RESEARCH

PHENOTYPIC CHARACTERIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN TO FLUCONAZOLE IN CANDIDA SPECIES ISOLATED FROM VULVOVAGINAL CANDIDIASIS IN A TERTIARY CARE HOSPITAL

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Received : 02/04/2022
Received in revised form : 16/07/2022
Accepted : 25/07/2022

Keywords:
Candida,
CHROMagar,
Antifungal Susceptibility Testing

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DOI: 10.47009/jamp.2022.4.3.39

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm,
2022; 4 (3); 175-179

Abstract

Background: Vulvovaginal candidiasis is a very common gynaecological fining in women worldwide. The aim of this study was carried out to determine the prevalence of candida species and their various antifungal susceptibility pattern in patients attending in Prasad Medical College and Hospital, 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 August 2018 to August 2020. The study group consisted of 180 populations between the age group of 15 to 56 years. The samples were collected from patient with vulvovaginal infections and also the consent of the patients was obtained. 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 Sabourauds dextrose agar (SDA) & 5% sheep blood agar and susceptibility testing to fluconazole was performed. **Result:** Among the 180 culture positive cases. There were 46.1% cases of C. albicans and 38.3% cases of C. tropicalis. The least number of cases i.e. 1.1% were of C. krusei and C. kefyr each respectively. There were 11.1% cases of C. parapsilosis. Only 2.2% cases had C. guillermondii and antifungal susceptibility test carried out on the all 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.

INTRODUCTION

Candida albicans is the most prevalent among Candida spp., which causes both superficial and systemic infections. Other pathogenic Candida species include C. tropicalis, C. glabrata, C. parapsilosis, and C. krusei accounting for 25%, 8%, 7%, and 4% of candidiasis, respectively.[1] Pathogenesis of candidiasis depends on the expression of virulence factors like germ tube formation, adhesions, phenotypic switching, biofilm formation, and the production of hydrolytic enzymes.[2]

Vulvo vaginal candidiasis (VVC) is clinically defined as inflammation of the vulva and vagina due to the presence of Candida and in the absence of other infectious etiology. It is due to the pH alteration of vagina in candida infection. Vaginal candidiasis is a common gynecological finding among women worldwide.[3] It has been found that up to 75% of the sexually active women have symptomatic vaginal candidiasis at least once.[4]

Candidiasis is the most common vaginal infection in most countries affecting 50 to 72% of women and 40 to 50% are having recurrent episodes. Nowadays candida constitutes the 3rd or 4th most common cause of blood stream infections.[5,6] 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.[7] 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 inflammation.[8] The commonest organism implicated is Candida albicans, and the predisposing factors are hormonal fluctuations in pregnancy, luteal phase of menstrual cycle, use of oral contraceptives and hormonal replacement therapy among others.[9]

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology), Prasad Medical College and Hospital, Lucknow, India during the period of August 2018 to August 2020. The study group consisted of 180 populations between the age group of 15 to 56 years. The samples were collected from patient with vulvovaginal infections and also the consent of the patients was obtained.

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 [1]. 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 35^0C and read at 24hrs and zone diameter were measured.

RESULTS & DISCUSSION

A total of 180 women in the age group of 15 to 56 years with clinically suspected vulvovaginal candidiasis referred from gynaecology out-patients department and the results were analysed 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: species prevalence based on 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: distribution of various candida Species

Types	N	%
C. albicans	83	46.1
C. tropicalis	69	38.3
C. parapsilosis	20	11.1
C. guillermondii	4	2.2
C. kefyr	2	1.1
C. krusei	2	1.1

Among the 180 culture positive cases There were 46.1% cases of C. albicans and 38.3% cases of C. tropicalis. The least number of cases i.e. 1.1% were of C. krusei and C. kefyr each respectively. There

ISSN (O): 2687-5365; ISSN (P): 2753-6556

were 11.1% cases of C. parapsilosis. Only 2.2% cases had C. guillermondii.

Table 4: Sensitivity and resistance of various antifungal drugs

Drugs	Sensitivity		Resistance	
	N	%	N	%
Fluconazole	73	40.6	107	59.4
Voriconazole	113	62.8	67	37.2
Nystatin	116	64.4	64	35.6
Itraconazole	86	47.8	94	52.2
Clotrimazole	81	45	99	55
Amphotericin B	180	100	0	0

There were 40.6% cases that were sensitive to fluconazole and 59.4% cases were resistance to it. There were 62.8% cases that were sensitive to Voriconazole and 37.2% cases were resistance to it. There were 64.4% cases that were sensitive to Nystatin and 35.6% cases were resistance to it. There were 47.8% cases that were sensitive to Itraconazole and 52.2% cases were resistance to it. There were 45% cases that were sensitive to Clotrimazole and 55% cases were resistance to it. All the cases were sensitive to Amphotericin B.

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.

Table 5: 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

Table 6: antifungal sensitivity pattern of various Candida species

Antifungal agent	No.	Fluconazole		Voriconazole		Nystatin		Itraconazole		Clotrimazole		Amphotericin B	
		R	S	R	S	R	S	R	S	R	S	R	S
C. albicans	83	2	81	28	55	3	80	31	52	62	57	0	83
c. tropicalis	69	14	55	3	66	0	69	5	64	11	58	0	69
C. paratropicalis	20	7	13	2	18	2	18	4	16	7	13	0	20
C. guillermondii	4	3	1	0	4	0	4	3	1	3	1	0	4
C. kefyr	2	0	2	1	1	0	2	1	1	2	0	0	2
C. krussei	2	1	1	1	1	1	1	1	1	1	1	0	2

The sensitivity pattern amongst different variants of Candida. Candida tropicalis showed a significant difference in the sensitivity pattern amongst the antiviral agents. Amongst the 83 cases of Candida albicans, there was a significant difference in the sensitivity patterns amongst the antiviral agents. C. krusei showed statistically insignificant difference in the sensitivity patterns amongst different antiviral agents. Amongst the 20 cases of C. parapsilosis there was a significant difference in the sensitivity patterns amongst the antiviral agents. There were only 2 cases of C. kefyr and there were was insignificant difference amongst the sensitivity.

DISCUSSION

Vulvo vaginitis Candidiasis (VVC) is an acute inflammatory disease and a frequent reason for gynaecological consultation as it can affect up to 75% of women of child-bearing age.[10] 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.[10] 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.[11]

In a study by Louis Jacob et al,[12] 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.[11] 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.[12] Risk factors for RVVC are currently unknown, although genome-wide

ISSN (O): 2687-5365; ISSN (P): 2753-6556

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.[13] 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.[14,15,16]

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.[18,19] 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.[15]

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.[13] 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.[16]

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 pharmacy.[17]

In a study by Sujit D. Rathod et al substantial proportions of the women reported vaginal itching (29%) or vaginal discharge (31%) or had vaginal erythema (9%) or vaginal discharge (35%) on examination. The positive predictive values of these signs and symptoms for predicting vulvovaginal candidiasis were low. In our study, approximately 58.3% of the females had vaginal discharge. Pain and itching was seen amongst 79.4% subjects. There were 79.4% females with acute disease and 20.6% females with chronic disease.[18]

Various local agents with similar effects are available, including clotrimazole, butoconazole, and miconazole. Agents that are used in short-term regimens contain higher doses of antifungal medicine, allowing higher concentrations for longer-lasting inhibitory effect. Topical azoles are more efficient than local nystatin in treating uncomplicated VVC.[19]

Recently, Mendling et al. performed a comparative study on 160 patients with VVC in which they compared treatment with clotrimazole vaginal suppositories alone and a combination of 2% clotrimazole cream for external use and clotrimazole vaginal suppositories. They concluded that the combination of both was better than the suppositories alone.[20]

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In our study, there were 40.6% cases that were sensitive to fluconazole and 59.4% cases were resistance to it. There were 62.8% cases that were sensitive to Voriconazole and 37.2% cases were resistance to it. There were 64.4% cases that were sensitive to Nystatin and 35.6% cases were resistance to it. There were 47.8% cases that were sensitive to Itraconazole and 52.2% cases were resistance to it. There were 45% cases that were sensitive to Clotrimazole and 55% cases were resistance to it. All the cases were sensitive to Amphotericin B. 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 pharmacy.[22]

Phillips studied the effectiveness of vaginal amphotericin B in women with non–C. albicans VVC that did not respond to the usual antimycotics. A 2-week regimen with 50 mg amphotericin B intravaginally was effective in 70% of cases. In a retrospective review, Sobel et al. evaluated the efficiency of topical treatment of C. glabrata VVC with flucytosine and boric acid .[134] Topical boric acid was used in a dose of 600 mg and was administered intravaginally for 14 to 21 days. In the two groups of patients with C. glabrata VVC, boric acid was effective in 64 to 71% of patients. When the patients did not respond to boric acid, flucytosine was used and was effective in 90%.[23]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

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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ISSN (O): 2687-5365; ISSN (P): 2753-6556