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Research Article

# Study of *Candida* Species in Various Clinical Specimens at UCMS-TH, Bhairahawa, Nepal

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## ABSTRACT

**Introduction:** The upward trends of fungal infections in the recent times contribute to high rates of morbidity and mortality. *Candida* species are commensal yeasts of skin and part of gut microbiota. The altered host immune system together with abnormal colonization and invasive medical technologies contribute to opportunistic infections. Candidiasis may be superficial or deep-seated. Increase in antifungal resistance recently has rendered treatment difficult due to restricted number of antifungal drugs.

**Methods:** This is a descriptive cross-sectional study done for a period of 18 months at a tertiary care center. Ethical clearance was obtained from institutional review committee with a reference number (UCMS/IRC/036/18). Two hundred isolates of *Candida* species were identified from various clinical samples by using phenotypic tests such as chrom agar, sugar fermentation test, sugar assimilation test and germ tube test. Antifungal susceptibility test was performed for five drugs namely ketoconazole (10 µg), fluconazole (10 µg), itraconazole (10 µg), nystatin (100 µg) and amphotericin-B (20 µg). All the statistical evaluation was done by using SPSS version 20.0 software (IBM Corp., Armonk, NY).

**Results:** Among 200 isolates of *Candida*, the most frequently isolated species was *C.albicans* which was seen in 69% of total isolates followed by *C. tropicalis*, *C. krusei*, and *C. dubliniensis*. Maximum *Candida* isolates were from urine sample (41.5%) followed by sputum (22.5%). Amphotericin B was found to be the most sensitive drug with a sensitivity of 97.1% whereas ketoconazole was the least sensitive drug with a sensitivity of 40.5% among the isolates.

**Conclusion:** *C. albicans* were the major isolates in this study, however, there is an increased incidence of non-albicans *Candida* species. Antifungal susceptibility test revealed increased resistance to different antifungal drugs among *Candida* species. This may conclude that delay in diagnosis and increased resistance may lead to serious complications and depicts the need of new therapeutic options.

**Keywords:** Antifungal resistance; Antifungal susceptibility test; *Candida albicans*; Non albicans *Candida*

## INTRODUCTION

With advancement of invasive medical technologies and extending immune suppressed population, fungi are now recognized as a major cause of infections. *C.albicans* is one of the major fungal pathogen, causing both superficial and profound candidiasis. However, there is a progressive shift to non-albicans *Candida*. *Candida* spp is ubiquitous pathobiotic microorganisms and are the normal flora of mucocutaneous membrane of humans [1]. They are responsible for endogenous opportunistic infection in debilitated host due to their versatility to adapt in different host niches. *Candida* spp. is established pathogens for causing fulminant infections and nosocomial outbreaks [2]. These infections are the consequences of local or generalized defect in host defenses and underlying risk factors like injudicious use of antibiotic, steroid therapy, indwelling

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catheters etc. The primary challenge to the management of candidiasis includes early identification and rapid instillation of antifungal therapy. Owing to the paucity of diagnostic modalities, isolation and speciation of *Candida* spp. are often missed [3].

Species identification is routinely done using phenotypic tests. However, newer diagnostic assays have high sensitivity and include chrom agar, Analytical Profile Index (API) systems and molecular methods. Detection of *Candida* spp. using conventional phenotypic tests are time consuming and labor intensive. Moreover, phenotypic tests have low discriminating power and lack of reproducibility of results leading to faulty diagnosis [4]. Antifungal resistance is an emerging problem imposing a global threat worldwide and *Candida* spp. are the organisms of concern due to their high propensity to develop antifungal resistance. Management of candidiasis is challenging because of difficult diagnosis and availability of few numbers of antifungal agents. This study aims for the correct identification of candida spp. along with their antifungal susceptibility testing as it aids in early diagnosis and optimum selection of antifungal therapy. This study aims for the correct identification of *Candida* spp. along with their antifungal susceptibility testing as it aids in early diagnosis and optimum selection of antifungal therapy [5].

## MATERIALS AND METHODS

This descriptive cross-sectional study was conducted from January 2018 to July 2019 in the microbiology laboratory of Universal College of Medical Sciences (UCMS-TH), Bhairahawa. Ethical clearance was obtained from institutional review committee of UCMS-TH (Reference number UCMS/IRC/036/18) [6].

Total 200 samples were collected from patients having one or more risk factors such as ICU admission, hospital stay for more than seven days, steroid therapy, device implantation, immunosuppressive conditions such HIV/AIDS, tuberculosis, malignancy and diabetes mellitus. All grams positive, oval budding yeast like cells with or without pseudo-hyphae was considered for the study whereas gram positive capsulated yeast cells showing urease test positive were excluded from the study [7].

Under aseptic conditions, various clinical specimens were collected and inoculated on to blood agar and incubated aerobically at 37°C. Gram stain was performed to look for budding yeast like cells. Selected colonies showing gram positive budding yeast like cells were subcultured on sabouraud's cycloheximide chloramphenicol agar [8]. For isolation and speciation, gram staining, germ tube test, inoculation on chrom agar and biochemical tests such as sugar fermentation tests and sugar assimilation tests were performed. ATCC (American Type Culture Collection) strain of *C. albicans* (90028) was taken as a reference strain [9].

Sugar fermentation test was performed with liquid media containing 1% peptone, 0.5% sodium chloride with 1% andrade's indicator in which 2% of filter sterilized sugars (glucose, lactose, maltose and sucrose) were added to the medium [10]. The solution was then poured into the test tubes with Durham's tube and each test tubes were inoculated with 0.1 ml of inoculum. The tubes were incubated at 25°C up to 7 days and examined at every 24 hours for the production of gas in Durham's tube and acid (pink color). Production of both acid and gas was taken as sugar fermentation positive [11].

Sugar assimilation test was performed with 24 hours old culture. Heavy inoculum was added in 2 ml of yeast nitrogen base to make yeast suspension. Yeast suspension was added to 18 ml of molten agar (45°C), mixed well and poured into 90 mm petri plate and allowed to set at room temperature [12]. Sugar discs (glucose, lactose, maltose and sucrose) were placed onto the surface of agar plates and incubated at 37°C for 3-4 days. The presence of growth around the discs was interpreted as assimilation positive for that sugar. Chromagar *Candida* was used as a differential media for speciating *Candida*. Colonies of *Candida* isolates were inoculated onto the medium and incubated at 37°C for 48-72 hours. Results were noted on the basis of colors produced [13].

Disk diffusion method of antifungal testing was performed using Mueller-Hinton agar with 2% glucose and methylene blue and was performed as per the Clinical and Laboratory Standards Institute (CLSI) protocol. Itraconazole (10 µg), ketoconazole (10 µg), fluconazole (10 µg), nystatin (100 µg), amphotericin B (20 µg) were used for antifungal testing and results were interpreted as sensitive, susceptible dose dependent and resistant [14]. The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, NY). Microsoft excel and word (Microsoft Corporation, Redmond, WA) were used to prepare graphs and tables. Descriptive statistics such as mean, proportion (%) were calculated using SPSS software [15].

## RESULTS

The age and gender wise distribution of *Candida* species where maximum isolates were from 31-40 years (22.5%) and from female (56.5%) (Table 1).

**Table 1:** Demographic profile of patients with Candida infection.

| Variables | Number (%) |
|---|---|
| **Age (years)** | |
| 0-10 | 3 (1.5%) |

| | |
|---|---|
| 11-20 | 11 (5.5%) |
| 21-30 | 40 (20%) |
| 31-40 | 45 (22.5%) |
| 41-50 | 21 (10.5%) |
| 51-60 | 27 (13.5%) |
| 61-70 | 26 (13%) |
| >70 | 27 (13.5%) |
| Total | 200 (100%) |
| **Gender** | |
| Female | 113 (56.5%) |
| Male | 87 (43.5%) |
| Total | 200 (100%) |

Among a total of 200 isolates, maximum isolates 83 (41.5%) were from urine sample (Table 2).

**Table 2:** Specimen wise distribution of *Candida* isolates.

| **Specimen** | **Number** (%) |
|---|---|
| Urine | 83 (41.5%) |
| Sputum | 45 (22.5%) |
| High vaginal swab | 35 (17.5%) |
| Foley's tip | 10 (5%) |
| Wound swab | 9 (4.5%) |
| Endotracheal tube | 6 (3%) |
| Oral swab | 5 (2.5%) |
| Pus | 4 (2%) |
| Blood | 3 (1.5%) |
| Total | 200 (100%) |

Various risk factors contribute to *Candida* infection out of which hospital stay for more than 7 days was highly significant accounting for 93 (46.5%) cases in this study (Table 3) [16].

**Table 3:** Distribution of risk factors associated with *Candida* infection.

| **Risk factors** | **Number of isolates** (%) |
|---|---|
| Hospital stay>7 days | 93 (46.5%) |
| ICU admission | 19.5% |

| | |
|---|---|
| Diabetes mellitus | 20 (10%) |
| Prolonged antibiotic therapy | 17 (8.5%) |
| Tuberculosis | 13 (6.5%) |
| Hormonal therapy | 12 (6%) |
| Device implantation | 6 (3%) |
| Total | 200 (100%) |

The most frequently isolated species were *Candida albicans* which was seen in 138 (69%) cases and least isolated species was *Candida dubliniensis* seen in 10 (5%) cases (Table 4).

**Table 4:** Distribution of various *Candida* species.

| **Candida species (n=200)** | **Number** (%) |
|---|---|
| *C. albicans* | 138 (69%) |
| *C. tropicalis* | 38 (19%) |
| *C. krusei* | 14 (7%) |
| *C. dubliniensis* | 10 (5%) |

Table 5 exhibits the antifungal susceptibility of *Candida* spp. where *C. albicans* showed maximum sensitivity to amphotericin B (96%) followed by itraconazole (94.2%) and was least sensitive to ketoconazole (48.5%). *C. tropicalis* isolates were mostly sensitive to amphotericin B (92.1%) followed by nystatin (81.57%). Out of 14 isolates of *C. krusei*, 64.2% were sensitive to amphotericin B and none of the isolates were sensitive to fluconazole. All isolates of *C. dubliniensis* showed 100% sensitivity to itraconazole, 70% sensitivity to nystatin and 30% sensitivity to fluconazole. Among the 200 isolates of various *Candida* spp., fluconazole and ketoconazole were found to be the most resistant drugs (Table 5) [17].

**Table 5:** Antifungal susceptibility patterns of *Candida*.

| **Antifungal agents** | **Susceptibility pattern** | ***C. albicans*, n=138** (%) | ***C. tropicalis* n=38** (%) | ***C. krusei* n=14** (%) | ***C. dubliniensis* n=10** (%) | **Total, n=200** (%) | **p-value** |
|---|---|---|---|---|---|---|---|
| Fluconazole | S | 90 (65.2%) | 21 (55.2%) | 0 (0) | 3 (30%) | 114 (57%) | <0.01 |
| | S-DD | 10 (7.21%) | 3 (7.89%) | 0 (0) | 0 (0) | 13 (6.5%) | |
| | R | 38 (27.5%) | 14 (36.8%) | 14 (100%) | 7 (70%) | 73 (36.5%) | |
| Ketoconazole | S | 67 (48.5%) | 23 (60.5%) | 2 (14.2%) | 4 (40%) | 96 (48%) | |
| | S-DD | 15 (10.8%) | 0 (0) | 0 (0) | 0 (0) | 15 (7.5%) | |
| | R | 56 (40.5%) | 15 (39.4%) | 12 (85.7%) | 6 (60%) | 89 (44.5%) | |
| Itraconazole | S | 130 (94.2%) | 25 (65.7%) | 8 (57.14%) | 10 (10%) | 173 (86.5%) | |
| | S-DD | 6 (4.34%) | 3 (7.89%) | 0 (0) | 0 (0) | 9 (4.5%) | |
| | R | 2 (1.44%) | 10 (26.31%) | 6 (42.8%) | 0 (0) | 18 (9%) | |
| Nystatin | S | 120 (86.9%) | 31 (81.5%) | 8 (57.14%) | 7 (70%) | 166 (83%) | |
| | S-DD | 12 (8.69%) | 2 (5.2%) | 1 (7.14%) | 0 (0) | 15 (7.5%) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | R | 6 (4.34%) | 5 (13.1%) | 5 (35.7%) | 3 (30%) | 19 (9.5%) |
| Amphotericin -B | S | 134 (97.1%) | 35 (92.1%) | 9 (64.2%) | 6 (60%) | 184 (92%) |
| | S-DD | 3 (2.17%) | 1 (2.63%) | 2 (14.2%) | 1 (10%) | 7 (3.5%) |
| | R | 1 (0.72%) | 2 (5.2%) | 3 (21.4%) | 3 (30%) | 9 (4.5%) |

**Note:** S: Sensitive; S-DD: Susceptible Dose Dependent; R: Resistant

## DISCUSSION

In this study, maximum number of *Candida* infections were seen in the age group of 31-40 (42.21%) followed by the age group of 21-30 years (20%). This finding is consistent with one of the studies conducted in India. Females (56.5%) were infected more than males (43.5%) in this study. Similar finding was seen in a study conducted by where infections were more in females than in males. As *Candida* can cause superficial and invasive infections, various clinical specimens were collected to isolate the pathogens. Maximum isolates were from urine samples (41.5%), followed by sputum (22.5%) whereas least isolates were from blood (1.5%). Similar findings were observed in the study conducted [18].

Candidiasis is prevalent among immune compromised hosts, so, there were one or more predisposing factors associated with infections in this study. Similar to this observation, 77% of patients had one or more risk factors in a study conducted by in India. Patients in hospital settings are at higher risk of acquiring nosocomial infections as they are subjected to various risk factors such as mechanical ventilation, use of broad spectrum antibiotics, device implantation and the debilitated condition of the patient itself acts as a risk factor. In this study, admission to intensive care unit was the prominent risk factor for candidiasis. This association was seen in a study conducted in Japan. Similarly, in a study by showed that critically ill surgical patients had a high risk of *Candida* colonization. In a study by the frequency of *Candida* infection among oral contraceptive pills users were significantly more which explains the higher number of infections in females. The other important factor contributing to candidiasis in our study was diabetes mellitus. In a study by diabetes mellitus was associated with both acute and chronic candidiasis. The pathogenesis for *Candida* infection in diabetes patients may be multi-factorial such as increased salivary glucose leading to less salivary flow, microvascular degeneration and decreased phagocytic activity of neutrophils [19].

In this study, the most frequently isolated species was C. albicans (69%) followed by non-albicans *Candida* (31%). Non-albicans *Candida* include C. tropicalis (19%), *C. krusei* (7%) and *C. dubliniensis* (5%). Our study findings were similar to other studies conducted in Nepal where *C. albicans* were the predominant isolates. But contrary to our findings, non-albicans *Candida* predominated over *C.albicans* in some studies. *C. albicans* showed maximum sensitivity to amphotericin B (97.1%) and least susceptible to ketoconazole (65.2%). Some of the previously conducted studies showed higher sensitivity to amphotericin-B. In this study, *C. krusei* showed 100% resistance to fluconazole which was similar to the study conducted. This finding may be supported by the fact that *C. krusei* are intrinsically resistant toward azoles. *C. tropicalis* showed maximum resistance to azole group of drugs like ketoconazole, fluconazole and itraconazole. Also showed higher resistance patterns of non-albicans *Candida* towards fluconazole. These findings were in line in one of the studies where low resistance to amphotericin-B and high resistance to fluconazole among *Candida* spp. were seen [20].

Nystatin have a very good susceptibility result among various *Candida* spp. Our findings matched with the results of one of the studies carried in India which showed high sensitivity of nystatin for *Candida* spp. These findings were found to be discordant with the study by where only 25% isolates were susceptible to nystatin. The limitation of this study was the time variability required for the speciation of various *Candida* isolates using different phenotypic tests and due to limited resources molecular characterization was not possible. This variation in time may have serious implications in life threatening infections where rapid diagnosis and prompt treatment are extremely important in prescribing the correct therapeutic drug [21].

## CONCLUSION

The changing epidemiology of *Candida* infection is alarming, so close observation of *Candida* species distribution among patients is necessary. As rapid instillation of antifungal drugs is crucial for improving the treatment outcome, early diagnosis is of utmost importance. Growing resistance towards antifungal drugs is alarming as treatment failure may lead to life threatening complications and deliberately demand new treatment options.

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## ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The ethical approval for the study was obtained from the institutional review committee of Universal College of Medical Sciences, (UCMS-TH).

## AVAILABILITY OF DATA AND MATERIALS

The raw data will be available on request.

## COMPETING INTEREST

The authors declare they have no competing interest.

## FUNDING

The necessary supplies and reagents were provided by department of microbiology of UCMS-TH.

## AUTHOR'S CONTRIBUTION

KG was responsible for study design and sample processing. KG, KRR and SR contributed to data analysis and manuscript writing.

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