ORIGINAL ARTICLE

Value of antifungal susceptibility testing in recurrent vulvovaginal Candidiasis by Non -Albicans Candida

[1]Ghada A. Mokhtar*, [2]Mohamed Sh. Ramadan, [1]Shymaa Yahia
[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University
[2]Department of Gynaecology and Obstetrics, Faculty of Medicine, Zagazig University

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Corresponding Author:
Ghada Abd el moniem Mokhtar
Department of Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University
Tel.: 01001968019
ayat_233@yahoo.com

ABSTRACT

Background*: Vulvovaginal candidiasis (VVC) is regarded as a prevalent vaginal infection and mainly results from Candida albicans. Nevertheless, there has recently been a prominent shift in candidiasis etiology regarding non-albicans Candida (NAC) species with achieving importance. For women with more than three episodes annually are described as recurrent vulvovaginal candidiasis (RVVC).* ***Objectives:*** *To isolate, speciate, and determine the value of antifungal sensitivity pattern of candida species isolated from patients developed (RVVC).* ***Methodology:*** *High vaginal swabs (HVS) were taken from patients with RVVC and cultured on ordinary mycological media. Any significant candida growth was identified and speciated by VITEK 2 system. Their antifungal sensitivity was done by disc diffusion approach governed by CLSI guidelines.* ***Results****: A total of 110 Candida species from 250 high vaginal swabs were isolated. Among all candida species isolated from patients with RVCC, C.albicanis accounts for 44% while NAC accounts for 56% with C.glabrata most common species isolated. Voriconazole, amphotericin B, and nystatin showed high sensitivity rates (92 %, 89%, and 84% respectively) on all candida species (C.albicans and NAC) isolated from patients with RVVC.* ***Conclusion:*** *In RVCC there is increase in NAC (56%) with C.glabrata most common species isolated. Voriconazole, Nystatin, and amphotericin B have the best antifungal activity against all spp.*

INTRODUCTION

Candida is considered as a dimorphic fungus derived from the phyla Ascomycota, residing respiratory, gastrointestinal as well as genitourinary tracts of over 30% of healthy people throughout their lifespan [1,2] Vulvovaginal candidiasis (VVC) is a highly prevalent health problem among females globally and often shows quick response to topical or oral antifungal therapy. Nevertheless, recurrent vulvovaginal candidiasis (RVVC) is developed in some women and identified by several episodes annually. RVVC is a debilitating, long-term disorder with severe effect on women life quality [3]

The RVVC pathogenesis is still unanswered until now. Large number of females with RVVC is healthy, with competent immune system and have no risk factor or defined cause. Some associated factors are linked with RVVC as genetic (polymorphism, familial, ethnicity), immunological factors (HIV, uncontrolled diabetes, corticosteroids, antibiotics, hormonal therapy) and behavioral (oral sex, oral contraceptive, frequent intercourse)[4]

C. albicans is considered the main causative agent of VVC; Nowadays non-albicans Candida (NAC) species have been focused because of its increased prevalence, as NAC species are isolated in most cases of RVVC [5]. *C. glabrata* is the most predominant, other NAC species were detected such as *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *C. dubliniensis*[6,7]

Minimum inhibitory concentrations (MICs) for azole have been noticed to be higher in NAC isolates which lead to resistant infections [8]. This finding was supported in a study in which researchers noticed that over two-thirds of their *C. glabrata* isolated from vagina express higher fluconazole resistance than the isolates from bloodstream infections[9]. The MICs of some NAC were observed to grow many times if they were exposed to antifungal agents [10] Isolates from RVVC cases have experienced more resistance to azoles than those isolated from non-complicated cases[11]

With no mortality rates, RVVC causes high morbidity rate that with increasing costs of treatment. Hence, more effort is needed not only to understand the immunopathogenesis but also to treat VVC patients efficiently therefore prevent the recurrence[4]

Susceptibility tests are regarded as a beneficial tool aiding in treatment of patients subjected to treatment with azole antifungal agents and when resistance to antifungal agents is highly predicted. However, it is not easily performed as a routine testing and not usually accessible. Moreover, species identification exhibits a

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high prediction towards possible susceptibility, highlighting its further utilization as a therapy guide[12]

This study conducted to isolate, speciate, and determine the value of antifungal sensitivity pattern of candida species isolated from patients developed RVVC.

METHODOLOGY

This prospective study was done in the Department of Medical Microbiology and Immunology Department and Gynecology-Obstetrical Clinics, Faculty of Medicine, Zagazig University, from June 2019 and March 2020.Approval of the ethical committee was obtained from Faculty of Medicine, Zagazig University, number (6466).

Totally 250 vaginal swabs were collected from patients with symptomatic RVVC.Samples were mixed with 10% potassium hydroxide (10% KOH) and stained with Gram stain for direct microscopy. The swabs were cultivated on Sabourad's Dextrose Agar (SDA) (Oxoid, USA) with gentamicin, then incubated at 37°C and 25°C for 24-48 hours.

Candida species identification and speciation was done by VITEK 2 system. The inoculum suspensions were mounted in sterile saline, and the individual test cards were automatically filled with the previously mounted culture suspension, sealed, and incubated by the VITEK 2 instrument. Then, cards were placed in the incubator at a temperature of 35.5 °C for 18 hrs. , and optical density readings were detected automatically every 15 min. The final profile results were compared with the database, and identification was attained.

Antifungal sensitivity testing by disk diffusion method:

As per the "CLSI" (M44-A) procedures, the inoculum was prepared by selecting five similar colonies from a 24 hrs. old cultivated Candida species. Colonies were mixed in 5 ml of sterile 0.9% normal saline[13]. The suspension was mixed by vortexing to obtain optimum turbidity and adjusted to 0.5 McFarland standards. After 15 minutes, the suspension was distributed onto "Mueller Hinton Glucose Agar (MHGA)" supplied from (HiMedia, India) with 2% glucose and 0.5 µg/ml of methylene blue were used for antifungal sensitivity testing. HiMedia antifungal disks were utilized: fluconazole (10 µg), ketoconazole (30 µg), clotrimazole (10 µg), nystatin (100 U), voriconazole (1 µg), miconazole (30 µg) and amphotericin-B (20 µg). Antifungal disks are distributed equally so that there were not less than 24 mm from the centre to centre. The plates were incubated at 37 °C for 24-48 hr. *C.albicans* ATCC 90028, *Candida tropicalis* ATCC 750, *Candida krusei* ATCC6258 and *C.Parapsilosis* ATCC 22019 were used as a control [13]

Statistical analysis:

Data were collected, tabulated, and analyzed using SPSS version 16.0.

RESULTS

A total of 110 Candida species were isolated from 250 high vaginal swabs. From 110 Candida isolates, 62 (56%) were Non-albicans Candida (NAC) and 48 (44%) were *C. albicans*. Among NAC, 22 (20%) were *C.glabrata*, followed by 20 (18%) *C.tropicalis*, 12 (10%) *C. parapsilosis* and 8 (7%) were *C.krusei* (Figure-1).

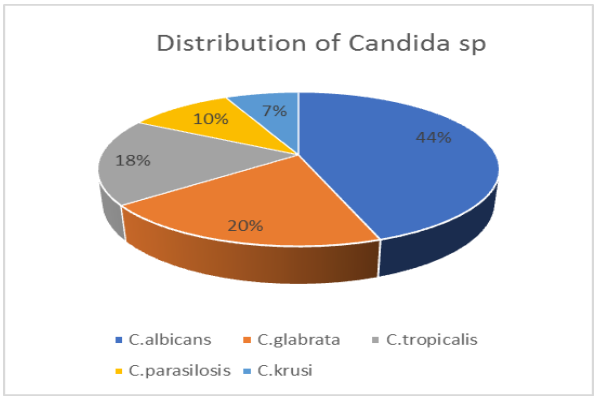

Fig. 1: Candida species distribution.

Sensitivity testing for 110 Candida species (48 isolates of *C.albicans* and 62 isolates of NAC) to was conducted using disk diffusion method (Table-1).

Table 1: Sensitivity pattern of different candida species to antifungal drugs

Antifungal agents		*C. albicans* n=48(44%)	Non-*albicans Candida* (NAC)				Total n=110(%)
			C.glabrata n=22(20%)	*C. tropicalis* n=20(18%)	*C. parapsilosis* n=12(11%)	*C. krusei* n=8(7%)	
FCZ	S	38(79)	17(77)	15(75)	10(83)	NA*	80/102(78)
	SDD	-	1(5)	-	1(8)	NA*	2/102(1.9)
	R	10(20)	4(18)	5(25)	1(8)	NA*	20/102(19.6)
VCZ	S	41(85)	21(95)	20(100)	12(100)	7(88)	101(92)
	SDD	2(4)	-	-	-	-	2(2)
	R	5(10)	1(5)	-	-	1(13)	7(6)
KCZ	S	36(75)	15(68)	14(70)	7(58)	5(63)	77(70)
	SDD	1(2)	1(5)	2(10)	1(8)	1(13)	6(5)
	R	11(23)	6(27)	4(20)	3(25)	3(38)	27(25)
CLOT	S	40(83)	17(77)	16(80)	9(75)	2(25)	85(77)
	SDD	1(2)	1(5)	-	1(8)	1(13)	4(4)
	R	7(13)	4(18)	4(20)	2(17)	5(63)	22(20)
MCZ	S	37(77)	15(68)	12(60)	7(58)	5(63)	76(69)
	SDD	4(8)	1(5)	2(10)	1(8)	-	8(7)
	R	7(15)	6(27)	6(30)	4(33)	3(38)	26(24)
AMP	S	44(92)	20(91)	17(85)	11(92)	6(75)	98(89)
	SDD	-	-	-	-	-	-
	R	4(8)	2(9)	3(15)	1(8)	2(25)	12(11)
NS	S	39(81)	20(91)	16(80)	10(83)	6(75)	91(83)
	SDD	2(4)	-	1(5)	-	1(12)	4(4)
	R	7(15)	2(9)	4(20)	2(17)	1(12)	16(15)

SDD= Susceptible Dose Dependent, NA*= *C.krusei* intrinsic resistance to fluconazole. FCZ=Fluconazole (10µg), KCZ=Ketoconazole (30µg), CLOT=Clotrimazole (10µg), NS=Nystatin (100U), VCZ=Voriconazole (1µg), MCZ=Miconazole (30µg) and AMP=Amphotericin-B (20µg).

Table 2: Interpretive values of different antifungal agents:

Drugs with concentration (µg)	Susceptible (mm)	Susceptible dose-dependent (mm)	Resistant (mm)
Fluconazole (10 µg)	≥19	15-18	≤14
Ketoconazole (30 µg)	≥28	21-27	≤20
Clotrimazole (10 µg)	≥20	12-19	≤11
Miconazole (30 µg)	≥20	12-19	≤11
Voriconazole (1 µg)	≥17	14-16	≤13
Amphotericin-B (20 µg)	≥15	10-14	≤10
Nystatin (100 u)	≥15	10-14	No zone

The inhibition zones interpreted as resistant (R), susceptible dose dependent (SDD) and susceptible (S) according to CLSI guidelines [13]

In our study Candida species had expressed elevated susceptibility to voriconazole (92 %) followed by amphotericin B 89%, nystatin 83%, fluconazole 78%, clotrimazole 77%, ketoconazole 70% and miconazole 69% as shown in (Table -1) with the interpretive values of various antifungal drugs listed in (Table 2).

In our study Candida species had expressed high resistance to ketoconazole 25% followed by miconazole 24%, clotrimazole 20%, fluconazole 19.6%, nystatin 15%, amphotericin B 11% and voriconazole 6%.

Candida albicans expressed the highest sensitivity to amphotericin B 92% and voriconazole 85% but the highest resistant to ketoconazole 23%.

C.glabrata had shown the highest sensitivity to voriconazole 95% while 27% resistance to Miconazole and ketoconazole. *C. tropicalis* had expressed the highest sensitivity 100% against Voriconazole and 30% resistance to Miconazole.

C. parapsilosis had shown 100% sensitivity against voriconazole and 30% resistance to Miconazole. *C.krusei* had showed 88% sensitivity to voriconazole while 38% resistance to both miconazole and ketoconazole.

DISCUSSION

Vulvo-Vaginal Candidiasis (VVC) is considered a highly prevalent health problem caused mainly by candida albicans [14,15]. Vulvo-Vaginal Candidiasis (VVC) usually responds rapidly to topical or oral antifungal therapy. However, some unlucky women develop RVVC, which is a long-term debilitating condition that can greatly affect the life quality of women. [4]

The pathogenesis of RVVC is multifactorial with some factors acting synergistically to facilitate and enhance *Candida* overgrowth, leading to clinical symptoms. The principal pathology in genital candidiasis is inflammation of the lower genital tract – vulva and vagina, secondary to an overgrowth or abnormal growth of *Candida* [4,11]

Recurrent vulvovaginal candidiasis (RVVC) is oftentimes diagnosed and treated on the ground of clinical symptoms and signs, without confirmatory laboratory tests. Majority of women self-diagnose and treat with over-the-counter anti-fungal drugs .Since vaginal infections are an extremely common reason for women to ask care from a clinician, VVC is often over-diagnosed or misdiagnosed, leading to inadequate treatment especially in resource-limited developing countries[16]

Effective treatment of RVVC, with adequate control of symptoms and eradication of the fungus, represents a challenge in daily clinical practice[4]

The prevalence of non-albicans Candida in RVVC is increasing nowadays specially in developing countries if compared with developed countries [17,18]

The rapid increase in resistance to antifungal drugs in NAC may be attributed to prolonged duration of antifungal therapy which results in treatment failure and development of azole resistance in those species, so performing antifungal susceptibility testing is a reasonable solution for this problem [19]Azole resistance may be caused by increased expression of gene encoding lanosterol demethylase (*ERG11*), alteration in the *ERG11*, the gene coding the multidrug efflux pumps, *CaMDR1*, *CDR1* and 2 [20]

C. albicans has been detected as the predominant causative pathogen in VVC. Nevertheless, Non - albicans candida species are more predominant in RVVC [21]

In our study *C.albicans* accounts for 44% of RVVC .This is in accordance with Chong and co-workers [21] who observed that *C.albicans* species isolated from RVVC patients were different from the strains causing sporadic VVC. Soll and his colleagues [22] supposed that there may be phenotypic switching of *C. albicans* in RVVC with no genotypic changes.

Schroppel et al.[23] postulated that repeated administration of antifungal drugs resulted in yeast genetic instability which is considered an important virulence factor.

Whereas the prevelance of *C.albicans* in our study was 44% of RVVC, similar results were reported by Al-Hedaithy et al.[24], Al-mamari et al [25] and Alfouzan et al [26]. The prevalence of *C. albicans* in VVC falls somewhere in the range of 47% and 89% in previous reports [27,28,29]

In our work, we highlighted the increased rate of NAC species, this finding could be explained long term administration of antifungal drugs early. The increasing prevalence of NAC in RVVC has been shown to be more prevalent in developing countries is disturbing situation as *C. glabrata* has been shown to have high minimum inhibitory concentration (MIC) to azole, and *C. krusei* exhibit intrinsic resistance to fluconazole[5]

We revealed that RVVC is most likely because of non-albicans Candida (56%) like C. glabrata, and this is nearly similar to the result obtained by Ahmad and Khan [30] who stated that non-albicans represent 53% and 37% of them was due to *C. glabrata*. This is also in accordance with Okungbowa and coworkers [31]who indicated that non-albicans Candida represent 80% and featured 34% of *C. glabrata*.

In the present study, *C. glabrata* accounts for 20% followed by 18% of C tropicalis, 11% of *C. parapsilosis* and 7% of *C. krusei*, which was similar to other previous reports [32,33]

To treat RVVC, well-tolerated antifungal agents are required with deeper awareness regarding its pathogenesis and natural history. This is to ensure the absence of a gold standard to treat RVVC [8]

Ketoconazole, clotrimazole, and miconazole are the first line azoles for the treatment of vulvovaginal candidiasis. In this work, there was noticeable high resistance for azoles except for voriconazole. Other drugs as amphotericin B and nystatin gave good effect against *Candida*.

The present finding revealed 19.6 % were fluconazole resistant by *Candida* species. This coordinates with other workers [24,33,34]. A higher resistance rate was reported[35] and no resistance [26]

C. tropicalis show highest fluconazole resistant (24%) in our study. Variable results were reported by several workers [35,36]

In the present study, sensitivity of *C. albicans* to fluconazole was (79%), which is higher than the study reported by *Babin et al.* [27] while Das et al [33] reported nearly the same result.

In this study, nystatin showed a good activity to NAC as well as *C. albicans* species with (83%) sensitivity to all Candida spp *C. glabrata showed* (91%) sensitivity to nystatin while, 7(15%) resistance against *C. albicans;* Similar finding was reported by a study done by *Sherin et al*[37]. In one research[38], the rate of resistance towards nystatin in their isolates showed

different values for *C. glabrata* (0.3%), *C. krusei* (3.8%) and *C. tropicalis* (0%).

In our work, voriconazole showed excellent activity against all Candida spp (92%) sensitivity. Both *C.tropicalis* and *C.parapsilosis* were (100%) sensitive to voriconazole .While, *C.glabrata*, *C.krsei*, and ,*C.albicans* were (95 %),(88%),and (85%) sensitive to voriconazole respectively . *C.albicans* reported (10%) resistance to voriconazole which is comparable to study done by Das et al [33]

In this work, the overall Clotrimazole was (77%) sensitive and ketoconazole was 70% which were comparable to a study done by Dharmik et al. [39]

Regarding to amphotericin B *C. glabrata* showed (91%) sensitivity while, C.krusei showed (25%) resistance which is higher than the rate reported by Ajitha et al.[40]

CONCLUSION

In our study RVCC showed increased incidence of NAC. Voriconazole, Nystatin, and amphotericin B have the best antifungal activity against all spp. We do recommend anti-fungal sensitivity testing for patients with RVVC. In this setting, species identification and susceptibility testing may enhance antifungal selection and patient response to treatment.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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