Research Article

Vulvovaginal Candidiasis and its Antifungal Susceptibility Pattern: Single center experience

Rati R[1], Patel J[2], Rishi S[3]

[1]Dr Ruchi Rati, 3rd Year, Post graduate student of Microbiology, [2]Dr Jaya Patel, 3rd Year, Post graduate student of Obstetric & Gynecology, [3]Dr Suman Rishi, Professor, Department of Microbiology. All are affiliated with National Institute of Medical Sciences, Shobhanagar Jaipur, Rajasthan, India.

Address for correspondence: Dr Jaya Patel, Email: sunshinejaya@gmail.com

Abstract:

Introduction: Candidiasis is the most common vaginal infection affecting about 70% of women. Our aim was isolation and identification of *Candida* from high vaginal swabs and in vitro antifungal activity of Fluconazole, Voriconazole, Nystatin, Amphotericin B. **Materials and Methods:** This study was carried out in NIMS Medical College, Jaipur during June 2013 to May 2014. The study group consisted of 100 women of reproductive age having symptomatic vaginal discharge, vulval purities or lower abdominal pain. High vaginal swabs were collected from each and processed by Gram Staining, culture on Sabroud's dextrose agar & CHROM agar. Antifungal susceptibility was performed by disc diffusion method as per CLSI guidelines. **Results:** Vulvovaginal candidiasis accounted for 22 % of cases. Species distribution is as follows: *C.albicans* 12, *C tropicalis* 7, *C krusei* 2, *C glabrata* 1. Senstivity to Voriconazole was 91.6% for *C albicans*, 71.42 % for *C.tropicalis*, 50% for *C.krusei*. Senstivity to Fluconazole was seen in 91.6 % of *C.albicans* & 42.85 % of *C.tropicalis*. *C. glabrata* was resistant to both Voriconazole & Fluconazole. Senstivity to Amphotericin B was 75 % for *C. albicans*, 100 % for *C.tropicalis*, 100% for *C.krusei* & *C. glabrata*. Senstivity to Nystatin was 91.6% for *C.albicans* & 100% for *C.tropicalis*, *C.krusei* & *C.glabrata*. **Conclusions:** There is an increase in non-albicans *Candida* infection and few like *C.krusei* are intrinsically resistant to azoles. This signifies the need of species identification & antifungal susceptibility as a routine laboratory procedure in diagnosis of vaginal candidiasis.

Key words: Vaginal Candidiasis, Candida, Vulvovaginal candidiasis.

Introduction

Vulvovaginal candidiasis (VVC) remains one of the most common infections of the female genital tract, which is classified by the world health organization (WHO) as a sexually transmitted disease [1, 2]. Candidiasis is the most common vaginal infection in most countries affecting about 50-72% of women, 40-50% having recurrent episodes[3,4]. The majority of cases of vulvovaginal candidiasis are caused by *Candida albicans*; however, episodes due to non-albicans species of *Candida* appear to be increasing [5,6]. *Candida* species, mostly *C. albicans*, can be isolated in the vaginal tracts of 20 to 30% of healthy asymptomatic non-pregnant women at any single point of time and in up to 70% if followed longitudinally over a 1-year period [7]. In about 5 per cent of cases, the disease has a chronic course, showing frequent and refractory episodes [5,8]. Fortunately the infection is rarely life threatening, whereas it is usually associated with such morbidities like discomfort, pain, sexual dysfunctions, vulvar dryness, cracks, itching, burning, soreness and finally health care costs [9-11]. The predisposing factors include: hormonal fluctuations in pregnancy, luteal phase of menstrual cycle, use of oral contraceptives, and hormone replacement therapy among others [12]. Antifungal agents commonly used to treat yeast infections include flucytosine, fluconazole, amphotericin B, voriconazole, clotrimazole, nystatin, capsofungin and ketoconazole. The problem with the use of antifungal agents, apart from safety and cost is the development of drug resistant strains [13]. There is evidence, however of an increased azole resistance among isolates of Candida Species isolated from women with VVC, other Candida are generally more resistant to

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azoles than *C.albicans* [14]. It has been estimated that antifungal therapy was inappropriately prescribed in 54% of culture negative cases, such unnecessary use of antifungal therapy contributes to the development of antifungal resistance and the emergence of infections associated with other Candida spp. and other opportunistic fungi [15]. Prolonged therapy and increased use of antifungals for recurrent candidiasis are the most common risk factors for azoles resistance among Candida isolates from vulvovaginitis candidiasis patients. Azoles have the advantage of being taken orally, which increase their potency [9,11,16]. Most nonalbicans *Candida* species have higher inhibitory concentrations (MICs) to the azole antifungal agents, and infections they cause are often difficult to treat [17,18]. This phenomenon emphasizes the importance of identification and surveillance of the *Candida* species in the clinical settings. The aim of the study is to find out the prevalence of vulvovaginal candidiasis with special reference to speciation of *Candida* and susceptibility profile of the *Candida* species to antifungal agents like amphotericin B, fluconazole, voriconazole & Nystatin.

Materials and methods

The study was carried out in the Department of Microbiology, NIMS Medical College and Hospital, Jaipur during the period of 1 year from June 2013 to May 2014. The study group consisted of 100 women of reproductive age group (18-45years), who presented with complaints of symptomatic vaginal discharge, vulval/vaginal pruritis, pain lower abdomen to the department of Obstetrics & Gynaecology of NIMS. After taking written informed consent, two high vaginal swabs were collected from each of them under all aseptic precautions and immediately sent to the department of Microbiology. Gram staining was performed using one swab while the other swab was used to inoculate Sabourauds Dextrose Agar tubes with Chloramphenicol and CHROM agar plates. These were incubated at 37°C for 48 hours. The isolates were identified and speciated using germ tube test and colour of the colonies on CHROM agar plates (HiMedia, India). All the isolates were subjected to antifungal susceptibility testing for amphotericin B, fluconazole, voriconazole, nystatin by disc diffusion method with M44-A Clinical Laboratory Standards(CLSI) guidelines. Disc supplied by Hi-Media, Mumbai were placed on Muller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue. Disk used included Amphotericin B (100 IU), Fluconazole (25µg), Voriconazole (1µg), Nystatin (50µg). After overnight incubation, zone of inhibition were noted. Categorized in susceptible, intermediate and resistance compared with the standard zones interpretive breakpoints published by CLSI M44-A2 [19] Dota KFD et al[20], Rosco Diagnostica[21].

Antifungal disc	Susceptible Zone(mm)	Susceptible dose dependent Zone(mm)	Resistant Zone (mm)
Amphotericin b	>15	10-14	<10
Fluconazole	>19	15-18	<15
Voriconazole	17	16-14	<13
Nystatin	>15	10-14	<10

Results

During the study period it was found that 22 out of 100 vaginal swabs obtained from women with vulvovaginitis were culture positive for Candida (22%) ,while 78 women's vaginal swabs had other causes of vaginitis (Bacterial vaginosis, Trichomoniasis etc). Majority of the women infected belonged to 26-35 years group.

Table 1: Distribution of Candida species isolated from vaginal discharge

Species identification	Number
C.albicans	12
C.tropicalis	7
C.krusei	2
C.glabrata	1

The predominant species isolated was *C.albicans* (n=12). Non-albicans species consisted of *C.tropicalis* (n=7), *C.krusei* (n=2) and *C.glabrata* (n=1).

Table: 2 Antifungal susceptibility pattern

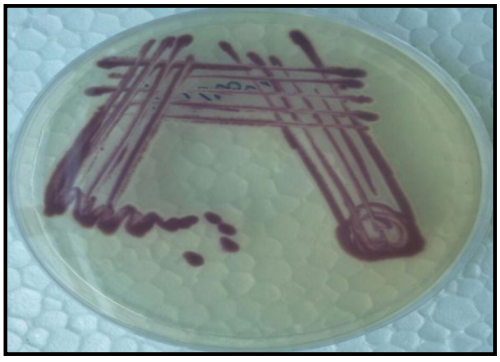
Antifungal agent	No.	Amphotericin B		Fluconazole		Nystatin		Voriconazole	
		Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
C.albicans	12	9	3	11	1	11	1	11	1
C.tropicalis	7	7	0	3	4	7	0	5	2
C.glabrata	1	1	0	0	1	1	0	0	1
C.krusei	2	2	0	0	2	2	0	1	1

Most of the isolates are sensitive to antifungal like Amphotericin B, Fluconazole, Nystatin & Variconazole.

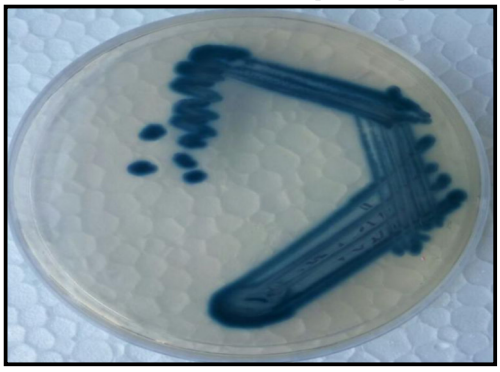
Table 3: Predisposing factors for Vaginal Candidiasis

Predisposing Factors	Number Of Patients	Percentage of Isolation
Pregnancy	8	37
Oral contraceptives	6	27
Intrauterine devices	4	18
Post antibiotic therapy	2	9
Urinary catheter	1	4.5
Diabetes Mellitus	1	4.5

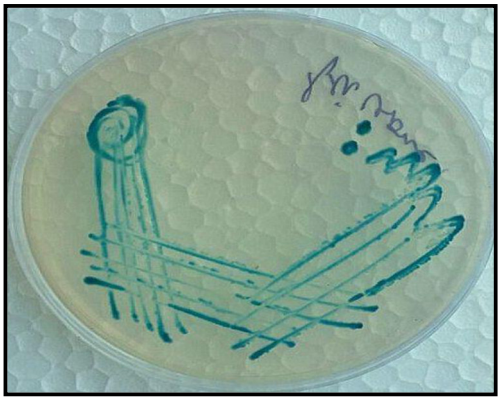
Pregnancy was the commonest risk factor for VVC 8(37%) followed by use of oral contraceptive pills 6(27%). Other risk factors were usage of Intrauterine devices, broad spectrum antibiotics, Diabetes mellitus.[Table 3]



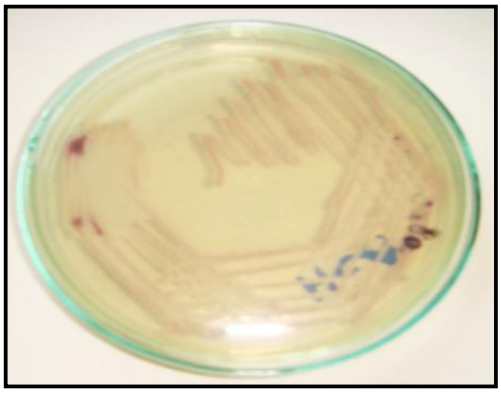
C. Tropicalis on Chrome Agar



C. Albicans on Chrome Agar



C. Krusei on Chrome Agar



C. Glabrata on Chrome Agar

Discussion

Vaginal candidiasis is an extremely common infection. 60-70% women during their reproductive age have at least one episode of VVC in their lives [22,23]. In our study the highest frequency of vaginal candidiasis was observed in the age group of (26- 35) years, followed by age group of (36-45) years. Similar findings were observed by Ako et al [24] who found that vaginal candidiasis occurs most frequently in the age group of 20- 25 years. Sehgal et al also showed the age group 21-30 years had the highest incidence of VVC [25]. This shows that the women of childbearing age groups are more vulnerable to vaginal candidiasis. Reason for the high incidence in this age group includes low levels of protective cervical antibodies, increased sexual activity and new influence of reproductive hormones that may lead to increased susceptibility to reproductive tract infections . Our result is in accordance with other studies [26]. In the present study 37% of the females who presented with the complaint of symptomatic vaginal discharge were pregnant. These are in agreement with studies of Sobel [22], Okungbova et al [27] and Ahmet et al [28]. Use of oral contraceptive pills was found to be associated with 27% cases of vulvovaginal candidiasis in our study. In this study women of lower socioeconomic strata, poor genital hygiene & illiteracy showed significantly higher incidence of VVC, use of synthetic clothes could be contributing factor by increasing perineal moisture. Our study is in consistent with work done by Jindal et al [29]. Increased incidence of VVC in the present study was observed with the use of broad spectrum antibiotics. Antibiotic agents increase vaginal yeast colonization and are thought to act by eliminating lactobacilli, thereby facilitating *Candida* to grow, adhere and germinate. The concept of interaction between lactobacilli and *Candida* includes competition for nutrients and stearic interference of adherence to vaginal epithelial cells [30,31]. Other important risk factors include Diabetes, use of intrauterine devices and urinary catheters. In this study, culture for *Candida* species was positive in 22% of cases. This study is in agreement with the work conducted by Bauters et al [32], who isolated *candida* species in 20.1%. Mohanty et al.[33], reported 18.5% prevalence of vulvovaginal candidiasis in a community setting. *C.albicans* was the most common spp.associated with vulvuvaginitis. This finding is in agreement with other studies who reported that *C.albicans* is the most predominant species causing vaginal candidiasis.

Table No 4: Candida species isolated in various studies

Candida species	*C. albicans*	*C. tropicalis*	*C. krusei*	*C. glabrata*
Srujana Mohanty et al, (2003) (%)	35.1	10.8	2.7	50.4
Neerja et al, (2005) (%)	69.5	6.55	6.55	8.7
Oviasogie et al, (2009) (%)	61.5	9.4	-	25.6
Latha Ragunathan et al, (2010) (%)	65	7.5	-	22.5
Present study, (2014) (%)	54.54	31.81	9.09	4.54

Antifungal susceptibility testing results in our study revealed that none of the non-Candida albicans isolates tested were resistant to AMB and NY. This high AMB and NY susceptibility rate is in agreement with other studies who reported that resistance of Candida species to AMB and other polyenes appear to be extremely uncommon [33,34,35], partly because of their irreversible fungicidal action once they are bound to the yeast cells [36]. The occurrence of resistance to azole antifungal agents were greater for group of other Candida spp. especially *C.glabrata* [37,38,34,39]. Many studies emphasized that *C.glabrata* and *C.krusei* are intrinsically less sensitive to azoles and some strains of *C.tropicalis* were resistant to azoles [39] , because these organisms are haploid unlike *C.albicans* which is diploid and drug resistance is more likely to develop during treatment. In the present study 8.4% *C.albicans* isolates were resistant to fluconazole, which is in contrast to a study from Brazil involving 56 yeast isolates obtained from women with candidal vaginitis which had no resistant strains to fluconazole [40].

Conclusion

To conclude, speciation using CHROM agar will help in early speciation and detection of VVC. *C. albicans* continues to be the major causative agent of vulvovaginal candidiasis. However there is an increase in infections with non-albicans *Candida* species. The

emergence of azole resistant *C. albicans* and non albicans species emphasises the need of species identification and antifungal susceptibility as a routine in the diagnosis and management of vaginal candidiasis. The emergence of fluconazole resistance in non albicans *Candida* may limit its use as a prophylactic agent in hospitals. Early and effective antifungal treatment is the need of the hour for treating the candidial infections. Therefore, routine antifungal susceptibility testing is essential to monitor the emergence of drug resistance. As only a limited number of *Candida* isolates could be tested in this study, further clinical studies need to be performed involving more number of isolates to confirm the findings.

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