

## Effect of Antifungal Drugs against *Candida* Isolates from Diabetic Women with Vaginitis

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### Abstract

Vaginitis is an inflammation of the vagina characterized by a whitish or whitish gray discharge, often with a curd-like appearance, odor, itching and irritation. Yeasts are always present in the vagina in small numbers, and symptoms only appear with overgrowth. The vaginal swab specimens were collected from the diabetic women with signs and symptoms of vaginitis. The isolated *Candida* species were identified by the morphological and cultural characteristics. The isolated and identified *Candida* strains were included *C. albicans*, *C. dubliniensis*, *C. krusei*, and *C. tropicalis* from diabetic women with signs and symptoms of vaginitis. The isolates were subjected to determine the *in vitro* anticandidal activity against antifungal drugs. All *Candida* strains isolated from diabetic women with vaginitis were 100% sensitive to Amphotericin-B and *C. krusei* strains showed 100% sensitivity to ketoconazole drug. Overall 32% of *Candida* strains showed multidrug resistance against the antifungal drugs we used.

**Keywords:** Antifungal drugs; *Candida*; Germ tube production; Multidrug resistance; Vaginitis

### Introduction

Vaginitis, also known as vaginal infection and vulvovaginitis, is an inflammation of the vagina and possible vulva. It can result in discharge, itching, and pain, and is often associated with an irritation or infection of the vulva. The discharge may be excessive in amounts or abnormal in color (such as yellow, gray, or green). One of the problems of women with diabetes is resistant vulvovaginitis, which is related to some factors such as hyperglycemia, allergy, and atopy. Diabetes is a known predisposing factor of vulvovaginal candidiasis, primarily because of hyperglycemia-induced alterations, including decreased random motion of neutrophils, chemotaxis, phagocytosis, and microbial killing. In addition, increased glucose levels in genital tissues enhance yeast adhesion and growth. Several factors are associated with increased symptomatic infection in women, including pregnancy, iron deficiency anaemia, uncontrolled diabetes mellitus, and the use of oral contraceptives or antibiotics [1,2]. Diabetes is a proven predisposing factor for vulvovaginal candidiasis, along with pregnancy, use of broad-spectrum antibiotics, high-estrogen-dose oral contraceptives, obesity and drug addiction [3]. Symptomatic vulvovaginal candidal infections have been shown to be more prevalent in patients with diabetes than in the general population [4] and are usually attributed to "yeast infection" by the women themselves [5].

In clinical settings, candidiasis is commonly treated with antimycotics-the antifungal drugs commonly used to treat candidiasis are topical clotrimazole, topical nystatin, fluconazole, and topical ketoconazole. It is possible for *Candida albicans* to develop a resistance to the drugs used to treat it, as seen from research done involving fluconazole, one of the drugs that is used to treat candidiasis and noted about the emergence of fluconazole resistant *Candida* species and quite remarkable percentage of yeasts and molds developed multidrug

resistance [6]. In this study, the effect of antifungal drugs against *Candida* isolates from diabetic women with vaginitis was assessed.

### Materials and Methods

#### Selection criteria

The inclusion criteria for the study, Patients with signs and symptoms of diabetic women (n=170) and Non diabetic normal healthy women without any signs and symptoms of vaginitis as control (n=50) were selected and took part in this study.

#### Specimen collection and transportation

The vaginal swab specimens were collected from the diabetic women with signs and symptoms of vaginitis. The specified routine procedure was followed; two vaginal swabs were collected from the posterior fornix of the vagina. One swab was used for smear preparation for gram staining and 10% KOH examination and the second swab were used for culture. Care was taken to avoid the collection of specimen from the diabetic women undergone any prior antibiotic therapy for atleast one week. The samples were safely transported to the laboratory under aseptic conditions for further proceedings.

#### Direct microscopic examination

**10% KOH wet mount:** A small portion of vaginal specimen was placed on clean glass slide, one or two drops of 10% Potassium hydroxide (KOH) solution was added and allowed to stand after 10-15 minutes and then examined for the presence of budding yeasts with or without pseudohyphae.

**Gram stain:** The smear made on the glass slide was allowed for air and heat fixation and submitted for gram staining procedure. Presence

of gram-positive budding yeasts with or without pseudohyphae, number/grades of the yeast cells and the adherence pattern of the yeast cells on the epithelial cells were noted and recorded.

### Isolation of *Candida*

The Vaginal specimen collected from the diabetic patients and normal healthy women were cultured on Sabouraud's Dextrose Agar (SDA) with antibiotic Chloramphenicol (50 µg/ml) and incubated at 37°C for one week. *Candida* growth on Sabouraud's Dextrose Agar (SDA) was examined from second day onwards and recorded.

### Identification of *Candida* species

The isolated *Candida* species were identified by the routine methods [7] i.e., Colony morphology in Sabouraud's Dextrose Agar (SDA), Growth characteristics in Sabouraud's Dextrose Broth (SDB), Germ tube production, Chlamyospore formation on corn meal agar, Sugar fermentation and Sugar assimilation [7-11]. The isolated and identified *Candida* strains from the patients and controls were included *C. albicans*, *C. dubliniensis*, *C. krusei*, and *C. tropicalis* were subjected to determine the *in vitro* anticandidal activity of antifungal drugs.

### In vitro antifungal drug susceptibility testing

**Inoculum preparation:** *Candida* species/strains inoculum was prepared by suspending several colonies of grown cultures on Sabouraud's Dextrose Agar (SDA) in phosphate buffered saline pH 7.2. Inoculum standardization was done using standard procedure in which the fungal suspensions were adjusted spectrophotometrically to an absorbance of 0.6 at 530 nm, which corresponds with the 0.5 McFarland standards.

**Disk diffusion method:** The commercially available following antifungal discs were Clotrimazole, Ketoconazole, Fluconazole, Nystatin and Amphotericin-B were used in this study. Each SDA plate was inoculated with the standard inoculum suspensions by soaking a swab and rotating it over the agar plate. The antifungal discs were placed over the inoculated agar. After 48 hours of incubation at 31°C zone of inhibition of growth was measured and recorded. A duplicate was also carried out [7]. The *Candida* strains resistant to four or more antifungal drugs were considered as multidrug resistant strains.

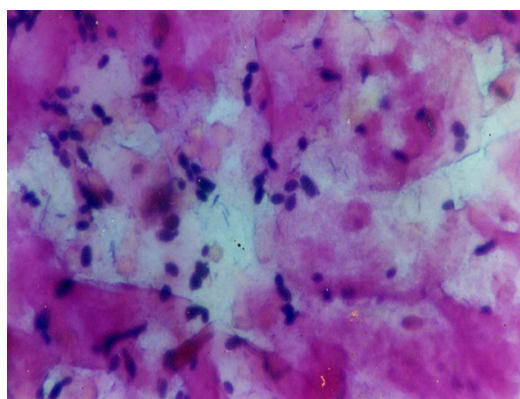
## Results

### Direct microscopic examination

**10% KOH wet mount examination:** All 170 (100%) vaginal specimens of patients showed the presence of either budding yeasts

alone or with pseudohyphae. Only 15 (30%) of control specimens showed only budding yeasts.

**Gram staining:** Among 170 patients vaginal specimens, 85% (n=145) of them showed gram positive budding yeasts heavily adhered on vaginal epithelial cells (Figure 1) and 9% (n=15) of the smear showed the presence of pseudohyphae along with the gram positive budding yeasts and 6% (n=10) of the smear showed less number of yeast cells adhered on vaginal epithelial cells. Only 12% (n=6) of the vaginal smear of the controls showed the presence of gram positive budding yeasts (rare cells) without pseudohyphae and non-adherent to the vaginal epithelial cells.



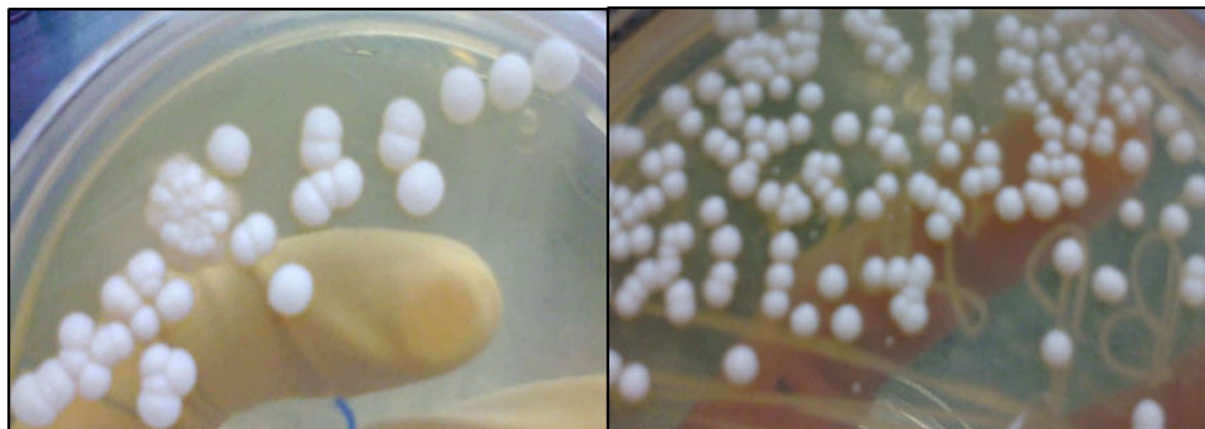
**Figure 1:** Gram stained vaginal smear from the diabetic women with vaginitis—Showing heavy load of gram positive budding yeasts adhered on the vaginal epithelial cells.

### Culture results

All the 170 patients vaginal specimens yielded 100% *Candida* growth whereas only 24% (n=12) *k* growth observed in the control specimens (50).

### Significant and insignificant *Candida* growth

All 170 vaginal samples from patients yielded 100% significant *Candida* growth (more than 10 colonies) on SDA (Figure 2). Among 50 control specimens, 24% (n=12) yielded *Candida* growth on SDA. Among which 8% (n=4) showed significant *Candida* growth, 16% (n=8) showed insignificant *Candida* growth (less than 10 colonies) and 76% (n=38) showed no growth.

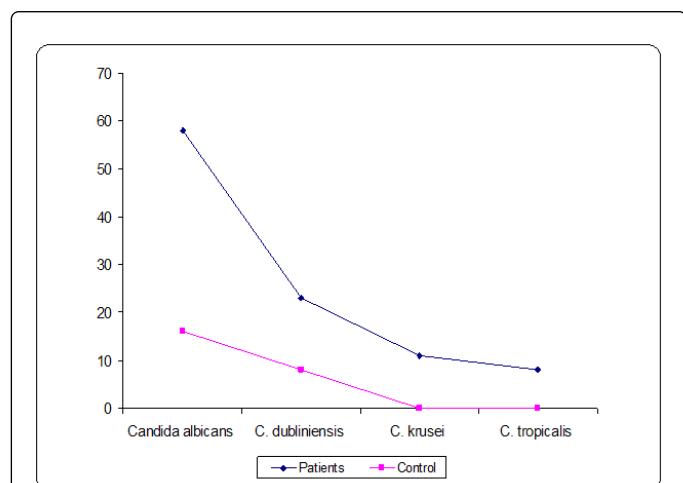


**Figure 2:** Significant *Candida* growth—Culture of vaginal specimen from the diabetic women with vaginitis showing the appearance of more than 10 *Candida* colonies on SDA.

### Predominant *Candida* species identification

The isolated *Candida* strains from patients were identified as *Candida albicans* (58%), *Candida dubliniensis* (23%), *Candida krusei* (11%) and *Candida tropicalis* (8%). *Candida* strains isolated from controls were identified as *C. albicans* (16%) and *C. dubliniensis* (8%) (Figure 3).

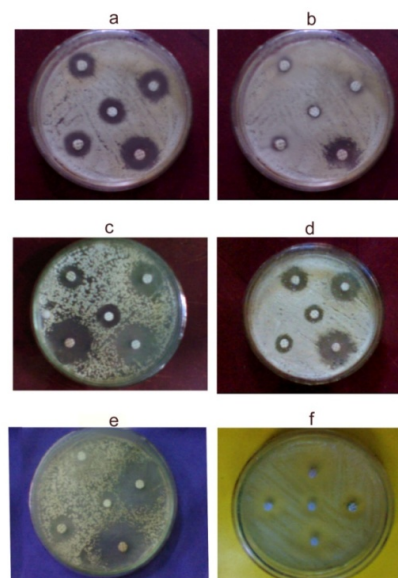
and *C. krusei* strains (all) showed 100% sensitivity to ketaconazole drug (Table 1 and Figure 5). Overall 32% of *Candida* strains showed multidrug resistance against the antifungal drugs we used.



**Figure 3:** Predominant *Candida* species from patients and controls.

### Antifungal drug sensitivity test

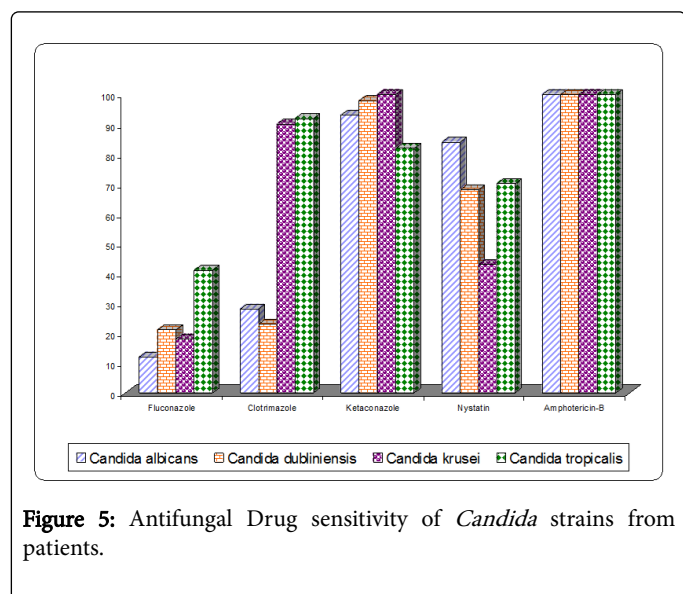
Totally 170 *Candida* strains (different species) from patients and 50 strains from healthy controls were subjected to anti-fungal sensitivity test. Out of 170 *Candida* strains, 26% (n=45) of them showed resistance to more than three or four anti-fungal drugs used (Figure 4). *C. albicans* (n=12) 12%, *C. dubliniensis* (n=24) 60%, *C. krusei* (n=6) 33%, *C. tropicalis* (n=3) 21%, showed multidrug resistance to five antifungal drugs used. All *Candida* species/ strains isolated from diabetic women with vaginitis were 100% sensitive to Amphotericin-B



**Figure 4:** *Candida* strains isolated from the diabetic women with vaginitis—Showing the different types of susceptibility pattern to the different antifungal drugs.

S.No	Candida isolates n=170	Antifungal drugs				
		Fluconazole	Clotrimazole	Ketaconazole	Nystatin	Amphotericin-B
1.	<i>C. albicans</i> (n=98)	12% S	28% S	93% S	84% S	100% S
2.	<i>C. dubliniensis</i> (n=40)	21% S	23% S	98% S	68% S	100% S
3.	<i>C. krusei</i> (n=18)	18% S	90% S	100% S	43% S	100% S
4.	<i>C. tropicalis</i> (n=14)	41% S	92% S	82% S	70% S	100% S

**Table 1:** Antifungal drug sensitivity tests of *Candida* isolated from the diabetic women with signs and symptoms of vaginitis.



**Figure 5:** Antifungal Drug sensitivity of *Candida* strains from patients.

## Discussion

Candidiasis is the general term used to indicate the different types of *Candida* infections and the vaginal candidiasis is the condition in which the *Candida* involves and cause vaginitis. Hyperglycemia is the major cause of increased susceptibility of the diabetic patients to vulvovaginal candidiasis. In our study all (100%) vaginal specimens showed either budding yeasts or budding yeasts with pseudohyphae. This indicates the association of *Candida* in vaginitis and the presence of pseudohyphae indicates the diseased status of the patients and the involvement of the *Candida* in vaginitis. It has been published that the production of germ tube and the pseudomycelium is due to the virulent and pathogenic nature of the *Candida* [6].

Udhaya et al. in their study note that the only 78% of the vaginal specimens (collected from the cancer cervix cases) and yielded *Candida* growth on SDA. But in our study we could able to get different results that, all vaginal specimens (100%) yielded *Candida* growth on SDA. Our result gains the support of the earlier publications that it is not uncommon the presence and the colonization of these *Candida* yeasts in the hyperglycemic conditions [12-17]. And the earlier study of Janani et al. confirms and supports our result evidently that she could able to isolate 100% *Candida* growth from the vaginal specimens of the asymptomatic diabetic women.

Janani et al. stated that the *Candida* strains isolated from asymptomatic diabetic women with vaginal candidiasis showed 57% of them resistance to more than three or four antifungal drugs used. *C. albicans* (92%) showed multidrug resistance. *C. krusei* (33%) and *C. dubliniensis* (42%) showed multidrug resistance. But our study result indicates that the *C. albicans* (12%), *C. dubliniensis* (60%), *C. krusei* (33%) and *C. tropicalis* (21%) showed the multidrug resistance against the antifungal drugs we used. It was quite obvious to note that the resistance towards clotrimazole and fluconazole was seems to be more when compare to other antifungal drugs we used (Table 1). There was another distinguishable result, we obtained that all *C. krusei* strains were sensitive to the drug ketaconazole (100%) (Table 1).

In our study, we also state that all vaginal specimens of the diabetic women with vaginitis yielded 100% significant *Candida* growth and the predominating species noted were *C. albicans* (58%) and 32% of multidrug resistance was noted to the multidrug resistant *Candida* strains. Most study data indicates that overall yeast carriage and infection rates increase in diabetes patients and correlate with degree of glucose control. Thus, good control of blood glucose and treatment with an appropriate antifungal agent are important in the management of vaginal candidal infections in diabetic women. Because most of the oral and topical azoles used to treat vulvovaginitis result in comparable efficacy rates, selection of therapy should be based on factors such as causative organisms, safety and side effects, the potential for drug interactions, and patient preferences.

Since the risk of infection and healing process with diabetic patients are more, the prophylactic measures and frequent assessment of the therapy regimen is seems to be much more essential. For which the regular and revised study of the antibiotic or antimicrobial sensitivity is essential. And to overcome the problem of multidrug resistance, the search for the new antimicrobial is also need in this field.

## Summary and Conclusion

Totally 220 vaginal swabs were collected from the patients and controls were subjected in this study and categorized as diabetic women with signs and symptoms of vaginitis - 170 and non-Diabetic normal healthy women without signs and symptoms of vaginitis - 50. 100% of the diabetic women yielded *Candida* growth whereas only 24% of non-diabetic normal healthy women vaginal specimen yielded *Candida* growth on SDA. Significant *Candida* growth (more than 10 *Candida* colonies) was obtained from the both diabetic women with vaginitis and non-diabetic normal healthy women. *Candida* strains isolated from the patients were identified as *Candida albicans* (58%), *Candida dubliniensis* (23%), *Candida krusei* (11%) and *Candida tropicalis* (8%) and in controls the isolated *Candida* strains were

identified as *Candida albicans* (16%) *Candida dubliniensis* (8%). Among the *Candida* species isolated, *Candida albicans* (58%) and *Candida dubliniensis* (23%) was the predominant *Candida* species isolated from the diabetic women with signs and symptoms of vaginitis.

All *Candida* species/strains isolated from diabetic women with vaginitis were 100% sensitive to Amphotericin-B and *C. krusei* strains (all) showed 100% sensitivity to ketoconazole drug. Overall 68% of *Candida* strains isolated from the diabetic women with vaginitis shown sensitivity towards antifungal drugs and 32% showed multidrug resistance against antifungal drugs we used.

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