

## Study of Antifungal susceptibility for Fluconazole and Clotrimazole of Candida Species by disc diffusion method in a Tertiary care centre in Karnataka

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### Abstract

**Background:** Vulvo-vaginitis is a common day to day problem in gynecological practice. *Candida albicans* is the commonest infectious cause. There is limited data regarding the antifungal susceptibility of yeast causing Vulvo-vaginal candidiasis, since isolation by cultures are rarely performed, hence the present study was undertaken. The main objective of this study is to find out the Antifungal susceptibility for Fluconazole and Clotrimazole of *Candida* Species by disc diffusion method among patients visiting Obstetrics and Gynecology Department of AJ Institute of Medical Sciences, Mangalore.

**Methods:** Vaginal swabs were collected from 100 patients visiting the hospital with history of white discharge and subjecting it to microscopy and culture. *Candida* isolates were further identified to species using conventional methods. Disc diffusion testing of each isolate with Fluconazole and Clotrimazole was performed as described in CLSI (formerly NCCLS) document M44-A.

**Results:** In our study antifungal susceptibility test was by disc diffusion method. The sensitivity of the isolates in our study to Clotrimazole was 81% and to Fluconazole was 83.7%.

**Conclusion:** To conclude, as the antifungal resistance does not seem to be a major problem in the general population, it may be appropriate to use courses of antifungal treatment in women with vulvovaginal candidiasis.

**Keywords:** Vulvovaginal Candidiasis; Antifungal Resistance; Antifungal Susceptibility.

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### Introduction

Vulvo-vaginitis is a common day to day problem. Vaginal candidiasis is known variously as Vulvo-Vaginal Candidiasis (VVC), candidal vaginitis, monilial vaginitis, monilia infection, vaginal yeast infection or thrush. *Candida albicans* is the commonest infectious cause producing symptoms and signs of vulvovaginal pruritus, burning irritation, soreness, burning on micturition, dyspareunia, and whitish cheesy discharge. The important feature of candidal vulvovaginitis is the recurrence of the infection, although majority of the cases will respond to treatment<sup>1-4</sup>. With the introduction of antifungal drugs, the causative agents of *Candida* infections shifted from an almost complete dominance of *C. albicans* to the common involvement of other species. Recognition of this change is clinically important, since the various species differ in susceptibility to the newer antifungal agents<sup>2,5</sup>.

Current chemotherapy relies on azoles most used in clinical practice. Members of this family comprise miconazole, ketoconazole, fluconazole, itraconazole

etc., which share common mechanisms of action but display complementary pharmacokinetics.

Fluconazole, first introduced in 1988, is widely used for the prophylaxis and treatment of fungal infections; however, resistance to this drug is an emerging clinical problem and may limit its use in the near future. Resistance has been demonstrated in clinical isolates from patients receiving long-term therapy and is mediated either by alterations in the Ergosterol biosynthetic pathway or by drug efflux mechanisms<sup>6-9</sup>.

Two types of resistance to azoles have been described

1. Primary or 'intrinsic' resistance results from the natural interaction between an organism and the antifungal agent and is independent of previous drug exposure. The most notable example of intrinsic resistance involving azoles is the universal resistance to fluconazole among isolates of *C. krusei*.
2. Secondary or 'acquired' resistance to the azoles remains uncommon except in immunocompromised patients, who are receiving prolonged and/or frequent courses of therapy.

The most common mechanisms include alteration or over expression of the fungal target enzyme (C-14demethylase) of azole drugs, exclusion of drug from fungal cells, and reduction or loss of function of 5-6 desaturase, thereby preventing intracellular accumulation of toxic 14-methoxysterols. Of these mechanisms, prevention of drug entry or extrusion of

the azole via activation of energy-dependent drug-efflux pumps is thought to be the most common. Two groups of multidrug efflux pumps have been identified in clinical isolates:

- a. Major facilitators, which are encoded by the MDR1 (BEN<sub>1</sub>) gene
- b. ATP-binding cassette transporters (ABCT), which are expressed by CDR1 and CDR2 genes in Candida spp.

All azoles, including Ketoconazole, are substrates for ABCT efflux pumps, but current data suggest that only Fluconazole and Ravuconazole are substrates for the major facilitator transport system. Reports of cross resistance to Itraconazole and other azoles in isolates of Candida spp. with acquired resistance to Fluconazole are at least partially explained by the up-regulation of CDR1 genes<sup>10</sup>.

Despite considerable evidence of azole resistance in oral candidiasis due to Candida species, little is known about the azole susceptibilities of the Candida isolates from genital tract isolates responsible for vaginitis. There is limited data regarding the antifungal susceptibility of yeast causing vulvo-vaginal candidiasis, since cultures are rarely performed. Hence the present study was undertaken to determine the Antifungal susceptibility to Fluconazole and Clotrimazole by disc diffusion method.

### Objectives

1. To determine the Antifungal susceptibility to Fluconazole and Clotrimazole of Candida species by disc diffusion method.

### Materials and Methods

The present study was done in the Department of Obstetrics and Gynaecology, AJ Medical College, Mangalore.

**Patient:** A detailed history was taken with a particular emphasis on the pregnancy, high risk behaviours, contraceptive methods used, recent antibiotic treatment, past history of chronic diseases such as Diabetes mellitus, economical status, hygiene etc. Thorough physical examination was carried out. Vital signs were noted. Presence of signs of other opportunistic infection was looked for and associated diseases if any were recorded. Each woman was subjected to per speculum examination, and the clinical characteristics of the vaginal discharge were noted. Written informed consent was obtained from each patient.

**Inclusion Criteria:** The inclusion criteria for the study were married women, between 18-65 years of age who presented to the health care center with self-reported symptoms of vaginal discharge and/ Oro-genital itching and/or genital burning during the study period.

**Exclusion Criteria:** Women with severe medical disorders requiring immediate referral to higher level of healthcare, women who were currently menstruating, who have never been sexually active, women who had

taken a course of antifungal during past three weeks and who had been previously enrolled in this study were excluded.

**Control:** Hundred age matched controls were included in the study to know the prevalence of colonization of Candida in healthy individuals. Samples of the vaginal discharge collected were processed as follows-

**Sample collection:** Two samples of the high vaginal secretions were taken with aseptic precautions with the help of sterile cotton swabs. One swab was used for pH evaluation and for direct microscopy and the other swab was used for inoculation onto Sabourauds Dextrose Agar with Chloramphenicol (Himedia)

**Culture:** SDA slants were incubated at 37<sup>o</sup> C for 48-72 hours and observed for growth. Those slants which were showing no growth after 72 hours were recorded as no growth and discarded. The SDA slants with growth were observed for colony morphology and were further processed for speciation.

### Antifungal Susceptibility Test

Disc diffusion testing of each isolate with Fluconazole and Clotrimazole was performed as described in CLSI (formerly NCCLS) document M44-A (2).

Mueller-Hinton agar plates supplemented with 2% glucose and methylene blue (HIMEDIA, Antimycotic Sensitivity Test Agar) was used. The agar surface was inoculated using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. The plates were incubated at 35°C and read at 24 h. Zone diameter endpoints were read manually with calipers at 80% growth inhibition.

The interpretive criteria for Fluconazole disc diffusion testing were those published by the CLSI and were as follows:

- Susceptible, zone diameter of >19 mm;
- Susceptible dose dependent, zone diameter of 15 - 18 mm;
- Resistant, zone diameter of <14 mm.

Provisional interpretive criteria were used for Clotrimazole,

- C.albicans (ATCC 90028) - 18-32mm
- C.parapsilosis (ATCC 22019) - 16-30mm
- C.tropicalis (ATCC 750)-10-20mm
- C.krusei (ATCC 6258) - 14-24mm

A total of 100 patients (study group) visiting A.J. Institute of Medical Sciences to the outpatient block and also admitted to Gynecological wards, having complaints of white discharge per vagina were included in this study based on the inclusion and exclusion criteria. Hundred age matched controls were also included for the study. The data was analyzed using appropriate statistical methods and discussed here after.

### Results and Observation

The age (in years) ranged from 18 to 65 years. The majority of the cases with white discharge per vagina were seen in the age group of 30 to 39 years (38%) followed by 20-29 years (32%) as shown in (Table 1). The mean+ SD of age (in years) in the study group was 33.27+10.7 years and in the control group was 34+10.2 years ( $p > 0.05$ ). Among the locale distribution of study group, about 55% of the patients belonged to rural area, and 45% were from urban area (Table 2). The literacy status of the study group shows that majority of the study group were illiterates or studied upto 5<sup>th</sup> STD (Table 3). Among the study group, about 29% of the cases had infection due to Candida and 28% showed bacterial causative. 5% of cases showed both mixed growth of Candida and bacteria. About 38% of the cases showed no growth (Table 4).

100 High vaginal swab samples collected from the study group, when subjected to Gram staining 32 samples showed Gram positive yeast like budding cells with/ or without pseudo-hyphae. On culture 34 samples grew Candida. Of 100 samples collected from the control group 2 samples showed Candida on Gram staining and 4 samples were culture positive. On inoculating the Candida isolates on the CHROM agar media, 24 isolates showed light green color indicative

of *C.albicans*, Dark blue color was seen 9 isolates suggestive of *C.tropicalis*, Pale pink colored colonies of *C.krusei* was seen in 2 cases and Two of isolates showed cream – pale white color colonies, suggestive of *C.glabrata/ C.parapsilosis/ C.gulliermondii/ C.stellatoidea* species. Among these, isolates from 3 samples showed colonies of more than one color, suggestive of mixed infection. Hence from 34 cases of vaginal candidiasis, total number of isolates was 37.

Antifungal susceptibility test was conducted by the disc diffusion method. The drugs tested were Clotrimazole and Fluconazole. Of 24 isolated, 19 *C.albicans* were sensitive to the drug Clotrimazole, 5 of the isolates showed resistance. All the 9 isolates of *C.tropicalis* were sensitive to Clotrimazole. Both isolates of *C.krusei* showed resistance pattern. One isolate of each *C.glabrata* and *C.parapsilosis* were sensitive to the drug (Table 5). Of the 24 isolates of *C.albicans*, 20 were sensitive to Fluconazole (83%) and 4 were resistant. All the 9 isolates of *C.tropicalis* (100%) were found to be sensitive. *C.glabrata* (100%) and *C.parapsilosis* (100%) were sensitive to Fluconazole (Table 6). Both the isolates of *C.krusei* (100%) were resistant to the drug. *C.albicans* isolates from the control group were sensitive to both the drugs (100%).

**Table 1: Age (in years) distribution among study and control group**

Age(in years)	Study group		Control	
	Number	Percentage	Number	Percentage
Below 20	8	8 %	8	8%
20-29	32	32%	32	32%
30-39	38	38%	38	38%
40-49	12	12 %	12	12%
50 and above	10	10%	10	10%

**Table 2: Locale distribution in the study group**

Locale	Number	Percentage
Urban	45	45%
Rural	55	55%

**Table 3: Literacy status of the study group**

Literacy status	Number	Percentage
Illiterate	13	13%
Literate up to 5 <sup>th</sup> std	60	60%
Up to 12 <sup>th</sup> std	21	21%
Graduate and above	06	6%

**Table 4: Leucorrhoea due to various causatives based on culture**

	Number	Percentage
Candida infection	29	29%
Bacterial infection	28	28%
Mixed infections	5	5%
No growth	38	38%

**Table 5: Different species of candida on CHROM agar**

Colony color on CHROM agar	Species identification	Number	Percentage
Light green	<i>C. albicans</i>	24	65
Dark blue with a halo	<i>C. tropicalis</i>	9	24
Pale pink	<i>C. krusei</i>	2	5
Cream / pale white	<i>C. glabarata</i>	1	3
	<i>C. parapsilosis</i>	1	3

**Table 6: Antifungal susceptibility of isolates to Clotrimazole**

Species	Sensitive		Resistant		Total
	No	Percentage	No	Percentage	
<i>C.albicans</i>	19	79%	5	21%	24
<i>C.tropicalis</i>	9	100%	0	0%	9
<i>C.krusei</i>	1	50%	1	50%	2
<i>C. glabarata</i>	1	100%	0	0%	1
<i>C.parapsilosis</i>	1	100%	0	0%	1

**Table 7: Antifungal susceptibility of isolates to Fluconazole**

Species	Sensitive		Resistant		Total
	No	Percentage	No	Percentage	
<i>C.albicans</i>	20	83%	4	17%	24
<i>C.tropicalis</i>	9	100%	0	0%	9
<i>C.krusei</i>	0	0%	2	100%	2
<i>C. glabarata</i>	1	100%	0	0%	1
<i>C.parapsilosis</i>	1	100%	0	0%	1

### Discussion

Our study showed that maximum number of cases presenting with leucorrhoea belonged to the age group of 30-39 years, which is comparable to the study done by Puri KJ et al<sup>6</sup> (20-40 years). The mean age distribution in our study was 33.27+10.7 years which can be compared to the mean age 30 years in a study done by Norma T. Gross et al<sup>7</sup>. Since this hospital serves free of cost to the patients and is accessible to the rural population, more number of cases from rural cases are seen in our study as compared to the urban population.

In our study Candida was found to be the causative organism in 29 cases (29%) and in 5 (5%) cases both Candida and bacterial causes were identified. Hence the incidence of vaginal candidiasis was 34%. The incidence of Candida in cases with white discharge per vagina is 34% which is significantly higher than Candida isolated from healthy group (4%) (p<0.05). This can be compared with the study done by Puri KJ, Madanet al<sup>6</sup> (31%), Stephanie Weissenbacher et al<sup>8</sup> (31.1%) and Kamara et al<sup>9</sup> (30.7%).

Studies have been done by Semra Kustimur et al<sup>11</sup> to evaluate Disc diffusion method in comparison with MIC for Fluconazole susceptibility to *Candida* strains. Disc diffusion correlation was 91% between the Minimal Inhibitory Concentrations obtained from macrodilution and disc diffusion zone diameters. The disc diffusion test was evaluated as a low-cost,

reproducible, and efficient way of assessing the in vitro susceptibility of *Candida* strains to Fluconazole.

In our study antifungal susceptibility test was by disc diffusion method. The sensitivity of the isolates in our study to Clotrimazole was 81% and to Fluconazole was 83.7%.

Our study showed, 19 isolates of 24 *C.albicans* (79%), all 9 isolates of *C.tropicalis* (100%), 1 isolate of both *C.glabrata* (100%) and *C.parapsilosis* (100%) were sensitive to the drug Clotrimazole. Both the isolates of *C.krusei* showed resistance to Clotrimazole. *C.albicans* (83%), *C.tropicalis* (100%), *C.glabrata* (100%) and *C.parapsilosis* (100%) were found to be sensitive to Fluconazole. *C.krusei* is inherently resistant to the Fluconazole our study also showed similar results. In the study done by S S El-Din et al<sup>12</sup>, none of the isolates recovered from the women with vaginal candidiasis demonstrated resistance to either Clotrimazole or Fluconazole. In a study by Yuan Xin-rong et al<sup>13</sup> showed that sensitivity of candida isolates from recurrent vaginal candidiasis to Clotrimazole was 92.5%, to Fluconazole was 81.3 %. The study done by Norma T. Gross et al<sup>14</sup>, showed majority of vaginal *Candida* isolates were susceptible to ketoconazole (91%), Fluconazole (96.5%), and Itraconazole (98%). In the study done by Sandra S. Richter et al<sup>15</sup>, all *C.albicans* vaginal isolates were sensitive to Fluconazole (100%).

## Conclusion

Most of the *C.albicans* species isolated showed sensitivity to antifungal drugs Clotrimazole and Fluconazole. Among the non-albicans species *C.tropicalis*, *C.glabarata* and *C.parapsilosis* were found to be sensitive whereas *C.krusei* showed resistance to Clotrimazole and Fluconazole. As antifungal resistance does not seem to be a problem in the general population, it may be appropriate to use courses of antifungal treatment in women with vulvovaginal candidiasis.

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**Conflict of interest:** Nil

**Ethical Clearance:** Taken from the Institute

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