Infection and Drug Resistance

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ORIGINAL RESEARCH

Antifungal susceptibility testing of vulvovaginal *Candida* species among women attending antenatal clinic in tertiary care hospitals of Peshawar

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Background: Vulvovaginal candidiasis (VVC) is considered as a pervasive gynecological problem among women worldwide. Owing to this fact, in the current study, we aimed at assessing the prevalence rate of *Candida* spp. causing VVC in symptomatic pregnant women and their antifungal susceptibility pattern.

Methods: This study was carried out in the tertiary care hospitals of Peshawar during the period of July 1, 2016 to December 31, 2016. The study group included 450 pregnant women in the age group of 17–44 years with symptoms of excessive vaginal discharge, pain and pruritis. In all, 108 pregnant women were culture positive for *Candida*. Antimicrobial susceptibility testing (AST) was conducted on specimens against various azoles and polyene F group of antifungals.

Results: Out of 108 *Candida* spp. isolated from vaginal swabs, there were 45 (41.7%) *Candida albicans*, 18 (16.7%) *Candida tropicalis*, 18 (16.7%) *Candida krusei*, 16 (14.8%) *Candida glabrata* and 11 (10.2%) *Candida dubliniensis*. According to age distribution, 27 years was the mean age. Pregnancy trimester distribution among patients was as follows: 21 (19.4%) patients were in their first trimester, 65 (60.2%) patients were in their second trimester and 22 (20.4%) patients were in the third trimester. Susceptibility of fluconazole was determined as follows: 33.3% of the *Candida* isolates were sensitive, 4.6% were susceptible dose dependent (SDD) and 62% were resistant. Susceptibility of *Candida* spp. with respect to nystatin in patients with VVC was as follows: 25% were sensitive, 16.7% were SDD and 58.3% were resistant. Susceptibility of clotrimazole was analyzed, and it was sensitive in 21.3% of patients, SDD in 19.4% of patients and resistant in 59.3% of patients. Voriconazole susceptibility was recorded to be sensitive in 85.2% of patients, SDD in 4.6% of patients and resistant in 10.2% of patients suffering from VVC. Susceptibility results for itraconazole in patients with VVC were as follows: 42.6% of patients were sensitive, 16.7% of patients were SDD, and 40.7% of patients were resistant.

Conclusion: In this study, frequency of VVC was noted to be high in the second trimester of pregnancy, with the highest frequency of *C. albicans* isolated, followed by *C. tropicalis* and *C. krusei*. Antifungal susceptibility testing revealed that fluconazole was exceedingly resistant against *Candida* species (62%), followed by clotrimazole (59.3%) and nystatin (58.3%). On the contrary, voriconazole had the highest antimicrobial activity against *Candida* species (85.2%).

Keywords: vulvovaginal candidiasis, fluconazole, voriconazole, itraconazole, ketoconazole, nystatin

Plain language summary

Problem: Resistance to antifungal agents has represented a major challenge for the clinic and a major public health problem, especially if the patient has been treated previously with antifungal agents.

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What is already known? Vulvovaginal candidiasis (VVC) is the most frequent gynecological ruling among women of childbearing age. Among sexually active women, 75% have at least once experienced symptomatic VVC, which usually presents as soreness, burning, itching and abnormal curd-like vaginal discharge.

What this paper adds? With multiple antifungals and varying susceptibility patterns of *Candida*, the study was conducted to determine the frequency of common *Candida* species among women with VVC and also the antifungal susceptibility pattern of identified *Candida* species causing VVC and make information accessible to the clinicians for effectual therapeutic outcome.

Introduction

Globally amid the fungal infections in human beings, candidal infections are predominantly reported. More than 200 species of *Candida* have been reported.[1] In healthy human beings, *Candida* is considered as a commensal, and its scope to produce either superficial or systemic infections depends on the host immune system and various risk factors.[2] Vulvovaginal candidiasis (VVC) is the most frequent gynecological ruling among women of childbearing age.[3] Among sexually active women, 75% have at least once experienced symptomatic VVC, which usually presents as soreness, burning, itching and abnormal curd-like vaginal discharge.[4,5] Pregnancy was the commonest factor (55%) followed by usage of broad-spectrum antibiotics (8%). Other risk factors were use of oral contraceptive pills, diabetes mellitus and tuberculosis.[6] In pregnant women, VVC complications include abortion, chorioamnionitis and subsequent preterm delivery.[7] Transmission of *Candida* can take place from the infected mother's vagina to the newborn, leading to *Candida* congenital infection.[8] It is perceived that *Candida albicans* presented for VVC cases of 70–90%, with a recent surfacing of non-*albicans* species.[9]

The improvement of standardized antimicrobial susceptibility testing (AST) has been the area under discussion of several studies in the past few decades. Reference scheme for yeast susceptibility testing (National Committee for Clinical Laboratory Standards [NCCLS]) is available. Agar-based susceptibility testing procedure has been a focus of interest for many researchers and comprises the classical disk diffusion method along with novel E-test method.[10,11] Antifungal susceptibility testing may specify clinical response, predict treatment failure and develop local antibiograms, aiding in empirical selection of antifungals.[12] Resistance to antifungal agents has represented a major challenge in public health problems, particularly if the patient has been in the past treated with an azole group of antifungal agent; the likelihood of microbiological resistance should likely be considered. With a diverse range of antifungals use and anecdotal susceptibility patterns of *Candida*, it has at the present turn out to be indispensable to perform AST and make information accessible to the clinicians for effectual therapeutic outcome.

Methods

The study was conducted at antenatal units of tertiary care hospitals in Peshawar. Samples were processed at the Department of Microbiology, Institute of Basic Medical Sciences (IBMS), Khyber Medical University, Peshawar, from July 2016 to December 2016. Permission from the institutional ethical committee of Khyber Medical University was obtained before commencement of the study. An informed consent form was obtained from the participants after the contents of the form were clearly explained. Furthermore, they were assessed on the basis of age, pregnancy trimester and signs/symptoms during the gynecological examination. The participants younger than 18 years were able to sign informed consent on their own behalf, and this was acceptable to the institutional ethical committee of Khyber Medical University. Both symptomatic and non-symptomatic pregnant women's samples that were colonized or presenting with VVC, i.e., pruritus, leukorrhea, edema and vulvovaginal erythema presenting with whitish plaques on the mucosa were used for mycological cultivation. Samples were collected upon inserting a sterile vaginal speculum into the 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 labeled 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 second swab was streaked onto culture plates of SDA (Oxoid, Basingstoke, UK) and Sabouraud dextrose agar with chloramphenicol (SC; Oxoid), which was then incubated for 48 hours at 27°C. The isolated pure colonies were confirmed on Gram staining for yeast cells.

Candida spp. was isolated on CHROMagar Candida (Oxoid) which is a differential and selective medium. Isolated *Candida* colonies on SDA were subcultured onto CHROMagar using an inoculating loop and incubated at 37°C for 24 hours. Presumptive identification was done based on colony color of the growing *Candida* strains. Identification of *Candida* was based on the color of individual colony. API 20C AUX (bioMérieux, Marcy-l'Étoile, France) was further used for species identification and confirmation, which is based on 19 carbohydrate assimilation tests plus a negative control, read by assessing cupules for turbidity. The kit was used in accordance with the guidelines given by the manufacturer. Reading of the strips was done after 48 and 72 hours of incubation at 30°C. Using this method, the following *Candida* species were identified: *C. albicans* (green colonies), *Candida krusei* (pink colonies) *Candida glabrata* (purple colonies), *Candida tropicalis* (blue colonies) and *C. dublinensis* (darker green colonies).

Antifungal susceptibility testing

A suspension was prepared by picking five to six colonies from the SDA culture plate of ~1 mm diameter from a 24-hour old culture of *Candida* species. 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. Müller-Hinton agar (MHA) surface was streaked to make a lawn of the isolate.

Antifungal susceptibility testing was undertaken by the disk diffusion method. Using disk dispenser (Oxoid™), fluconazole disk (10 µg), itraconazole (10 µg), voriconazole (10 µg), clotrimazole (10 µg) and nystatin (100 IU) antifungal discs (Thermo Scientific™ Oxoid™) were applied on MHA (Thermo Scientific™ Oxoid™) as recommended by the Clinical Laboratory Standard Institute (CLSI) M44A document.

The plates were incubated in ambient air at 35°C and read at 24 hours. The diameters of zones of inhibition were measured in millimeters using a ruler for each antifungal disk. Interpretation of all antifungal susceptibility (susceptible S, susceptible dose dependent [SDD], and resistant R) was done according to CLSI standards (Table 1). Quality control was undertaken by using quality control strains, American Type Culture Collection (ATCC) 90028.

Table 1 Interpretative breakpoints of antifungal agents

Antifungals	Disk concentration	Zone of activity (mm)		
		Sensitive	Intermediate/ SDD	Resistant
Nystatin	100 U	≥15	10–14	≤10
Clotrimazole	10 µg	≥20	12–19	≤11
Fluconazole	10 µg	≥19	15–18	≤14
Voriconazole	10 µg	≥19	15–18	≤14
Itraconazole	10 µg	≥15	10–14	≤9

Abbreviation: SDD, susceptible dose dependent.

Results

A total of 108 patients were observed to determine frequency of in vitro susceptibility of *Candida* spp., and the results were analyzed.

Age distribution among 108 patients was analyzed as follows: 11 (10.1%) patients were in age range 17–20 years, 28 (25.9%) patients were in age range 21–24 years, 31 (28.7%) patients were in age range in 25–28 years, 20 (18.52%) patients were in age range 29–32 years, 12 (11.11%) patients were in age range 33–36 years, three (2.7%) patients were in age range 37–40 years and three (2.7%) patients were in age range 41–44 years. The minimum age calculated was 17 years, and the maximum age was 44 years. Mean age was 27 years with standard deviation (SD) ±5.61012 (Figure 1).

Pregnancy trimester distribution among patients was analyzed as follows: 21 (19.4%) patients were in their first trimester, 65 (60.2%) patients were in their second trimester and 22 (20.4%) patients were in their third trimester (Figure 2).

Frequency of *Candida* spp. distribution among patients with VVC was analyzed as follows: 45 (41.7%) patients had isolated *C. albicans*, 18 (16.7%) patients had *C. tropicalis*, 18 (16.7%) patients had *C. krusei*, 16 (14.8%) patients had *C. glabrata* and 11 (10.2%) patients had isolated *C. dubliniensis*. In all, 65% of the patients had a past history of antifungal use.

Susceptibility of *Candida* spp. with respect to fluconazole was analyzed as follows: fluconazole was sensitive in 36 (33.3%) of patients, SDD in five (4.6%) of patients and resistant in 67 (62%) of patients with VVC (Table 2). Mean zone of inhibition for fluconazole disk was 13 mm with SD ±6.65060.

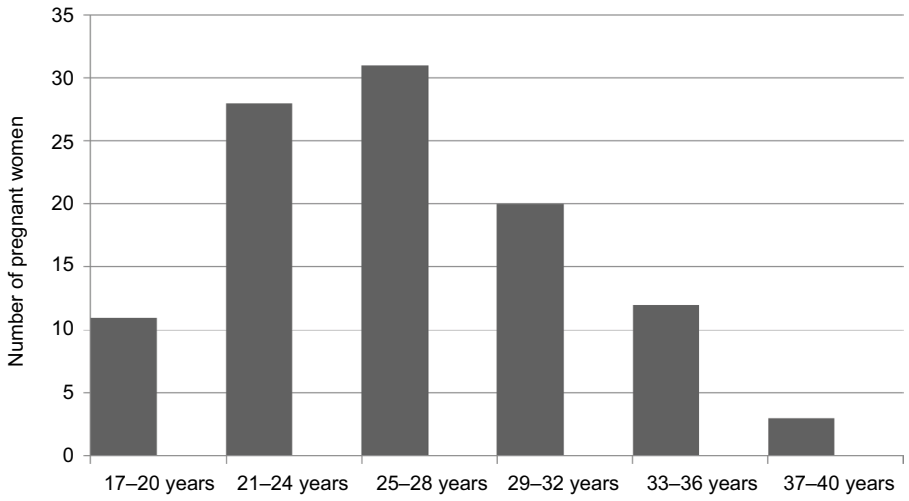

Figure 1 Age distribution of patients.

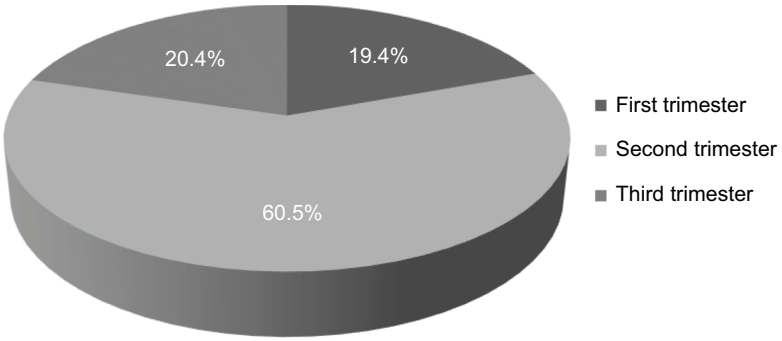

Figure 2 Pregnancy trimester distribution for vulvovaginal candidiasis.

Table 2 Antifungal susceptibility pattern of isolated *Candida* spp.

Drugs	Sensitive (%)	SDD (%)	Resistant (%)
Fluconazole	33.3	4.6	62
Nystatin	25	16.7	58.3
Clotrimazole	21.3	19.4	59.3
Voriconazole	85.2	4.6	10.2
Itraconazole	42.6	16.7	40.7

Abbreviation: SDD, susceptible dose dependent.

Susceptibility of *Candida* spp. with respect to nystatin was analyzed as follows: nystatin was sensitive in 27 (25%) of *Candida* isolates, SDD in 18 (16.7%) of *Candida* isolates and resistant in 63 (58.3%) of *Candida* isolates causing VVC (Table 2). Mean zone of inhibition for nystatin disk was 10 mm with SD ±4.58981.

Susceptibility of *Candida* spp. with respect to clotrimazole was analyzed as follows: clotrimazole was sensitive in 23 (21.3%) of *Candida* isolates, SDD in 21 (19.4%) of *Candida* isolates and resistant in 64 (59.3%) of *Candida* isolates in patients with VVC (Table 2). Mean zone of inhibition for clotrimazole disk was 12 mm with SD ±6.56882.

Susceptibility of *Candida* spp. with respect to voriconazole was analyzed as follows: voriconazole was sensitive in 92 (85.2%) of *Candida* isolates, SDD in five (4.6%) of *Candida* isolates and resistant in 11 (10.2%) of *Candida* isolates (Table 2). Mean zone of inhibition for voriconazole disk was 21 mm with SD ±4.23074.

Susceptibility of *Candida* spp. with respect to itraconazole was analyzed as follows: itraconazole was sensitive in 46 (42.6%) of *Candida* isolates, SDD in 18 (16.7%) of *Candida* isolates and resistant in 44 (40.7%) of *Candida* isolates (Table 2). Mean zone of inhibition for itraconazole disk was 13 mm with SD ±5.51711.

Susceptibility of 45 *C. albicans* isolates with respect to each of the antifungal was analyzed as follows: fluconazole was sensitive in 17 (37.7%) of *Candida* isolates, intermediate

in four (8.8%) of *Candida* isolates and resistant in 24 (53.3%) of *C. albicans* isolates. Nystatin was sensitive in 12 (26.6%) of *C. albicans* isolates, intermediate in eight (17.7%) of *C. albicans* isolates and resistant in 25 (55.5%) of *C. albicans* isolates. Clotrimazole was sensitive in eight (17.7%) of the isolated *C. albicans*, SDD in 10 (22.2%) of the isolated *C. albicans* and resistant in 27 (60%) of the isolated *C. albicans.* Voriconazole was sensitive in 39 (86.6%) of the isolated *C. albicans*, SDD in three (6.6%) of the isolated *C. albicans* and resistant in three (6.6%) of the isolated *C. albicans.* Itraconazole was sensitive in 24 (53.3%) of the isolated *C. albicans,* SDD in eight (17.7%) of the isolated *C. albicans* and resistant in 13 (28.8%) of the isolated *C. albicans* (Figure 3).

Susceptibility of 18 *C. tropicalis* isolates with respect to each of the antifungal was analyzed as follows: fluconazole was sensitive in 11 (61.1%) of *Candida* isolates, SDD in none of the isolated *C. tropicalis* and resistant in seven (38.8%) of the isolated *C. tropicalis*. Nystatin was sensitive in seven (38.8%) of the isolated *C. tropicalis*, SDD in four (22.2%) of the isolated *C. tropicalis* and resistant in seven (38.8%) of the isolated *C. tropicalis*. Clotrimazole was sensitive in five (27.7%) of the isolated *C. tropicalis*, SDD in six (33.3%) of the isolated *C. tropicalis* and resistant in seven (38.8%) of the isolated *C. tropicalis.* Voriconazole was sensitive in 15 (83.3%) of the isolated *C. tropicalis*, SDD in two (11.1%) of the isolated *C. tropicalis* and resistant in one (5.5%) of the isolated *C. tropicalis.* Itraconazole was sensitive in 11 (61.1%) of the isolated *C. tropicalis*, SDD in three (16.6%) of the isolated *C. tropicalis* and resistant in four (22.2%) of the isolated *C. tropicalis* (Figure 4).

Susceptibility of 18 *C. krusei* isolated with respect to each of the antifungal was analyzed as follows: fluconazole was sensitive in none of the patients, SDD in none of the patients and resistant in 18 (100%) of the patients isolated with *C. krusei*. Nystatin was sensitive in five (27.7%) of the isolated *C. krusei*, SDD in four (22.2%) of the isolated *C. krusei* and resistant in nine (50%) of the isolated *C. krusei*. Clotrimazole was sensitive in five (27.7%) of the isolated *C. krusei*, SDD in three (16.7%) of the isolated *C. krusei* and resistant in 10 (55.5%) of the isolated *C. krusei.* Voriconazole was sensitive in 16 (88.8%) of the isolated *C. krusei*, SDD in none of the isolated *C. krusei* and resistant in two (11.1%) of the isolated *C. krusei.* Itraconazole was sensitive in five (27.7%) of the isolated *C. krusei,* SDD in one (5.5%) of the isolated *C. krusei* and resistant in 12 (66.6%) of the isolated *C. krusei* (Figure 5).

Susceptibility of 16 isolates of *C. glabrata* with respect to each of the antifungal was analyzed as follows: fluconazole was sensitive in six (37.5%) of the *Candida* isolates, SDD in none of the *Candida* isolates and resistant in 10 (62.5%) of the *Candida* isolates isolated with *C. glabrata*. Nystatin was sensitive in three (18.7%) of the isolated *C. glabrata*, SDD in two (12.5%) of the isolated *C. glabrata* and resistant in 11 (68.7%) of the isolated *C. glabrata*. Clotrimazole was sensitive in five (31.25%) of the isolated *C. glabrata*, SDD in one (6.25%) of the isolated *C. glabrata* and resistant in 10 (62.5%) of the isolated *C. glabrata.* Voriconazole was sensitive in 13 (81.25%) of the isolated *C. glabrata*, SDD in none of the isolated *C. glabrata* and resistant in three (18.7%) of the isolated *C. glabrata.* Itraconazole was sensitive in four

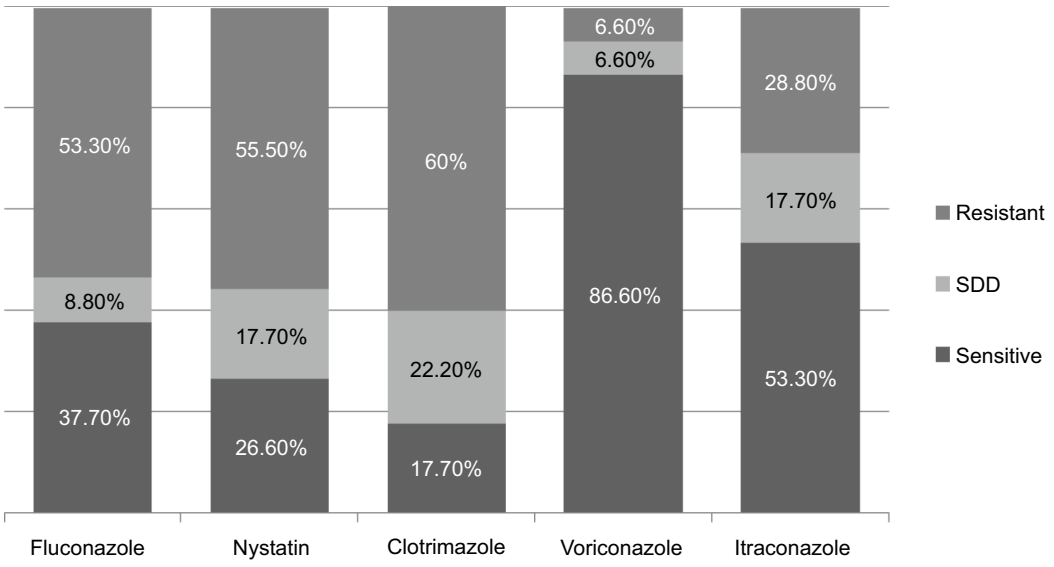

Figure 3 Antifungal susceptibility pattern in *Candida albicans*.
Abbreviation: SDD, susceptible dose dependent.

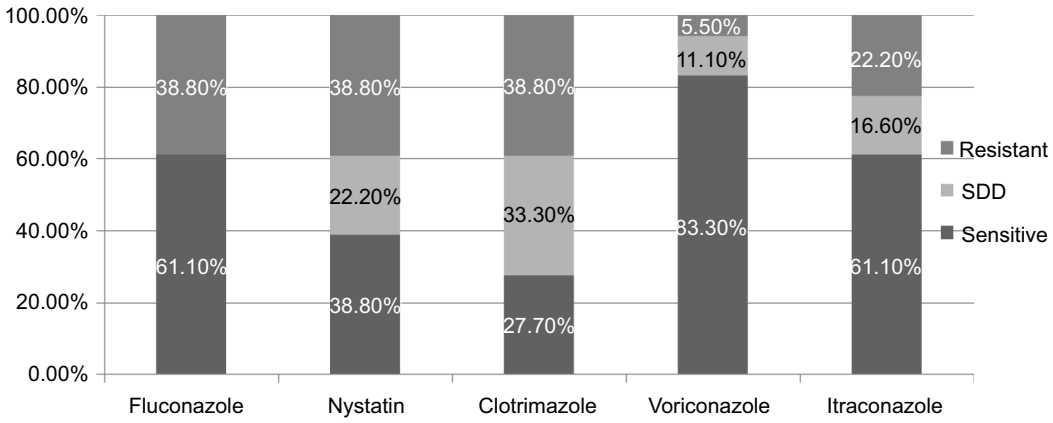

Figure 4 Antifungal susceptibility pattern in *Candida tropicalis*.
Abbreviation: SDD, susceptible dose dependent.

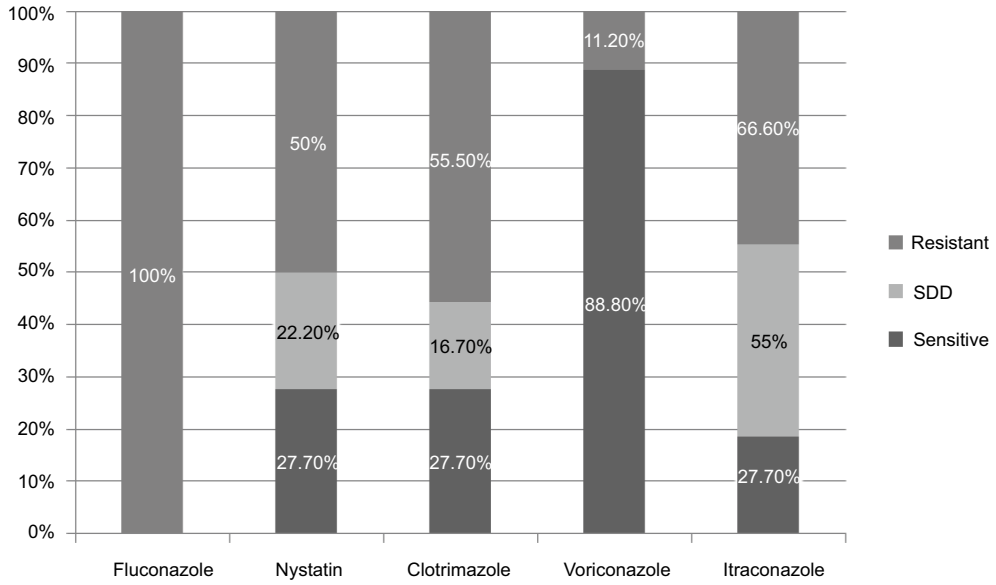

Figure 5 Antifungal susceptibility pattern in *Candida krusei*.
Abbreviation: SDD, susceptible dose dependent.

(25%) of the isolated *C. glabrata*, SDD in five (31.25%) of the isolated *C. glabrata* and resistant in seven (43.7%) of the isolated *C. glabrata* (Figure 6).

Susceptibility of 11 isolates of *C. dubliniensis* with respect to each of the antifungal was analyzed as follows: fluconazole was sensitive in two (18.2%) of the patients, SDD in one (9.1%) of the patient and resistant in eight (72.7%) of the patients isolated with *C. dubliniensis*. Nystatin was sensitive in none of the isolated *C. dubliniensis*, SDD in none of the isolated *C. dubliniensis* and resistant in 11 (100%) of the isolated *C. dubliniensis*. Clotrimazole was sensitive in none of the isolated *C. dubliniensis*, SDD in one (9.1%) of the isolated *C. dubliniensis* and resistant in 10 (90.9%) of the isolated *C. dubliniensis.* Voriconazole was sensitive in nine (81.8%) of the isolated *C. dubliniensis*, SDD in none of the isolated *C. dubliniensis* and resistant in two (18.2%) of the isolated *C. dubliniensis.* Itraconazole was sensitive in two (18.2%) of the isolated *C. dubliniensis*, SDD in one (9.1%) of the isolated *C. dubliniensis* and resistant in eight (72.7%) of the isolated *C. dubliniensis* (Figure 7).

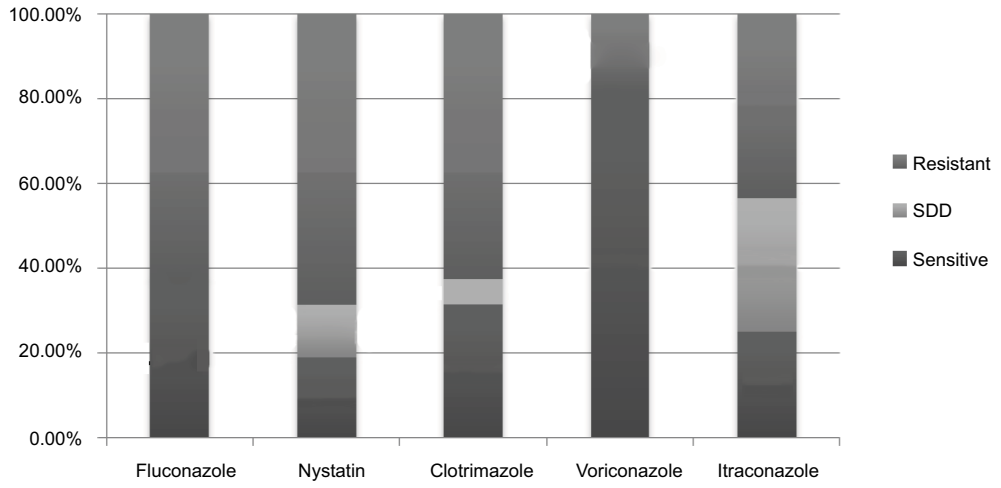

Figure 6 Antifungal susceptibility pattern in *Candida glabrata*.
Abbreviation: SDD, susceptible dose dependent.

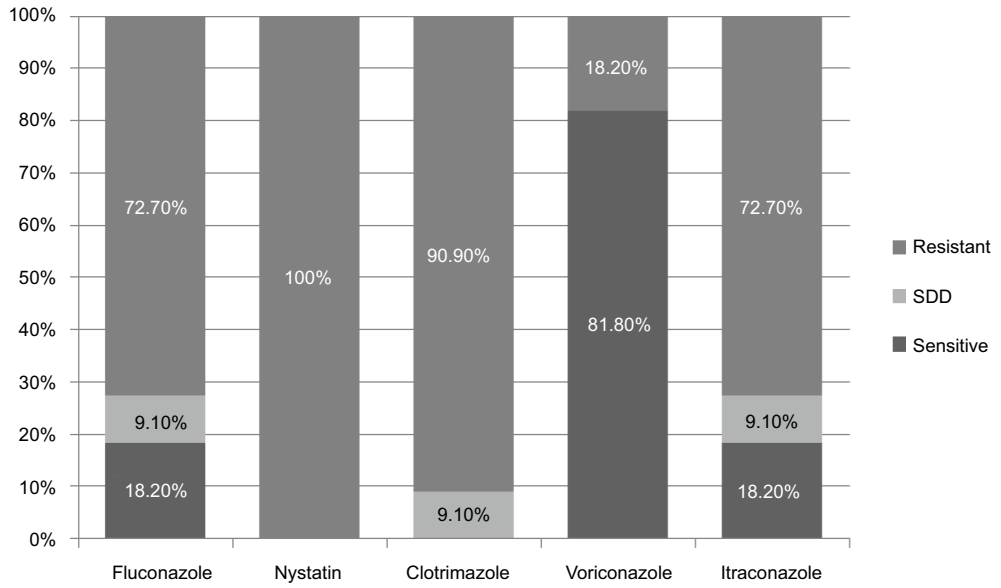

Figure 7 Antifungal susceptibility pattern in *Candida dubliniensis*.
Abbreviation: SDD, susceptible dose dependent.

Discussion

Worldwide, many studies have revealed that *Candida* spp. can switch from a commensal state into a pathogen causing infections, in reaction to transformation in the host leading to infections of oral mucosa, gastrointestinal lining and genital tract epithelium. One of the studies demonstrated that prevalence of candidiasis was more (33.8%) in women in the age range of 20–29 years, followed by those (24.3%) in the age range of 30–39 years, and the overall prevalence of VVC was 25%.[13] The prevalence rate is parallel to 26% recorded in Ibadan[14] and is more or less twofold to that reported in Burkina Faso (14%) and approximately half to that in (55.4%) Cameroon.[15] In another study, the prevalence reported was 30.7% in Jamaica[16] and 30% in Nigeria.[17] A range of studies have revealed asymptomatic vaginal colonization of *Candida* as 5–30%.[18] Many investigators noted a high infection rate in the 20–29 age group, which was consistent with our study, as many of the patients were in the age range of 25–28 years,

most probably due to indiscriminate drug usage, especially contraceptives.[19] Various researchers declared women of age group 15–45 years to have the highest incidence, which is almost the same in the present study in women in the age range of 17–44 years. The cause for this high incidence could be due to decreased levels of protective cervical antibodies in the reproductive tract.[20]

Spinillo et al[21] reported that a high rate of *Candida* infections occurs in married women between 30 and 45 years with a reproductive history, repeated sexual activity and taking oral contraceptive pills. Rural women had an elevated rate of infectious vaginitis, owing mostly to conditions of poor medical care, lack of health education, scarce economic resources and difficulty in timely medical treatment.[22]

The presenting complaints in 31% patients were itching, 29.4% suffered from vaginal discharge as the primary complaint, 15.6% of women presented with pain only and 13.3% presented with a triad of discharge, itching and pain. Among other complaints described were vaginal/vulvar, erythema, dysuria and dyspareunia. In another study, females (55%) who reported to clinics with VVC had pregnancy. This most likely owes to raised level of hormones during pregnancy, which is an energy source for *Candida* growth.[6] An increased propensity to infection is due to both an elevated level of vaginal colonization and a higher prevalence of symptomatic vaginitis.[23] Our findings were comparable with this study; 19.67% of *C. tropicalis* was isolated from vaginal swab, which was second to urine.[24]

Oyewole et al[25] also detected the highest occurrence of VVC in the second trimester (61%) of pregnancy, which is almost similar to our study with the highest frequency (60.2%) in the second trimester. A Brazilian study reported *C. albicans* isolated in 92.3%, *C. krusei* isolated in 3.3%, *C. glabrata* isolated in 2.2%, *Candida parapsilosis* isolated in 1.1% and *C. tropicalis* isolated in 1.1% of pregnant women with VVC.[26] A study in Kenya showed that *C. albicans* was the most frequent isolated (73.7%) followed by *C. glabrata* (13%), *Candida famata* (5%), *C. krusei* (3%) and *C. parapsilosis* (1%).[27]

Our findings were somewhat similar to another study publishing the highest occurrence of *C. albicans* (54.3%), followed by *C. glabrata* (25.7%), *C. tropicalis* (5.7%) and *C. dublinensis* (14.3%), identified by the CHROMagar test.[13] Nelson et al[19] also reported *C. albicans* as the commonest vaginal species followed by *C. glabrata* in VVC cases in pregnancy. Another report by Oyewole et al[25] showed the maximum rate of *C. albicans* as 50%, followed by *C. glabrata* 21.4%, *C. tropicalis* 14.3%, *C. krusei* 11.9% and *Candida pseudotropicalis* 2.4%. In colonized women, the predominant species isolated was *C. albicans*, corresponding to 62.9% from the women with VVC. Other colonizing species in the vaginal mucosa of asymptomatic women were *C. glabrata* 14.3%, followed by *Candida sphaerica* 8.6% and *C. parapsilosis* 2.9%.[28] In different countries, studies reported *C. albicans* being most widespread species in VVC (76–89%), followed by *C. glabrata* (7–16%). The proportion of non-*albicans* spp. related to VVC among these countries varied from 11% to 24%.[29]

In India, five *Candida* species were isolated and identified in a study, where a minor proportion of *C. albicans* (35.5%) was noted, followed by increased occurrence of non-*albicans* species, with *C. tropicalis* (26.4%), *C. glabrata* (20.6%), *C. krusei* (15.7%) and *C. dubliniensis* (1.6%).[30] One study has described a higher level of non-*C. albicans* species occurrence over time.[31] As stated by Ferrazza et al[32,] almost 25% of VVC cases were caused by non-*albicans* species. Comparing the results of our study with those other studies which demonstrated, *C. glabrata* to be the second most significant species among VVC cases because of its higher frequency and increased rate of clinical resistance to antifungals.[33] In the present study, 41.7% of VVC cases were due to *C. albicans,* followed by *C. tropicalis* (16.7%), *C. krusei* (16.7%), *C. glabrata* (14.8%) and *C. dubliniensis* (10.2%).

A study in Uganda showed species frequency as follows: *C. albicans* 78.9%, *C. glabrata* 14.3%, *C. krusei* 3.3%, *C. tropicalis* 1.4%, *C. famata* 0.96%, *Candida lusitaniae* 0.4% and *C. parapsilosis* 0.4%. Resistance among antifungals was noted as nystatin 0.61% of *C. albicans*. *C. krusei* showed fluconazole resistance of 71.43%. *C. lusitaniae, C. krusei*, *C. famata* and *C. glabrata* were observed to be 100% resistant to itraconazole. *C. famata* and *C. glabrata* showed 50% and 36.7% resistance to clotrimazole correspondingly. *C. albicans* displayed resistance of 20.6% to itraconazole and 6.6% to voriconazole.[34] In an 8-year study, fluconazole resistance increased from 2.4% to 55.4% (2006–2012), but the rate dropped to 8.9% in 2013. Considering miconazole and itraconazole, resistance increased from 2.4% and 7.1% to 59.8% and 58.9% (2006–2013), repectively.[35] Among 100 *Candida* isolates, *C. albicans* (30%), *C. parapsilosis* (10%), *C. tropicalis* (21%), *C. glabrata* (8%), *Candida parakrusei* (8%) and *C. krusei* (3%) were isolated by workers. In vitro antifungal activity points to clotrimazole minimum inhibitory concentration of 16 and 8 µg/mL to be effective against 70% of *Candida* spp., fluconazole MIC of 64 and 32 µg/mL to be effective against 36.2% of isolates and nystatin disk to be 63.5% effective.[36] In a study conducted in India,

Candida isolates showed 97.2% sensitivity to fluconazole, 80% sensitivity to clotrimazole, 57% sensitivity to itraconazole and 37% sensitivity to miconazole.[37] In most reports, regarding fluconazole and itraconazole, resistance was noted in 42% and 48%, respectively, of the *Candida* isolates.[28] In the current study, *C. krusei* showed 100% resistance to fluconazole, followed by 66.6% resistance to itraconazole. Prostitutes with vulvovaginitis were evaluated in a study that reported an increased susceptibility of *C. albicans* to fluconazole compared to *C. glabrata*. One resistant strain and four SDD strains of *C. glabrata* were isolated.[38] On the other hand, when isolates of *Candida* were tested in a study by Mishra et al[39], all *C. glabrata*, 50% *C. tropicalis* and 12% *C. albicans* isolates were found to be resistant to fluconazole. Recently, some authors reported that among all *C. tropicalis* isolates, amphotericin B was 100% sensitive, whereas 37.7% were found to be resistant to fluconazole, which is similar to the finding in our study with fluconazole resistance of 38.8%.[24] Our study indicated that most *Candida* spp. tested were resistant to fluconazole (62%), followed by clotrimazole (59.3%), nystatin (58.3%) and itraconazole (40.7%); the lowest was recorded for voriconazole (10.2%). *C. glabrata* in our study showed resistance to nystatin (68.7%), followed by fluconazole (62.5%), itraconazole (43.7%) and voriconazole (18.7%). The most resistant *C. dubliniensis* isolate in the current study was nystatin (100%), followed by clotrimazole (90.9%), itraconazole (72.7%) and voriconazole (18.2%).

Conclusion

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.

Disclosure

The authors report no conflicts of interest in this work.

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