliermondii</i>	05	0
<i>Candida krusei</i>	06	06
<i>Candida kefyri</i>	03	0
<i>Candida zeylanoides</i>	01	0
<i>Candida lusitaniae</i>	01	0
<i>Candida lipolytica</i>	01	0
Total	80	80

Table 2. Showing sensitivity and specificity of Hicrome agar against the cornmeal agar

Species	Sensitivity %	Specificity %
<i>Candida albicans</i>	100%	96.42%
<i>Candida tropicalis</i>	100%	96.77%
<i>Candida parapsilosis</i>	70.58%	100%
<i>Candida glabrata</i>	100%	84.21%
<i>Candida krusei</i>	100%	100%

Table 3. Showing sensitivity, specificity of candida of Hicrome agar in comparison with other similar studies

Species	Our study	VP Baradkar ⁵	Manisha ⁹
<i>C albicans</i>	100%, 96.42%	96.55, 96.42	100%, 100%
<i>C tropicalis</i>	100%, 96.77%	100%, 100%	92.9, 100%
<i>C parapsilosis</i>	70.58%, 100%	80%, 98.03%	—
<i>C glabrata</i>	100%, 84.21%	90.9%, 88.23%	100%, 96.42%
<i>C krusei</i>	100%, 100%	—	100%, 98.42 %

Table 4. Appearance of different candida isolates on Hicrome , cornmeal agar

Species	Colony colour on Hicrome	Morphology on Corn meal agar (CMA)	Identification with Hicrome	Identification with Hicrome vs CMA
<i>C. albicans</i> (24)	Light green colored smooth colonies	Pseudohyphae with terminal chlamydospores; clusters of blastoconidia at septa.	Identified all strains as <i>C. albicans</i>	Accurate identification all 24
<i>C. tropicalis</i> (18)	Blue to purple coloured raised colonies	Blastoconidia anywhere along pseudohyphae	Identified all strains as <i>C. tropicalis</i>	Accurate identification all 18
<i>C. parapsilosis</i> (17)	Cream colored colonies with mauve tinge	Blastoconidia along curved pseudohyphae; giant mycelial cells	Only 12 identified, other 5 strains misidentified as <i>C. glabrata</i>	Identified all 17
<i>C. glabrata</i> (4)	Cream to white smooth colonies	No pseudohyphae; cells small; terminal budding	16 strains were identified , morphology essential	4 strains correlated by both methods
<i>C. krusei</i> (6)	Purple fuzzy colonies	Pseudohyphae with cross – match sticks or treelike blastoconidia	Identified all strains as <i>C. krusei</i>	Accurate identification all 6
<i>C. guilliermondii</i> (5)	Cream colonies	Fairly short, fine pseudohyphae, clusters of blastoconidia at septa	misidentified as <i>C. glabrata</i>	5 strains correlated by both methods
<i>C. kefyr</i> (3)	Cream colonies	Elongated blastoconidia resembling logs in a stream along pseudohyphae	misidentified as <i>C. glabrata</i>	3 strains correlated by both methods
<i>C. zeylanoides</i> (1)	Cream colonies	Pseudohyphae give feather- like appearance at low power	misidentified as <i>C. glabrata</i>	1 strains correlated by both methods
<i>C. huistaniae</i> (1)	Cream colonies	Short chains of elongate blastoconidia along curved pseudohyphae	misidentified as <i>C. glabrata</i>	1 strains correlated by both methods
<i>C. lipolytica</i> (1)	Cream colonies	Elongated blastoconidia in short chains along pseudohyphae	misidentified as <i>C. glabrata</i>	1 strains correlated by both methods

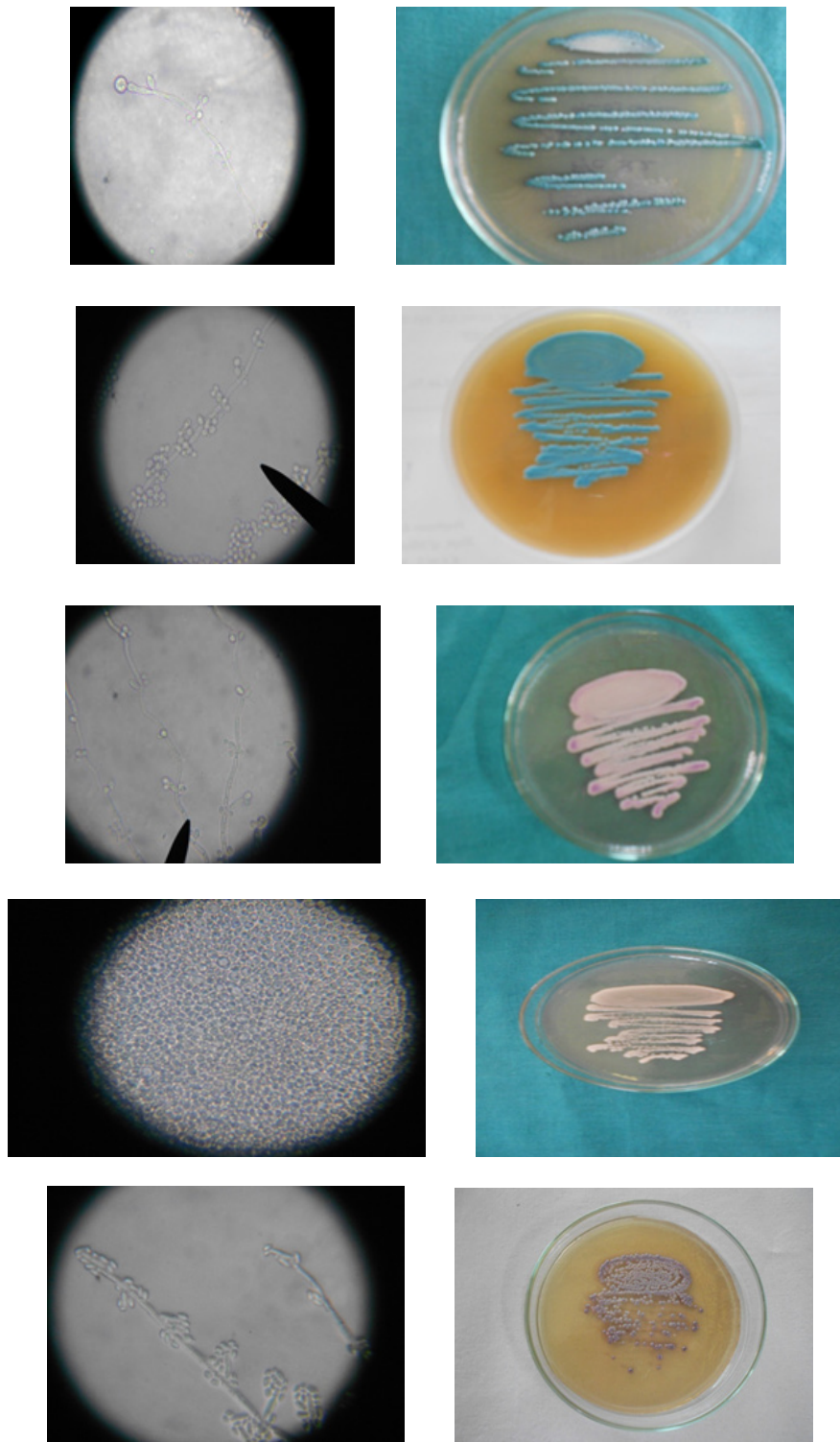


Fig. 1. Microscopic appearance on corn meal agar (left; magnification ,x400) and colony colors on Hicrome agar for candida after 48 hours of incubation. (A) *C. albicans* (B) *C. tropicalis* (C) *C. parapsilosis* (D) *C. glabrata* (E) *C. krusei*

in *C.parapsilosis*, *C. krusei*. 96.77% & 96.42% specificity was observed in *C. tropicalis*, *C.albicans* respectively. 84.21% specificity was observed in *C.glabrata*.

Table 3 Showing sensitivity, specificity of Hicrome as observed in other similar studies. The sensitivity, specificity of *C.albicans*, *C.tropicalis*, *C. krusei* are in agreement with the similar studies done by VP Baradkar⁵, Manisha⁹.

Table 4 Appearance of different candida isolates on Hicrome, Cornmeal agar Hicrome agar for candida falsely identified *C.parapsilosis* as *C.glabrata* based on colony colour. *Candida kefyr*, *C.zeylanoides*, *C.lusitaniae* *C.lipolytica* were identified by morphology on corn meal agar which were misidentified as *C.glabrata* by Hicrome. The appearance of candida isolates on Hicrome agar, microscopic appearance on Cornmeal agar are given in Fig. 1.

DISCUSSION

Approximately five Candida species were considered pathogenic in the 1960s, recent reviews listed at least 17 Candida species as being pathogenic⁴. When studies are limited to this genus, the most frequently isolated species were *C.albicans*, *C.tropicalis*, *C.glabrata*, *C.parapsilosis*⁵⁻⁹.

Isolation of other Candida species, such as *C. krusei*, *C. guilliermondii*, *C.lipolytica*, *C. kefyr*, along with other unspecified species were also increased¹⁰. These results are reflected by increase in the case reports concerning new and emerging yeasts¹¹.

Many tests with different techniques from conventional to molecular methods are available for yeast identification. But selection of the method by a lab depends on its affordability (sample size etc.), reliability of the test result, and also the time factor.

Many studies states that the potential advantage of chromogenic media is the straight forward identification of mixed yeast infections¹². Now a day's Hicrome agar is most commonly employed for the yeast identification in clinical microbiology laboratories.

In our study 10 different species of candida were identified by morphological study on corn meal agar. On Hicrome only 5 species

of candida were identified i.e., *C.albicans*, *C.tropicalis*, *C.glabrata*, *C.krusei*, *C. parapsilosis*. (Table 1)

Hicrome agar was useful in identification of *C.albicans*, *C.tropicalis*, *C.glabrata*, *C.krusei* with 100 % sensitivity, moderate sensitivity of 70.58 % was observed in *C.parapsilosis*. 100 % specificity was observed in *C.parapsilosis*, *C.krusei*. Moderate specificity was observed in *C.glabrata* 84.21%. (Table 2)

The sensitivity, specificity of *C.albicans*, *C.tropicalis*, *C. krusei*, *C.glabrata*, *C.parapsilosis* are in agreement with the similar studies done by VP Baradkar⁵, Manisha⁹ (Table 3). As observed in the previous studies done on speciation of candida using Hicrome^{5,9} only the above mentioned species were identified, new emerging candida species identification were not stated.

In our study we noted that while identifying candida species based on the color description by manufacturer⁸, 21 isolates which appeared as white colonies were interpreted as *C. glabrata*. When morphological identification by corn meal agar was done on these isolates, *C. parapsilosis*(5), *Candida kefyr*(3), *C.zeylanoides*(1), *C.lusitaniae*(1), *C.lipolytica*(1), *C. guilliermondii* (5) were identified. (Table 4)

Hicrome agar falsely identified *C.parapsilosis* as *C.glabrata* was stated in the study done by Sagar et al¹³ which is similar to our study.

The advantage of Hicrome agar in identification of candida species i.e., ease of the test method compared to conventional methods and rapidity of identification cannot be ignored in the era of emergence of NAC. Identification on Hicrome agar poses a problem as it is based on colour, features like fuzzy, hue etc and variations in intensity of color with passage of time. The interpretation becomes subjective, besides the media only recommends identification of *C.albicans*, *C.tropicalis*, *C. glabrata*, *C.krusei*, *C.parapsilosis*.

The study by Hazen¹⁰ states the variable efficacy for fluconazole is evident with *C.glabrata*, *C.parapsilosis*, *C.rugosa*, *C.tropicalis*, *S. cerevisiae*, and *T.beigelii* which adds to the importance of accurate identification.

As observed in our study many new species identification were missed when identification on

only Hicrome considering its, ease and rapidity.

The turnaround time taken for identification by morphology on corn meal agar is 48hr⁷ is similar to that on Hicrome as per the manufacturer's instructions⁶.

But identification by morphological study on corn meal agar demands the skill from the lab personnel, which can be mastered.

Koehler¹⁴ et al opines that careful observation of the yeast morphology on corn meal agar, adds confidence in the identification of candida species, which will also alert the microbiologist about the presence of unusual isolates.

CONCLUSION

In view of accurate identification, the limitations of Hicrome in yeast identification not to be ignored by a clinical microbiology laboratory. The incorporation of corn meal agar in routine yeast identification prevents misidentification, adds confidence, improves the mycology skills among the lab personnel without compromising cost, or time factor.

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