

A STUDY ON PREVALENCE OF CANDIDA ALBICANS IN VULVOVAGINAL CANDIDIASIS AND THEIR PHENOTYPIC CHARACTERIZATION WITH ANTIFUNGAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL, LUCKNOW, INDIA

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Abstract:

Background: Vaginal candidiasis are a common gynaecological finding among women worldwide. This study was carried out to determine the prevalence of vulvovaginal candidiasis (VVC) along with speciation of *Candida* with special reference to its antifungal susceptibility pattern to fluconazole and also to evaluate the risk factors responsible for VVC in patients attending our tertiary care hospital in Lucknow, India. **Materials and Methods:** This study was conducted in the Department of Microbiology, Prasad Medical College and Hospital, Lucknow, India during the period of 2018 to 2020. The study group consisted of clinically suspected 180 cases of vulvovaginitis candidiasis the age group of between 15 to 56 years. The collection of data include name, age, complaints like vaginal discharge, pain and itching, duration of illness whether acute or chronic, any past history of presenting episode, any systemic diseases such as diabetes/hypertension/tuberculosis. An informed written consent was obtained from all patients, attending Obstetrics & Gynecology out patients Department. Samples were collected upon inserting a sterile vaginal speculum into vagina; two high vaginal swabs, one after the other, were taken by a sterile cotton wool swab into the posterior vaginal fornix and rotated gently and High vaginal swabs were subjected to direct 10% KOH wet mount microscopy, Gram stain, culture onto Sabouraud's dextrose agar (SDA) & 5% sheep blood agar and susceptibility testing to fluconazole was performed

Results: *Candida albicans* were isolated in 83(46%) of total clinically suspected case and 97(54%) were non candida albicans and antifungal susceptibility test carried out on the 83 isolates as recommended by Clinical and Laboratory Standards Institute (CLSI)

Conclusion: *Candida albicans* was the predominant species responsible for VVC infection and commonly used antifungal drugs clotrimazole, voriconazole and fluconazole were most effective.

Keywords: *Candida*, CHROMagar, Antifungal susceptibility testing

INTRODUCTION

Vulvo vaginal candidiasis is clinically defined as inflammation of the vulva and vagina due to the presence of *Candida* and in the absence of other infectious aetiology. Vaginal candidiasis is a common gynaecological finding among women worldwide^{1,2} It has been found that up to 75% of the sexually active women have symptomatic vaginal candidiasis at least once³. Candidiasis is the most common vaginal infection in most countries affecting 50 to 72% of women and 40 to 50% are having recurrent episodes. Vaginal candidiasis if untreated can lead to chorioamnionitis and which can result in subsequent abortion and prematurity in pregnant women and pelvic inflammatory disease which results in infertility in non-pregnant women^{4,5}. *Candida* species mostly *Candida albicans*, can be isolated in the vaginal tracts of 20 to 30% of healthy asymptomatic non-pregnant women. If the balance between colonization and the host is temporarily disturbed, *Candida* can cause disease such as VVC, which is associated with clinical signs of inflammation⁶. The commonest organism implicated is *Candida albicans*¹, and the predisposing factors are hormonal fluctuations in pregnancy, luteal phase of menstrual cycle, use of oral contraceptives and hormonal replacement therapy among others^{7,8}

MATERIALS AND METHOD

This study was conducted in the Department of Microbiology, Prasad Medical College and Hospital, Lucknow, India during the period of 2018 to 2020. The study group consisted of 180 populations between the age group of 15 to 56 years. The collection of data include name, age, complaints like vaginal discharge, pain and itching, duration of illness whether acute or chronic, any past history of presenting episode, any systemic diseases such as diabetes/hypertension/tuberculosis. An informed written consent was obtained from all patients. Samples were collected upon inserting a sterile vaginal speculum into vagina; two high vaginal swabs, one after the other, were taken by a sterile cotton wool swab into the posterior vaginal fornix and rotated gently. The swabs were then inserted into its outer casing and were labelled with the patient's case number, name and date. One of the swabs was used for direct smear examination, and the second one was inoculated on Sabouraud dextrose agar (SDA) and incubated aerobically at 37°C. Examination of high vaginal swabs was done by the wet preparation method, i.e., 10% potassium hydroxide (KOH) preparation and Gram staining. Sterile normal saline drops were added to one of the tubes and shaken to extricate materials from the swab. Then, a wet film was prepared by putting a drop of the saline deposit onto a clean glass slide, which was then covered with a cover slip and examined under the microscope for budding yeast cells and pseudohyphae using 40× objective lens. Thereafter, a sterile inoculating loop was used to transfer small amount of the deposit onto a clean glass slide to form a smear. The smear was air-dried, and then, Gram staining was performed. The slide was then examined under the microscope using the oil

immersion objective lens for yeast cells. For phenotypic identification, The other swab was used to inoculate Sabourauds dextrose agar (SDA) and CHROM agar plates. The inoculated plates were incubated at 37 C and 35 C, respectively for 48 hours. Isolates on SDA plates were identified and speciated using conventional methods i.e. Germ tube test, Sugar assimilation test and morphology on Corn meal agar as per standard methods ^{9,10}. The isolates on CHROM agar were identified by colour of the colonies (*Candida albicans* produce green colony).

ANTIFUNGAL SUSCEPTIBILITY TESTING

A suspension was prepared by picking 5 to 6 colonies from the SDA culture plates of *Candida albicans*. Colonies were then inoculated in 5 mL of sterile saline, and its turbidity was adjusted to 0.5 McFarland standards visually. A sterile cotton wool swab was moistened in the adjusted inoculum suspension, and then, excess fluid was rinsed by rolling the swab on the inside surface of the tube above the fluid surface. Muller-Hinton agar (MHA) surface was streaked to make a lawn of the isolate. Antifungal susceptibility testing was undertaken by the disk diffusion method. fluconazole disk (10 µg), itraconazole (10 µg), voriconazole (10 µg), clotrimazole (10 µg) and nystatin (100 IU) antifungal discs were applied on MHA as recommended by the Clinical Laboratory Standard Institute (CLSI) and the plate were incubated in ambient air at 35°C and read at 24hrs and zone diameter were measured.

RESULTS

A total of 180 women in the age group of 15 to 56 years with clinically suspected vulvovaginal candidiasis referred from gynecology out-patients department and the results were analyzed as:

Table 1: age distribution the study subjects

Age Group (in years)	N	%
26-30	27	15
31-35	94	52.22
36-40	35	19.44
>40	24	13.33
Total	180	100
Mean±SD	34.88±4.64	

Table 1 demonstrate the age distribution amongst the subjects. There were 15% (n=27) subjects between 26-30 years of age. There were 52.22% (n=94) subjects between 31-35 years of age. There were 19.44% (n=35) subjects between 36-40 years of age. There were 13.33% (n=24) subjects more than 40 years of age. Majority of subjects were amongst middle age group. The mean age range amongst the subjects was 34.88±4.64 years.

Table 2: prevalence of candida albicans on the basis of germ tube test (N=180)

Germ tube test positive	%	Germ tube negative	%
83	46	97	54

Total 180 isolated candida species were subjected to germ tube test. It was in 83 specimens and they were considered as *candida albicans* and 97 specimens were negative for germ tube test and were considered as Non albicans candida.

Table 3: analysis of drug sensitivity pattern in *candida albicans*(n=83)

Drugs	Sensitive	Percentage	Resistant	percentage
Fluconazole	67	81%	16	19%
Itraconazole	52	63%	31	37
Nystatin	80	96%	3	4
Voriconazole	55	66%	28	34
Clotrimazole	57	69%	26	31
Amphotericin B	81	98%	2	2

Among the 83 cases of candida albicans, Fluconazole, Nystatin and Amphotericin B were highly sensitive. 81(98%) cases were sensitive to Amphotericin B, 80(96%) sensitive to Nystatin and 67(81%) was sensitive to fluconazole.

Discussion

Vulvo vaginitis Candidiasis (VVC) is an acute inflammatory disease and a frequent reason for gynecological consultation as it can affect up to 75% of women of child-bearing age.¹⁰⁴ Clinical signs and symptoms include intense pruritus, vaginal discharge, an erythematous vulva and dyspareunia. A number of predisposing factors such as oral contraceptive usage, pregnancy, uncontrolled diabetes mellitus and long-term broad spectrum antibiotic treatment have been identified as predisposing factors. Many others are suspected, including changes in the composition and function of the vaginal microbiota.¹¹ VVC is usually readily treated, provided that risk factors are removed or controlled, and VVC remains for many women an infrequent experience. RVVC is a much more serious clinical condition due to the recurrences of symptoms (four or more episodes per year) and for its refractoriness to successful treatment. Recent epidemiological investigations have suggested that the prevalence of RVVC may be higher than previously estimated and can be as high as 7–8% of women who experience a first episode.¹²

In a study by Louis Jacob et al¹³, the highest prevalence rates were found in the age groups of 18-25 years (7.1%), 26-30 years

(6.8%), and 31-35 years (6.9%). Overall, 22.8%, 15.8%, and 13.8% of VVC patients were aged 18-25, 26-30, and 31-35 years old, respectively. In our study, there were 15% (n=27) subjects between 26-30 years of age. There were 52.22% (n=94) subjects between 31-35 years of age. There were 19.44% (n=35) subjects between 36-40 years of age. Majority of subjects belonged between 36-40 years of age. A study published in 2013, which was based on survey data from women in five European countries and the United States, estimated that VVC was reported at least once by 29-49% of the population.¹²

Risk factors for VVC are the use of antibiotics, sexual activity, high-estrogen containing oral contraceptives, pregnancy, use of sodium glucose cotransporter 2 (SGLT2) inhibitors, and uncontrolled diabetes mellitus.^{13,14} Risk factors for RVVC are currently unknown, although genome-wide association studies have begun to unravel some genetic determinants of susceptibility. In contrast to invasive and oral candidiasis, R/VVC is a disease of immunocompetent and otherwise healthy women.¹⁵

Thus, the global disease burden is much higher for VVC than these other infectious routes. Using rough estimates of susceptible global populations and incidence rates for each of these disease states, invasive candidiasis causes ~700,000 cases per year, oral candidiasis results in ~15.5 million infections per year, and RVVC alone causes approximately 140M cases per year. The incidence rate for acute VVC is practically impossible to estimate, given that it is underreported to clinicians due to largely selective over-the-counter treatment options. While VVC is non-lethal, the sheer enormity of disease burden results in ~\$1.8B in medical costs each year and the economic impact due to lost work hours was recently extrapolated to approach an additional \$1B per annum in the US alone.¹⁶

C. albicans, which most commonly causes VVC, is part of normal vaginal microflora.¹⁷ The second most common pathogen identified in women with VVC is *C. glabrata*, which is isolated in 7 to 16% of cases.¹⁸ *C. albicans* is polymorphic, adopting two major morphological forms: the ovoid yeast and elongated hypha.¹⁹ It has long been observed that the capacity to transition between these morphologies is the primary virulence attribute of *C. albicans*, as strains unable to undergo this switch are severely attenuated in pathogenicity or colonization.²⁰

A comparison of vaginal nystatin and oral fluconazole for treating RVVC was performed in a recent study on 293 patients by a Chinese research group.¹³² Standard oral fluconazole regimens for treating RVVC were compared with 2 weeks of vaginal nystatin every month. The results showed that both oral fluconazole and vaginal nystatin are effective in treating RVVC and that in cases of fluconazole-resistant *C. albicans* or *C. glabrata* RVVC nystatin can also be efficient.²¹

In our study, out of 83 cases of *Candida albicans* 81% that were sensitive to fluconazole and 19% cases were resistance to it. There were 66% cases that were sensitive to Voriconazole and 34% cases were resistance to it. There were 96% cases that were sensitive to Nystatin and 4% cases were resistance to it. There were 63% cases that were sensitive to Itraconazole and 37% cases were resistance to it. There were 69% cases that were sensitive to Clotrimazole and 31% cases were resistance to it and 98% were sensitive to Amphotericin B & only 2% were resistant to it. Therapy with azoles is less effective in treating non-*C. albicans* VVC. All preparations used for treating non-*C. albicans* VVC have to be made in the pharmacy²².

CONCLUSION

C. albicans, known to be the most virulent species among *Candida*, showed highest percentage adherence as well as highest number of fungal cell attachment. The diversity in expression of various extracellular enzymes leads to exaggerated synergistic effect showing better adaptability of the fungi to its new found environment. *Candida* species are capable of producing virulence factors such as proteinase, esterase and hemolysin enzymes and biofilm. The present study demonstrated the importance of species identification and susceptibility testing for antifungals in pregnant women attending antenatal units. The predominant cause of vulvovaginal candidiasis in this study was *C. albicans*. An escalating number of *Candida* spp. From clinical isolates were resistant to antifungal agents that are routinely used for the treatment of VVC due to the fact that majority of the women had previous history of antifungal use. A significant number of non-*albicans Candida* were recognized, which demonstrated decreased susceptibility to all drugs, particularly the azoles, which are generally used for the management of vaginal candidiasis. Isolation of non-*albicans* yeasts may have clinical implication due to their reduced susceptibility to various antifungals. Antifungal susceptibility testing may possibly be used to calculate clinical response, to forecast malfunction in management, and accordingly, local antibiograms can aid in empirical assortment of antifungals, guiding options for long-term therapy, and are meant for alternative regimens in testing of isolates from recurrent infections

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