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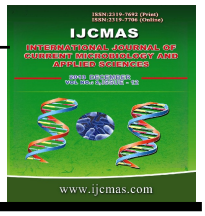

Original Research Article

Vulvovaginal Candidiasis due to non *albicans Candida*: its species distribution and antifungal susceptibility profile

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Keywords

Antifungal susceptibility; *Candida albicans*; Non *albicans Candida* species; species identification; vulvovaginal candidiasis.

A B S T R A C T

Candida species are usually commensals but at many times may cause opportunistic infection. Vulvovaginal candidiasis (VVC) is the most common manifestation of genital candidiasis. *Candida albicans* is the cause of VVC in majority of cases, but recent studies document the increasing episodes of vulvovaginitis due to non *albicans Candida* (NAC) species. As most clinical microbiology laboratories do not perform species identification and antifungal sensitivity testing of *Candida* spp. on routine basis there is paucity of informative data regarding the role of NAC spp. in VVC. This study investigated the species distribution and antifungal susceptibility profile of NAC spp. isolated from VVC cases. Of 271 *Candida* spp isolated from high vaginal swab collected from clinically suspected cases of vulvovaginitis, 197 (72.7%) belonged NAC spp. Among these *C. tropicalis* and *C. glabrata* were the major isolates. Azole resistance was more common in *C. tropicalis*, *C. glabrata* and *C. kefyr*. Our study highlights the importance of isolation, species identification and antifungal susceptibility of *Candida* prior to initiation of therapy for proper selection of antifungal agent and to prevent emergence and spread of drug resistant *Candida* spp.

Introduction

Candida, though a normal microbiota of human body is capable of causing a wide spectrum of clinical manifestations ranging from mucocutaneous overgrowth to life threatening disseminated infections like candidemia (Eggimann *et al*.,2003).

Vulvovaginal candidiasis (VVC) is the most common manifestation of genital candidiasis (Achkar and Bettina 2010).

It is defined as signs and symptoms of inflammation of the vulva and vagina in the presence of *Candida* spp (Achkar and Bettina, 2010). Clinical manifestations of VVC are pruritus, hyperemia, vaginal discomfort and leucorrhea, burning, soreness, dyspareunia and vaginal or vulvar erythema (Moreira and Paula, 2006). VVC is diagnosed in upto 40% of females with vaginal complaints.

According to the data available, VVC affects three quarters of all females at least once during their lifetime and nearly half of them experience recurrence (Mohanty *et al*., 2007).

The distribution of *Candida* spp. in VVC cases varies widely depending on the geographical locations as well as the population studied (Achkar and Bettina, 2010). Although *Candida* albicans is the most common cause of VVC, non *albicans Candida* (NAC) species can also induce Vulvovaginitis (Dan M *et al*., 2002). VVC due to NAC spp. is clinically indistinguishable from that caused by *C. albicans*; moreover it is more resistant to antifungal treatment (Sobel, 1985).

The dramatic rise in VVC caused by NAC spp. is claimed but not proved by many studies (Cauwenbergh, 1990). This may be because species identification and antifungal sensitivity testing of *Candida* spp is not routinely done in most diagnostic microbiology laboratories.
The use of single dose oral and topical formulation together with low dosage azole maintenance regimen and the availability of over-the-counter antimycotics are risk factors suggested for the increase of VVC due to NAC spp (Sobel *et al*., 1998). These factors eliminate the more sensitive *C. albicans* which results in selective proliferation of NAC spp that are resistant to most of commonly used antifungal drugs (Ferrer, 2000). The present study was conducted with an aim to determine prevalence, species distribution and antifungal profile of NAC spp. isolated from VVC cases.

Materials and Methods

This was a cross sectional study and is part of a PhD thesis conducted in the Department of Microbiology, Rural Medical College and hospital of Pravara Institute of Medical Sciences Loni, Maharashtra. The protocol of the study was approved by Institutional ethics committee.

Inclusion criteria - NAC spp isolated from high vaginal swabs collected from clinically suspected cases of vulvovaginitis.

Exclusion criteria - NAC spp from mixed cultures.

The clinical history of patients was recorded from the laboratory requisition forms.

Species identification- Species identification of *Candida* isolates was done following standard mycological protocol including germ tube test, fermentation and assimilation of various sugars and colony color on Hichrom *Candida* agar. Hi-Candida identification kit supplemented the speciation of *Candida* isolates (Sachin *et al*., 2012).

Antifungal susceptibility testing – Antifungal susceptibilty testing of the isolates was performed by Hicomb MIC test (Himedia Laboratories Pvt Ltd, Mumbai). The antifungal agents used were fluconazole (range 0.016-256 µg), itraconazole (range 0.002-32 µg) and ketoconazole (range 0.002-32 µg). The test was performed by the method described by Deorukhkar and Saini (Deorukhkar and Saini, 2013a).

The antifungal susceptibility of the isolates was interpreted as sensitive (S), dose dependent-susceptible (DDS) and resistant (R). The results of fluconazole and

itraconazole were interpreted from the Clinical and Laboratory Standards Institute (CLSI) (formerly known as National Committee for Laboratory Standards (NCCLS) M27-A2 standard guidelines (CLSI, 2002). Due to the lack of defined break points for ketoconazole, arbitrary values based on the studies of the other researchers were used (Priscilla *et al*., 2002, Deorukhkar and Saini, 2013a).

Result and Discussion

During the study period a total of 271 *Candida* spp were isolated from 307 high vaginal swab processed for isolation and identification of *Candida*. Out of 271 *Candida* spp., 74 (27.3%) isolates were identified as *C. albicans*, whereas 197 (72.7%) belonged to NAC spp.

The risk factors identified for vulvovaginitis due to NAC spp. were similar to that of *C. albicans*. But diabetes and prior treatment with fluconazole were identified as major risk factors for VVC due to *C. glabrata*.

Figure 1, shows the species distribution of NAC spp. *C. tropicalis* (30.9%) followed by *C. glabrata* (27.9%) were the major isolates. As shown in Table 1, fluconazole resistance was more common in *C. tropicalis* (29.5%), *C. glabrata* (27.3%) and *C. kefyr* (25%) isolates. A total of 7 (12.7%) *C. glabrata* isolates were dose dependent susceptible to fluconazole. Fluconazole resistance was less in *C. parapsilosis* (10%).

Itraconazole resistance was more in *C. tropicalis* (42.6%), *C. glabrata* (40%) and *C. kefyr* (25%). Dose dependent susceptibility to itraconazole was shown by 18.8% of *C. kefyr* isolates (Table 2). As shown in Table 3, *C. glabrata* (43.7%) isolates showed maximum resistance to ketoconazole followed by *C. tropicalis* (39.4%) and *C. krusei* (25%).

NAC spp. once overlooked as contaminants or non pathogens have emerged as potential pathogens (Deorukhkar and Saini, 2013b). In our study 72.7% of *Candida* isolates from VVC cases belonged to NAC spp. Other researchers from India and abroad have also documented the increase isolation of NAC spp. from sporadic and recurrent VVC cases (Paulitsch *et al*., 2006, Mohanty *et al*., 2007).

Among the NAC spp. *C. tropicalis* was the predominant isolate. Our observation is in contrast to that of Mohanty *et al* (Mohanty *et al*., 2007), where *C. glabrata* was reported as the most common isolate. *C. tropicalis* has been identified as the most prevalent pathogen of NAC group. The drastic increase in *C. tropicalis* infections worldwide has resulted in this organism being labeled as an emerging pathogenic yeast (Kothavade *et al*, 2010).

In the present study *C. glabrata* was the second most common isolate. *C. glabrata* VVC represents a complicated form of disease. VVC due to *C. glabrata* is common in women receiving long term maintenance low dose fluconazole prophylactic regimens (Fidel *et al*., 1999). Risk factors for VVC due to *C. glabrata* include old age, underlying medical conditions like uncontrolled diabetes mellitus and douching (Lynch *et al*., 1996). Diabetes mellitus was also a major

Table.1 Fluconazole susceptibility profile of *Candida* isolates

Species	Sensitive (%)	Dose-dependent susceptible (%)	Resistant (%)
C. tropicalis	37 (60.7)	06 (9.8)	18 (29.5)
C. glabrata	33 (60)	07(12.7)	15 (27.3)
C. krusei	25 (78.1)	01 (3.1)	06 (18.8)
C. guilliermondii	19 (82.6)	-	04 (17.4)
C. kefyr	12 (75)	-	04 (25)
C. parapsilosis	09 (90)	-	01 (10)
Total	135 (68.5)	14 (7.1)	48 (24.4)

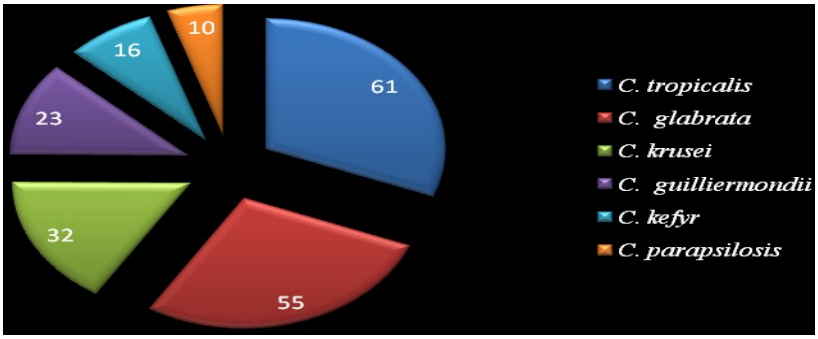
Table.2 Itraconazole susceptibility profile of *Candida* isolates

Species	Sensitive (%)	Dose-dependent susceptible (%)	Resistant (%)
C. tropicalis	29 (47.5)	06 (9.8)	26 (42.6)
C. glabrata	26 (47.3)	07(12.7)	22 (40)
C. krusei	18 (56.2)	07 (21.9)	07 (21.9)
C. guilliermondii	20 (86.9)	-	03 (13.1)
C. kefyr	09 (56.2)	03 (18.8)	04 (25)
C. parapsilosis	08 (80)	01 (10)	01 (10)
Total	110 (55.9)	24 (12.2)	63 (31.9)

Table.3 Ketoconazole susceptibility profile of *Candida* isolates

Species	Sensitive (%)	Dose-dependent susceptible (%)	Resistant (%)
C. tropicalis	28 (45.9)	09 (14.7)	24 (39.4)
C. glabrata	25 (45.4)	06 (10.9)	24 (43.7)
C. krusei	20 (62.5)	04 (12.5)	08 (25)
C. guilliermondii	18 (78.3)	01 (4.3)	04 (17.4)
C. kefyr	12 (75)	-	04 (25)
C. parapsilosis	09 (90)	-	01 (10)
Total	112 (56.9)	20 (10.2)	65 (32.9)

Figure.1 Species distribution of Non *albicans Candida* isolates



risk factor associated with *C. glabrata* VVC in our study. *C. glabrata* is the only *Candida* spp. that is haploid and hence do not form hyphae and pseudohyphae in vivo. This feature of *C. glabrata* limits the use of direct microscopy in laboratory diagnosis (Geiger *et al*., 1995). C. glabrata VVC co-exists with bacterial vaginosis and frequently occurs at a higher pH (Fidel *et al*., 1999).

The acquired or instric reduced susceptibility shown by NAC spp. towards commonly used antifungal agents has underlined the need of antifungal susceptibility testing to predict therapeutic outcome. Antifungal susceptibility testing is still not well developed and utilized as antibacterial testing. The CLSI broth macrodilution, a standard reference method is labor-intensive and time consuming, therefore is not applicable as a routine method in most clinical microbiology laboratories (Vandenbossche *et al*., 2002).

In our study we used MIC test for antifungal testing of isolates. This test can detect the DDS category in addition to sensitivity and resistance to a particular antifungal agent. DDS is a novel category and is unique to antifungal susceptibility testing, which emphasizes the need to maximize the dose of given antifungal agent. It also indicates maximum blood or tissue concentration for a given drug that can be achieved (Rex and Pfaller, 2002).

In our study ketoconazole resistance was noted in 65 (32.9%) isolate, itraconazole resistance in 63 (31.9%) isolates and fluconazole resistance was seen in 48 (24.4%) isolates. Antifungal resistance was more common in *C. tropicalis* and *C. glabrata* isolates. Azole resistance in *Candida* spp. is of concern because these drugs are frequently used as therapeutic alternatives to amphotericin B. Azole antifungal agents are easy for administration and are less toxic. These antifungal agents have high bioavailability, good water solubility, wide volume of distribution into tissue and body fluids and long half- life (Dismukes, 2000).

From our study it can be concluded that the frequency of VVC due to NAC spp. has increased. This underscores the importance of isolation, species identification and antifungal susceptibility of *Candida* prior to initiation of therapy for proper selection of antifungal agent. The judicious use of antifungal agents is very important for the prevention of emergence and spread of drug resistant *Candida* species.

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