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Vulvovaginal Candidiasis: Isolation and Identification of *Candida* from Reproductive Age group Woman

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Abstract

Vulvovaginal candidiasis (VVC) is an oppertunistic mucosal infection caused by varies candida species that affects large number of healthy women of childbearing age. It is estimated that 75% of women will experience at least one episode in their lifetime. candida albicans is a dimorphic commensal organism of urogenital tract and has been identified as main pathogenic agent in VVC, accounting for approximately 85-90% of patient with positive cultures. A prospective study of female genital swabs collected from gynecological clinic and analysed for microscopy, culture, speciallized test. Most common VVC caused by candida albicans and non albican species were also detected like candida tropicalis, candida glabrata in 50 samples collected from pregnant and non pregnant women. Microscopic analysis was performed by 10% KOH wet preperation technique and gram staining. Sabouraud dextrose broth was used as primary growth medium and than candida species were identified by growth on HiCrome candida speciallized medium. further identification was done by special test- germ tube formation, chlamydospore production test, carbohydrate assimilation and fermentation test.

Keywords: VVC, Germ tube formation, chlamydospore production.

Introduction

Vulvovaginitis, vulvitis, and vaginitis are general terms that refer to the inflammation disease of the vagina and/or vulva(the external genital organs of a woman). These conditions can be caused by bacteria, fungi or parasite. candida vulvovaginitis also has been called 'vulvovaginal candidiasis', 'candidal vaginitis', 'Monilial infection', 'vaginal yeast infection'[1].

20-25% of vaginitis cases are candida vulvovaginitis. It has been estimated that about 75% of all women get a vaginal yeast infection at least once[1,2]. In 80-90% of cases, VVC is caused by an overgrowth of yeast *Candida albicans.* The remaining cases are caused by other *Candida* species such as *Candida glabrata, Candida krusei and Candida parapsilosis*, which show more resistance to first line antifungal drug treatment[3].

VVC tends to occur more frequently in women who are pregnant, diabetic and not controlling their disease, taking birth control pills, or antibiotics[4,5]. The differentiation of diverse species of *Candida* in the laboratories seems necessary. The identification and classification of *Candida* species done by methods such as colony morphotyping[6], conventional culture techniques, and morphological and biochemical analysis[7]. To identify *Candida* species by the traditional methods such as germ tube formation in serum, chlamydospore production on Corn Meal Agar (CMA), and carbohydrate absorption.

Symptoms suggestive of episodic VVC include external dysuria, vulval pruritus, swelling, or redness. Signs include vulval oedema, fissures, excoriation, or thick curdy discharge. The vaginal pH is usually normal range between 3.8 to 4.5[8].

Material and Methods

Vaginal yeast cultures were collected from patients, pregnant and non-pregnant women, with vaginal discharge, suggestive of vulvovaginal candidiasis by using sterile cotton-tipped swab. Briefly, from June 2013 to May 2014, the total of 85 samples were collected from patients, attended at the gynecological hospital (Megh Clinic and Me and Mummy Hospital. Surat, Gujarat, India).

Specimen Collection and Retrieval of Organisams No special practice for collection, the swab should be repeatedly rubbed firmly over that area. Swabs are immediately transferred to Sabouraud's Dextrose Broth(SDB). That culture tubes were incubated at 30°C for 24-48 hour**s.** Precautions should be take that transportation of specimens should be completed in less than 2 hours.

Direct Evidence for VVC is performed by Gram staining and 10% KOH wet mount for observation of budding yeast formation and pseudohyphae.

Indirect Evidence by pellicle formation on the surface of the liquid media like SDB. It is an ancillary test to identify mainly *C.tropicalis* and *C.krusei.*

Cultural Techniques were used to identify the different *Candida* species. For that the enriched SDB is used to streak the Sabouraud Dextrose Agar Plate, Hichrom Candida Identification Agar Plate(Himedia), and the BiGGy Agar Plate, (Himedia).

Further Confirmation By Biochemical Assay for the differentiation of different *Candida* species. Traditional methods are used such as Germ tube formation test, Chlamydospore production test,Carbohydrate assimilation and Fermentation test. i. Germ tube is an initial hyphae from a sporulating yeast. Germ tube test was performed by inoculating horse serum with culture and incubated at 37°C for minimum of 2 hours, then drop will examined under the microscope for demonstration of germ tube. ii. Chlamydospore production test was performed by inoculating corn meal agar plate. iii. Carbohydrate Assimilation (auxanogram) test by using carbohydrate free yeast nitrogen base agar on which different filter paper discs containing carbohydrate were placed. iv. Carbohydrate fermentation (Zymogram) test by using sugar fermentation media. observation of color change and air bubble in durham's tube.

Results and Discussion

The smear made from suspension is processed by gram staining method and the observation under oil immersion lens is done. As a result the violet color gram positive budding yeast is seen (figure-1) The suspension was observed by taking the drop of it on slide n put coverslip on it under low and high power objectives. The clear structure of yeast was observed. (figure-2)

On SDA *c.albicans* shows typical cream colored, smooth colonies (figure-3).

Culture of *candida* having mix culture of different species were identified by using the selective media, HiChrom agar. This shows different color colony for different species. *C.albicans*-green, *C.tropicalis*-purple, *C.krusei*-pink (figure-4).

BiGGY agar shows the dark brown color colony of *C.albicans* (figure 5).

Germ tube is only formed by *C.albicans* and other *Candida* species are not able to form germ tube (figure-6).

Chlamydospore production test gives possitive test only for *C.albicans* (figure-7).

Different species of *candida* has difference in ability to assimilate and to ferment different sugars (figure-8).

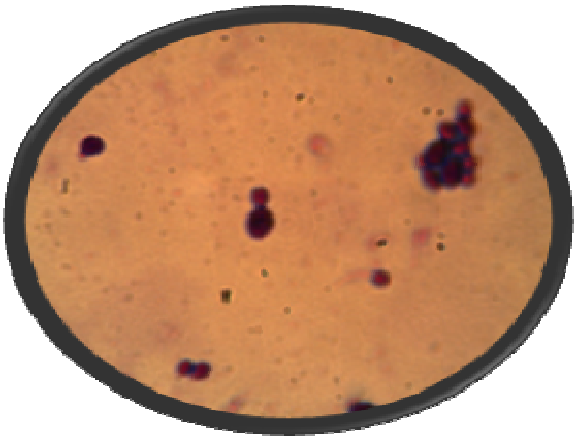


Figure-1
Gram Staining

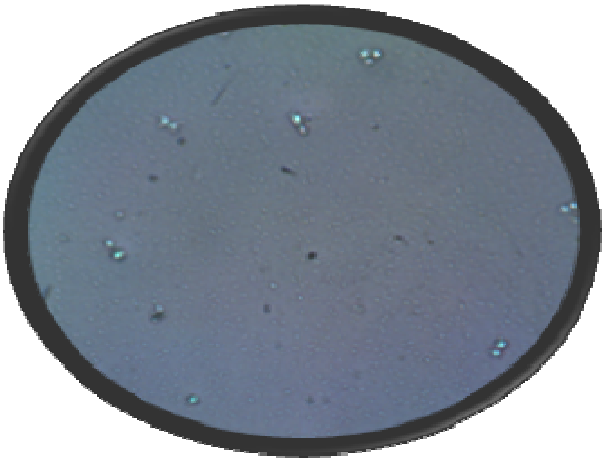
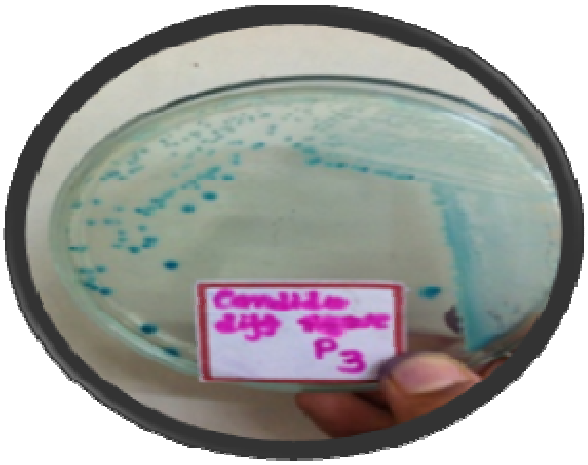


Figure-2
KOH wet mount

Figure-3
Growth on SDA

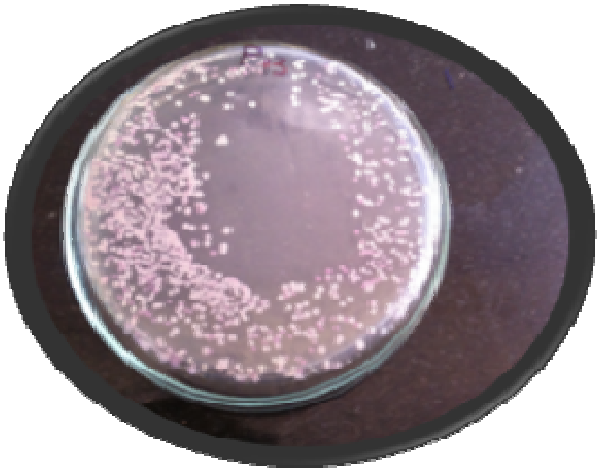


C.albicans

Figure-5
Growth on BiGGY agar

C.krusei

Figure-6
Germ Tube formation



C.tropicalis ***C.glabrata***
Figure-4
Growth on HiChrom agar medium

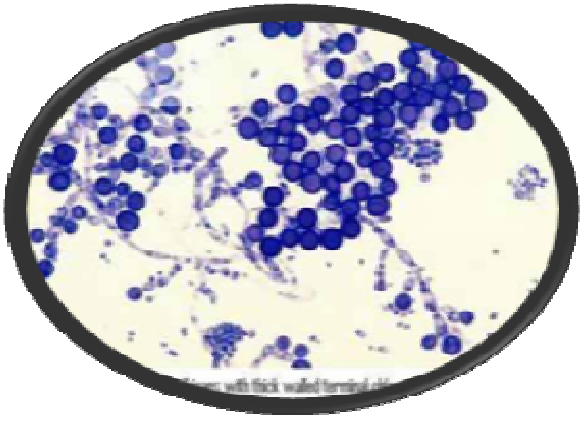


Figure-7
Chlamydospore Production

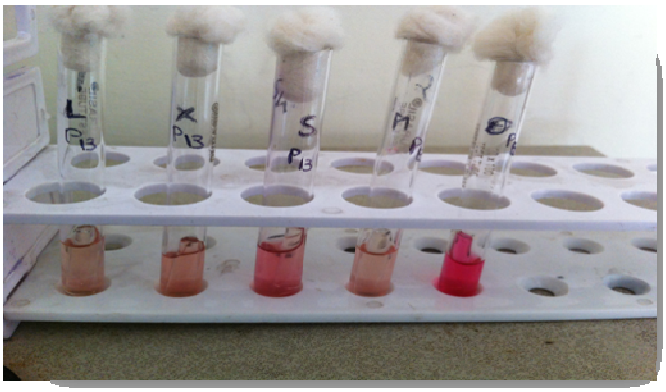


Figure-8
Sugar fermentation

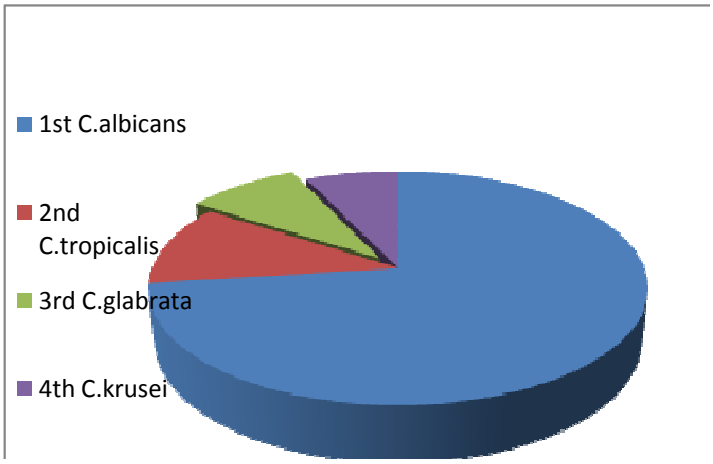


Figure-9
Prevalance of different Candida species

Conclusion

Chrom agar when used to differentiate can give excellent results within short time. Presumtive identification becomes easier especially in case of non-albicans *candida*. Hence Chromagar can be routinely used instead of Sabouraud's Dextrose agar. If this is corroborated with additional tests like germ tube and sugar assimilation test, identification upto species level can be made appropriately. Thus their optimum identification and isolation will help the clinicians to know the pathogen and their susceptibility pattern will help them to instruct proper drugs thereby avoiding any treatment failures.

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