ORIGINAL ARTICLE

ISOLATION OF CANDIDA SPECIES FROM CLINICAL SPECIMENS WITH THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN (AN OBSERVATIONAL STUDY FROM PAKISTAN)

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ABSTRACT

Objectives: This study aimed to observe the prevalence of Candida infection in hospital-admitted patients and observe their antifungal susceptibility to treat these admitted patients according to their sensitivity,

Methods: This study was conducted on 105 isolates of *Candida species* obtained from various clinical samples, including urine, cerebrospinal fluid, invasive catheter tips, blood, and tracheal secretions of patients admitted to a Tertiary Care Hospital in Lahore, Pakistan. The patients taking antifungal and outpatient department samples were excluded from this study. Each sample was processed according to standard operating procedures in the microbiology laboratory of Lahore General Hospital, Lahore, Pakistan, affiliated with Post Graduate Medical Institute, Ameer ud din Medical College, Lahore, Pakistan.

Results: The frequency of *Candida species* is found to be 5.71% in patients admitted to tertiary care. The most common isolated *Candida spp.* was *Candida tropicalis*, followed by *Candida albicans.* A 27.6% Fluconazole resistance was observed in the isolates. There was no Amphotericin resistance in the Candida isolates both by disc diffusion and on minimum inhibitory concentrations MIC method.

Conclusion: Resistance against the antifungal azole group was observed in the current study. More studies on fungal infection should be conducted in the healthcare sector of developing countries to add more information regarding new emerging resistant fungal infections.

Keywords: Candida albicans, Candida tropicalis, Antifungals, Fluconazole, Amphotericin B

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INTRODUCTION

Candidiasis is an infection caused by *Candida* species. Candidemia is responsible for opportunistic mycosis and is a leading cause of nosocomial infections[1]. According to the Center for Disease Control and Prevention, Candida infections are the 6th most common source of nosocomial infections[2]. It presents various diseases, from single-organ involvement to multi-systemic organ presentation[1]. *Candida spp* encompasses more than 400 species, many of which cause invasive human infections[3].

Species distribution has changed over the past three decades. More than 95% of *Candida* infections are caused by the five most common species, which are *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida krusei, and Candida glabrata*[4].

Candida is causing a wide-ranging portion of invasive infections, and the incidence of Candida non-albicans species increases over time. The infections by Candida non-albicans species are associated with a high mortality rate and are linked with high antifungal resistance among these infections[3,5]. *Candida spp* accounts for around 10%

of BSI and 25% of urinary tract infections UTI, and its mortality rate is 20 to 40%[3,6]. These nosocomial infections are due to prolonged hospital stays and increased healthcare costs[6].

For Candida infection, multiple risk factors are involved, including non-judicial use of broad-spectrum antibiotics, malignancy, immunocompromised patients, uncontrolled diabetes mellitus, surgical interventions, and prolonged stay in hospitals[7]. Besides these risk factors, adherence to abiotic and biotic substances and the release of hydrolytic enzymes have played an essential role in candida spp pathogenesis. These enzymes are responsible for tissue penetration, host cell destruction, and invasion of human bodies[8]. Candida colonization occurs due to this adherence to host cells, and this adherence reduces the clearance of Candida by the host defense system. Around half of the hospital-acquired infections are associated with invasive medical devices[6,9].

Nosocomial infections of Candidemia can be prevented by infection prevention and control measures, including hand hygiene, standard precautions, aseptic techniques, adjusting the nurse-patient ratio, and surveillance[10].

IdentificationCaCandida at the strain level is crucial for determining their antifungal susceptibility in hospital-acquired infection. This will be helpful for species tracking and their resistance pattern against antifungal drugs, as the high mortality rate with candida infections nowadays is suggestive of ineffective antifungal treatments[11,12]. Hence, identification at the species level and selection of appropriate antifungal drugs is crucial; even though resistance is rare in C. Albicans 1- 2%[12], they are acquiring new resistance patterns and making their treatment more challenging[12]. In this study, we aimed to identify hospitalized candida species infections from various clinical specimens and securitized antifungal susceptibility patterns to track resistance patterns and treatment options for candidiasis.

METHODS

This study was conducted from February 2018 to February 2019 on 105 isolates of *Candida species* obtained from various clinical samples, including urine, cerebrospinal fluid, invasive catheter tips, blood, and tracheal secretions of patients admitted in a Tertiary Care Hospital of Lahore, Pakistan. The patients taking antifungal and outpatient department samples were excluded from this study. Each sample was processed according to standard microbiological operating procedures in the microbiology laboratory of Lahore General Hospital (LGH), Lahore, Pakistan, affiliated with Post Graduate Medical Institute (PGMI), Ameer ud din Medical College (AMC), Lahore, Pakistan. Ethical approval was taken for the research from the Institutional Review Board (IRB) of the research department of PGMI/AMC/LGH.

The clinical samples were received in the Microbiology unit of the pathology department for microbial examination. Gram staining was done. Cultures were put up on Blood, MacConkey, and CLED agars as per the request of the sample. The next day, plates were observed, and samples displaying the supposed growth of *Candida species* were included in the study. One hundred five consecutive, non-repeated isolates from 1837 clinical samples were further handled for final identification of species of *Candida* antifungal susceptibility testing.

Candida species were examined on wet-mount preparation, a drop of normal saline and *Candida* colonies were mixed and emulsified on a slide, and a cover slip was placed gently to avoid air bubbles forming. The condenser was lowered, and the iris diaphragm was adjusted. The wet mount preparation was examined first under 10X to detect oval budding yeast cells and pseudohyphae. These budding oval cells and pseudohyphae presumed to be *Candida species* were examined under 40X.

Gram staining was used for presumptive identification of *Candida species.* The dried smear was examined under 10X for screening the slide. An oil drop was added to the slide and viewed under a 100X oil immersion lens. In Gram-stained smears, *Candida species* appear as Gram-positive oval budding yeast cells (blastoconidia) and pseudohyphae.

All *Candida species* isolated from clinical samples were subculture on Sabouraud Dextrose Agar (SDA) plates and aerobically incubated at 37 °C for 24–48 hours. SDA is usually used to isolate yeasts and fungi; its pH is acidic, i.e., around 5.0, which resists the growth of bacteria but authorizes yeast growth. *Candida species appear* as white to cream-colored, smooth colonies.

Identification of *Candida species* was made by API Rapid yeast plus system (Remel®). The test was run according to the manufacturer's guidelines. Well-isolated colonies of Candida species were taken from Sabouraud's agar and then inoculated in an API RAPID Yeast Plus panel containing 18 reaction cavities. Enough colonies were taken to make Inoculation fluid until the turbidity obliterated the black lines on the inoculation card. Fluid was then transferred into the panel, resealed, and incubated at 30 C for 4 hours. The panel was read from left to right after adding reagents to mentioned cavities. The score was recorded on the report form, and the microcode was entered in ERIC to get results.

Antifungal susceptibility testing was done through Disc Diffusion and E-test methods. Antifungal susceptibility testing was performed and interpreted for all the isolates of *Candida species* by using the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI), against two antifungal impregnated disks, i.e., 20mg amphotericin B (Liofilchem, Italy) and 25mg fluconazole (Liofilchem, Italy). The interpretation of the results according to CLSI

E–TEST: In this method, predefined gradient concentrations of Amphotericin B and Fluconazole (Liofilchem®) plastic E-strip were used to determine the MIC (CLSI M27-S4).

RESULTS

The frequency of *Candida species* is found to be 5.71% in patients admitted to the tertiary care hospital. Out of 1837 clinical specimens collected in the microbiology lab, those 105 samples showed growth of Candida. The rest of the results are displayed in table 1 and Figures 1, 2, and 3.

No antifungal resistance was seen against Amphotericin B in Candida spp isolated. There was 27.6% resistance against Fluconazole in the *Candida spp* isolated from the samples of this study. The mean Amphotericin B on MICs was statistically the same in all Candida species, p-value > 0.05.

Interpretation of MIC breakpoints for amphotericin B is as follows:

Susceptible: ≤ 1µg/mL, Resistant: ≥1µg/ml

The mean Fluconazole MICs were statistically higher in *Candida krusei* than in all other groups, p-value < 0.001.

interpretation of MIC breakpoints for Fluconazole is as follows:

Susceptible: ≤ 8 µg/mL, Susceptible dose-dependent: 16 ~ 32µg/mL, Resistant: ≥ 64µg/mL

The mean Fluconazole MICs in *C.albicans* was 19.77 ± 31.40, in *Candida tropicalis* was 25.07 ± 39.90, in *Candida zeylanoides* was 18 ± 26.20 and in *Candida, Krusei* was 123.20 ± 78.30.

The range of Amphotericin b MICs for *Candida species* was 0.064-0.750 $\mu g/mL$, while the mean MIC of Amphotericin B was 0.36 $\mu g/mL$. The range of zone diameter of Fluconazole for *Candida species* was 5-40mm, with a mean of 24.3mm. The mean fluconazole MIC was statistically higher in *Candida krusei*, with a mean MIC of 123.20µg/ml. The range of MIC for *Candida species* was found to be 0.25-256 µg/ml with a mean MIC of 27.65 µg/ml.

Table 1*Candida species* is found to be in 5.72% of patients admitted in tertiary care hospital n=1

Variables	No of samples	Positive for Candida spp.	Percentage
Age			
Up to 19	474	22	4.6%
20-39	542	21	3.9%
40-59	503	35	7.0%
60 & above	318	27	8.5%
Total	1837	105	5.7%
Gender			
Male	1047	46	4.39%
Female	790	59	7.46%*
Ward			
Gynae/Obs	170	10	5.9%
ICU	335	17	5.1%
Medicine & Allied	271	31	11.4%*
Pediatrics	253	8	3.2%
Surgery & Allied	808	39	4.8%
Total	1837	105	5.7%
TYPE OF SPECIMENS			
Blood	773	5	0.6%
HVS	17	7	41.2%
Pus/Wound Swab	264	7	2.7%
Tips (ETT tip, CVP tips etc)	37	9	24.3%
Urine & Catheter tips	548	77	14.1%
Sputum/Tracheal secretion	123	0	0.0%
others (fluids, tissues etc)	75	0	0.0%
Total	1837	105	5.7%

Figure 1: Distribution of *Candida spp.* according to Gender and age

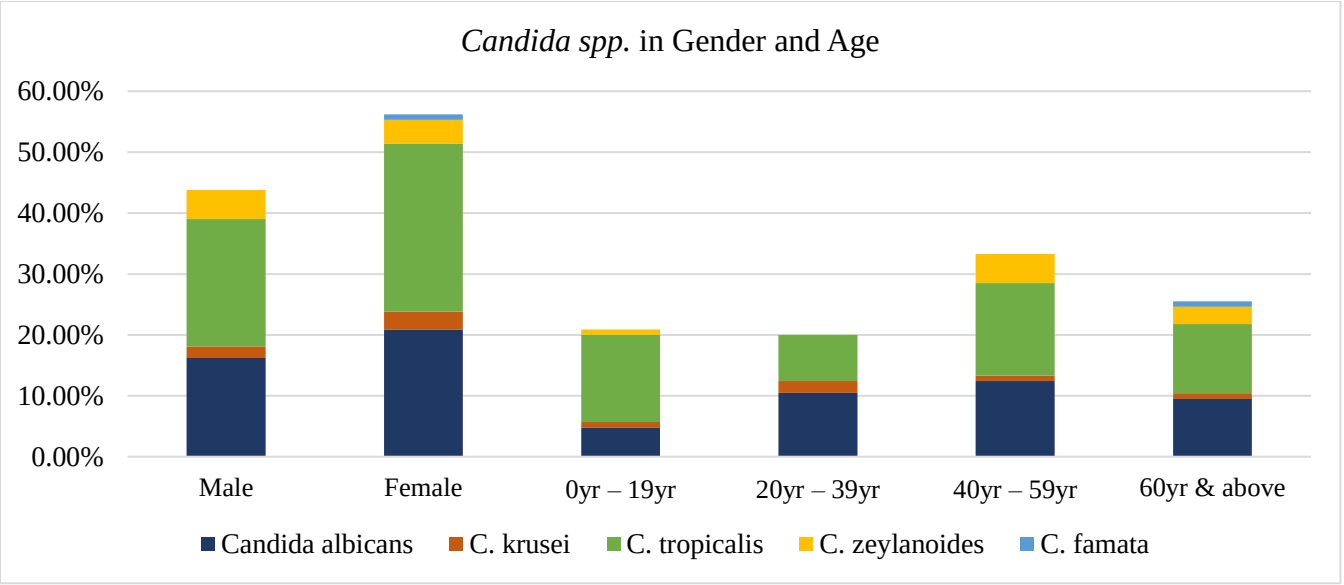


Figure 2: Distribution of *Candida spp*. according to ward and specimens' type

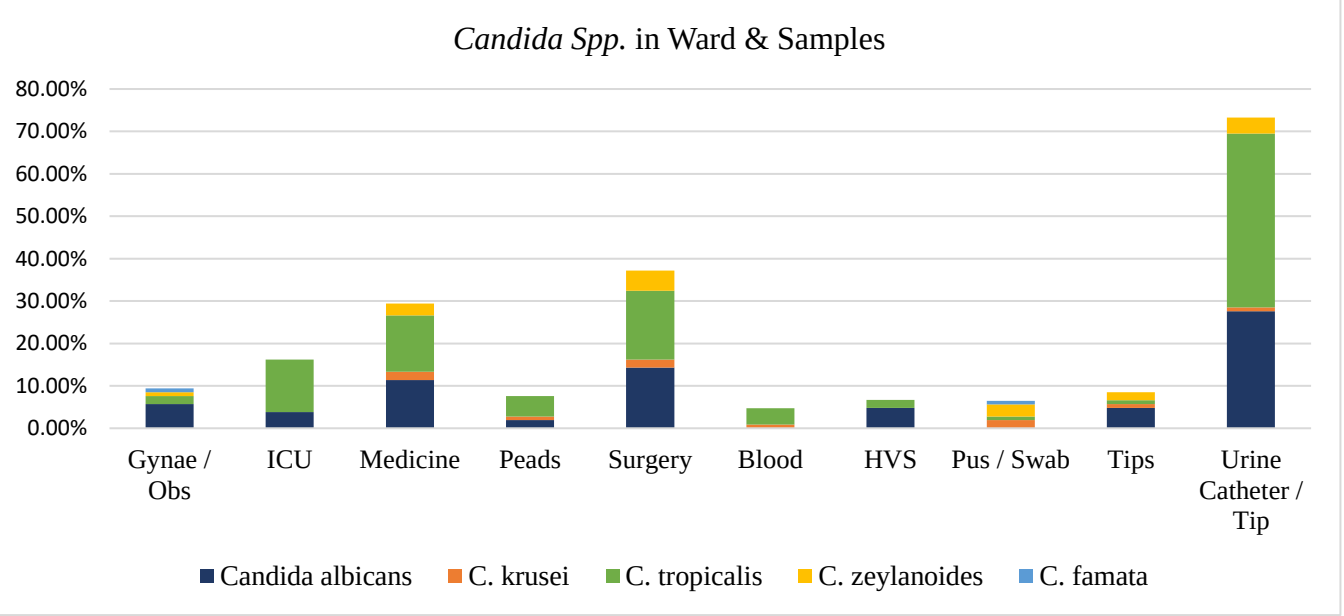
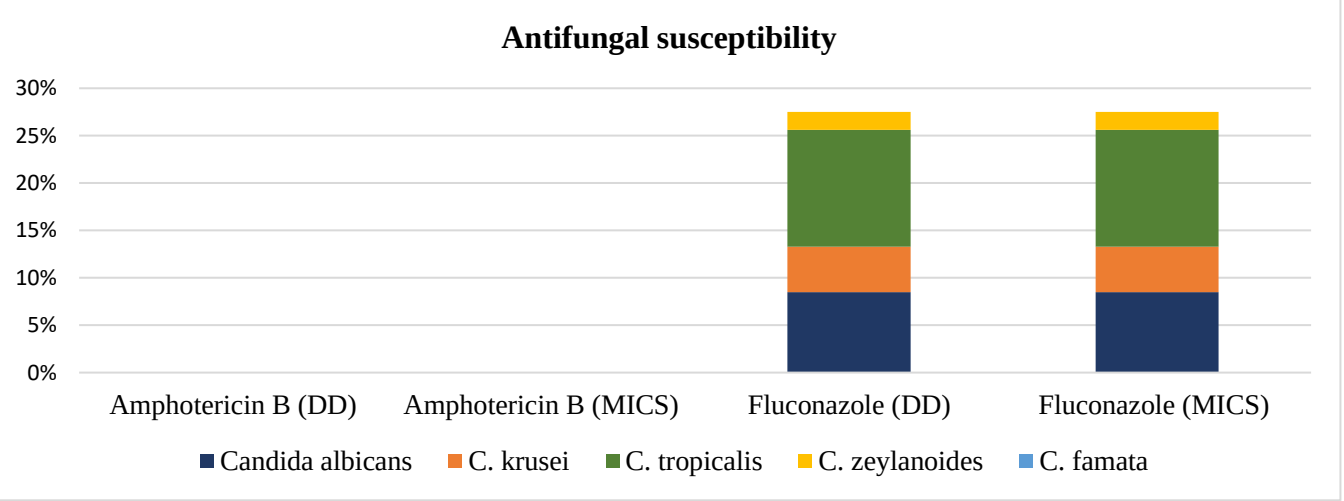


Figure 3: Antifungal susceptibility pattern of different *Candida spp.*



DISCUSSION

Hospital-acquired infections result in significant morbidity and mortality among hospitalized patients and put a tremendous financial burden on patients (Saliba et al., 2018). Incidences of hospital-acquired infections are reported to be 5-10% in developed countries, while in underdeveloped countries like India and Pakistan, it is said to be about 25% (Baviskar et al., 2019)

In the current study, 1837, various clinical specimens of admitted patients were examined, where 105 samples were found to be positive for *Candida species*. The frequency of *Candida species* in this study was 5.72%. Candida species' prevalence was 6% in another study in Karachi (Kumar et al. 2014). In our study, the age group was divided into four categories, and the results were significant (p-value: 0.0153). According to the results of this study, the highest percentage (8.5%) of *Candida species* was seen in group 60 and above years of age. Similarly, more frequency of *Candida species* was observed in elderly patients by other researchers (Jeon et al.,2019). In this study, there was a predominance of females (56.19%) where *Candida species* were more frequently isolated than males (43.8%), and this study is consistent with the study of Tasneem et al. (2017), where they reported 65.8% cases of females and 30.9% males.

Among various wards, the highest frequency of *Candida species* in our study was seen in medicine and allied wards (p value:0.000498) which were 11.4%, as highlighted in Table 3. Here we keep in mind that there are four medical units of the General Hospital, and chronic patients are admitted to medicine and allied wards. A high incidence (53.5%) and mortality were observed in medical and allied wards compared to surgery and ICUs.

In our study, the frequency of various *Candida species* according to the type of specimen was found to be significant (p value:0.0004998), where the highest percentage of *Candida species* was observed in high vaginal swabs (41.2%), followed by other clinical specimens. Studies conducted by Toure et al. (2016) and Scapaticci et al. (2018) found results that are comparable to our findings, where the highest percentage of *Candida species* was seen in high vaginal swabs (48%). In our study, *Candida tropicalis* was the most frequently isolated species, consistent with studies conducted by Mohamed et al (2018) in Malaysia where Candida tropicalis was the most isolated species (28.9%).

In the current study, susceptibility testing was done by two methods, i.e., Disc Diffusion and E-strip Testing. Both methods showed similar results in which all 105 (100%) isolates were sensitive to Amphotericin B. According to our study, out of 105 isolates, only 76 were susceptible to Fluconazole, and 29 isolates were resistant to it by both methods, i.e., Disc diffusion and E test strip method). Antifungal resistance was a notable finding in our study and was mainly restricted to Fluconazole, where the frequency of fluconazole resistance by *Candida species* was 27.61%. In a study conducted in Karachi by Rizwan et al. Pakistan, resistance to Fluconazole was reported as 57.7% (Rizwan et al.,2018). This study was subject to certain limitations; we only worked on *Candida spp.*, and the API for Candida we had used in the study was unable to isolate *Candida auris*, the emerging resistant pathogen nowadays. The study was also limited to only Candida fungal infections; more studies could be conducted on fungal molds isolated from clinical specimens of the patients.

CONCLUSION

Resistance against the antifungal azole group was observed in the current study. This study emphasizes that more such studies should be conducted to see the frequency of *Candida species* and resistance to the azole group of antifungals, especially Fluconazole. We urgently need a strategy to control antifungal drugs' inappropriate and widespread use. More studies on fungal infection should be conducted in the healthcare sector of developing countries to add more information regarding new emerging resistant fungal infections.

CONFLICT OF INTEREST

No conflict of interest among the authors of this study.

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