# Species Distribution and Antifungal Susceptibility Profile of Candida Isolated from Various Clinical Specimens

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## Abstract

***Background:*** *Candida spp. is one the most common causative agent of mycoses. The severity of candidiasis ranges from moderate to fatal and is dependent on the site of infection and the immune status of the host. Although Candida albicans is the most common cause of candidiasis, the shift towards treatment resistant non albicans Candida (NAC) spp. is evident in recent years.*

***Aim:*** *The present study was conducted with an aim to determine species distribution and antifungal susceptibility profile of Candida isolated from various clinical specimens.*

***Materials and methods:*** *Candida spp. isolated from various clinical specimens were included in the study. The isolates were identified upto species level as per standard mycological protocol. The antifungal susceptibility profile of Candida isolates was determined by disc diffusion method.*

***Results:*** *During the study period a total of 732 Candida spp. were isolated from various clinical specimens. NAC spp. was the predominant isolates. C. tropicalis and C. glabrata were the major isolates from NAC spp. NAC spp. demonstrated more resistance to both polyene and azole group of antifungal agents as compared to C. albicans.*

***Conclusion:*** *Continuous monitoring of trends of species distribution and antifungal resistance pattern is essential to control and optimize therapy of Candida infections.*


**Keywords:** *Antifungal resistance, Candida albicans, non albicans Candida, species identification.*

## Introduction

Until 19th century most of infectious diseases were attributed to bacterial, viral and parasitic origin.[1] The role of fungus in infection was rarely documented. Over the last few decades, the scenario of infectious diseases has changed and fungi, which were once studied only as "microbiological curiosities" with less or no pathogenic role have emerged as important cause of opportunistic and health-care associated infections.[2] The common targets of opportunistic fungal pathogens are immunocompromised hosts such as patients suffering from HIV/AIDS, diabetes and cancer.[3] Although the target group of mycoses is restricted as compared to bacterial and viral infections, it is often associated with high morbidity and mortality.[3]

*Candida* spp. is one the most common causative agent of mycoses.[3] In recent years, factors like increased use of intravenous catheters, total parenteral nutrition, broad spectrum antibiotics and advent of HIV/AIDS have increased the incidence of candidiasis.[4] The clinical spectrum of candidiasis is wide and ranges from mucocutaneous overgrowth to disseminated infections like candidemia.[4] The severity of candidiasis ranges from moderate to fatal and is dependent on the site of infection and the immune status of the host.

In humans, *Candida* spp. is a part of normal commensal flora of gastrointestinal and genitourinary tract.[5] The transition of *Candida* spp. from a non-pathogenic commensal to a potent pathogen is contributed both by host predisposing factors and virulence factors of infecting species.[4] Although *Candida albicans* is the

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most common cause of candidiasis, the shift towards treatment resistant non albicans *Candida* (NAC) spp. is evident in recent years.[6]

The present study was conducted with an aim to determine species distribution and antifungal susceptibility profile of *Candida* isolated from various clinical specimens.

## Materials and methods

The present study 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, India. The protocol of the study was approved by the Institutional Ethics Committee. *Candida* spp. isolated from various clinical specimens were included in the study.

The repeated isolation of *Candida* spp from clinical specimens collected from oropharyngeal, vaginal, urinary and bronchial candidiasis was considered significant, while a single isolation was considered significant from sterile body fluids like blood, peritoneal fluid, pleural fluid and cerebrospinal fluid (CSF).

Colonies appearing pasty, opaque, slightly domed or flat, smooth and pale coloured (white, off-white or beige) with a sweet smell reminiscent of ripe apples were suspected to be colonies of *Candida*. [5] The suspected colonies of *Candida* isolates were identified by wet film, Gram stain and India ink preparation.

The mycological workup for speciation of *Candida* isolates started with the germ tube test. Isolates producing germ tubes within 2 hours of incubation were further subjected to temperature studies, chlamydospores formation and biochemical tests for differentiation of *C. albicans* from *C. dubliniensis*. Germ tube negative *Candida* isolates were classified on the basis of sugar assimilation and colony colour on Hichrom *Candida* agar. Hi*Candida* identification kit (Himedia Laboratories Pvt. Ltd., Mumbai, India) supplemented the identification of isolates.

The antifungal susceptibility profile of *Candida* isolates was determined by disc diffusion method on Mueller Hinton agar supplemented with 2% glucose and 0.5 ìg methylene blue (MH-GM) (Figure 1). The antifungal agents used were amphotericin B (20 ìg), fluconazole (10 ìg), itraconazole (10 ìg) and ketoconazole (10 ìg). The antifungal susceptibility of the isolates were interpreted as sensitive (S) and resistant (R) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[7]

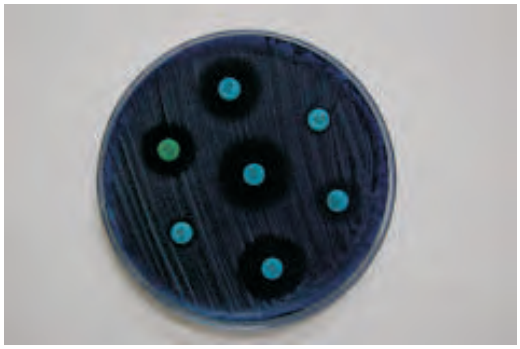

**Figure 1: Antifungal susceptibility testing of *Candida* spp. by disc diffusion method**

## Results

During the study period a total of 732 *Candida* spp. were isolated from various clinical specimens. The year wise isolation of Candida spp. from various clinical specimens is shown in figure 2. The increase in the rate of isolation of *Candida* was noted in our study. As shown in figure 3 majority of *Candida* spp. were isolated from urine samples followed by vaginal and oropharyngeal swabs.

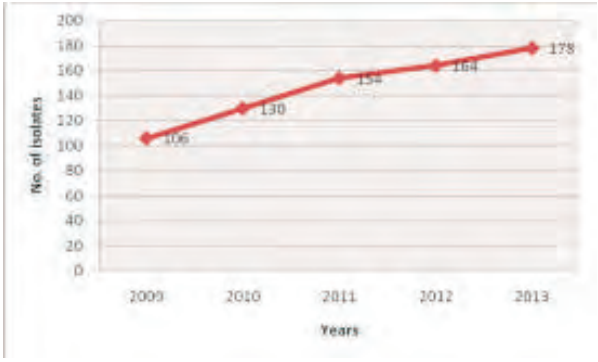


**Figure 2: Year wise distribution of Candida spp. isolated from various clinical specimens.**

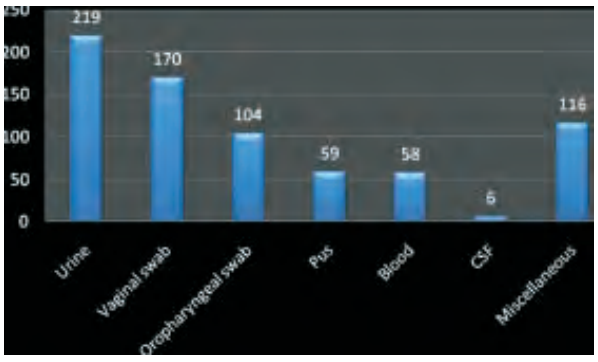


**Figure 3: Clinical specimen wise distribution of Candida spp.**

Presence of indwelling urinary catheter, diabetes, pregnancy and advanced age were major risk factors associated with *Candida* urinary tract infection (CUTI), whereas pregnancy and diabetes were most common predisposing factors associated with vulvovaginal candidiasis (VVC). HIV infection and diabetes were major risk factors for oropharyngeal candidiasis (OPC).

The species wise distribution of *Candida* isolates is shown in figure 4. *C. albicans* was isolates from 213 clinical specimens. NAC spp. was the predominant isolates. *C. tropicalis* and *C. glabrata* were the major isolates from NAC spp.

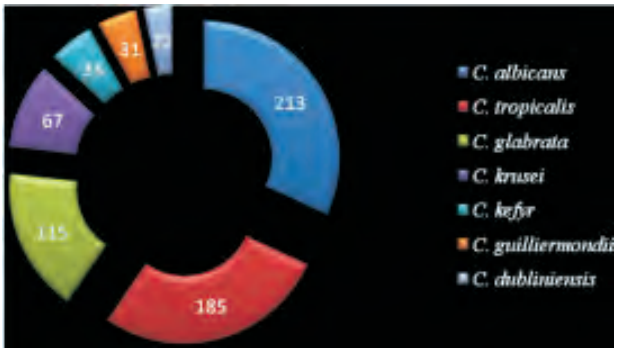


**Figure 4: Species wise distribution of Candida isolates**

Antifungal susceptibility profile of *Candida* isolates is tabulated in Table 1. NAC spp. demonstrated more resistance to both polyene and azole group of antifungal agents as compared to *C. albicans*. Among NAC spp., *C. glabrata* demonstrated high resistance to all antifungal drugs used in the study.

## Discussion

Candidiasis is considered as the most common opportunistic mycoses and a rising problem worldwide. In this study, the isolation pattern and species distribution of *Candida* isolates from various clinical specimens over a 5-year period were investigated and the *in-vitro* susceptibilities of the isolates to amphotericin B, fluconazole, itraconazole and ketoconazole were determined. Majority of *Candida* spp. were isolated from urine samples. The major predisposing factors for CUTI included indwelling catheters, recent antibiotic therapy, advanced age, pregnancy and diabetes mellitus. Candiduria is rarely seen as a community acquired in a structurally normal urinary tract and in healthy people. [8] Presence of indwelling urinary catheters facilitates the entry and colonization of *Candida*. Diabetes increases *Candida* colonization by promoting stasis of urine in neurogenic bladder.[9] Use of broad spectrum antibiotics favors colonization of *Candid*a by suppressing commensal bacterial flora of gastrointestinal and lower genital tract.[8]

VVC is among the most common health problems in women seeking gynecological care.[10] In this study, diabetes and pregnancy were major predisposing factors associated with VVC. In pregnancy, the high levels of reproductive hormones increases glucogen content of the vaginal environment and provide a carbon source for *Candida* growth and germination.[11] In our study, 104(14.2) *Candida* spp. were isolated from oropharyngeal swab collected from suspected cases of OPC. HIV and diabetes were major risk factors associated with OPC. Oropharyngeal candidiasis (OPC) is the most common

**Table 1: Antifungal susceptibility profile of Candida spp**

| *Candida* spp. (n) | Amphotericin B | | Fluconazole | | Itraconazole | | Ketoconazole | |
|---|---|---|---|---|---|---|---|---|
| | S (%) | R (%) | S (%) | R (%) | S (%) | R (%) | S (%) | R (%) |
| *C. albicans* (213) | 194 (91.1) | 19 (8.9) | 176 (82.6) | 37 (17.4) | 178 (83.5) | 35 (16.5) | 165 (77.4) | 48 (22.6) |
| *C. tropicalis* (185) | 164 (88.2) | 21 (11.4) | 128 (69.2) | 57 (30.8) | 131 (70.8) | 54 (29.2) | 121 (65.4) | 64 (34.6) |
| *C. glabrata* (115) | 101 (87.8) | 14 (12.2) | 62 (53.9) | 53 (46.1) | 84 (73.1) | 31 (26.9) | 46 (40) | 69 (60) |
| *C. krusei* (67) | 63 (94.1) | 04 (5.9) | 42 (62.6) | 25 (37.4) | 44 (65.6) | 23 (34.4) | 36 (53.7) | 31 (46.3) |
| *C. kefyr* (64) | 60 (93.7) | 04 (6.3) | 46 (71.8) | 18 (28.2) | 44 (68.7) | 20 (31.3) | 35 (54.6) | 29 (45.4) |
| *C. guilliermondii* (35) | 32 (91.4) | 03 (8.6) | 26 (74.2) | 09 (25.8) | 27 (77.1) | 08 (22.9) | 23 (65.7) | 12 (34.3) |
| *C. parapsilosis* (31) | 28 (90.3) | 03 (9.7) | 24 (77.4) | 07 (22.6) | 25 (80.6) | 06 (19.4) | 19 (61.2) | 12 (38.2) |
| *C. dubliniensis* (22) | 20 (90.9) | 02 (9.1) | 14 (63.7) | 08 (36.3) | 16 (72.7) | 06 (27.3) | 16 (72.7) | 06 (27.3) |

opportunistic mycoses among HIV infected patients. It develops in 80-90% of HIV infected patients at some point during the progression of the disease.[12]

Advances in medical practice have increased the incidence of mycotic infections worldwide. New fungal pathogens have emerged in unprecented numbers, while others that were common have decreased in number or almost eradicated. NAC spp. once overlooked as mere contaminants or non pathogenic commensals have emerged has important pathogens. In the present study, predominance of NAC spp. over *C. albicans* was noted. The apparent increased emergence of these species as human pathogens can be attributed to improved identification methods to improved identification methods and also associated with the degree of diseases of the patients, the interventions that they were subjected and the drugs used.[13]

In the present study, *C. tropicalis* and *C. glabrata* were the most common isolates from NAC group. For many years, *C. tropicalis* and *C. glabrata* was considered a relatively nonpathogenic saprophyte or commensal.[13,14] However, following the widespread use of immunosuppressive drugs together with broad spectrum antibiotic therapies, the incidence of mucosal and disseminated candidiasis caused by *C. tropicalis* and *C. glabrata* has significantly increased. As compared to other NAC spp. infection, the mortality rate associated with *C. glabrata* is the highest.[13]

The increased incidence and change in the epidemiology of candidiasis has highlighted the importance of species identification and antifungal susceptibility testing of *Candida* isolates for initiation of appropriate antifungal therapy.

The Clinical and Laboratory Standards Institute (CLSI) recommends broth microdilution method (M27-A2) as reference standard procedure for determining antifungal susceptibility of *Candida* and other medically important yeasts. [6] However, this method is time consuming and costly affair for diagnostic services having low antifungal testing volume. On the other hand, disc diffusion method is relatively rapid and technically simpler. It has reduced preparation time, decreased time to reporting, improved ease of use and requires minimal technical expertise. Disc diffusion method also has excellent correlation when compared with MIC values derived from the M27-A2.[6]

In the present study, we used GM-MH media for disc diffusion method for determining antifungal susceptibility profile of *Candid*a isolates. Antifungal resistance was high in NAC spp. as compared to *C. albicans*. Among NAC spp. antifungal resistance was more in *C. glabrata* isolates. *C. glabrata* is either intrinsically resistant to antifungal agents or may acquire resistance during course of treatment. [15] Various mechanisms like modification of ergosterol biosynthesis, efflux of antifungal and alteration in the affinity of the drug target are suggested for resistance to antifungal drugs in *C. glabrata*.[15] Due to drug resistance, treatment failure and high mortality rate, *C. glabrata* has gained attention of clinical mycologists and clinicians.[15]

## Conclusion

Just a decade ago, *C. albicans* was considered as the most pathogenic member of the genus *Candida*. The isolation of NAC spp. from clinical specimens was ignored as the isolates were considered as non pathogenic commensals or contaminants. Our study, documents a shift from *C. albicans* to NAC spp. as cause of various clinical types of candidiasis. Although the clinical manifestations of infections caused by different members of NAC spp. are usually indistinguishable, but several NAC spp. are inherently resistant or acquire resistance, or both, to commonly used antifungal drugs. Therefore continuous monitoring of trends of species distribution and antifungal resistance pattern is essential to control and optimize therpy of *Candida* infections.

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## Conflict of interests

The authos declare that there is no conflict of interests regarding the publication of this article.

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