

Mycological Profile of *Candida tropicalis* and its Virulence Factors from Candidemia Patients at A Tertiary Care Facility

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Abstract

Candida tropicalis is the fourth main infective agent of *Candida* species in several developing nations and leads to the greatest fatality rate among the *non-albicans Candida* (NAC) species that cause candidemia. Seventy clinically known *Candida* isolates are isolated in positively flagged blood samples from BacT/ALERT 3D from various wards. Virulence factors like hemolysin production, coagulase activity, phospholipase activity, and biofilm formation were studied and antifungal susceptibility testing was and Minimum Inhibitory Concentration (MIC) values were interpreted. Of the total 70 clinical *Candida* isolates, the most predominant organism isolated was found to be *C. tropicalis* 27 (38.57%) which is succeeded by 19 (27.14%) *C. albicans*, 13 (18.57%) *C. parapsilosis*, 6 (8.57%) *C. glabrata*, and 5 (7.14%) *C. krusei* respectively. Among the 70 *Candida* isolates, 49 (70%) showed hemolysin production, 43 (61.42%) isolates demonstrated phospholipase activity, 34 (48.57%) showed coagulase activity and 55 (78.57%) isolates showed biofilm production by crystal violet assay. A high level of Fluconazole resistance has been observed in 23 (32.85%) *Candida* isolates in comparison with other antimicrobials utilized in this study. The higher MIC value of: ≥ 64 $\mu\text{g/mL}$ Fluconazole was shown by 4 (5.71%) isolates of *C. tropicalis* by broth microdilution method. The interpretation of various virulence factors and antifungal drug resistance were seen mostly among NAC species, thus hence signifying its pivotal role in immunocompromised individual treatment.

Keywords: *Candida tropicalis*, Candidemia, Virulence Factors, Antifungal Drug Resistance

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INTRODUCTION

Candida species causing bloodstream infections (BSIs) are also called candidemia which exists mostly as a pervasive type of invasive candidiasis.¹ Candidemia is one of the most prevalent reasons for BSIs among hospitalized patients in the United States, and it frequently leads to prolonged hospitalization as well as mortality.² *C. tropicalis* is the fourth main infective agent of *Candida* species in several developing nations and contributes the greatest fatality rate among the NAC species that cause candidemia. The extensive utilization of antifungal agents increases *C. tropicalis* antifungal resistance, especially to azoles, which would ultimately result in the treatment failure of candidemia.^{3,4}

C. tropicalis is considered the first and most predominant species causing candidemia in growing nations, wherein an enormous number of cases are treated by Fluconazole as a consequence of the outrageous price of echinocandins.⁵ In comparison to other continents, tropical Asia and Latin America have the relatively greatest proportion of candidemia caused by *C. tropicalis*.⁶ As stated by the Centers for Disease Control and Prevention (CDC), around 25,000 candidemia incidences were reported nationwide each year. *C. tropicalis* (38%) is the commonest organism in India among candidemia cases.⁷ On the other hand, isolates of *C. tropicalis* have spread globally, and since 2010, this situation has particularly become debatable in the Asia-Pacific area. However, a dramatic rise in pan azole-resistance and Amphotericin B resistance of *C. tropicalis* was shown in the majority of studies conducted on candidemia studies.⁸⁻¹¹

Several virulence factors of *Candida* species cause BSIs including hemolytic activity, coagulase, proteinase, phospholipase, esterase, lipase, and biofilm formation. All of these multiple virulence factors could raise BSIs of *Candida* species by escape mechanism which is part of the defence system in order to impair the host tissue.¹² The manifestation of virulence factors of *Candida* species can be differentiated based on species, type of infection, geographical region, host reaction and stage, and the site of infection. Understanding these virulence factors is crucial to know the pathophysiology, and also assists

researchers to discover novel antifungal targets for more effective therapeutic regimens.¹³

As per the CDC monitoring statistics, the percentage of Fluconazole-resistant *Candida* isolates remained relatively stable over the last 20 years.¹⁴ In India, nationwide candidemia research has been held revealing that the origination of multidrug-resistant (MDR) *C. tropicalis* was in an equivalent percentage to what was perceived for MDR *C. auris*.¹⁵ The progression in drug tolerance enables the organism to procure persistent modifications of genes that result in the development of antifungal resistance, therapeutic failure, and death.¹⁶ Indeed, various research suggests *C. tropicalis* are tolerant to azoles as the efficiency of Fluconazole on these isolates from blood samples was diminished, which would ultimately result in Fluconazole therapeutic failure.¹⁷ Despite the progression of resistance, one or more antimicrobial agents should represent a severe concern among candidemia cases caused by *C. tropicalis* since there are so few antifungals available to treat, especially in underdeveloped nations, where Fluconazole is one of the most often antifungals used.¹⁸

Despite the fact that we comprehend how azole resistance developed in *C. tropicalis*, molecular researchers suggest that azole resistance in *C. tropicalis* may mostly be raised due to mutations in Cdr1 and Mdr1 efflux pumps, as well as in ergosterol production gene (*ERG11*).¹⁹ Alternative azole resistance pathways have also been linked to biofilm formation mitochondrial abnormalities and other virulence factors.²⁰ Hence, this present work is done for analyzing the complete mycological profile of *Candida* species and emphasizing virulence factors as well as the antimicrobial sensitivity pattern of *C. tropicalis* isolates in order to determine effective ways of management to curb candidemia based on the new regimen.

MATERIALS AND METHODS

A cross-sectional study has been held in a tertiary care centre and ethical approval was obtained from the Institute Ethical Committee (Human Studies) between August 2021 to March 2022. Seventy clinically known *Candida* isolates were isolated from positively flagged blood

samples incubated in Bact/ALERT 3D collected from various wards and gram stained directly to determine the existence of gram-positive budding yeast-like cells (Figure 1). The standard culture techniques were carried out on Sabouraud Dextrose Agar (SDA) with antibiotics, MacConkey agar, and Blood agar and were incubated at 25°C and 37°C for 24 to 48 hours. After that, every plate was studied macroscopically and the colony characteristics were examined. Colonies that appeared on SDA were smooth, creamy, convex, and pasty colonies (Figure 2). Then, the colonies were Gram stained and examined under the microscope.

Following the culture characterization, species identification was performed by inoculating the colonies onto *Candida* CHROM agar plates which were incubated at 37°C for 48 hours and they were identified and speciated based on the color produced by the organisms on the chromogenic medium, i.e. *C. albicans* - Apple green, *C. tropicalis* - Metallic Blue,

C. krusei - Pink, *C. parapsilosis* - Cream to pink, and *C. glabrata* - purple. Further, germ tube formation test and chlamydospore formation by Cornmeal agar was also performed to identify the *Candida* species that could not be distinguished by color on *Candida* CHROM agar medium [26] (Figure 3).

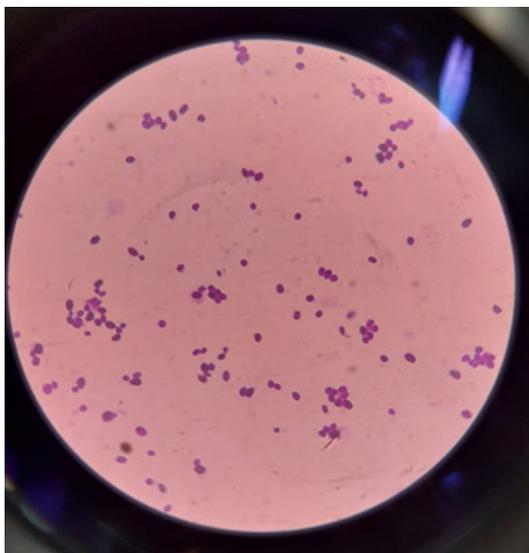


Figure 1. Gram Staining Showing Budding Yeast Cells

Virulence factors like hemolysin production, coagulase activity, phospholipase activity, and biofilm formation were studied and the outcomes were compared with the antifungal susceptibility testing along with the detection of Minimum Inhibitory Concentration (MIC) values which may be linked with the azole resistance pathway.

Germ tube formation method

A colony of yeast was suspended with 0.5 ml of human, sheep, or fetal bovine serum in a sterile Eppendorf tube and incubated at 37°C for 2-4 hours. Subsequently, a single drop of serum was placed onto a glass slide and a cover slip was kept over it. Then, it was examined microscopically under a low-power objective. The appearance of long tube-like projections attached to the yeast cells was called germ tube formation which is typically present in *C. albicans* or *C. dubliniensis*.

Haemolysin Production

Haemolysin production was demonstrated on SDA containing sheep blood along with gentamicin as described by Manns et al.²¹ The medium was aseptically injected with 10 µL of inoculum prepared with the isolates. Petri plates containing the above-described medium were cultured for 48 hours at 37°C and ATCC 90028 *C. albicans* is used as a control. Hemolysin activity



Figure 2. Colonies of *C. tropicalis* on Sabouraud Dextrose Agar

was demonstrated by the existence of a halo zone of hemolysis surrounding a colony. The colony diameter to the transparent hemolysis zone (in mm) ratio is calculated as hemolytic activity (Hz).

Coagulase Activity

Using the method developed by Yigit et al.,²² coagulase production was identified. A 500 50µL of rabbit plasma was added to a tube and it was aseptically inoculated with 0.1mL of an inoculum that has been kept overnight aseptically. After incubation of 2, 4, 6, and 24 hours at 35°C, the tubes were checked. A positive coagulase test shows clot formation which can not be revived by mild shaking. Positive control: ATCC 25923 *Staphylococcus aureus* and negative control: ATCC 14990 *S. epidermidis*, respectively.

Phospholipase Production

The method developed by Samaranayake et al.,²³ was used to identify phospholipase production. A standard test strain inoculum containing a volume of 5µL was inoculated aseptically onto agar containing egg yolk. Petri

plates are dehydrated at ambient temperature, they were incubated for 48 hours at 37°C. The formation of a zone of precipitation circling the colony was checked and the existence of a precipitation zone suggested that the phospholipase enzyme was exhibited. The positive control was used as ATCC 10231 *C.albicans*. The colony diameter to the combined and precipitation zone ratio is calculated as phospholipase index (Pz). When Pz was 1, it indicates no phospholipase production.; Pz< 1 denotes positive activity. The test was carried out three times in triplicate for each isolate to reduce experimental error.

Biofilm formation

A growth from SDA has suspended in a sterile brain-heart infusion (BHI) broth, and cultured overnight. The next step was a 1:100 dilution in BHI. Then, a commercially available polystyrene, pre-sterilized, round-bottomed, 96-well microtiter plate (HiMedia) was used to incubate 100µL of diluted broth at 37°C overnight for the development of biofilm, and with distilled water, the microtiter plates were cleaned.

Table 1. Mycological profile of Candida isolates isolated from Candidemia cases (n=70)

| Candida species | Species Distribution (%) | Phospholipase activity (%) | Haemolysin production (%) | Coagulase activity (%) | Biofilm production (%) |
|------------------------|--------------------------|----------------------------|---------------------------|------------------------|------------------------|
| <i>C. tropicalis</i> | 27 (38.57%) | 25 (58.14%) | 27 (55.10%) | 11 (32.35%) | 25 (45.45%) |
| <i>C. albicans</i> | 19 (27.14%) | 11 (25.58%) | 12 (24.48%) | 13 (38.24%) | 13 (23.64%) |
| <i>C. parapsilosis</i> | 13 (18.57%) | 2 (4.65%) | 5 (10.20%) | 6 (17.64%) | 9 (16.36%) |
| <i>C. glabrata</i> | 6 (8.57%) | 4 (9.30%) | 3 (6.12%) | 3 (8.82%) | 3 (5.45%) |
| <i>C. krusei</i> | 5 (7.14%) | 1 (2.32%) | 2 (4.08%) | 1 (2.94%) | 5 (9.09%) |
| Total (n=70) | | 43 (61.43%) | 49 (70%) | 34 (48.57%) | 55 (78.57%) |

Table 2. Antifungal susceptibility pattern of Candida isolates by Kirby Bauer disc diffusion method (n=70)

| Candida species | FLC | | ITR | | VRC | | AMP B | |
|-------------------------------|--------|--------|-----|-----|--------|-------|--------|-------|
| | S | R | S | R | S | R | S | R |
| <i>C. tropicalis</i> (n=27) | 20 | 7 | 23 | 4 | 24 | 3 | 26 | 1 |
| <i>C. albicans</i> (n=19) | 17 | 2 | 16 | 3 | 18 | 1 | 19 | 0 |
| <i>C. parapsilosis</i> (n=13) | 9 | 4 | 11 | 2 | 13 | 0 | 13 | 0 |
| <i>C. glabrata</i> (n=6) | 1 | 5 | 3 | 3 | 5 | 1 | 6 | 0 |
| <i>C. krusei</i> (n=5) | 0 | 5 | 3 | 2 | 4 | 1 | 5 | 0 |
| Total (n=70) | 47 | 23 | 56 | 14 | 64 | 6 | 69 | 1 |
| Percentage (%) | 67.14% | 32.86% | 80% | 20% | 91.43% | 8.57% | 98.57% | 1.43% |

*S – Sensitive, *R – Resistant, *FLC – Fluconazole, *ITR – Itraconazole, *VRC – Voriconazole, *AMP B – Amphotericin B

Crystal violet assay

Crystal violet assay was performed to demonstrate the biofilm production as described by Kuhn et al.²⁴ The microtiter plate was cleaned with distilled water before being stained at ambient temperature for 15 minutes with 0.1% aqueous solution of crystal violet(120 μ L). The wells were cleaned with sterile distilled water for 4 times. Then, 125 μ L of 95% methanol was used to de-stain, and the wells were incubated at room temperature for 15 minutes. An enzyme-Linked

Immunosorbent Assay (ELISA) reader was used to read the de-stained wells spectrophotometrically at 570 nm. 12 Duplicate runs of each sample were done.

The biofilm formation was read as follows:

OD - Optical Density

ODC – Optical Density cutoff

SD – Standard Deviation

Avg NC - Average OD value of negative control

Avg NC=0.194, SD=0.013

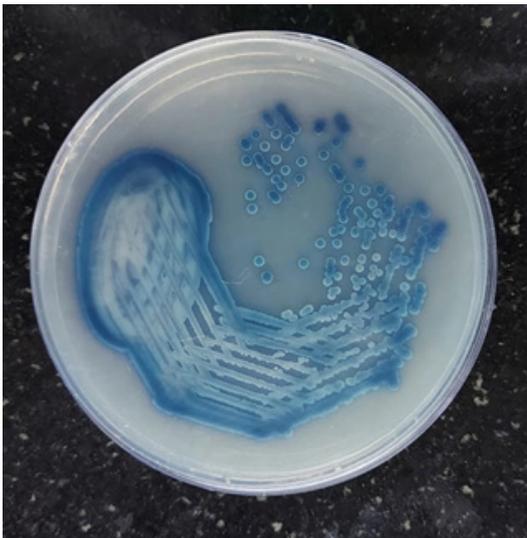


Figure 3. *Candida tropicalis* on Candida CHROMAGAR

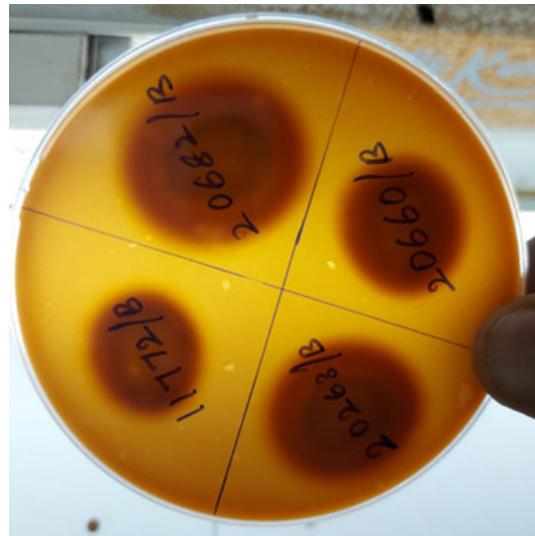


Figure 4. Hemolysin Production by *C. tropicalis*



Figure 5. Coagulase activity by *C. albicans*

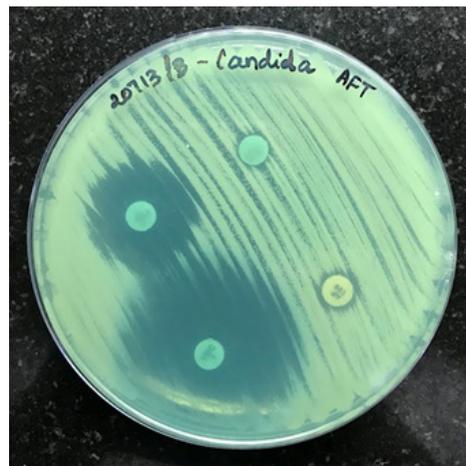


Figure 6. Fluconazole-resistant *C. tropicalis* isolate - Antifungal Susceptibility Testing

Table 3. MIC of Fluconazole-resistance *C. tropicalis* isolates (n=7)

| Name of the Isolate | MIC of Fluconazole (0.25- ≥ 64 µg/mL) | | | | | | | | |
|----------------------------|---------------------------------------|-------------|-------------|------------|------------|------------|------------|--------------|-----------------|
| | ≥ 64 µg/mL | 32 µg/mL | 16 µg/mL | 8 µg/mL | 4 µg/mL | 2 µg/mL | 1 µg/mL | 0.5 µg/mL | ≤ 0.25 µg/mL |
| <i>C. tropicalis</i> (n=7) | 4 | - | 1 | - | 1 | 1 | - | - | - |

ODC=Avg NC+3'SD, ODC=0.194+0.039, ODC=0.233

OD≤ODC= non-biofilm producers

ODC<OD≤2 ODC=weak biofilm producers

2ODC<OD≤4 ODC=moderate biofilm producers

4ODC<OD=strong biofilm producers

Antibiogram

The antibiogram pattern for antifungals like Fluconazole (25µg), Amphotericin B (100 U), Itraconazole (10µg), and Voriconazole (1µg) (Hi-Media Laboratories) was performed by the Kirby-Bauer disk diffusion method. To improve the determination of growth margins, it was carried out on Mueller-Hinton agar which was added to 0.5 g of methylene blue per milliliter and 2% glucose. The zone sizes were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Then, Fluconazole resistant isolates were subjected to broth microdilution M27-A3 in order to determine MIC values, and results were interpreted according to the CLSI guidelines.²⁵

RESULTS

In the course of the research period (16 months), blood samples were received from various wards, and those flagged with positive signals were subjected to the examination by microscope and culture findings (SDA, Blood agar, and MacConkey agar) for *Candida* species. A total of 70 consecutive and non-duplicate *Candida* species were comprised in our study. Organisms other than *Candida* isolates were excluded.

Among the 70 *Candida* isolates, 13 (18.57 %) isolates indicated a positive result for germ tube formation test, and 57 (81.42%) isolates of *Candida* species did not show germ tube formation. Of the total 70 clinical *Candida* isolates, the most predominant organism isolated was *C. tropicalis* 27 (38.57%) succeeded by 19 (27.14%) *C. albicans*, 13 (18.57%) *C. parapsilosis*, 6 (8.57%) *C. glabrata*, and 5 (7.14%) *C. krusei* respectively (Table 1). However,

all the isolates indicated negative findings on the urease test.

In the interpretation of virulence factors of the 70 *Candida* isolates, phospholipase activity was demonstrated by 43 (61.43%) isolates, out of which 25 (58.13%) were *C. tropicalis* followed by 11 (25.58%) were *C. albicans*, 4 (9.30%) were *C. glabrata*, 2 (4.65%) isolates of *C. parapsilosis* and 1 (2.32%) isolate of *C. krusei* (Table 1).

With respect to hemolysis, 49 (70% out of the 70 *Candida* isolates) showed hemolysin production (Table 1) (Figure 4), from which 27 (55.10%) were *C. tropicalis*, 12 (24.48%), *C. albicans*, *C. parapsilosis* 5 (10.20%), *C. glabrata* 3 (6.12%), and *C. krusei* 2 (4.08%). Coagulase activity was demonstrated by 34 (48.57%) *Candida* isolates, of them, 13 (38.24%) *C. albicans* produced the maximum coagulase activity followed by *C. tropicalis* 11 (32.35%), 6 (17.64%) *C. parapsilosis*, 3 (8.82%) *C. glabrata* and 1 (2.94%) *C. krusei* respectively (Table 1) (Figure 5).

Biofilm production was conducted by crystal violet assay for all the 70 isolates of *Candida* species and was interpreted as follows, 55 (78.57%) isolates showed biofilm production and among them, 25 (45.46%) *C. tropicalis* isolates showed biofilm production, subsequently followed by 13 (23.63%) *C. albicans*, 5 (9.09%) *C. krusei*, 9 (16.36%) *C. parapsilosis*, and 3 (5.45%) *C. glabrata*. *C. tropicalis* stood out among them as the most frequent cause of biofilm development. Of them, 18 isolates were found to produce strong biofilm production (3+), 10 isolates were observed to show moderate biofilm production (2+). Also, 8 *Candida* species showed weak biofilm production (1+) (Table 1).

The antibiogram profile of the 70 isolates of *Candida* was demonstrated for Voriconazole, Amphotericin B, Fluconazole, and Itraconazole. A high level of resistance among Fluconazole has been observed in 23 (32.86%) *Candida* species in comparison with other antimicrobials utilized

in this study. Among 23 (32.86%) Fluconazole resistant *Candida* species, *C. tropicalis* (7 out of 27 isolates) were more predominant and all *C. krusei* (5 out of 5 isolates) were resistant to Fluconazole. With respect to Itraconazole, 14 (20%) out of 70 *Candida* isolates showed resistance and 56 (80%) were sensitive. For Voriconazole, 6 (8.57%) isolates showed resistance, and 64 (91.43%) were sensitive. While the Amphotericin B resistance was interpreted in only 1 (1.43%) isolate of *C. tropicalis* and the rest 98.57% of *Candida* species were sensitive (Table 2) (Figure 6).

Fluconazole MICs for all *C. tropicalis* isolates were determined using microdilution method and the results were interpreted based on CLSI guidelines in which 1 (14.28%) *C. tropicalis* isolate showed MIC of $\leq 2\mu\text{g/mL}$ and 2 (28.57%) isolates showed intermediate resistance to Fluconazole exhibiting MIC from 4-32 $\mu\text{g/mL}$. The higher Fluconazole MIC value of $\geq 64\mu\text{g/mL}$ was shown by 4 (57.14%) isolates of *C. tropicalis* (Table 3).

DISCUSSION

Candidemia is a major concern, especially among immunocompromised patients. The most prevalent causative agent of candidemia has shifted in recent years over *C. albicans* to NAC species. In Southern India, the most prevalent isolates of candidemia were *C. tropicalis* and *C. parapsilosis*.²⁶ However, when compared to other *Candida* species, candidemia brought on by *C. tropicalis* isolates is a potentially fatal infection that increases patient mortality. There are risk factors that might enhance a person's vulnerability to candidemia including: increase usage of antibiotics and corticosteroids, repeated hospitalisation, neutropenia, malignancy AIDS, intravascular catheterization, chemotherapy, and other illnesses that impair immunity.^{27,28}

Over the last several years, a steady rise in the isolation NAC species from candidemia patients has been noticed.²⁸⁻³⁰ Numerous investigations have isolated and noted similar patterns of NAC species, but other research indicates that *C. glabrata* and *C. albicans* predominate.³¹⁻³⁴ In this study, 70 consecutive, non-repetitive *Candida* isolates were speciated by the conventional method. In this study, NAC species account for

about 72.85% while *C. albicans* (27.14%) and similar results were demonstrated by Sachin C et al.³⁵ Among them, 27 (38.57%) *C. tropicalis* were the most frequent isolates of *Candida* species and similar results were also reported in the study findings of Tak V et al. and Chakrabarti et al.^{36,37}

Since *C. albicans* was reported as the predominant harmful organism of candidemia, there have been much research on these virulence factors. However, a lot of research publications discuss the development of virulence among NAC species.³⁸⁻⁴⁰ The demonstration of virulence factors shows that 43 (61.43%) isolates showed phospholipase activity and 25 isolates of *C. tropicalis* showed maximum phospholipase activity. These results were correlated with the study findings of Sachin C et al. which showed that 60.9% of isolates demonstrated phospholipase activity and 76% and phospholipase activity was predominantly produced by 19 isolates of *C. tropicalis*.³⁵ Conversely, Khater et al. resulted that phospholipase activity was more predominant among *Candida albicans* (45.62%) which was higher than that of the NAC species.⁴¹ However, on the contrary, Figueiredo-Carvalho et al. reported no phospholipase activity among NAC species.⁴²

Among 70 *Candida* isolates, 49 (70%) showed hemolysin production and the present study results were correlated with the study findings of Luo et al. and M. A. Galen-Ladero et al. which showed 85.7% and 77.2%.^{43,44} Although studies have revealed that *C. tropicalis* produces hemolysin, it is crucial to know if the hemolytic activity shown is actually occurring or whether it is the consequence of phospholipase synthesis.⁴⁵ Therefore, it appears necessary for conducting more sophisticated research based on the molecular study in order to elucidate the hemolysin production which contributes to the pathogenesis of *C. tropicalis*.²² Coagulase activity was demonstrated by 34 (48.57%) *Candida* isolates, of them, 13 *C. albicans* produced the maximum coagulase activity and these results were similar to the findings reported by Yigit N et al. which showed that 50.6% *Candida* isolates had produced coagulase activity. Among them 14 *C. albicans* produced coagulase activity.²²

The development of a mature, highly organized biofilm happens when the yeast cell attaches to a surface and starts to multiply.

Biofilm development is believed to be a powerful infectious feature causing therapeutic flops and infections that are recurring.^{46,47} Out of 70 *Candida* isolates, 55 (78.57%) strains showed biofilm production, and these findings correlated with the study results of Sanyuktha Tulasidas et al. which showed biofilm activity in 74% of *Candida* isolates.⁴⁸ It was interpreted that 25 (45.46%) of *Candida tropicalis* isolates showed maximum biofilm production. This finding is in concordance with the results obtained in a study conducted by Sasani E et al. and similarly reported *C. tropicalis* (47%) as the prevalent isolate that produced biofilm activity.⁴⁹ On many medical devices, especially intravascular ones, *Candida* isolates more easily colonize to create *in vitro* biofilms that might result in catheter-associated bloodstream infections. The development of biofilms may have a major impact on how patients with candidemia respond to therapy.⁵⁰ The invasiveness and spread potential of *C. tropicalis* are influenced by biofilm formation, an essential virulence component. However, our study findings also demonstrate that most of the biofilm-producing *C. tropicalis* isolates were showing a high degree of Fluconazole resistance than non-biofilm producers as it is considered an essential virulence factor endowing pathogenicity among candidemia patients.⁵¹

With respect to an antibiogram pattern, maximum of isolates were sensitive for all drugs used: Amphotericin B (98.57%), Voriconazole (91.43%), Itraconazole (80%), and Fluconazole (67.14%). These findings were similar to other studies.⁵²⁻⁵⁴ A high level of resistance among Fluconazole has been observed in 23 (32.86%) *Candida* isolates in comparison with other antimicrobials in the current study. Of them, *C. tropicalis* was found to show maximum resistance to Fluconazole and only 1 (1.43%) isolate of *C. tropicalis* was identified as resistant. This was similar to the study findings of Giri et al.⁵⁵ Notably, all strains of *C. krusei* were Fluconazole-resistant as these species are intrinsically resistant to Fluconazole. A few more isolates also showed resistance to Itraconazole (20%), Voriconazole (8.57%), and lower rate of resistance was reported in Amphotericin B (1.43%) which were in concordance with various study reports.⁵⁶⁻⁵⁸

The maximum resistance pattern was

demonstrated by *C. tropicalis* as it is emerging as one of the most significant and alarming *Candida* isolates causing antifungal resistance.⁵⁹ So, MIC of all Fluconazole-resistant *C. tropicalis* isolates were demonstrated and interpreted by the Micro dilution method as per CLSI guidelines.⁶⁰ The current study results were similar to the study findings of Gandham et al. and it showed that Fluconazole showed higher MIC of ≥ 2 $\mu\text{g/ml}$ in 4 isolates of *C. tropicalis* and 1 of *C. parapsilosis*, and 2 of *C. albicans* were resistant.⁶¹ The most likely cause of this change appears to be the overuse of Fluconazole, which allowed for the survival of resistant species like NAC species.⁶² The changing epidemiology and rising antifungal resistance highlight the importance of constant monitoring of these candidemia cases. Therefore, the management of the infections and reducing death rates may be aided by a proper diagnosis of *Candida* species and a strict antifungal stewardship program.^{63,64}

CONCLUSION

In this study, the presence of candidemia among the patients from various wards occurs with the predominance *C. tropicalis*. Amphotericin B has the highest antifungal action, whereas Fluconazole has the least activity expressed in the highest antifungal resistance seen. The interpretation of various virulence factors and antifungal drug resistance were seen mostly among NAC species, thus hence signifying its pivotal role in the immunocompromised individual treatment. To enhance outcomes, particularly for critically sick newborns and immunocompromised people, timely identification of *Candida* BSI and knowledge of its resistance profile are very essential. Thus, this information on antifungal susceptibility and virulence variables are required to minimise the overall impact of *Candida* infections that are rising, therapeutic failures, and financial burden severely.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethical Committee of SRM Medical College Hospital and Research Centre, India, with reference number 2896/IEC/2021.

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