

Original Research Article

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Isolation and Sensitivity Pattern of Genital *Candida* Species in STD OP Female Attendees of Tertiary Care Hospital, Chennai, India

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ABSTRACT

Keywords

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The study was aimed to isolate *Candida* species from vaginal discharge of female patients with complaints of itching and to determine susceptibility pattern of vaginal isolates of *Candida* to antifungal drugs. A total of 400 vaginal discharge samples from all the female patients attending STD OP, Institute of Venereology, RGGGH / Madras Medical College, Chennai were included in this study. All the samples were subjected to KOH mount, cultured on Sabouraud's Dextrose Agar, incubated overnight at room temperature, further gram stain and germ tube test were performed and subcultured on Hichrome *Candida* differentiation agar (M1297A). The species were differentiated by the morphology and colour of colonies. Antifungal susceptibility testing was done for all the species. Out of 400 samples, 83 patients had *Candida* isolations: *C.glabrata* – cream coloured colonies 37(45%), *C.albicans* – green coloured colonies 25(30%), *C.tropicalis* – blue coloured colonies 14(17%), *C.krusei* – pink coloured colonies 7(8%). All 83 isolates showed sensitivity to miconazole and nystatin, 39 to itraconazole, 6 to clotrimazole, 1 to fluconazole and nil with ketoconazole.

Introduction

Fungal infections have been increasingly reported worldwide. The emergence of fungal infections has been attributed to advancement in medical management and increasing load of severely ill patients. The last few decades have seen a steady increase in the incidence of fungal infections especially *Candida* species.

Candida species are normal commensals of human skin, throat, gastrointestinal tract, female genital tract and urinary tract with

catheters. *Candida* is one of the common opportunistic fungal infections.

Candidiasis is the most common fungal disease with wide spectrum of clinical presentation. The infections may be acute or chronic, superficial or deep involving various systems.

Vaginal candidiasis is a common problem for women and may affect their physical and emotional health. Several predisposing

factors such as diabetes mellitus, uterine contraceptives, pregnancy and broad spectrum antibiotics are reported as risk factors for candidial infection. Vulvovaginal candidiasis remains a common cause of morbidity among adult women. While the main etiological agents of vaginal candidiasis is being changing from *C.albicans* to other non-albicans species, Knowledge of identification of patterns of genital *Candida* species and drug resistant candidial isolates is important for management.

The present study was carried out to isolate the species and test the susceptibility pattern of *Candida* isolates from the vaginal discharge.

Materials and Methods

A total of 400 vaginal discharge samples were collected from all the female patients attending STD OP, Institute of Venereology, RGGGH/ Madras Medical College, Chennai. The swabs were subjected to KOH mount, cultured on Sabouraud's Dextrose Agar and incubated at room temperature overnight. SDA culture showing confluent heavy growth with cream colored, pasty colonies suggesting *Candida* were further characterized. Gram stain and germ tube test were done for all the isolates. The colonies were further subcultured on to Hichrome agar and incubated at room temperature (25°C) overnight. The plates were further incubated for 48 hours to get better developed colored colonies. Presumptive identification of *Candida* species was made by distinctive color and morphology of the colonies as per the manufacturer's instructions. The antifungal susceptibility testing was done on SDA by disc diffusion method (Zahra Salehei *et al.*, 2012). A suspension of overnight cultures of *Candida* isolates was prepared in sterile normal saline. The suspension was inoculated on

SDA plates and rolled on the surface of the agar medium. The plates were dried for 15 minutes at room temperature in laminar hood and then the antifungal discs were placed on the inoculated agar with sterile forceps. The plates were incubated at room temperature for 24 hours and zone diameters were measured manually [Table 3].

The antifungal discs used were Nystatin 100 units/disc, Miconazole 50µg, Itraconazole 10µg, Clotrimazole 50µg, Fluconazole/ Ketoconazole 10µg each (Hi Media).

Results and Discussion

In the present study, among 400 samples - 83(21%) had candidiasis. These clinical isolates namely *C.glabrata* – cream coloured 37 (45%), *C.albicans* – green coloured 25(30%), *C.tropicalis* – blue coloured 14(17%) and *C.krusei* – pink coloured 7(8%) colonies were identified (table 1). Some isolates showed pseudo hyphae and yeast cells, and some isolates showed only yeast cells without pseudo hyphae. There were few differences in microscopic morphology of yeast cells. *C.tropicalis* and *C.krusei* showed large ovoid yeast cells. *C.albicans* and *C.glabrata* species showed round budding yeast cells.

All the isolates were sensitive to miconazole and nystatin. 39 isolates showed sensitivity to itraconazole, 6 to clotrimazole, 1 to fluconazole and none to ketoconazole. (Table 2).

For *C.albicans*, out of 25/83 isolates 1 isolate showed sensitivity to fluconazole, 25 showed sensitivity to miconazole and nystatin, 16 to itraconazole and 4 to clotrimazole.

All the *C.glabrata* isolates 37/83 showed sensitivity to miconazole and nystatin, 2 for clotrimazole and 13 for itraconazole.

(Figure.1). All the 14/83 isolates of *C.tropicalis* showed sensitivity to miconazole and nystatin and 7 isolates to

itraconazole. None of the isolates were sensitive to ketoconazole, fluconazole and clotrimazole.

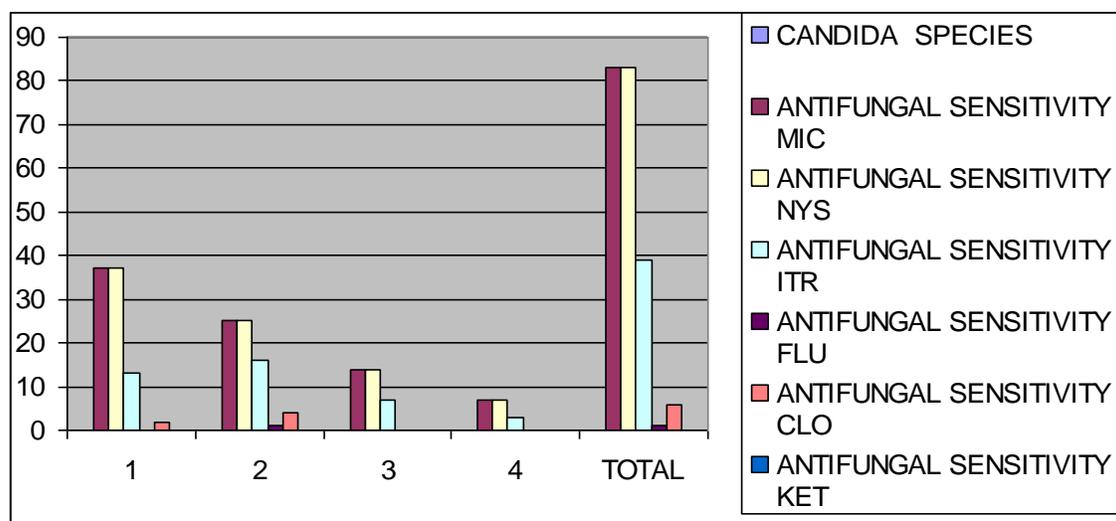
Table.1 Candida speciation using Hi Chrome Candida differentiation media

S.NO	NAME OF THE SPECIES	COLOUR	NO.OF ISOLATES	%
1	<i>C.glabrata</i>	Cream	37	45%
2	<i>C.albicans</i>	Green	25	30%
3	<i>C.tropicalis</i>	Blue	14	17%
4	<i>C.krusei</i>	Pink	7	8%

Table.2 The sensitivity pattern of Candida species to various antifungal agents.

S.No	CANDIDA SPECIES	ANTIFUNGAL SENSITIVITY					
		MIC	NYS	ITR	FLU	CLO	KET
1	<i>C.glabrata</i>	37	37	13	0	2	0
2	<i>C.albicans</i>	25	25	16	1	4	0
3	<i>C.tropicalis</i>	14	14	7	0	0	0
4	<i>C.krusei</i>	7	7	3	0	0	0
TOTAL		83	83	39	1	6	0

Fig.1 The antifungal susceptibility pattern of Candida species.



Out of 7/83 isolates of *C.krusei*, all 7 showed sensitivity to miconazole and nystatin and 3 to itraconazole. Candidial vaginitis is a common fungal infection among adult women during reproductive age. Fortunately, the infection is rarely life threatening but usually associated with morbidities like discomfort, pain, sexual

dysfunctions, vulval dryness, cracks, itching, burning, soreness and finally health care cost (Paulitsch *et al.*, 2006).

Earlier studies found that *C.albicans*, the most common isolate as a main agent of vaginal candidiasis (Richter *et al.*, 2005). However; it seems that non albicans species

(*C.glabrata*, *C.tropicalis*, *C.krusei*) appear to be in increase (Defontaine *et al.*, 1999). This is correlating with our study. In other studies *C.krusei* and *C.glabrata* of *Candida* species appear to be increasing. However (Mohanty *et al.*, 2007) has reported *C.glabrata* as the main etiology of VVC which is correlating with our study.

Clotrimazole and Fluconazole are the two antifungal drugs widely used in the treatment of VVC. The sensitivity patterns of *Candida* isolates varies among studies in different countries. Quindos *et al.*, (1999) study showed that the *Candida* species were sensitive to Fluconazole and Ketoconazole. Zahra Seifi *et al.*, (2012) study showed *Candida* species were resistant to Fluconazole. Our results showed that the *Candida* isolates were resistant to Fluconazole and Ketoconazole. This study indicated that *C. glabrata* was the predominant isolate and that the drug pattern are changing. Fluconazole the systemic drug of choice for genital candidiasis is less sensitive and resistant whereas Miconazole and Nystatin are highly sensitive.

Further studies are required for confirming the drug sensitivity pattern, so that appropriate antifungal drugs could be selected to manage candidiasis and to prevent its recurrence and the morbidity in the patients. Further critical studies with large sample size are needed.

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