Original Article

Antifungal profile of vulvovaginal candidiasis in sexually active females from a tertiary care hospital of Western Rajasthan

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Abstract

Introduction: Vulvovaginal candidiasis (VVC) is the commonest form of sexually transmitted infection especially in sexually active females. Various species of *Candida* i.e., *Candida albicans* and *non-albicans Candida* are associated with VVC. More than 75% of women experiences vulvovaginal candidiasis at least once in their lifetime and 10% of it can lead to recurrent VVC. So, this study was planned to evaluate the clinico-mycological profile and antifungal profile of VVC in sexually active female attending tertiary care hospital. **Materials and Methods:** The present two months study was conducted in sexually active females attending Obstetrics –gynecology OPD with VVC in tertiary care hospital. Two high vaginal swabs were taken and fungal culture was done on SDA agar by standard methods. Identification and antifungal susceptibility testing of candidial isolates were done by standard mycological methods. **Results:** Most of the patients belonged to younger age group between 18 and 29 years (55%). Lower abdominal pain was the most common symptom after vaginal discharge followed by burning sensation and pruritis. *Candida glabrata* (15) with 58% of all the isolates was the most common *Candida* species associated with VVC in this study, followed by *Candida albicans* (5, 19%). Highest antifungal resistance was observed to itraconazole (81%) followed by amphotericin B (35%) and fluconazole (31%). 81% resistance to itraconazole among Candida glabrata and Candida albicans. Voriconazole was maximum susceptible to all Candida species. **Conclusion:** This study highlights the incidence of VVC among sexually active females of reproductive age group as its recurrence may result into obstetric complications and even infertility and also enlightens the common *Candida* species and their antifungal profile, which would help the treating clinicians to formulate local antifungal treatment policy for VVC.

Keywords: Antifungal profile, *Candida* species, conventional method, vulvovaginal candidiasis

Introduction

Candidiasis is one of the most common fungal infections in humans. It's an opportunistic pathogen which are mostly considered commensal flora but when it knobs as a pathogen can cause serious manifestation. Various species of *Candida* i.e., *Candida albicans* and *non-albicans Candida* are associated with spectrum of diseases from cutaneous to disseminated candidiasis.

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Vulvovaginal candidiasis (VVC) is among the commonest form of candidiasis especially in sexually active females. VVC is a sexually transmitted infection of vaginal mucosa caused by various species of genus *Candida, Candida albicans* being the commonest of all.[1] It is typically described as "white cottage cheese" discharge per vagina with associated vulval and vaginal inflammation.[2] It is the second most common cause of vaginitis after bacterial vaginosis.[3] *Candida* species are considered part of normal microbiome of vagina in most of the women apart from other bacterial species. Every year approximately 5-10 million females worldwide seeks advice for vaginitis. Several studies have reported that nearly 75% of women experiences vulvovaginal candidiasis at least once in their lifetime, 50% of them have second episodes and around 5-10% of them complains of recurrent VVC that is defined as more than 4 episodes in a year.[4,5]

Alteration of the normal vaginal microbiome may lead to *Candida* overgrowth resulting into active *Candida* infection.[6] Often the reasons for its occurrence and recurrence is still unclear, use of antibiotics and oral contraceptive pills, diabetes mellitus, dietary practices, specific immune defect, sexual activities, personal hygiene and socio-demographic characteristics have been implicated as possible risk factors.[4,7] Inflammation of vaginal mucosa apart from white curdy discharge, presents with itching, burning sensation and lower abdominal pain as other non-specific symptoms, which becomes the reason for seeking Obstetrics and Gynecological advice worldwide.[8] Although non-specific symptoms of VVC are frequently ignored, they are often associated with significant morbidity and if left untreated, can result into various obstetric complications especially among pregnant women.[9]

Though, *Candida albicans* is the most common cause of VVC accounting for 85-90% of the isolates, other *Candida* species such as *C. tropicalis, C. glabrata* and *C. krusei* are not so uncommon particularly among HIV-infected women.[10,11] Some studies from India, reports *C. tropicalis* as second most frequently isolated *Candida* species after *C. albicans*.[9] Resistance to antifungal agents have been increasing and is likely to increase further in near future. Resistance to azoles, in *C. albicans* and *non-albicans Candida* species has become more common. This is likely because of prolonged treatment and widespread over the counter availability.[12]

Adequate knowledge of potential risk factors, mycological profile and antifungal susceptibility pattern of *Candida* species could help the treating clinician in devising the most effective empirical therapy. Since, there is dearth of such vital information from this part of the country, this study was planned in an attempt to bridge that gap.

Materials and Methods

This is a two months duration (July-August 2018) prospective study conducted in the Department of Microbiology and Department of Obstetrics and gynecology of a tertiary care hospital of western Rajasthan among the sexually active women of reproductive age group (18-49 years) including pregnant women attending the outpatient department of Obstetrics and gynecology during the study period. However, menstruating women, women who had received any antifungal therapy in last one month and patients who had delivered or aborted in last six weeks were excluded from the study.

Proper ethical clearance prior to the commencement of study was taken from the Institutional Ethic Committee (June'2018).

All the statistical analysis was done using Microsoft Excel spread sheet (Microsoft Corporation, US) and results were demonstrated in tabular and graphical form wherever required.

A total of 60 patients were enrolled in this study after taking a proper written consent. Vital information regarding patient's socio-economic status, personal hygiene, sexual behavior, chief presenting complaints and medical and surgical history were collected. General physical examination including per speculum and bimanual per-vaginal examination were performed. All the relevant information of clinical history and physical examination were recorded on a Case Record Form.

Discharge from the posterior vaginal fornix (high vaginal swab) was collected on a pair of sterile swabs per patient and was sent to the Department of Microbiology without delay. Direct microscopy using Potassium hydroxide (KOH) wet mount preparation and Gram's staining was done from one swab to screening for presence of budding yeast cells [Figure 1], while second swab was used to put fungal culture on Sabouraud's Dextrose Agar (SDA) tube supplemented with 50 mg of Chloramphenicol to inhibit bacterial growth and was incubated at 37°C for 48 hours. *Candida* identification was done using conventional methods of identification on the basis of colony morphology (smooth, white, creamy and pasty colony), colour produced on Candida Differential Chromagar (CHROMagar, Hi Media Pvt Ltd., Mumbai) [Table 1 and Figure 2], Gram's Staining, germ tube formation test and morphology on corn meal agar (Dalmau's method) [Figure 3].

Antifungal susceptibility testing was performed by using E-strip method for fluconazole (0.016-256 mcg/ml),

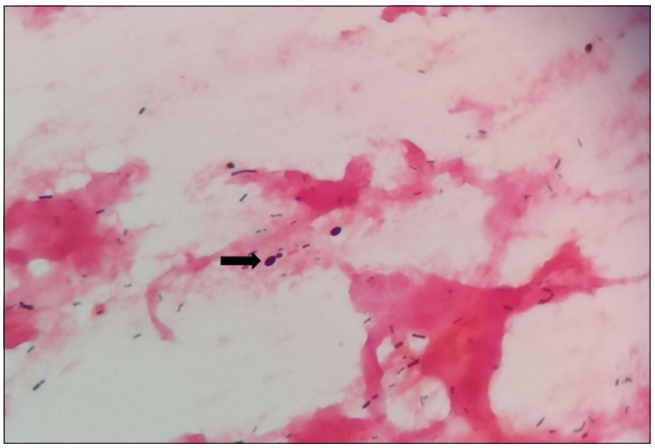

Figure 1: Gram's Staining of the high vaginal swab showing Gram positive budding yeast cells (Arrow head)

voriconazole (0.002-32 mcg/ml), itraconazole (0.002-32 mcg/ml) and amphotericin B (0.002-32 mcg/ml) (HiMedia Laboratory Pvt. Ltd., Mumbai) on Muller Hinton's Agar (MHA) (HiMedia Laboratory Pvt. Ltd., Mumbai) supplemented with 2% glucose and Methylene blue dye 0.5 µg/ml. Minimum Inhibitory Concentration (MIC) of fluconazole, itraconazole and voriconazole were determined as per Clinical and Laboratory Standard Institute guidelines (CLSI M27-A3) while MIC for amphotericin B was calculated as proposed in literature [Table 2].[13,14]

Results

A total of 60 patients were enrolled during the study period of 2 months duration. Most of the patients belonged to younger age group between 18 and 29 years (55%) followed by 30 and 39 years (18%) and 40 and 49 years (9%) [Figure 4]. Every patient had vaginal discharge as one of the common presenting complaint. Other associated symptoms were lower abdominal pain, burning sensation, pruritis, dysuria and dyspareunia [Figure 5]. Lower abdominal pain was the most common symptom after vaginal discharge followed by burning sensation and pruritis while dysuria and dyspareunia were less commonly associated symptoms. Among patients with complaints of lower abdominal pain, 50% were laboratory confirmed to be case of VVC. Fifteen patients had triad of vaginal discharge, pruritis and burning sensation, of which 11 (73%) were confirmed to be a case of VVC.

Approximately 43% (26) of all the total sample (60) collected were positive for one or the other species of *Candida*. On the basis of conventional methods of species identification *Candida glabrata* (15) with 58% of all the isolates was the most common *Candida* species associated with VVC in this study, followed by *Candida albicans* (5, 19%), *Candida tropicalis* (3, 11%), *Candida kefyr* (2, 8%) and *Candida krusei* (1, 4%). Majority of the *Candida* species isolated were of *non-albicans Candida* [Figure 6].

Antifungal susceptibility was performed to determine MIC using E-strips. Overall, the highest resistance was observed

Table 1: Colour produced on Chromagar by different *Candida* species.

Candida Species	Colony Colour
Candida albicans	Light green
Candida dubliniensis	Pale green
Candida parapsilosis	White to cream
Candida tropicalis	Blue with pink halo
Candida krusei	Purple, fuzzy
Candida glabrata	Cream to white
Candida kefyr	Cream to white with slight purple centre

Table 2: Interpretive breakpoints of MIC adopted in the present study

Antifungal agent	Susceptible	Susceptible-dose dependent	Resistant
Fluconazole	≤8 µg/ml	16 µg/ml≤ MIC ≤32 µg/ml	≥64 µg/ml
Voriconazole	≤0.125 µg/ml	-	≥0.5 µg/ml
Itraconazole	≤0.125 µg/ml	0.25 µg/ml≤ MIC ≤0.5 µg/ml	≥1 µg/ml
Amphotericin B	≤1 µg/ml	NA	>1 µg/ml

MIC: Minimum Inhibitory Concentration; NA: Not Applicable

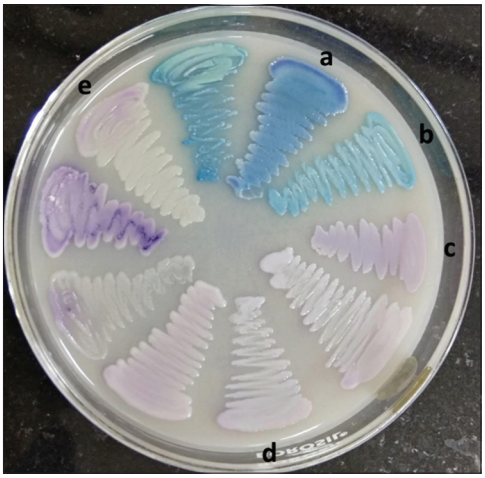

Figure 2: Colour produced on Candida CHROMagar. **a.** *C. tropicalis;* **b.** *C. albicans,* **c.** *C. krusei,* **d.** *C. glabrata,* **e.** *C. kefyr*

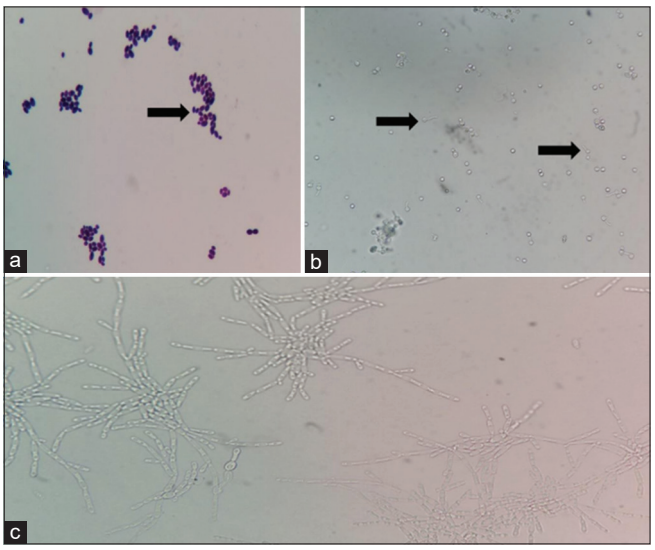

Figure 3: a. Gram's Stain of isolate showing budding yeast cells (Arrow head); **b.** Germ tube formation test (Arrow head); **c.** Morphology on Corm Meal agar (Dalmau's method)

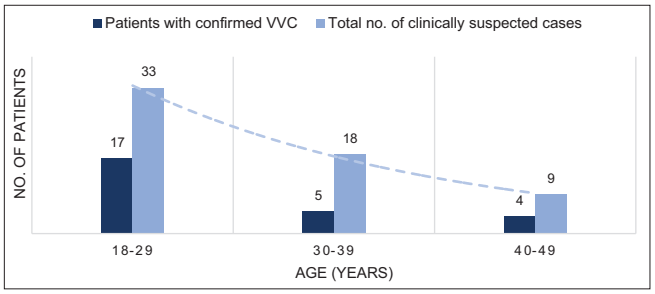

Figure 4: Age wise distribution of cases

against itraconazole (81%) followed by amphotericin B (35%) and fluconazole (31%). Approximately one fourth (23%) of the isolates showed resistance against voriconazole. *C. glabrata* was the most commonly isolated species, and 80% of them were resistant to itraconazole, 53% were resistant to amphotericin B. In the present study itraconazole resistance was maximum among all antifungal drugs to all Candida species as shown in Table 3.

Discussion

Candidiasis is one of the most common fungal manifestation in humans. *Candida* species though considered to be the normal flora of vagina, but its overgrowth can lead to a condition called Vulvovaginal candidiasis (VVC) especially among adult females of reproductive age group. The incidence of VVC mostly associated *non-albicans Candida* is higher among 17- and 18-year-old group than younger group.[15] Majority of the patients included in this study were of age group 18–29 years, consistent with other studies by Samal R *et al.*, Masand DL *et al.*, and Dharmik PG *et al.*[1,8,16] Various factor plays an important role in the development of VVC. Since the majority of the patients are from sexually active group, sexual activity is likely to be associated with increased risk. During pregnancy, high level of estrogen and progesterone are associated with the alteration of normal vaginal microbiome resulting into overgrowth of *Candida* species leading to active symptomatic infection. In this study, no correlation was found between parity and occurrence of VVC.

In this study, the rate of VVC was observed to be 43%, similar to the study done by Mukasa KJ *et al.* in 2015 where the rate was about 45% and in study from Ethiopia published in 2018 with rate of 41%.[17,18] Few studies from India reported VVC rate of 48% and 50%.[1,19] However, there are few studies reporting quite lower rate of VVC ranging from 14 to 30%.[4,8,19,20]

In the present study, more than 80% of the isolates were of *non-albicans Candida* species with *C. glabrata* accounting for 58% of the isolates alone followed by *C. albicans* (19%) and *C. tropicalis* (12%). Mohanty *et al.* also observed the similar result in their study with more than 50% of *C. glabrata* followed by *C. albicans* and *C. tropicalis*.[21] In contrast to our results, various studies have reported *C. albicans* as commonest species to be associated with VVC while overall incidences of *non-albicans Candida* species together were higher than *C. albicans,* suggesting that although *C. albicans* continues to be the most common associated *Candida* species but *non-albicans Candida* species incidences are on rising trend. The higher incidences of *non-albicans Candida* species may be attributed to the excessive use of antifungal agents which inhibits the growth of *C. albicans* but fails to suppress the growth of few *non-albicans Candida* species.

The *in-vitro* antifungal susceptibility testing revealed higher resistance to commonly used antifungal agents including itraconazole. Approximately 81% of the isolates including 57% of *C. glabrata* alone were resistant to itraconazole, while high resistance to fluconazole and amphotericin B were also observed. All *C. albicans* were sensitive to amphotericin B in our study, correlating with the study done by Babin D. *et al.*[19] Relatively lesser resistance was seen among *Candida* species to voriconazole (23%), while all the *C. albicans* isolates were sensitive to it, suggesting voriconazole to be the most effective antifungal agent in our setting while a study among Saudi women showed terbinafine as the most effective antifungal agent.[22] *C. krusei* is intrinsically resistant to certain azoles like fluconazole, and in our study too, the only isolate was resistant to various azoles. The higher incidence of *non-albicans Candida* species in our

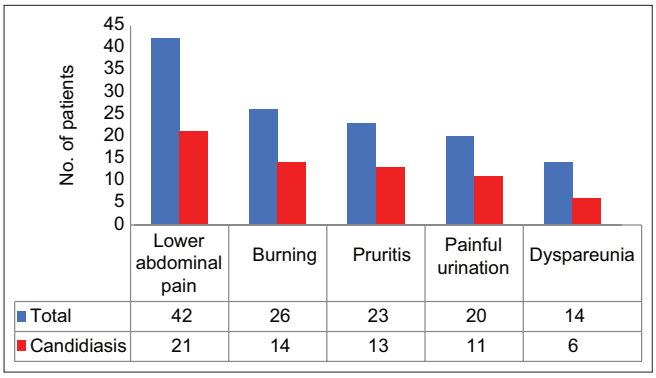

Figure 5: Symptoms wise distribution of cases

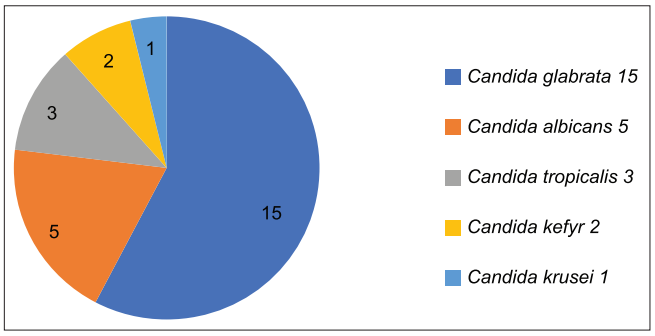

Figure 6: Distribution of various *Candida* species

Table 3: Antifungal resistance of Candidial isolates (*n*=26)

Drugs	*Candida albicans*	Non-albicans *Candida* species				Total
	C. albicans n=5 (%)	*C. glabrata n*=15 (%)	*C. tropicalis n*=3 (%)	*C. kefyr n*=2 (%)	*C. krusei n*=1 (%)	*n*=26 (%)
Amphotericin B	0	8 (53.33)	0	0	1 (100)	9 (34.62)
Itraconazole	4 (80)	12 (80)	2 (66.67)	2 (100)	1 (100)	21 (80.77)
Fluconazole	1 (20)	4 (26.67)	0	2 (100)	1 (100)*	8 (30.77)
Voriconazole	0	5 (33.33)	0	1 (50)	0	6 (23.08)

**Candida krusei* is intrinsically resistant to Fluconazole. (CLSI)

study might be the reason behind relatively higher resistance as *non-albicans Candida* species are prone to show higher resistance against antifungal agents. Another reason could be the irrational use due to over-the-counter availability of these antifungal agents. Empirical prescription of various topical and oral azoles entirely based on clinical suspicion could be another reason. These factors are possible reasons for the excessive exposure of fungi to these agents resulting into emergence of resistant strains.

Conclusion

Vulvovaginal Candidiasis is a common condition among sexually active young adult females. *Candida glabrata* is the commonest species to be associated with the vulvovaginal candidiasis in our study. E-strip method for determination of MIC against commonly used antifungal agents is an alternative and simple method. Highest resistance to itraconazole, while least with voriconazole was noted in almost all the species of *Candida*. The main purpose of this study is to highlight the incidence of VVC among sexually active females of reproductive age group as its recurrence may result into obstetric complications and even infertility. Moreover, this study also enlightens the common *Candida* species associated with VVC and their antifungal profile of commonly used antifungal agents, thus would help the treating clinicians to formulate and select the most effective local antifungal treatment policy for VVC.

The major limitations of this study are firstly, the small sample size, and thus the result cannot reflect the actual burden of the disease in the community. Secondly, *in-vivo* testing for various antifungal agents were not done to rule out any possible patient to patient variation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Samal R, Vaithy A, Kotasthane DS, Ghose S. Prevalence and clinico-mycological profile of vulvovaginal candidiasis in a tertiary care hospital. Int J Reprod Contracept Obstet Gynecol 2015;4:1142-7.
2. Sheary B, Dayan L. Recurrent vulvovaginal candidiasis. Aust Fam Physician 2005;34:147-50.
3. Nyirjesy P, Sobel JD. Vulvovaginal candidiasis. Obstet Gynecol Clin North Am 2003;30:671-84.
4. Ahmad A, Khan AU. Prevalence of Candida species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. Eur J Obstet Gynecol Reprod Biol 2009;144:68-71.
5. Amouri I, Sellami H, Borji N, Abbes S, Sellami A, Cheikhrouhou F, *et al.* Epidemiological survey of vulvovaginal candidosis in Sfax, Tunisia. Mycoses 2011;54:e499-505.
6. Peters BM, Yano J, Noverr MC, Fidel PL Jr. Candida vaginitis: When opportunism knocks, the host responds. PLoS Pathog 2014;10:e1003965.
7. Olowe OA, Makanjuola OB, Olowe R, Adekanle DA. Prevalence of vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis among pregnant women receiving antenatal care in Southwestern Nigeria. Eur J Microbiol Immunol 2014;4:193-7.
8. Masand DL, Patel J, Gupta S. Utility of microbiological profile of symptomatic vaginal discharge in rural women of reproductive age group. J Clin Diagn Res 2015;9:QC04-7.
9. Vijaya D, Dhana lakshmi TA, Kulkarni S. Changing trends of vulvovaginal candidiasis. J Lab Physicians 2014;6:28-30.
10. Ying C, Zhang H, Tang Z, Chen H, Gao J, Yue C. Antifungal susceptibility and molecular typing of 115 Candida albicans isolates obtained from vulvovaginal candidiasis patients in 3 Shanghai maternity hospitals. Med Mycol 20161;54:394-9.
11. Sobel JD, Ohmit SE, Schuman P, Klein RS, Mayer K, Duerr A, *et al.* The evolution of Candida spp. and fluconazole susceptibility among oral and vaginal isolates recovered from human immunodeficiency virus (HIV)-seropositive and at-risk HIV-seronegative women. J Infect Dis 2001;183:286-93.
12. Marchaim D, Lemanek L, Bheem Reddy S, Kaye KS, Sobel JD. Fluconazole resistant Candida albicans vulvovaginitis. Obstet Gynecol 2012;120:1407-14.
13. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, M27-A3. Vol. 28. No. 14. 3rd ed.. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
14. Negri M, Henriques M, Svidzinski TI, Paula CR, Oliveira R. Correlation between Etest, disk diffusion, and microdilution methods for antifungal susceptibility testing of Candida species from infection and colonization. J Clin Lab Anal 2009;23:324-30.
15. Xu L, Hu Z, Yu F, Tang Y. Analysis of characteristics of vulvo-vaginal infections in 14- to 18-year-old girls in late puberty. J Int Med Res 2020;48:300060520946506.
16. Dharmik PG, Gomashe AV, Upadhyay VG. Susceptibility pattern of various azoles against Candida species causing vulvovaginal candidiasis. J Obstet Gynecol India 2013;63:135-7.
17. Mukasa KJ, Herbert I, Daniel A, Sserunkuma KL, Joel B, Frederick B. Antifungal susceptibility patterns of vulvovaginal Candida species among women attending antenatal clinic at Mbarara regional referral hospital, South Western Uganda. Br Microbiol Res J 2015;5:322-31.
18. Bitew A, Abebaw Y. Vulvovaginal candidiasis: Species distribution of Candida and their antifungal susceptibility pattern. BMC Womens Health 2018;18:94.
19. Babin D, Kotigadde S, Rao PS, Rao TV. Clinico-mycological profile of vaginal candidiasis in a tertiary care hospital in Kerala. Int J Res Biol Sci 2013;3:55-9.
20. Khan M, Ahmed J, Gul A, Ikram A, Lalani FK. Antifungal susceptibility testing of vulvovaginal Candida species among women attending antenatal clinic in tertiary care hospitals of Peshawar. Infect Drug Resist 2018;11:447-56.
21. Mohanty S, Xess I, Hasan F, Kapil A, Mittal S, Tolosa JE. Prevalence & susceptibility to fluconazole of Candida species causing vulvovaginitis. Indian J Med Res 2007;126:216-9.
22. Yassin MT, Mostafa AA, Al-Askar AA, Bdeer R. *In vitro* antifungal resistance profile of Candida strains isolated from Saudi women suffering from vulvovaginitis. Eur J Med Res 2020;25:1.