Research Article

IJCRR
Section: Healthcare
Sci. Journal
Impact Factor
4.016

ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA AGAINST SIX ANTIFUNGAL DRUGS BY DISK DIFFUSION METHOD ISOLATED FROM VULVOVAGINAL CANDIDIASIS

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ABSTRACT

Introduction: Vulvovaginal candidiasis is the second most common cause of vaginitis after bacterial vaginosis and is diagnosed in 40 % women with vaginal discharge. Incidence of fungal infections has increased dramatically over the past few decades. Epidemiologic data from the past decade reveal a paradigm shift in *candida* species from albicans to non -albicans *candida* species. The aim of this study was to determine incidence of vulvovaginal candidiasis, identify the species and test their in vitro susceptibility profile against six antifungal agents – Fluconazole, Itraconazole, Ketoconazole, Clotrimazole, Amphotericin B and Nystatin.

Material and Methods: Two high vaginal swabs were collected from patient with complain of vaginal discharge. One sample is processed for direct microscopic examination and other sample is used for culture on Sabourad's dextrose agar and Blood agar. Isolates identified by battery of test and antifungal susceptibility testing of Candida species done by Disk Diffusion method.

Results: A total of 122 isolates of candida species were obtained. *Candida albicans* was found to be the most frequently isolated species 81(66.39%) of the total isolates, followed by *C. glabrata* 19 (15.65%),*C. tropicalis* 12(33.61%), *C. parapsilosis* 6(4.91%), *C. krusei* 3(2.4%) and *C. gullermondii* 1(0.8%).Non-albicans candida constituted 41(33.61%).Antifungal susceptibility pattern showed that candida isolates were more sensitive to Nystatin and Amphotericin –B, compared to that of Clotrimazole, Fluconazole and Ketoconazole. 78% *C. albicans* were sensitive to Fluconazole, 5% were dose dependent susceptible, and 17% were resistant. *C. krusei* had shown 33% resistance against fluconazole and67% resistance against Clotrimazole.

Conclusion: The majority of *C. albicans* isolates were susceptible to Fluconazole. *C. krusei* had shown maximum resistance among all the isolates. Identification of Candida to species level and their antifungal susceptibility testing should be done to achieve better clinical results.

Key Words: Vulvovaginal candidiasis (VVC), Antifungal susceptibility testing, Disk diffusion method

INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common fungalinfection that affects healthy women of all ages.[1] At least75% of women will develop one or more infections once during their lifetime, with 5 to 8% of those individuals developing recurrent infections.[1,11]Symptoms generally include itching, burning, soreness and abnormal vaginal discharge.[4]

The principal agent of VVC is *Candida albicans*, but other species known generally as *C.* non-*albicans* (*C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C.gullermondii)* are also isolated.[19]*C. glabrata* is the second most common yeast, and its treatment is considered a serious clinical challenge. [2]*Candida albicans* and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and fungal susceptibility, therefore *Candida* species identification is important for successful management.[3]Prolonged therapy and increased use of antifungal for recurrent candidiasis are the most common risk factors for azoles resistance among *Candida* isolates from vulvovaginitis candidiasis patients.[11]Azoles have the advantage of being taken orally, which increase their potency.[5]

The emergence of drug resistant strains, both chromosomal and plasmid borne, reinforce the need for the study of these pathogens and the surveillance of its susceptibility to drugs commonly used for therapy.[2,9]Close

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Received: 16.04.2015 **Revised:** 02.05.2015 **Accepted:** 25.05.2015

monitoring of the antimicrobial susceptibility and its resistance mechanism is essential in an environment of rapidly changing resistance patterns.[4]

Methods for evaluating the susceptibility of yeasts to antifungal agents have been the subject of numerous studies during the last decade.[15,16]A standard reference procedure has been described by the National Committee for Clinical Laboratory Standards (NCCLS).[13] The reference procedure is a macrotube dilution technique which is too cumbersome for use in most clinical laboratories.[2,15] A broth microdilution adaptation of that procedure has been found to be acceptable.[13,16]Although microdilution tests can usually be read after 24 h, 48-h readings are specified for the NCCLS macrotube test.[14,15]The E test is a proprietary test that has also been found to be capable of giving reliable results that can often be read after 24 h of incubation.[2,16]

For use in a clinical laboratory, a simplified disk diffusion test has some important advantages.[6,8,18]For practical reasons, that disk technique should be similar to the disk procedure that is being used to test antibacterial agents.[8,12,10]

Objectives of this study were to isolate *Candida* from VVC patients, characterization and *in vitro* antifungal susceptibility against six antifungal drugs by the Disk Diffusion method.

MATERIAL AND METHODS

This study was conducted during July 2010 to October 2011 in the Department of Microbiology, SMIMER medical college Surat, Gujarat, India.A total number of 410 OPD patients with complains of vaginal discharge attending department of Obstetrics & Gynaecology are included in the study.Two swabs were collected from each patient with the help of sterile cotton swabs. Specimen was collected from the vagina or cervix avoiding the contamination of other organism. Out of two swabs, one was subjected to direct smear examination, and the other was inoculated on Sabouraud's dextrose agar (SDA) and Blood agar and incubated at 37˚C aerobically. The growth of *Candida*on SDA was confirmed based on colony morphology and gram stain examination. After growth species of Candida were identified.

Species identification- Species identification of Candida isolates was done following standard mycological protocol including germ tube test, fermentation and assimilation of various sugars, chlamydospores production on Corn meal agar and colony color on Hi Chrome Candida agar. Antifungal susceptibility testing- Antifungal susceptibility testing of the isolates was performed by six antifungal drugs disk Fluconazole (25 mcg), Ketoconazole (10 mcg), Itraconazole (10 mcg), Clotrimazole (10 mcg), Nystatin(100 U) and Amphotericin-B (100 U).

Antifungal susceptibility testing was performed by disk diffusion method using Mueller-Hinton Agar + 2% Glucose and 0.5 μg/ml Methylene Blue Dye (GMB) Medium as per CLSI guidelines (C.L.S.I. document M44-A2, 2009.). 0.5 McFarland standard was used to standardize the inoculums density. *C.albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were used as quality control. All the culture media, Antifungal disk, and control strains were obtained from Himedia Laboratories, India.

The antifungal susceptibility of the isolates was interpreted as sensitive (S), dose dependent-susceptible (DDS) and resistant (R). The results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS AND ANALYSIS

During the study period a total of 122 *Candida* spp. were isolated from 410 high vaginal swab processed for isolation and identification of *Candida*.

Out of 122 isolates, 81(66.39%) were *C. albicans*, 19(15.65%) were *C. glabrata*, 12(9.85%) *C. tropicalis*, 6(4.91%) *C. parapsilosis*, 3(2.4%) *C. krusei* and 1(0.8%) were *C. gullermondii*.

In our study All the *Candida* spp. had shown 100% sensitivity against Nystatin and almost 100% sensitivity against Amphotericin-B (Except *C. tropicalis* which showed 83.4% sensitivity to Amphotericin-B). (Table-1, 2)

C. albicans had shown maximum sensitivity of 79% and 78% against Clotrimazole and Fluconazole respectively. It had shown 17% resistance against Fluconazole.

C. glabrata had shown 95% sensitivity against Clotrimazole and 15.78%, 15.70% resistance against Itraconazole and Fluconazole respectively.

C. tropicalis had shown maximum 83% sensitivity against Fluconazole. It had shown 17% resistance against Fluconazole, Ketoconazole & Clotrimazole and 16.60% resistance against Amphotericin-B.

C. parapsilosis had shown 100% sensitivity against Ketoconazole and Amphotericin-B, followed by 83% sensitivity against Clotrimazole and Fluconazole. It had shown 16.60% resistance against Itraconazole.

C.krusei had shown 67% sensitivity against Fluconazole and 67% resistance against Clotrimazole.

C. gullermondii had shown 100% sensitivity against all

the six antifungal drugs used in the study.

In our study overall sensitivity of Candida species against Fluconazole was 79%, Ketoconazole76%, Itraconazole 52%, Clotrimazole 79.5%, Nystatin 100% and Amphotericin-B was 98.36 %.(Table-7)

DISCUSSION

Vulvovaginal candidiasis is one of the most common fungal infections among adult women during their lifetime.[3,20]Clotrimazole and Fluconazole are the two antifungal drugs that are widely used in the treatment of vulvovaginal candidiasis.[5] The main agent of vulvovaginal candidiasis is *C. albicans*; however, it seems non-*albicans* species *(C. glabrata and C. tropicalis)* of *Candida* appear to be increasing.[5,7] *C. glabrata*is the second commonest agent in vaginal infections in most regions.[5]The sensitivity patterns of *Candida* isolates varies among studies in different countries.[2]

In our study Fluconazole had shown 78% sensitivity against *C. albicans*, which is quite comparable with study of *Babin et al*[17](76%),while study done by *Ajitha et al*[3] and *Emam et al*[8] showed higher sensitivity rate of 93.3% and 96.7% respectively. Fluconazole was 33% resistant against *C. krusei*in this study while in study conducted by *Sachin et al*[7] it was 18.8% resistance and in study by *Adesji et al*[1] it was 100% resistance. (Table 3)

Clotrimazole had highest sensitivity 100 % against *C. gullermondii* followed by C. *glabrata* 95%in our study, while 66.67% sensitivity was found in study of *Ajitha et al*[3] against *C. glabrata*. (Table 4)

Ketoconazole had shown maximum resistant 33.5% against *C.krusei* in our study,while it was 25% in study of *Sachin et al*[7]. (Table 5)

Itraconazole was 13.5% resistant among *C. albicans* in our study which is 13.95% in *Babin et al*[17]and15.09% resistance in *Salehei Z et a*l.[5] (Table 6)

Nystatin was 100% sensitive in all *Candida* spp. in our study which is quite comparable with *Emam et al*[8] (100%).

Amphotericin –B was almost 100% sensitive in all *Candida* spp., except *C.tropicalis*83.4% sensitive in our study. Data in *Ajitha et al* [3] was 81.8% and in *Lata et al* [9]75.6%.

Overall, Fluconazole was 79% sensitive, while in study of *Kelen Dota et al*[2] Fluconazole showed 35.5% and in *Dharmik et al*[4] 97.2% sensitivity.(Table 7)

In this study the overall sensitivity of Clotrimazole and Itraconazole were 79.5% and 52% respectively, which were comparable to study of *Dharmik et al*[4] 80% and 57% respectively. (Table 7)

CONCLUSION

This study provides information on species pattern and antifungal susceptibility of Candida species isolated from VVC cases attending Obstetrics and Gynecology OPD of SMIMER Hospital, Gujarat, India. In the present study, though *C. albicans* was the commonest spp. isolated, there was a slight increase in the prevalence of non-albicans Candida spp. Among the non-albicans Candida, *C. glabrata* was the commonest species. Majority of *C. albicans* isolates were susceptible to Fluconazole.

Antifungal susceptibility pattern showed that *Candida* isolates were more sensitive to Amphotericin –B and Nystatin, compared to that of Clotrimazole and Azoles. *C. krusei* had shown maximum resistance among all the Candida species. In vitro susceptibility testing of the yeast to antifungal agents will play a vital role in appropriate selection of antifungal agents for the treatment of fungal infections.

ABBREVIATIONS

VVC-Vulvovaginal Candidiasis

SDA-Sabourad's Dextrose agar

NCCLS-National Committee for Clinical Laboratory Standards

ACKNOWLEDGEMENT

Authors would like to thank Dean and Medical Superintendent, SMIMER Medical College and hospital for allowing us to carry out this study and for providing the facilities and help.

They are also thankful to the Head of Department Obstetrics and Gynecology, SMIMER Medical College and hospital for allowing us to collect the specimens of their patients.

Authors would also like to extend their gratitude to authors whose articles are cited and included in the references of the present study.

They are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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Table 1: Azoles (Fluconazole, Ketoconazole, Itraconazole) susceptibility profile of Candida isolates

	Antifungal agents								
Candida species	**Fluconazole No,%**			**Ketoconazole No,%**			**Itraconazole No,%**		
No,%	**S**	**SDD**	**R**	**S**	**SDD**	**R**	**S**	**SDD**	**R**
C. albicans 81	63	4	14	59	13	9	40	30	11
66.39%	78%	5%	17%	73%	16%	11%	49.38%	37.03%	13.50%
C. glabrata 19	15	1	3	17	1	1	10	6	3
15.65%	79%	5.30%	15.70%	90%	5.00%	5.00%	52.63%	31.57%	15.78%
C. tropicalis 12	10	0	2	9	1	2	8	3	1
9.85%	83%	-	17%	75%	8.00%	17%	66.66%	25%	8.34%
C. parapsilosis 6	5	1	0	6	0	0	4	1	1
4.91%	83%	17%	-	100%	-	-	66.66%	16.66%	16.66%
C. krusei 3	2	0	1	1	1	1	0	2	1
2.4%	67%		33%	33%	33.50%	33.50%		66.66%	33.33%
C. guilliermondii 1	1	0	0	1	0	0	1	0	0
0.8%	100%	-	-	100%	-	-	100%	-	-
Total 122(100 %)	96 79%)	06 (5%)	20 (16%)	93 (76%)	16 (13%)	13 (11%)	63(52%)	42(34%)	17(14%)

R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

Table 2: Clotrimazole, Nystatin, Amphotericin B susceptibility profile of Candida isolates

Candida species No,%	Antifungal agents								
	Clotrimazole No,%			Nystatin No,%			Amphotericin-B No,%		
	S	SDD	R	S	SDD	R	S	SDD	R
C. albicans 81	64	10	7	81	0	0	81	0	0
66.39%	79%	12%	9%	100%			100%		
C. glabrata 19	18	0	1	19	0	0	19	0	0
15.65%	95%		5%	100%			100%		
C. tropicalis 12	9	1	2	12	0	0	10	0	2
9.85%	75%	8%	17%	100%			83.40%		16.60%
C. parapsilosis 6	5	1	0	6	0	0	6	0	0
4.91%	83%	17%		100%			100%		
C. krusei 3	0	1	2	3	0	0	3	0	0
2.4%		33%	67%	100%			100%		
C. guilliermondii 1	1	0	0	1	0	0	1	0	0
0.8%	100%			100%			100%		
Total 122 (100 %)	97 (79.5%)	13 (10.5%)	12 (10%)	122 (100 %)	0	0	120 (98.36%)	0	2 (1.64%)

R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

Table 3: Comparison of Fluconazole susceptibility among Candida isolates

Candida species	Present Study		Pfaller et al[10]		Sachin et al [7]		Adesji et al [1]		Ajitha et al [3]		Emam et al [8]		Salehei Z et al.[5]	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
C. albicans	78	17	98	1.4	-	-	7.69	84.6	93.3	6.66	96.7	0	9.43	81.13
C. glabrata	79	15.7	68.7	15.7	60	27.3	0	50	33.3	66.7	66.6	16.7	0	100
C. tropicalis	83	17	91	4.1	60.7	29.5	16.7	66.7	63.7	36.6	50	0	0	100
C. parapsilosis	83	0	93.2	3.6	90	10	-	-	50	50	-	-	-	-
C. krusei	67	33	8.6	78.3	78.1	18.8	0	100	-	-	0	100	0	100
C. gullermondii	100	0	73.5	11.4	82.6	17.4	-	-	-	-	-	-	-	-

R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

Table 4: Comparison of Clotrimazole susceptibility among Candida isolates

Candida species	Present Study			Ajitha et al [3]			Salehei Z et al.[5]		
	S	Ms	R	S	MS	R	S	Ms	R
C. albicans	79	12	9	80	-	20	77.4	20.8	1.88
C. glabrata	95	0	5	66.67	-	33.3	50	50	0
C. tropicalis	75	8	17	54.54	-	45.5	50	0	50
C. parapsilosis	83	17	0	50	-	50	-	-	-
C. krusei	0	33	67	100	-	-	100	0	0
C. gullermondii	100	0	0	-	-	--	-	-	-

R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

Table 5: Comparison of Ketoconazole susceptibility among Candida isolates

	Present Study			Sachin et al [7]			Salehei Z et al.[5]		
Candida species	**S**	**Ms**	**R**	**S**	**MS**	**R**	**S**	**Ms**	**R**
C. albicans	73	16	11	-	-	-	24.52	32.07	43.39
C. glabrata	90	5	5	45.4	10.9	13.7	37.5	50	12.5
C. tropicalis	75	8	17	45.9	14.7	39.4	0	75	25
C. parapsilosis	100	0	0	90	-	10	-	-	-
C. krusei	33.33	33.5	33.5	62.5	12.5	25	100	0	0
C. gullermondii	100	0	0	78.3	4.3	17.4	-	-	-

R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

Table 6: Comparison of Itraconazole susceptibility among Candida isolates

	Present Study			Sachin et al [7]			Salehei Z et al.[5]		
Candida species	**S**	**Ms**	**R**	**S**	**MS**	**R**	**S**	**Ms**	**R**
C. albicans	49.38	37.03	13.5	-	-	-	7.54	77.35	15.09
C. glabrata	52.63	31.57	15.78	47.3	12.7	40	0	100	0
C. tropicalis	66.66	25	8.34	47.5	9.8	42.6	0	100	0
C. parapsilosis	66.66	16.66	16.66	80	10	10	-	-	-
C. krusei	0	66.66	33.33	56.2	21.9	21.9	0	100	0
C. gullermondii	100	0	0	86.9	-	13.1	-	-	-

R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

Table 7: Comparison of Antifungal susceptibility among Candida isolates

Sensitive	**Present Study**	**K Dota et al [2]**	**Dharmik et al [4]**
Fluconazole	79	35.5	97.2
Ketoconazole	76	83.9	-
Itraconazole	52	22.6	57
Clotrimazole	79.5	-	80
Nystatin	100	100	-
Amphotericin B	98.36	100	-