# Prevalence and Antifungal Susceptibility of Candida species at a Tertiary Care Hospital in North India

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## Abstract*:*

**Background:** The introduction of Candida species other than *Candida albicans* as the primary agents has caused a recent shift in the epidemiology of candidiasis, which is extremely concerning on a global scale. Some species of Candida are naturally resistant to azoles. For directing therapy, in vitro susceptibility testing is crucial.

**Aim**: This study was conducted to determine the prevalence of Candida spp. and evaluate its susceptibility profile against antifungal agents at a tertiary care center in North India.

**Methods**: A total of 140 confirmed cases of Candida infection were enrolled. Demographic and epidemiological details like gender and specimen type were noted. Species identification was done by phenotypic methods and Antifungal susceptibility testing was done by E test.

**Results**: Majority of patients were females (n=87; 62.1%). Urine (n=65; 46.4%), was the most common specimen followed by Sputum (n=30; 21.4%) %), Blood (n=12; 8.5%) Body fluids (n=10; 7.14%) oral swab (n=10; 7.14%), High vaginal swab (n=8; 5.7%) and Nails (n=5; 3.5%) respectively. *C. tropicalis* (n=48 , 34.3%) was the most common species followed by *C.albicans* (n=35; 25%) *C. glabrata* (n=21; 15%), *C krusei*. (n=14; 10%), *C. parapsilosis* (n=12; 8.5%), *C. guilliermondii* (n=6; 4.2%) and *C. rugosa* (n=4; 2.8%) respectively. Regarding antifungal sensitivity Caspofungin and Amphotericin B were found to be most sensitive (100%) against all the species. Fluconazole was the most resistant antifungal agent followed by Itraconazole. Urine was the major source for Candida infection in women suggesting their high susceptibility to candida infection.

**Conclusion**: Our study highlights the predominance of NAC species. Caspofungin and Amphotericin B were the most sensitive antifungal agents against all candida species whereas, Fluconazole was least effective. Species Identification and antifungal susceptibility should be done to help the treatment process of Candidal infection.

***Keywords:****Candida, Antifungal susceptibility, E-test, Caspofungin, Voriconazole.*

## Introduction:

Evidence suggests that more than one billion people are affected by fungal infections annually, and nearly 1.5 million are expired due to infection severity (1). This suggests that fungal infections have a high mortality rate. Among various fungal species, Candida is the most common type. It is an endogenous species and its spread is marked as an opportunistic infection (2). Candida spp. is normally distributed in the flora of skin, mucous membrane and gastrointestinal tracts of healthy individuals. However, with the increasing trends of immunocompromised persons being at high risk, there is an upward trajectory in the rate of fungal infection, specifically, due to Candida (3). Although, there are over 200 species of Candida. *C.albicans* is the most common. However, a number of other species including *C.glabrata, C.parapsilosis, C.tropicalis* and *C. krusei*

are also commonly isolated from hospital specimen. Out of 200 species, nearly 20 species cause serious infection in humans(4).When these fungi grow, the result in Candiasis/Candidosis/Candidemia causing superficial, subcutaneous and deep mycosis(5).Candidiasis is a life threatening with a mortality rate as high as 38%. It also responsible for prolonging duration of hospital stay (6).In order to offer effective treatment, it is essential that fungal species affecting the patient should be identified correctly because; sensitivity profile of a fungal species against various antifungal agents varies. Moreover, in view of the emergence of antifungal resistant species, it is essential that the antifungal susceptibility profile of different *candida* species should be studied. With this objective, the present study was carried out to describe distribution of various *Candida* spp. among clinical isolate and antifungal susceptibility profile of various *candida* species at a tertiary care centre in north India.

**Materials and Methods**

The approval for the study was obtained from the Institutional Ethics Committee and permission to analyze the data was obtained from respective authorities. As the study did not directly involve inclusion of patients and/or their clinical profile, hence there was no need to obtain informed consent from the patients.

**Collection of Specimens**

A total of 140 isolates of *Candida* obtained from different clinical specimen such as Blood, Urine, Sputum, Body fluids, High vaginal swab, Oral swab and Nails were collected from patients attending various departments of Era's Lucknow Medical College Lucknow. Samples were collected in sterile collection devices and containers with appropriate label. Institutional Ethics Committee of Era's Lucknow Medical College and Hospital (ELMC & H) approved the study. Since this study did not involve direct inclusion of patients and/or their clinical profile, Informed consent from the patients was not required.

## Processing of Specimens

Primary identification was performed by direct smear examination by Gram's staining and KOH mount. Samples were collected using aseptic precautions and inoculated on Sabouraud's Dextrose Agar SDA (Hi media) screw caped bottles and incubated at 37°C for48-72hours.After growth, species identification was done by Germ tube test,HiCromeAgar media(Himedia),carbohydrate fermentation test and sugar assimilation test.

**Antifungal susceptibility testing done by E-TEST-**The E-test gradient strips of Amphotericin B,Fluconazole,Voriconazole,Itraconazole and Caspofungin was obtained from HIMEDIA.
The concentration gradient for Fluconazole ranged from (0.016 -256 µg/mL) while for other drugs was0.002-32µg/mL,The E-testwasperformedbyfollowingthemanufacturer's instructions. E strips were applied and the plates were incubated at 35ºC and read after 48hours. The MIC was determined from the inhibition ellipse that intersected the scale on the strip (7) C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 were included as quality controls.The interpretative criteria for Fluconazole(FCZ),Itraconazole(ICZ),and Voriconazole (VCZ) susceptibility of Candida species in vitro were derived from the CLSI M27-A3 and M60-ED 1 documents(8,9) while the interpretative criteria for Amphotericin B (AMB) were taken from the literature (10,11). For each group, the lowest concentrations at which 50% (MIC 50) and 90% (MIC 90) of the isolates were inhibited were determined.

**DataAnalysis**

Data was tabulated and presented as frequency and proportions. The antifungal efficacy was evaluated in terms of Sensitive, Susceptible Dose dependent and Resistant outcomes. MIC 50 and MIC 90 are also calculated.

**Results**

In this study, we analyzed 140 specimens including 62.1% (n=87) females and 37.9% (n=53) males.
The most common specimen was Urine (n=65; 46.4%) followed by Sputum (n=30; 21.4%), Blood (n=12; 8.5%) Body fluids (n=10; 7.14%) Oral swab (n=10; 7.14%), High vaginal swab(n=8;5.7%)and Nail(n=5; 3.5%)respectively (Table 2).

*C.tropicalis* (n=48, 34.3%) was the most common species followed by *C.albicans* (n=35;25%)

*C.glabrata*(n=21;15%),*C.krusei* (n=14;10%),*C.parapsilosis*(n=12;8.5%),*C.guilliermondii* (n=7;5%) and *C. rugosa* (n=4; 2.8%) respectively (Table 3).

Table 4 summarises the MIC ranges and MIC50 and MIC90 values obtained in susceptibility testing using the CLSI method. According to the MIC values obtained for Amphotericin B,and Caspofungin against all the isolates of candida species were classified as susceptible according to the described breakpoints.The MIC values for Itraconazole were 0.006to1μg/mL, indicating that 103 of the candida isolates were susceptible (73.5%), 29 were dose-dependently susceptible (20.7%), and 8 isolates were resistant (5.7%). Regarding Fluconazole79 of the candida isolates were susceptible (56.42%), 7 were intermediate (5%) and 56 were resistant (40%) Voriconazole showed sensitivity against 127 isolates of Candida (90.7%) and resistant against 13 isolates of Candida (9.2%) respectively. The highest MIC was showed by Fluconazole while lowest Caspofungin and Amphotericin B. Caspofungin and Amphotericin B were found to be most sensitive antifungal agent against all the Candida species (100%).Overall, Fluconazole was the most resistant antifungal agent followed by Itraconazole.

**Table1. Gender distribution of Patients**

| S.No. | Gender | Number | Percentage |
|---|---|---|---|
| 1 | **Female** | 87 | 62.1 |
| 2 | **Male** | 53 | 37.9 |
| 3 | **Total** | 140 | 100 |

**Table2.Distribution of collected numbers of samples**

| S.No. | Clinical Specimens | Number | Percentage (%) |
|---|---|---|---|
| 1 | **Urine** | 65 | 46.4 |
| 2 | **Sputum** | 30 | 21.4 |
| 3 | **Blood** | 12 | 8.5 |
| 4 | **Fluids** | 10 | 7.14 |
| 5 | **Oral Swab** | 10 | 7.14 |
| 6 | **High Vaginal Swab** | 8 | 5.7 |
| 7 | **Nail** | 5 | 3.5 |

**Table3.Species of *Candida* Isolated from clinical samples**

| S.No. | *Candida* species | Number | Percentage (%) |
|---|---|---|---|
| **1** | ***C.tropicalis*** | 48 | 34.3% |
| **2** | ***C.albicans*** | 35 | 25% |
| **3** | ***C. glabrata*** | 21 | 15% |
| **4** | ***C.krusei*** | 14 | 10% |
| **5** | ***C.parapsilosis*** | 12 | 8.5% |
| **6** | ***C.guilliermondii*** | 6 | 4.2% |
| **7** | ***C.rugosa*** | 4 | 2.8% |


**Fig1.*Candida* species growth on HiCromeMedia**


**Fig2.Caspofungin showing sensitivity against *candida albicans***

**Table4.Susceptibility patterns of *Candida* species isolated from patients**

| **Candida species(No.of strain)** | **Antifungal agent** | **Range** | **MIC50** | **MIC90** | **Susceptibility break points** | **SDD/I** | **R** |
|---|---|---|---|---|---|---|---|
| *C.albicans* (35) | Amphotericin B | 0.004–.25 | .032 | .19 | ≤1 | - | ≥1 |
| | Fluconazole | 0.125-12 | 0.25 | 1 | ≤2 | 4 | ≥8 |
| | Itraconazole | 0.125–1 | .25 | 1 | ≤0.125 | 0.25-0.5 | ≥1 |
| | Voriconazole | 0.006–1 | .008 | .032 | ≤0.12 | 0.25-0.5 | ≥1 |
| | Caspofungin | 0.002-.016 | 0.006. | .008 | ≤0.25 | 0.5 | ≥1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *C.tropicalis* (48) | AmphotericinB | 0.002–0.38 | .064 | 0.94 | ≤1 | - | ≥1 |
| | Fluconazole | 0.25-12 | 0.5 | 0.75 | ≤2 | 4 | ≥8 |
| | Itraconazole | 0.012-0.5 | 0.125 | 0.5 | ≤0.125 | 0.25-0.5 | ≥1 |
| | Voriconazole | <0.12–2 | .012 | 1 | ≤0.12 | 0.25-0.5 | ≥1 |
| | Caspofungin | .004-0.23 | .004 | .012 | ≤0.25 | 0.5 | ≥1 |
| *C.glabrata* (21) | AmphotericinB | 0.012-.25 | 0.125 | 0.75 | ≤1 | - | ≥1 |
| | Fluconazole | 0.023–64 | 6 | 8 | - | ≤32 | ≥64 |
| | Itraconazole | 0.125–0.5 | 0.125 | 0.5 | ≤0.125 | 0.25-0.5 | ≥1 |
| | Voriconazole | 0.006–2 | 0.125 | 0.38 | ≤0.5 | - | ≥1 |
| | Caspofungin | .002-.012 | .004 | .012 | ≤0.12 | 0.25 | ≥0.5 |
| *C.krusei* (14) | AmphotericinB | 0.064-.75 | .25 | .38 | ≤1 | -- | ≥1 |
| | Itraconazole | 0.094-1 | .25 | 0.5 | ≤0.125 | 0.25-0.5 | ≥1 |
| | Voriconazole | 0.064–4 | .064 | 0.125 | ≤0.5 | 1 | ≥2 |
| | Caspofungin | 0.006-.016 | 0.006 | 0.008 | ≤0.25 | 0.5 | ≥1 |
| *C.parapsilosis* (12) | AmphotericinB | 0.002–0.125 | .032 | .094 | ≤1 | - | ≥1 |
| | Fluconazole | 0.032–8 | 0.25 | 0.38 | ≤2 | 4 | ≥8 |
| | Itraconazole | 0.006-0.5 | .094 | .25 | ≤0.125 | 0.25-0.5 | ≥1 |
| | Voriconazole | 0.004–0.032 | 0.006 | 0.023 | ≤0.12 | 0.25-0.5 | ≥1 |
| | Caspofungin | 0.004-.032 | 0.004 | .016 | ≤2 | 4 | ≥8 |
| *c.rugosa*(4) | AmphotericinB | 0.19–0.5 | 0.25 | 0.5 | ≤1 | - | ≥1 |
| | Itraconazole | 0.125–.25 | .125 | .25 | ≤0.125 | 0.25-0.5 | ≥1 |
| | Voriconazole | .012-.094 | .012 | .064 | ≤0.12 | 0.25-0.5 | ≥1 |
| | Fluconazole | 8-16 | 8 | 8 | ≤2 | 4 | ≥8 |
| | Caspofungin | 0.002-.016 | 0.006 | .012 | ≤2 | 4 | ≥8 |
| *C.guilliermondii* (6) | AmphotericinB | 0.002–0.125 | .012 | .032 | ≤1 | - | ≥1 |
| | Itraconazole | 0.04-0.5 | .012 | 0.5 | ≤0.125 | 0.25-0.5 | ≥1 |
| | Voriconazole | .032-.094. | .032 | .064 | ≤0.12 | 0.25-0.5 | ≥1 |
| | Fluconazole | 8-16 | 8 | 8 | ≤2 | 4 | ≥8 |
| | Caspofungin | 0.004-.032 | .006 | .012 | ≤2 | 4 | ≥8 |

*Fluconazole,Itraconazole and Voriconazole range values according to Clinical and Laboratory Standards Institute(CLSI) literature

*Ranges:FCZ(0.016-256µg/mL),ICZ(0.002-32µg/mL),VCZ(0.002-32µg/mL),andAMB (0.002-32µg/mL)andCAS(0.002-32µg/mL)

**Table5.Susceptibilitypattern of*Candida*speciesbasedonMICvalues**

| ***Candidaspecies*** | **Antifungal agents** | **Susceptible** | **Dosedependents usceptible /intermediate** | **Resistant** |
|---|---|---|---|---|
| *C.albicans(35)* | A | 35(100%) | - | - |
| | F | 27(77.14%) | - | 8(22.85) |
| | I | 29(82.85%) | 4(11.42%) | 2(5.71%) |
| | V | 32(100%) | - | 3 |
| | C | 35(100%) | - | - |
| *C.tropicalis*(48) | A | 48(100%) | - | - |
| | F | 38(87.5%) | - | 10(12.5%) |
| | I | 44(91.6%) | 4(8.33%) | - |
| | V | 44(91.6%) | - | 4(8.33%) |
| | C | 48(100%) | - | - |
| *C. glabrata*(21) | A | 21(85.71%) | - | - |
| | F | 4(19.04%) | 7(52.38%) | 10(47.61%) |
| | I | 15(85.71%) | 6(14.28%) | |
| | V | 19(90.47%) | - | 2(9.5%) |
| | C | 21(100%) | - | - |
| *C.krusei*(14) | A | 14(100%) | | |
| | F | - | - | 14(100%) |
| | I | 3(71.42%) | 5(35.7%) | 6(28.57%) |
| | V | 10(71.42%) | - | 4(28.5%) |
| | C | 14(100%) | - | - |
| *C. parapsilosis* (12) | A | 12(100%) | - | - |
| | F | 10(83.3%) | - | 2(16.6%) |
| | I | 7(58.3%) | 5(41.6%) | - |
| | V | 12(100%) | - | - |
| | C | 12(100%) | - | - |
| *C.rugosa*(4) | A | 4(100%) | - | - |
| | F | 0(0) | - | 4(100%) |
| | I | 2(100%) | 2(50%) | - |
| | V | 4(100%) | - | - |
| | C | 4(100%) | - | - |
| *C. guilliermondii* (6) | A | 6(100%) | - | - |
| | F | 1(16.6%) | - | 5(83.3%) |
| | I | 3(100%) | 3(50%) | - |
| | V | 6(100%) | - | - |
| | C | 6(100%) | | - |

**Discussion**

In the present study, gender-specific differences in the prevalence of Candida infection were observed. In all specimen collected, percentage of women (62.1%) was higher than man(37.9%) suggesting that women have high risk for developing candida infection. In an earlier study, Guru and Raveendra also obtained majority of their candida specimen from women(54.5%)(2). Similarly, Pawar et al. and Prabhakaran et al. also reported higher proportion offemales compared to males in various specimen they studied (4,12). The reason for this could be high prevalence of Candida in vulvovaginal infections. Vulva and vagina provide the most conducive environment for fungal growth particularly Candida. More over,the affected women often complain of recurrent

Candidiasis suggesting their susceptibility of infection(13).The high prevalence of urine specimen(46.4%) also support the dominance of vulvovaginal source of infection. In the present study, sputum (21.4%) was the second most common specimen found positive for Candida, which suggests that oral Candidiasis is also common. Guru and Raveendran also reported urine specimen as the most common source for Candida (53%), with high prevalence in women (54.5%) (2). In recent years, oral candidiasis has been recognized as one of the most common mycoses in human beings (14). The present findings also suggest that oral candidiasis is amongst the most common mycoses in humans.

A number of previous studies have reported *C.albicans* as the most common Candida species. However, in the recent years several studies have reported a high prevalence of other species too. In present study, *C. tropicalis* (n=48,34.3%) was the most common Candida species followed by *C.albicans* (n=35,25%) followed by *C.glabrata* (n=21,15%), *C.krusei* (n=14;10%), *C.parapsilosis* (n=12; 8.5%), *C.guilliermondii* (n=6;4.2%)and *C.rugosa* (n=4;2.8%).Our findings are in agreement with the observations made by Chaudhary et al. who found *C.tropicalis* as the most prevalent (43%) species followed by *C. albicans* (41%), *C. krusei*(9%)and *C.parapsilosis* (7%) (15). In another study, Mathur et al. also did not find *C.albicans* to bethe most common isolate. They observed that *C.tropicalis* (39.4%) was the most common species, followed by *C.auris* (17.5%),*C.albicans*(14%)and *C.parapsilosis*(11.4%).These findings show that there is shifting the trend of Candida species prevalence (16).

Regarding the susceptibility of various Candida species to antifungal drugs, we found that all species of candida were highly susceptible (100%) to new generation antifungal drugs like Caspofungin and Amphotericin B whereas, however, maximum resistance was observed for Fluconazole(40%) followed by Itraconazole (5.7%) which also had a dose-dependent susceptibility to 29(20.7%) specimen. In their study,Guru and Raveendran also found Amphotericin B to be most sensitive (98%) however, they did not evaluate the antifungal susceptibility pattern against the newer generation antifungals like Caspofungin(2). However,Gandhi and Patel in their study reported the sensitivity of Amphotericin B to be maximum (97%) followed by Voriconazole(77%), Fluconazole(75%)and Itraconazole(64%) respectively(17).

In this study a hundred percent resistance was reported for *C. krusei* against Fluconazole because of its intrinsic resistance toward azoles. Also,the resistance of *C.glabrata* to Fluconazole was consistently higher (47.61%) resistant was observed however in their study, GeetaS.H. found *C. glabrata* was100%sensitivity against Fluconazole (18).

**Conclusion**

The findings of present study thus showed changing trends of *Candida* species and their antifungal susceptibility pattern at a tertiary care centre in North India. Keeping in view of the changing profile of *Candida* species and their antifungal susceptibility, there should be continuous monitoring of the prevalence of various fungal species in order to design effective treatment strategies Antifungal MIC determination is also essential in this period, as *Candida* species with higher MICs values are on the rise.

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**Ethical–**Ethical committee of ERA's Medical College, approved the study protocol

**Authors'contribution:**SK,drafted the manuscript, gathered information from the literature, performed the experiments ,designed the figures & tables and wrote the paper.MS, PK, MA and VK supervised and reviewed the manuscript.

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