



MICROBIOLOGICAL STUDY OF VARIOUS CANDIDA SPECIES AND ITS ANTIFUNGAL SENSITIVITY TESTING ISOLATED FROM ANTENATAL WOMEN WITH VAGINITIS, IN TERTIARY CARE TEACHING HOSPITAL, WESTERN INDIA

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Abstract-

Background and Objective: An estimated 75% of women will have at least one episode of VVC, and 40-45% will have two or more episodes within their lifetime and infection occurs more frequently in pregnant women-CDC. This study was conducted to estimate the prevalence of vulvovaginal candidiasis along with its antifungal susceptibility pattern among antenatal women attending Obstetric and Gynecology Department Sir T Hospital, Bhavnagar, Gujarat, India.

Methods: Vaginal swab specimens for culture were drawn from 100 female attending obstetric and gynecology Department, Sir T Hospital, Bhavnagar during August 2009 to August 2011. The specimen was inoculated into Sabouraud's dextrose agar and the isolated colonies were identified by battery of biochemical reactions. The specimen were also inoculated Chrom *Candida* differential agar. Antifungal sensitivity pattern of isolates was studied by Modified Kirby Bauer Disc diffusion technique on Muller Hinton agar with 2% glucose and Methylene blue.

Result: In this study, a total number of 100 patients of vaginal Candidiasis were included, among them 40 samples indicates positivity, out of them 22 samples (55%) indicate *Candida albicans* and 18 samples (45%) are Non albicans species.

Conclusion: Vaginal candidiasis is an extremely common infection in 60-70% women during their reproductive age at least once in their lives. There is an increase in infections with non-albicans *Candida* spp. and few of them are intrinsically resistant to azoles. Effective antifungal treatment is an important criterion in treating the candidal infections.

Keywords- Candida, vaginitis, pregnancy, chrom agar, antifungal susceptibility

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Introduction

Candida species are the second most common cause of vulvovaginitis worldwide [1]. The prevalence of vulvovaginal candidiasis (VVC) is increasing due to the extensive utilization of broad-spectrum antibiotics as well as increased cases of immunocompromised patients [2,3]. An estimated 75% of women will have at least one episode of VVC, and 40-45% will have two or more episodes within their lifetime and infection occurs more frequently in pregnant women. The three diseases most frequently associated with vaginal discharge are Bacterial Vaginosis, trichomoniasis and candidiasis. The subjective symptoms may be leucorrhoea typically curdy white discharge itching over vulva, burning micturition backache and/or dyspareunia [4]. As Vulvovaginal candidiasis is so common in women during their childbearing years, it is important to understand the pathology of this disease as well as the safety or risks of drugs used to treat it during pregnancy. The incidence of yeast infection

also rise in immune compromised patient due to disease like diabetes, sexual transmitted disease, endocrine disorder & leukemia also. Cure rate is 80% for uncomplicated cases. Approximately 10-20% of women will have complicated VVC that necessitates diagnostic and therapeutic considerations. The complications can be treatment failure in about 20%. Recurrent candidiasis can affect up to 50% of sufferers at a given period in their life, depression and psychosexual problems can occur in women who suffer recurrent episodes & treatment during pregnancy is more likely to fail.

This study was conducted to estimate the prevalence of vulvovaginal candidiasis along with its antifungal susceptibility pattern among antenatal women attending Obstetric and Gynecology Department, Sir T Hospital, Bhavnagar, Gujarat, India.

Materials and Methods

In this study antenatal women who were attending Obstetric and

Gynecology Department, Sir T Hospital, Bhavnagar, Gujarat, India in duration of August 2009 to August 2011 with complaints of discharge per vaginam were included. On per vaginam and per speculum examination, from the women with curdy white discharge Vaginal swabs were taken. 100 vaginal swabs were taken & were inoculated into Sabouraud's Dextrose Agar and Hichrom *Candida* agar [5,6]. The White to cream colored, pasty, smooth colonies were identified and confirmed by wet mount, Gram's staining, Germ tube testing and Dalmau plate (commenal agar) morphology, Sugar assimilation and fermentation tests. The isolates identified by biochemical tests were also compared with growth and color changes in Hichrom *Candida* agar. Antifungal sensitivity pattern of isolates were performed by Modified Kirby Bauer Disc diffusion technique on Muller Hinton agar + 2% glucose + Methylene blue [7].

Ethics

The permission for this study was taken from Institutional Ethical Committee of Government Medical College, Bhavnagar, Gujarat, India.

Results

In this study, 100 women of vaginitis were included. The vaginal swabs were processed for fungal culture, among which 45 swabs showed positive yeast like growth. Among them 45 samples indicates positivity, out of them 31 samples indicate *Candida albicans* and other are non-albicans species. Thus out of total 45 *Candida* positive specimens 31(68.88%) specimens were of *Candida albicans*, 1 was of (2.22%) *Candida parapsilosis*, 8 was of *Candida glabrata* (17.77%), 2 was of *Candida krusei* (4.4%), *Candida tropicalis* was 3 (6.66%) [Table-1].

Table 1- Age distribution within total samples

| Age in Years | Total specimen | <i>Candida</i> isolated |
|--------------|----------------|-------------------------|
| 21-30 | 59 | 35 |
| 31-40 | 33 | 6 |
| 41-45 | 8 | 5 |
| Total | 100 | 40 |

Most of the women from age group 21-30 i.e. n=59 and among these women vulvovaginal candidiasis was common and specimen collected showed the maximum positivity rate among these 60% of the women had curdy discharge and most of the isolated *Candida* were from this type of discharge [Fig-1], [Table-2], [Fig-2]. *Candida albicans* found 77% positivity and other species in another 23% positivity rate [Table-3].

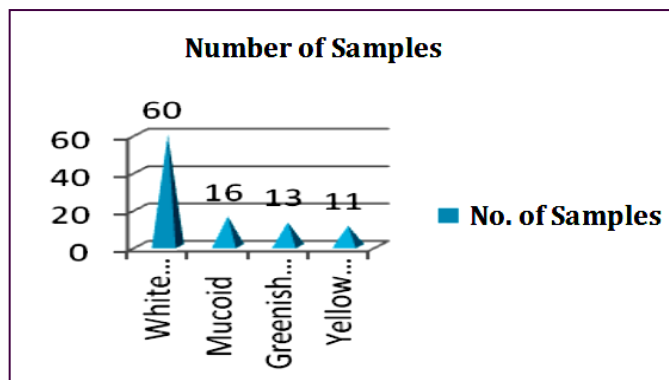


Fig. 1- Types of vaginal discharges in study antenatal women who were attending Obstetric and Gynecology Department Sir T Hospital, Bhavnagar, Gujarat, India

Table 2- Total sample distribution

| Total no. of Specimen | Growth obtained in Sabouraud's Dextrose Agar | Growth on Hichrom <i>Candida</i> Agar | No growth on Sabouraud's Dextrose Agar |
|-----------------------|--|---------------------------------------|--|
| 100 | 40 | 45 | 60 |

Percentage of positive *Candida* spp.

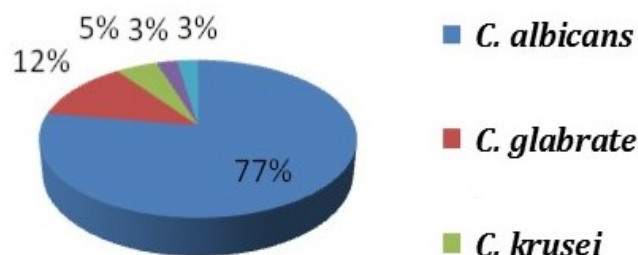


Fig. 2- Isolation rate of various *Candida* spp.

Table 3- Distribution of different species of isolated *Candida*

| <i>Candida</i> species | No. of <i>Candida</i> positive specimen (total 40) | Hichrom <i>Candida</i> agar positive specimen |
|-----------------------------|--|---|
| <i>Candida albicans</i> | 31 | 31 |
| <i>Candida glabrata</i> | 5 | 8 |
| <i>Candida krusei</i> | 2 | 2 |
| <i>Candida tropicalis</i> | 1 | 3 |
| <i>Candida parapsilosis</i> | 1 | 1 |

In this study Hichrom agar positivity rate was found more as compared to conventional method of fungal culture methods for isolation, so Hichrom agar is very effective media for rapid laboratory diagnosis of *Candida* species in clinical samples [Table-4].

Table 4- Antifungal testing sensitivity pattern of isolated *Candida* spp.

| Species | Fluconazole | Clotrimazole | Amphotericin-B | Nystatin |
|-----------------------------|-------------|--------------|----------------|----------|
| <i>Candida albicans</i> | 22(100%) | 18(81.8%) | 21(95.4%) | 22(100%) |
| <i>Candida glabrata</i> | 0(0%) | 0(100%) | 8(100%) | 8(100%) |
| <i>Candida krusei</i> | 0(0%) | 0(100%) | 5(100%) | 5(100%) |
| <i>Candida tropicalis</i> | 4(100%) | 3(75%) | 4(100%) | 4(100%) |
| <i>Candida parapsilosis</i> | 1(100%) | 1(100%) | 1(100%) | 1(100%) |

In this study fluconazole was effective for *C. albicans*. Nystatin & Amphotericin B approximate 95-100% sensitive for all isolated species of *Candida*.

Out of total 100 women, 17 were diabetic. Out of them 12(70.58%) had vulvovaginal candidiasis. 3 were HIV reactive and out of them 1 (33.3%) had vulvovaginal candidiasis.

Discussion

Candidal vulvovaginitis or vaginal thrush is an infection of the vagina's mucous membranes by *Candida albicans*. Up to 75% of women will have this infection at some point in their lives, and approximately 5% will have recurring episodes. It is the second most common cause of vaginal inflammation after bacterial vaginosis-CDC. It is most commonly caused by a type of fungus known as *Candida albicans*. The *Candida* species of fungus is found naturally in the vagina, and is usually harmless. However, if the conditions in the vagina change, *Candida albicans* can cause the symptoms of

thrush. Symptoms of thrush can also be caused by *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*. Non-albicans *Candida* is commonly found in complicated cases of vaginal thrush such that first line treatment is ineffective. These cases are more likely in immunocompromised patients-CDC.

Jorizzo [8] and Sobel, et al [9] has observed that vulvovaginitis patients presented with white curdy vaginal discharge which is comparable to our study. In a large study of women attending a sexually transmitted disease clinic, only 28% of 545 women with pruritis, burning or a vaginal discharge were *C. albicans* culture positive. Thus, in symptomatic women, a positive wet mount or culture for *Candida* is necessary to assess whether this organism is present in the vagina. The findings in the our study shows association between a positive *Candida* culture & symptomatology along with current oral contraceptive and antibiotic usage and pregnancy parallel earlier reports [10-12].

The swabs were taken & were inoculated into Sabouraud's Dextrose Agar and Hichrom *Candida* agar [5,6]. The White to cream colored, pasty, smooth colonies were identified and confirmed by wet mount, Gram's staining, Germ tube testing and Dalmau plate (cornmeal agar) morphology, Sugar assimilation and fermentation tests. The isolates identified by biochemical tests were also compared with growth and color changes in Hichrom *Candida* agar [5,6]. Antifungal sensitivity pattern of isolates were performed by Modified Kirby Bauer Disc diffusion technique on Muller Hinton agar + 2% glucose + Methylene blue [7]. In our study, a total number of 100 patients of vaginitis were included, among them 45 samples indicates positivity, out of them 31 samples indicate *Candida albicans* and other are non-albicans species. Thus Out of total 45 *Candida* positive specimens 31(68.88%) specimens were of *Candida albicans*, 1 was of (2.22%) *Candida parapsilosis*, 8 was of *Candida glabrata* (17.77%), 2 was of *Candida krusei* (4.4%), *Candia tropicalis* was 3 (6.66%). *Candida albicans* was the most common species isolated (77.5%) & *C. glabrata* (10.6%), *C. tropicalis* (3.8%), *C. krusei* (2.7%) on rapid identification on chrom agar inoculation. Prevalence and susceptibility to *Candida* spp. with fluconazole suggested that yeasts were isolated in 111 (18.5%) women and these consisted of *C. albicans* (39, 35.1%), *Candida glabrata* (56, 50.4%), *C. tropicalis* (12, 10.8%), *C. krusei* (3, 2.7%) and *C. parapsilosis* (1, 0.9%). Multiple epidemiologic studies have indicated that *Candida albicans* is responsible for in excess of 90% of episodes of VVC, although rare reports indicate that non- albicans *Candida* (NAC) species are responsible for 10-30% of episodes in certain geographic regions [13-15].

In our study most of the patients belongs to age group 21-30 (59.32%). In a study of clinical pattern of Candidiasis infections in Bombay, Dalal and Kelkar [16] had noticed more number of patients belonging to the age group of 21-40 years (35%). Clayton and Noble [17] in a study on observations on the epidemiology of *Candida albicans* found majority of patients belonging to the age group of 20-60 years.

Montes, et al [18] and Macneill & Carey [19] have found diabetes mellitus as one of the common predisposing factors for Candidiasis of various types which is also seen in this study. It was found that 11(20.4%) of 54 Candidal cases, had diabetes as the risk factor.

Talib & Singh [20] and Baradkar & Karyakarte [21] have observed positive culture in 65% and 63.33% respectively, all of whom were HIV positive patients.

In Our study Hichrom agar was found to be more sensitive in comparison to conventional culture and identification tests. In studies by Odds and Bernaerts [22], Louwagie, et al [23] and Moyer, et al [24], a major advantage of Chrom agar was the ability to detect mixed cultures of yeasts in clinical specimens. In all three previous studies, Chrom agar was superior to other routine and selective media in detecting multiple *Candida* species in both clinical and stock cultures. Our results were in agreement with these prior studies.

Conclusion

Vaginal candidiasis is an extremely common infection in 60-70% women during their reproductive age at least once in their lives [1,2]. Epidemiological profile of genitourinary candidiasis varies globally depending upon socioeconomic and health factors. The associated risk factors in pregnancy are - poor socioeconomic strata & poor hygiene, late pregnancy, multigravida, Immunocompromised status like presence diabetes mellitus and HIV infection [15]. There is an increase in infections with non-albicans *Candida* spp. and few of them are intrinsically resistant to azoles like *C. krusei*. This specifies the need of species identification and antifungal susceptibility as a part of the laboratory diagnosis of vaginal candidiasis. Effective antifungal treatment is an important criterion in treating the candidial infections [25].

Conflict of Interest : None declared.

References

- [1] Geiger A.M., Foxman B. & Gillespie B.W. (1995) *Am. J. Public Health*, 85, 1146-1148.
- [2] Sobel J.D. (1993) *Clin. Obstet. Gynecol.*, 36, 153-165.
- [3] Jovanovic R., Congema E. & Nguyen H.T. (1991) *J. Repro. Med.*, 36, 593-597.
- [4] Fauci A.S. (1997) *Harrison's Principles of Internal Medicine*, 14th ed.(1), 805.
- [5] Pfaller M.A., Houston A., Coffmann S. (1996) *J. Clin. Microbiol.*, 34, 58-61.
- [6] CLSI (2009) *Methods of Antifungal Susceptibility Testing of Yeast*, Approved guidelines, 29(17), M44-A2.
- [7] Houang E.T., Chu K.C., Koehler A.P., Cheng A.F. (1997) *J. Clin. Pathol.*, 50, 563-565.
- [8] Jorizzo J.L. (1984) *Dermatol. Clin.*, 2, 19-27.
- [9] Sobel J.D., Faro S., Force R.W., Foxman B., Ledger W.J., Nyirjesy P.R., Reed B.D., Summers P.R. (1998) *Am. J. Obstet. Gynecol.*, 178, 203-211.
- [10] Spinillo A., Capuzzo F., Nicola S. (1995) *Am. J. Obstet. Gynecol.*, 51, 293-297.
- [11] Morton R.S., Rashid S. (1977) *Proc. R. Soc. Med.*, (4), 3-6.
- [12] Ryley J.F. (1986) *J. Med. Vet. Mycology*, 4, 5-22.
- [13] Sobel J.D. (2007) *Lancet*, 369, 1961-1971.
- [14] Vermitsky J.P., Self M.J., Chadwick S.G. (2008) *J. Clin. Microbiol.*, 46, 1501-1503.
- [15] Mohanty S., Xess I., Hasan F. (2007) *Indian J. Med. Res.*, 126, 216-219.
- [16] Dalal P.J., Kelkar S.S. (1980) *Ind. J. Der. Ven. Lep.*, 46(1), 31-32.
- [17] Clayton Y.M., Noble W. (1966) *J. Clin. Pathol.*, 19, 76-78.

- [18]Montes L.F., Moschella S.L., Hurley H.J. (1992) *Text Book of Dermatology*, 3rd ed.(1), 913-923.
- [19]Witkin S.S., Jeremias J., Ledger W.J. (1998) *J. Allergy Clin. Immunol.*, 81, 412-416.
- [20]Talib S.H., Singh J. (1997) *Indian J. Pathol. Microbiol.*, 40(3), 377-382.
- [21]Baradkar V.P., Karyakarte R.P. (1999) *Indian J. Med. Microbiol.*, 17(1), 42-44.
- [22]Odds F.C., Bernaerts R. (1994) *J. Clin. Microbio.*, 32, 1923-1929.
- [23]Louwagie B., Surmont I., Verhaegen J., Odds F. (1995) *Eur. J. Clin. Microbiol. Infect. Dis.*,14, 406-411.
- [24]Veena M., Vidya G.S., Sharma A., Prakash M.R. (2012) *Int. J. Biol. Med. Res.*, 3(2), 1778-1782.
- [25]Babin D., Kotigadde S., Rao S., Rao T.V. (2013) *Int. J. Biol. Med. Res.*, 3(1), 55-59.