

Vulvovaginal Candidiasis- An Opportunistic Infection in Childbearing Age Group Women, Its Isolation, Identification and Antibiotic Profile

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Abstract: VVC is an opportunistic mucosal infection. *Candida vulvovaginitis* also has been called "vulvovaginal candidiasis," "candidal vaginitis," "monilial infection," or "vaginal yeast infection." It has been estimated that about 75% of all women get a vaginal yeast infection at least once. *Candida* species were isolated from pregnant and non-pregnant women's clinical sample in gynecological clinic. culture test for vaginal swab & scrapings were conducted on sabouraud's dextrose broth & sabouraud's dextrose agar respectively. Hichrome candida agar culture was used for differential identification of candida. Smears from vaginal swabs were prepared for gram staining, 10% KOH wet mount & pellicle formation on liquid medium. The suspected strain of candida was inoculated in horse serum for germ tube formation. Carbohydrate fermentation tests were also conducted. Antibioresistogram of isolated & identified candida species was performed on M.H.Agar by taking different antifungal azole drugs. Different candida species showed susceptibility & resistance towards various drugs.

Keywords: VVC[VulvoVaginal Candidiasis], Germ tube formation, chlamydospore production

1. Introduction

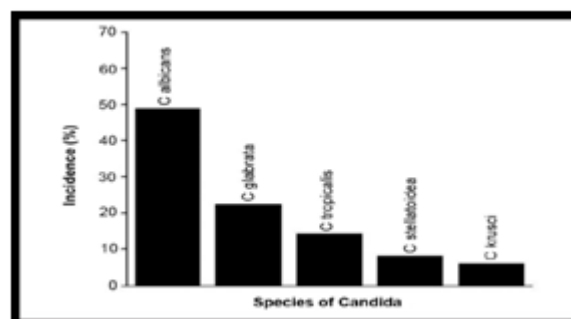
VVC often referred to as a yeast infection and it is a common gynecologic ailment, affecting 3 out of 4 women in their lifetimes [Das Neves et al., 2008]. Greater than 40% of affected women will have 2 or more VVC episodes [Ferrer., 2000; Eschenbach., 2004] and infection occur more frequently in pregnant women. VVC is caused by overgrowth of yeast candida species in the vagina and is characterized by curd like vaginal discharge, erythema & itching [Achkar et al., 2010]. Yeast *candida* is commonly inhabits in oral & vaginal mucosa and gastrointestinal tract of human beings as one of the commensal organism. It causes opportunistic infection in immuno-compromised patients, produces allergic reaction and rarely cause morbidity and mortality [Douglas., 1988]. It also cause variety of infection that range from mucosal candidiasis to life-threatening disseminated candidiasis [Dixon et al., 1996; O'Dwyer et al., 2007; Terrier et al., 2007]. It also has been associated with considerable direct & indirect economic cost [Foxman et al., 2000], enhanced susceptibility to HIV infection [Røttingen et al., 2001] and it is also being investigated for a potential relation with preterm birth [Roberts et al., 2011].

Much of the epidemiologic literature concerning VVC reports on studies in which women were queried on their self reported history of VVC [Geiger et al., 1995] but without laboratory confirmation of infection by candida. As the VVC is common during childbearing age, it is important to understand the pathology of this disease as well as safety or risks of drugs used to treat it during pregnancy.

The diagnosis of this infection is a great challenge. One of the major reason for the increase in candida infection is the

development of its resistant strain to azole drugs used in the prophylaxis & treatment of candidiasis [Diaz-Guerra et al., 1998; Shahid et al., 2006]. The identification of invasive or disseminated candidiasis is based on clinical symptoms that are diffuse and not easily differentiated from those manifested by other infectious agent. Therefore different methods are used in the diagnosis of the infection. *Candida albicans* is a commonest cause of vulvitis & vaginitis. It is not the only cause and clinician must be aware of common conditions which produce similar symptoms. Vaginal swab & vulval biopsy are the most useful tools for differentiating these conditions.

Candida albicans infection occurs in the vast majority (80 to 90%) of diagnosed VVC cases, while infection with other species such as *Candida glabrata* or *Candida tropicalis* occurs less frequently. [Baron et al., 1993] [Graph:1].



Graph 1: showing frequency of diseases

VVC and associated symptoms resolve in a short period of time. 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 [Zdolsek et al., 1995].

There are several treatment options for candida infection such as antifungal and antiseptics with corticosteroids as a useful addition for pruritus and erythema. Short course of azole based treatment regimes are considered and affordable in most cases [Sobel., 2007].

2. Materials 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 135 samples were collected from patients, attended at the gynecological hospital (Megh Clinic & Me and Mumy Hospital. Surat, Gujarat, India)

2.1 Specimen Collection & Retrieval Of Organisms

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



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

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

2.4 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), & the BiGGy Agar Plate.(Himedia).

2.5 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 Fermentation test.

2.5.1 Germ tube is an initial hyphae from a sporulating yeast. Germ tube test was performed by inoculating horse serum with culture & incubated at 37°C for minimum of 2 hours, then drop will be examined under the microscope for demonstration of germ tube.

2.5.2 Chlamydospore production test was performed by inoculating corn meal agar plate.

2.5.3 Carbohydrate fermentation (Zymogram) test by using sugar fermentation media. observation of color change & air bubble in Durham's tube.

2.6 Antibiogram, Isolated and identified cultures were checked for their antibiotic profile. For that, Muller Hinton agar and Himedia antifungal discs were used [fluconazole, itraconazole, clotrimazole, amphotericin B, nystatin, ketconazole], after incubation by measuring the zone size of different antibiotics we can predict that either the specific *Candida* species is resistant or sensitive for that particular drug.

3. Results and Discussion

Candida is a dimorphic organism, means it can exist in two shapes and forms simultaneously. One form is a yeast-like state that is a non-invasive, sugar fermenting organism. The other is a fungal form that produces very long root-like structures, called rhizoids that can penetrate the mucosa and it is invasive.

Healthy immune system prevents yeast from becoming an infectious fungus. It is when our bodies lose their proper immune protection or the intestinal pH is altered unfavorably, that the organism can change from the yeast form to the fungal form. When this happens, the non-parasitic fungal form penetrates the gastrointestinal mucosa and breaks down the boundary between the intestinal tract and the rest of the circulation in our bodies. This allows partially digested dietary proteins to travel into the blood stream, where they exert a powerful antibody-stimulating response on the immune system. It has been officially estimated that about 80% of the population may have candidiasis that is out of control.

The immune system is attacked by *Candida* as a result of the prolonged use of antibiotics, taking steroids or oral contraceptives on a regular basis, or due to high sugar diet. It is also known that *Candida* increases its numbers during periods of stress and lowered immune states. Fungal infection of vagina is sometimes called a thrush. Candidiasis is also known as yeast infection is a common fungal infection that occurs when there is overgrowth of the fungus called *Candida* which is always present in the body in small amounts. However, when an imbalance occurs, such as when the normal acidity of the vagina changes or when hormonal balance changes, *Candida* can multiply. When this happens, symptoms of candidiasis appear. This may include depression, dry, itchy, flaky skin, anxiety, recurring irritability or mood swings, heartburn, indigestion, lethargy, food and environmental allergies, joint soreness, chest pain or other skin problems, recurring cystitis/vaginal infections, premenstrual tension and menstrual problems. Immune suppression, AIDS, diabetes and thyroid disorder may also cause candidiasis.

Vaginal thrush was characterized by typical white lesions developed on the epithelial surfaces of vulva, vagina and cervix.

- In this study, the Sabouraud's dextrose broth inoculated with swab from the lesions of vagina showed turbid growth after incubation.
- Samples from the lesions of vagina were examined in the Gram stained smear.[Fig:1]

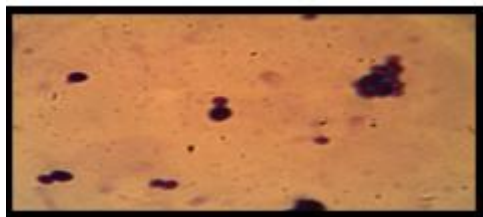


Figure 1: Gram staining

- The fungus appeared as budding yeast cells and pseudomycelium was present in most case. by performing KOH wet mount.[Fig:2]



Figure 2: 10% KOH Wet mount

- Pellicle formation that differentiate *Candida krusei*. [Fig:3]



Figure 3: 10% KOH Wet mount



Figure 4: Pellicle formation

- On Sabouraud's dextrose agar, the swab from lesions of vagina showed typical smooth creamy white colonies with a characteristic yeast odour.[Fig:4]



Figure 5: Growth on SDA

- On Hichrome candida agar, samples from vagina produced glistening green color colonies, purple color

colonies, rose color colonies & off white colonies.[Fig:5,6,7]

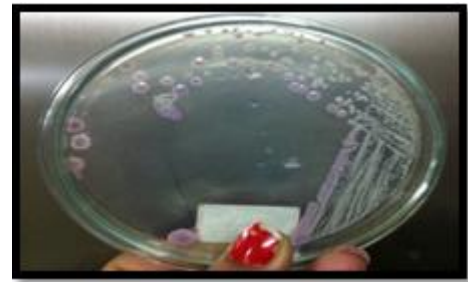


Figure 6: *Candida albicans*

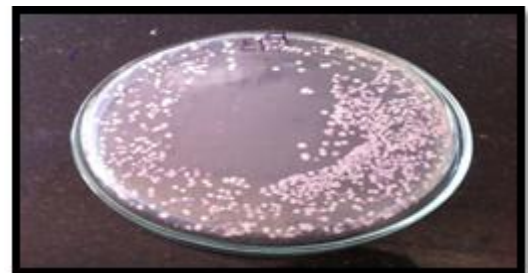


Figure 7: *Candida krusei*

- On BiGGY agar differentiation of *C. albicans*, *Candida krusei* & *Candida tropicalis* can be observed. [Fig:8]

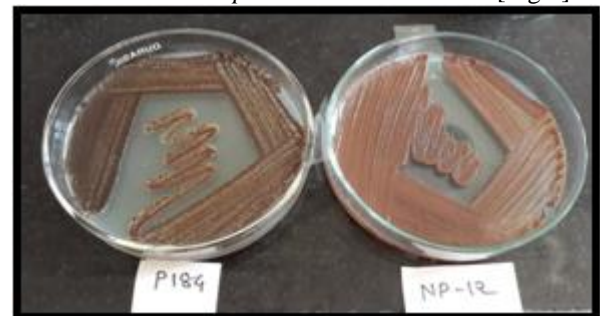


Figure 8: *Candida glabrata* *Candida tropicalis*

- The suspected stains of *Candida* isolates were grown on the corn meal agar. It showed the formation of large, highly refractive and thick walled terminal chlamydo spores. [Fig:9]

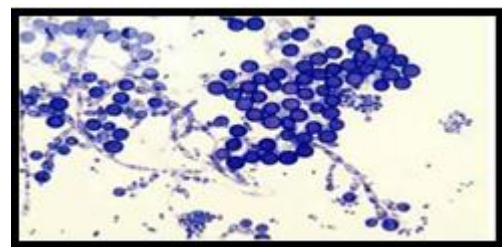


Figure 9: Chlamydo spore Production

- When the suspected *C. albicans* was inoculated with horse serum the formation of germ tubes was seen as long tube like projections extending from the yeast cells. There was no constriction at the point of attachment to the yeast cells. The germ tubes were formed within two hours of incubation in *C. albicans* [Fig. 10].

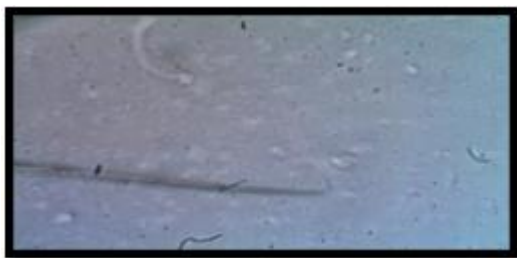


Figure 10: Germ Tube

- Carbohydrate fermentation tests for *Candida spp.* are presented in table. The tests for fermentation of dextrose, maltose, galactose and trehalose were positive but negative for fermentation of sucrose and lactose. [Fig:11]



Figure 11: Carbohydrate Fermentation

- The antibiotic susceptibility tests for the different isolates shows different results, some are susceptible and some shows resistant toward various antibiotics. [Fig:12]



Figure 12: Antibioresistogram

To summarize, *candida* were isolated from patients with invasive candidiasis and characterized. Suspected *Candida spp.* were selectively isolated on Hichrome candida agar and identified. After the identification, gynecological practitioner must go through the antibiotic test and then have to finalize the treatment.

4. Conclusion

After using the different culture methods for isolation of organism and than identification by latest HiCHROM chromogenic medium instead of other traditional method is quite expensive but give accurate results. If we identify the specific species level yeast than the specific drug for it, so are able to know the resistance and susceptibility of that organism toward that specific antibiotic which help medical practitioner to prescribe proper drug treatment and can prevent the patient from RECURRENT VULVOVAGINAL CANDIDIASIS.

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