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PRESUMPTIVE IDENTIFICATION OF CANDIDA SPECIES FROM VAGINAL SPECIMENS USING CHROMAGAR

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ABSTRACT

Background: Vulvovaginal candidiasis is caused by *Candida albicans* as well as non-albicans species of *candida*. The conventional methods of species identification by assimilation and fermentation are reported to be cumbersome. In order to facilitate rapid identification, several chromogenic substrate containing culture media have been developed which yield colonies with varying colours secondary to chromogenic substrates that react with enzymes secreted by microorganism.

Aim and objective : To evaluate the usefulness of CHROMagar over conventional methods for speciation of *candida* isolates.

Materials and methods : High vaginal swabs collected from 200 sexually active women [18- 65yrs] with symptoms who attended the outpatient gynaecology department in a tertiary care hospital from January 2012 to October 2012 were subjected to gram stain and culture. The isolated colonies were speciated using CHROMagar, germ tube test, sugar assimilation and fermentation tests. The species were identified by the colours produced by each isolate on CHROMagar in 24hours and by sugar assimilation and sugar fermentation tests in 48hours.

Results : A total of 71 *candida* isolates were obtained which included 26 *Candida albicans*, 23 *Candida parapsilosis*, 13 *Candida glabrata*, 5 *Candida tropicalis* and 4 *Candida krusei* in 24 hours on CHROMagar and in 48 hours by sugar assimilation and sugar fermentation tests. The sensitivity and specificity of CHROMagar candida for these 5 species was 100%.

Conclusion : The use of chromogenic medium is an easy, rapid and reliable method for the presumptive identification of *candida* species in 24 hours. CHROMagar is of great help for the clinical microbiologist to save time and cost for diagnosis.

INTRODUCTION

Candida is now the second commonest cause of vaginal infections. More than 75% of women experience atleast one episode of vulvovaginal candidiasis during their lifetime[1]. Vulvovaginal candidiasis is caused by *Candida albicans* in 85% of cases. However episodes due to non-albicans species of *candida* appear to be increasing.[2] Conversely, in many women a true vaginal candida infection may remain unrecognized.[3] In general detection of candida infection by microscopic examination of a specimen diluted in potassium hydroxide is relatively insensitive as *Candida* pseudohyphae may be difficult to distinguish from Aspergillus hyphae. False positive microscopic examination are also possible and are probably more common than generally suspected[4]. The conventional methods of yeast identification which mainly consist of sugar assimilation and fermentation characteristics, are reported to be cumbersome[5]. In order to facilitate rapid identification, several chromogenic substrate containing culture media have been developed. These special media yield microbial colonies with varying colours secondary to chromogenic substrates that react with enzymes secreted by microorganisms[6]. These enzymes are species specific, allowing organisms to be identified to the species level by their colour and colony characteristics[7,8]. CHROMagar *candida* is one such medium that can identify *candida* to species level.

AIM AND OBJECTIVE

To evaluate the usefulness of CHROMagar over conventional methods for speciation of *candida* isolates.

MATERIALS AND METHODS

This cross sectional study was conducted in department of microbiology, Vinayaka Missions Kirupananda Variyar Medical College and Hospital, Salem after obtaining

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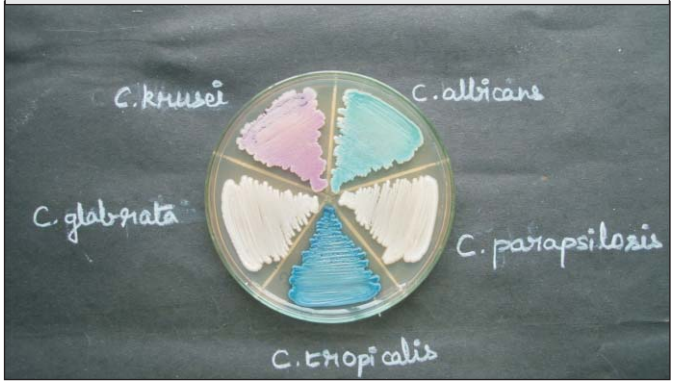
ethical committee approval. Two high vaginal swabs were collected from 200 sexually active women [18-65 yrs] with history of white discharge per vagina, itching, vaginal discomfort, dysuria and dyspareunia attending the outpatient gynaecology department from January 2012 to October 2012 after obtaining written informed consent. One swab was used for Gram stain and other used for culture. Those positive for gram positive budding yeast cells were cultured on Sabouraud's dextrose agar media with chloramphenicol and incubated at 37°C for 24 hours. Fresh cream-coloured smooth, pasty colonies of *candida* were speciated using CHROMagar, sugar assimilation and sugar fermentation tests. Candida isolates were inoculated onto CHROMagar candida medium which was prepared as per manufacturer instructions. About 42.72 gram of CHROMagar Candida was suspended in 1000 ml of distilled water. It was heated to boiling gently to dissolve the medium completely. Then it was allowed to cool to 50°C and poured into petri plates. After the media is set, fresh *candida* isolates were inoculated and incubated at 37° C for 24 hours and species were identified by colony morphology and colour (as shown in Fig 1,Table 1). *Candida* isolates were also subjected to germ tube test, sugar assimilation and sugar fermentation tests.[9] *Candida albicans* ATCC-90028, *Candida tropicalis* ATCC-750 and *Candida krusei* ATCC-6258 strains were used as control. Sensitivity and specificity of CHROMagar were calculated as below:

Sensitivity = (true positive) X 100 / (true positive + false negative)

Specificity = (true negative) X 100 / (true negative + false positive).

Fig 1 : CHROMagar candida showing different coloured colonies.



RESULTS

A total of 71 *candida* isolates were obtained. The results are shown in Table 1. Candida species were identified in 24 hours using CHROMagar, in 48 hours by sugar assimilation and upto 7days by fermentation tests.

Table 1 : Colony morphology of candida species after 24 hours of incubation on CHROMagar

S. No	Species	Total number of isolates (n= 71)	Colony Morphology on CHROMagar in 24 hrs.	Sugar fermentation test after 7 days of incubation	Sugar assimilation test after 48 hrs of incubation
1	*Candida albicans*	26	Green colonies	Glu,Mal fermented with acid and gas production	Glu,Lac,Mal, Suc, Gal, Tre assimilated.
2	*Candida parapsilosis*	23	Ivory to pink colonies. Small to medium , smooth to wrinkled	Glu fermented with acid and gas production	Glu,Lac,Mal, Suc, Gal, Tre assimilated
3	*Candida glabrata*	13	Pale pink to purple colonies. Small to medium, smooth, convex, creamy	Glu fermented with acid and gas production	Glu ,Tre assimilated.
4	*Candida tropicalis*	5	Steel blue, purple diffusion colonies	Glu, Mal, Suc fermented with acid and gas production	Glu,Lac,Mal, Suc,Gal, Tre ass imilated
5	*Candida krusei*	4	Pink, pale borders. Medium to large, flat , rough colonies	Glu fermented with acid and gas production	Glu alone assimilated

Note: glu- glucose, Mal- Maltose, Suc- Sucrose, Lac- Lactose,Gal- Galactose, Tre- Trehalose

DISCUSSION

In resource limited settings, rapid presumptive identification of yeast species is quite difficult. The sugar assimilation and sugar fermentation tests are not used in these laboratories due to lack of resources, expertise and the time required for these tests.[10] With the increasing incidence of vaginal candidiasis caused by non albicans *candida*, we were interested in testing the usefulness of CHROMagar *candida* in presumptive identification of *candida* species. CHROMagar easily identified several species of *candida* on the basis of colony colour and morphology and accurately differentiates between the three most common species of *candida* i.e. *Candida albicans*, *Candida krusei* and *Candida tropicalis* which has been reported by Murray et al[11]. It has the advantage of earlier and rapid identification of *candida* species, compared to technically demanding, time consuming and expensive conventional method of sugar assimilation and sugar fermentation tests.

The sensitivity and specificity of CHROM agar in this study

was 100% for *C.albicans* which correlates with studies by Willinger *et al (*98.8% sensitivity and 100% specificity)[15], Baradkar et al (100% sensitivity and 94.6% specificity)[14], Yucesoy et al (99.4% sensitivity and 100% specificity)[13] and Odds and Bernaerts et al (95% sensitivity and 95.3-100% specificity)[16].

The sensitivity and specificity of CHROM agar for *C.parapsilosis* in this study was 100% in contrast to the studies of Peng *et al (*90% sensitivity and 82.3% specificity)[12], Baradkar et al (100% sensitivity and 94.6% specificity)[14], Yucesoy et al (99.4% sensitivity and 100% specificity).[13] CHROM agar candida facilitates the detection and identification of yeasts from cultures and can provide results in 24 hours sooner than sugar assimilation and fermentation tests in which results are obtained after 48 hours and 7days respectively.

CONCLUSION

The use of CHROMagar *candida* medium is an easy, rapid, technically simple, cost effective and reliable method for the presumptive identification of most commonly isolated *candida* species compared to sugar assimilation and sugar fermentation tests. Hence we conclude that the species identification by CHROMagar is of great help for the clinical microbiologist to save time and cost for diagnosis and easy to use and is suitable for routine use in clinical mycology laboratories.

REFERENCES

1. Schroppei K, Rotman M and Galask R. Evolution and replacement of Candida albicans strains during recurrent vaginitis demonstrated by DNA fingerprinting.J.Clin.Microbiol., 1994;32(11):2646-54.
2. Richter SS, Galask RP and Pfaller MA. J.Clin.Microbiol. 2005;43:2155-62.
3. Nyirjesy P, Seeney SM and Grody MH. Chronic fungal vaginitis: the value of cultures. AM J Obstet Gynecol 1995; 173: 820-3.
4. Odds FC. Genital candidosis. Clin Exp Dermatol 1982; 7:345-54.
5. Freydiere AM, Guinet R and Boiron P. Yeast identification in the clinical microbiology laboratory: Phenotypical methods. Med Mycol. 2001;39:9-33.
6. Yucesoy M and Merol S. Performance of CHROMagar candida and BIGGYagar for identification of yeast species. Annals Clin Microbiol Antimicrobial 2003;2:1-8.
7. Lyme LH, Dnane RH, Eliriton KM and Dooley D. Direct isolation of candida spp from blood cultures on the chromogenic medium CHROMagar candida.J.Clin Microbiol, 2003; 41(6):2629-32.
8. Hospenthal DR. Presumptive identification of Candida species other than C. albicans, C. krusei and C. tropicalis with the chromogenic medium CHROMagar Candida. Annals of Clinical Microbiology and Antimicrobials. 2006; 5:1
9. Chander, Jagadish. Textbook of Medical Mycology. 3rd ed: Mehta publishers;2009. p.266-283.
10. Pfaller MA, Houstan A and Coffmann S. Application of CHROMagar candida for rapid screening of clinical specimens for candida albicans, C.tropicalis, C.krusei and C.glabrata.J Clin Microbiol.1996;34:58-61.
11. Murray CK, Beckins ML, Green Ja and Hospenthal DR. Use of Chromogenic medium in the isolation of yeasts from clinical specimens. J Med Microbiol.2005;54:981-5.
12. Peng CF, Lee KM and Lee SH. Characterisation of two chromogenic media of Candida ID_2 and CHROMagar Candida for preliminary identification of yeasts. J Biomed Lab Sci 2007;19:63-8.
13. Yucesoy M, Esen N and Yulung N. Use of Chromogenic agar for the identification of candida albicans strains. Kobe J Med Sci.2001; 47:161-7.
14. Baradkar VP, Mathur M and Kumar S. Hichrom Candida agar for identification of Candida species. Indian Journal of Pathology and Microbiology.2010;53(1):93-95
15. Willinger B and Manabi M. Evaluation of CHROMagar candida for rapid screening of clinical specimens for candida species. Mycoses 1999;42:61-5
16. Odds FC and Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. 1994;32:1923–29.