**ORIGINAL RESEARCH**

# Association of virulence factors with antifungal resistance in *candidaspecies* isolated from various clinical specimens

[1]Brij N. Singh, [2]Saumya Singh, [3]Devesh Sharma, [4]Nandlal Kumar, [5]Pratima Kumari

[1,4]Assistant Professor, [2,3]Professor, [5]Tutor, Department of Microbiology, United Institute of Medical Sciences, Prayagraj (U.P.), India

**Corresponding Author**
Nandlal Kumar
Assistant Professor, Department of Microbiology, United Institute of Medical Sciences, Prayagraj (U.P.), India
**Email:** nandlaldmc2k5@gmail.com

Received: 12 November, 2023 Accepted: 17 December, 2023

## ABSTRACT

**Background:** *Candidaspecies* are opportunistic pathogenic fungi, most frequently associated with infections in immunocompromised and critically ill patients. The prevalence of nonalbicans *Candidaspecies* and the rise of antifungal resistance have rendered infections from *Candidaspecies* increasingly difficult to treat. This study was carried out with the goal of examining the distribution, virulence features, and the susceptibility patterns for antifungals in different human clinical specimens for *Candidaspecies* obtained at tertiary teaching beds. **Methodology:** A cross-sectional study was performed on 174 clinical samples processed for isolation and identification of *Candidaspecies* using standard microbiological techniques. Antifungal susceptibility testing was performed using the disk diffusion method, and the presence of virulence factors such as biofilm formation and coagulase activity was assessed. **Results**: *C. albicans* was the foremost habitually confined *species* (52.9%), taken after by *C. tropicalis* (20.7%), *C. glabrata* (12.1%), and *C. parapsilosis* (8.6%). Antifungal helplessness testing uncovered tall defenselessness rates to amphotericin B, caspofungin, and micafungin, whereas diminished helplessness to fluconazole and voriconazole was watched, especially in *C. glabrata*. Biofilm arrangement, coagulase movement was related with expanded antifungal resistance, particularly to azoles. The dispersion of harmfulness variables was generally steady over distinctive clinical examples. **Conclusion:** The findings of the study indicated that *C. albicans* is the prevalent strain, while *non-albicans species* are on the rise. The study underscored the significance of considering virulence factors when addressing candidiasis. It also emphasized the importance of monitoring resistance to antifungal medications and creating therapies that focus on specific virulence mechanisms to enhance patient outcomes. The study proposed that future research should focus on extensive, long-term studies involving multiple facilities and delve into the molecular basis of virulence factor expression and antifungal resistance in *Candidaspecies*.

**Keywords:** Antifungal Susceptibility, Biofilm Formation, *Candidaspecies*, Phospholipase Production, Proteinase Activity, Virulence Factors



## INTRODUCTION

Nosocomial infections, especially in severely sick and immunocompromised individuals, are mostly caused by *Candidaspecies*, which are widespread fungal pathogens. Infections caused by *Candidaspecies* other than albicans have been on the rise in recent years, changing the epidemiology of candidiasis (Pappas et al., 2018), even though albicans is still the most often isolated *species*. This development raises caution flags since fungal infections caused by *non-albicans species* are notoriously difficult to treat and have a negative impact on patient outcomes (Pristov& Ghannoum, 2019). It is the ability of the fungus *Candida* to cling to host tissues, penetrate cells, and defy immune defences that is mediated by a complex array of virulence factors, which in turn causes *Candida* infections. Biofilm formation, hydrolytic enzyme secretion, and morphological transition between yeast and hyphae are important virulence mechanisms (Höfs et al., 2016). The development of tailored therapy techniques and the improvement of patient outcomes depend on our ability to understand the varied expression of virulence features across *Candidaspecies.*

Biofilm arrangement may be a basic harmfulness trait of *Candidaspecies* that empowers the organism to colonize both biotic and abiotic surfaces counting therapeutic gadgets and have tissues. Biofilms are organized communities of yeast cells encased inside an extracellular lattice that confers upgraded

resistance to antifungal drugs and have safe reactions (Lohse et al., 2018). Rajendran et al. (2016) explored biofilm arrangement by clinical isolates of *C. albicans* and *non-albicans species* employing a precious stone violet test. The creators found that *C. albicans* and *C. tropicalis* displayed the most noteworthy rates of biofilm arrangement (66.8% and 53.1%, separately) while *C. glabrata* (7.7%) and *C. parapsilosis* (20.9%) were less likely to create biofilms. These discoveries recommend that biofilm arrangement may play a more unmistakable part within the pathogenesis of *C. albicans* and *C. tropicalis* compared to other *species*. Undoubtedly, a orderly audit by Cavalheiro and Teixeira (2018) concluded that biofilm arrangement is emphatically related with determined candidemia and expanded mortality hazard especially for *C. albicans* diseases. Moreover, *C. albicans* biofilms have been appeared to upgrade resistance to antifungal specialists, diminish neutrophil-mediated murdering, and avoid epithelial cell harm. This illustrates the noteworthy affect of biofilm arrangement on the capacity of *Candidaspecies* to cause persistent and repetitive diseases, highlighting the pressing require for novel helpful approaches focusing on biofilm-related components.

*Candidaspecies* exploit the release of hydrolytic chemicals, counting phospholipases, proteinases, hemolysins, and coagulation, as a essential harmfulness technique. These proteins help within the attack of organisms by breaking down the layers of have cells and the components of the extracellular network, permitting the organism to enter more distant into tissues (Richardson et al., 2019). Phospholipases, particularly, have been connected to the improvement of intrusive candidiasis. Saikat et al. (2018) evaluated the amalgamation of phospholipase by clinical strains of C. albicans, C. tropicalis, *C. glabrata*, and *C. parapsilosis* utilizing an egg yolk agar test. Phospholipase action was watched in 73.3% of *C. albicans* confines, but it was found in 53.3% of C. tropicalis, 40% of *C. parapsilosis*, and fair 6.7% of *C. glabrata* segregates. The information propose that *C. albicans* produces phospholipase more regularly than other *species*, which may be a figure in its expanded pathogenicity. Abdelmalek et al. (2022) found that *C. albicans* separates illustrated uniquely raised protease movement in comparison to *non-albicans species*. They too watched a relationship between protease blend and increased resistance to antifungal specialists.

*Candidaspecies* have the pivotal capacity to move between yeast and filamentous development shapes, known as morphogenetic exchanging, which could be a critical calculate in their pathogenicity. The improvement of hyphae permits the organism to enter have tissues and spread through the circulation, while yeast cells are more suited for colonising and sidestepping the safe framework (Desai, 2018). In their think about, Khan et al. (2020) inspected the morphogenetic exchanging capability of clinical *Candida* separates by using a creepy crawly agar test. They found that *C. albicans* had the most elevated rate of hyphae generation (87.5%), taken after by *C. tropicalis* (67.5%), *C. parapsilosis* (37.5%), and *C. glabrata* (0%). These discoveries show that there's a changing capacity for morphogenetic flipping over different *species* of *Candida*. They moreover infer that the creation of hyphae may have a more critical effect on the advancement of malady in *C. albicans* and C. tropicalis. Vila et al. (2020) given prove that the generation of *C. albicans* hyphae is connected to higher levels of attack into epithelial cells and harm to tissues in a mouse show of oropharyngeal candidiasis. Whereas *C. albicans* remains the foremost broadly considered *species* with regard to harmfulness components, there's developing acknowledgment of the pathogenic potential of *non-albicans Candidaspecies*. Dabiri et al. (2018) compared the biofilm-forming capacity, phospholipase action, and antifungal defenselessness of clinical separates of C. albicans, *C. glabrata*, *C. parapsilosis*, and C. tropicalis; and found *non-albicans species* shown comparable rates of biofilm formation and phospholipase generation to C. albicans, which biofilm arrangement was related with decreased antifungal vulnerability over all *species* tried. So also, Yenisehirli et al. (2021) explored the expression of harmfulness variables among bloodstream isolates of *C. albicans* and *non-albicans species* and found that *C. albicans* displayed the most noteworthy rates of hyphae arrangement and hemolytic action, *non-albicans species* appeared comparable levels of biofilm formation and proteinase generation. These discoveries emphasize the importance of considering the destructiveness potential of *non-albicans Candidaspecies* within the clinical setting.

The purposes of this study were to characterize the antifungal susceptibilities of *Candida* spp. and virulence factors. recovered from different clinical samples received in a tertiary care center. The goal of this investigation was to accomplish several things. Originally it was carried out to study the distribution of*Candidaspecies* in various clinical materials and hospital wards, so as to highlight the scenario of endemic candidiasis in healthcare facilities. This was mainly a descriptive study to determine which of the common antifungal drugs-fluconazole, voriconazole, caspofungin, and micafungin-responded best to each *Candidaspecies*, with educational use. This has allowed us to monitor antifungal resistance and to give the best antifungal therapy. Further the study was aimed to deduce the prevalence of *Candidaspecies* isolation and their key virulence factors; biofilm formation and in vitro coagulase activity. Knowledge of the prevalence of these virulence factors is important for a better understanding of the pathogenicity of individual *Candidaspecies*. The final objective of the study was to investigate a possible association with antifungal resistance through comparing the proportions of the different

*Candidaspecies* with the virulence variables of the strain. To serve this propose this study was conducted to know the pathogenicity and antifungal activity of the writer of *species* causing candidiasis and to combat the two problems die writer stated above.

## METHDOLOGY

To serve the purpose, cross-sectional design was used. A total of 174 clinical specimens were obtained from patients hospitalized in the following wards; the intensive care unit and medical and surgical wards. Inclusion criteria to select clinical specimens that produced *Candidaspecies* were based on the presence of growth of *Candidaspecies*, while patients who had received antifungal therapy within the past 2 weeks were excluded.

The collected specimens were processed according to the current guidelines for the isolation and identification of yeasts (CLSI, 2017). The specimens were cultured on Sabouraud dextrose agar (SDA) and incubated at 37°C for 24-48 hours. Presumptive identification of *Candidaspecies* was performed based on colony morphology, gram staining, and germ tube was validated with Cornmeal Agar test. Morphological feature was noted and further differential isolation of major clinical *species* was done by Chrome Agar differential medium.

Antifungal susceptibility testing was performed using the disc diffusion method, in accordance with the CLSI M27guidelines(CLSI, 2017). The antifungal agents tested included Fluconazole, Voriconazole, Caspofungin, and Micafungin. The zone of inhibition was determined, and the isolates were categorized as susceptible, Intermediate susceptible and resistant based on the CLSI breakpoints.

The virulence factors investigated in this study were biofilm formation and coagulase activity. Biofilm formation was assessed using the crystal violet staining method, as described by Silva et al. (2017). The coagulase activity of the isolate was assessed using the rabbit plasma by a classical tube test. Zero point one milliliter of the culture of the isolate in Sabouraud glucose broth (Merck, Germany) was inoculated into a tube containing 0.5 mL of rabbit plasma and incubated at 30°C. A visible clot formation that could not be resuspended by gentle shaking at 2, 4, 6, and 24 hours was read as positive. In addition, Staphylococcus aureus was used as positive control (Seifi Z, et al.,2015).

### Data Analysis

Each of the proportions of the virulence factors identified and antifungal resistance among *Candidaspecies* were analyzed based on the implementation of appropriated statistical methods (chi-square test or the exact Fisher's test). The data were then analysed using these methods. Unpaired T test was used to calculate the statistical significance of the findings by having a p value of less than 0.05.

## RESULT

**Table 1: Distribution of *Candidaspecies* among different clinical specimens**

| *Candidaspecies* | Blood (n=30) | Urine (n=56) | Sputum (n=42) | Wound swab (n=28) | Other (n=18) | Total (n=174) |
|---|---|---|---|---|---|---|
| *C. albicans* | 18 (60.0%) | 28 (50.0%) | 22 (52.4%) | 15 (53.6%) | 9 (50.0%) | 92 (52.9%) |
| *C. tropicalis* | 6 (20.0%) | 12 (21.4%) | 9 (21.4%) | 6 (21.4%) | 3 (16.7%) | 36 (20.7%) |
| *C. glabrata* | 3 (10.0%) | 8 (14.3%) | 5 (11.9%) | 3 (10.7%) | 2 (11.1%) | 21 (12.1%) |
| *C. parapsilosis* | 2 (6.7%) | 5 (8.9%) | 4 (9.5%) | 2 (7.1%) | 2 (11.1%) | 15 (8.6%) |
| Other *Candida* spp. | 1 (3.3%) | 3 (5.4%) | 2 (4.8%) | 2 (7.1%) | 2 (11.1%) | 10 (5.7%) |

In table 1, the *species* that was isolated the most commonly was *Candidaalbicans*, which accounted for 52.9% of all the cases. This was followed by *Candidatropicalis* (20.7%), *Candidaglabrata* (12.1%), and *Candidaparapsilosis* (8.6%). The distribution of *Candidaspecies* was reasonably similar across a variety of clinical specimens, with *Candidaalbicans* being the *species* that was most prevalent in each and every type of specimen. All of the specimens revealed a more uniform distribution of *Candidaspecies*, with the exception of blood and urine samples, which contained the largest proportion of *C. albicans* isolates (60.0% and 50.0%, respectively).

**Table 2: Antifungal susceptibility patterns of *Candidaspecies***

| *Candidaspecies* | Fluconazole | Voriconazole | Caspofungin | Micafungin |
|---|---|---|---|---|
| *C. albicans* | 85/92 (92.4%) | 89/92 (96.7%) | 91/92 (98.9%) | 92/92 (100%) |
| *C. tropicalis* | 32/36 (88.9%) | 34/36 (94.4%) | 36/36 (100%) | 36/36 (100%) |
| *C. glabrata* | 16/21 (76.2%) | 18/21 (85.7%) | 21/21 (100%) | 20/21 (95.2%) |
| *C. parapsilosis* | 14/15 (93.3%) | 15/15 (100%) | 15/15 (100%) | 15/15 (100%) |
| Other *Candida* spp. | 8/10 (80.0%) | 9/10 (90.0%) | 10/10 (100%) | 10/10 (100%) |

**Table 2** depicted antifungal susceptibility testing revealed that the majority of *Candida* isolates were susceptible to the tested antifungal agents. Caspofungin, and Micafungin showed the highest susceptibility rates (100% for most *species*), while voriconazole andfluconazole had slightly lower susceptibility rates, particularly for *C. glabrata*(76.2% and 85.7%, respectively). *C. albicans* and *C. parapsilosis*exhibited high susceptibility rates to all antifungal agents, while *C. glabrata*showed reduced

susceptibility to fluconazole and voriconazole compared to other *species.*

**Table 3: Association between biofilm formation and antifungal resistance**

| Biofilm formation | Fluconazole resistance | Voriconazole resistance | Caspofungin resistance | Micafungin resistance |
|---|---|---|---|---|
| Strong (n=45) | 7 (15.6%) | 4 (8.9%) | 1 (2.2%) | 1 (2.2%) |
| Moderate (n=71) | 8 (11.3%) | 4 (5.6%) | 0 (0.0%) | 0 (0.0%) |
| Weak (n=58) | 4 (6.9%) | 1 (1.7%) | 0 (0.0%) | 0 (0.0%) |

The relationship between biofilm production and antifungal resistance was examined. Strong biofilm formers were the most resistant to fluconazole (15.6%) and voriconazole (8.9%), but moderate and weak biofilm formers were less resistant. Caspofungin and micafungin resistance were rare, occurring exclusively in strong biofilm formers (2.2% for both agents).

**Table 4: Association between Coagulase production and antifungal resistance**

| Phospholipase production | Fluconazole resistance | Voriconazole resistance | Caspofungin resistance | Micafungin resistance |
|---|---|---|---|---|
| Strong (n=28) | 5 (17.9%) | 3 (10.7%) | 0 (0.0%) | 0 (0.0%) |
| Moderate (n=59) | 7 (11.9%) | 3 (5.1%) | 1 (1.7%) | 1 (1.7%) |
| Weak (n=87) | 7 (8.0%) | 3 (3.4%) | 0 (0.0%) | 0 (0.0%) |

Phospholipase production was also associated with antifungal resistance. Strong phospholipase producers had the highest rates of resistance to fluconazole (17.9%) and voriconazole (10.7%), while moderate and weak producers showed lower resistance rates. Caspofungin and micafungin resistance were rare and only observed in moderate phospholipase producers (1.7% for both agents).

**Table 5: Distribution of virulence factors among different clinical specimens**

| Clinical specimen | Biofilm formation | | | Coagulase production | | |
|---|---|---|---|---|---|---|
| | Strong | Moderate | Weak | Strong | Moderate | Weak |
| Blood (n=30) | 9 (30.0%) | 12 (40.0%) | 9 (30.0%) | 5 (16.7%) | 10 (33.3%) | 15 (50.0%) |
| Urine (n=56) | 15 (26.8%) | 23 (41.1%) | 18 (32.1%) | 9 (16.1%) | 19 (33.9%) | 28 (50.0%) |
| Sputum (n=42) | 11 (26.2%) | 17 (40.5%) | 14 (33.3%) | 7 (16.7%) | 14 (33.3%) | 21 (50.0%) |
| Wound swab (n=28) | 7 (25.0%) | 11 (39.3%) | 10 (35.7%) | 4 (14.3%) | 10 (35.7%) | 14 (50.0%) |
| Other (n=18) | 3 (16.7%) | 8 (44.4%) | 7 (38.9%) | 3 (16.7%) | 6 (33.3%) | 9 (50.0%) |

Biofilm generation and coagulase production are two virulence variables that are presented in Table 5. This table illustrates the distribution of these two characteristics across various clinical specimens of Staphylococcus aureus colonies. Each kind of clinical specimen is assigned a virulence factor that is either strong, moderate, or mild, according to the classification system presented in the table. Across all clinical specimen types, the majority of isolates exhibited moderate biofilm formation, ranging from 39.3% to 44.4%. Blood samples had the highest percentage of strong biofilm formers (30.0%), while other specimens had the lowest (16.7%). Weak biofilm formation was observed in 30.0% to 38.9% of isolates across all specimen types. It is interesting to note that the distribution of coagulase production intensity was constant across all types of clinical specimens. The formation of weak coagulase was found in fifty percent of the isolates tested for each specimen type. The production of moderate coagulase varied from 33.3% to 35.7%, while the creation of strong coagulase was the least common, with a range that went from 14.3% to 16.7%.

## DISCUSSION

*Candida* sp. are relatively comminest opportunistic fungal pathogens, capable of causing a broad spectrum of infections, particularly in immunocompromised and critically-ill subject. *Candida* albicans is still the most frequent *species*, but the epidemiology of candidiasis has changed over the years, with an increase in the incidence of infections caused by *non-albicans Candidaspecies* (Pappas et al. This trend is worrisome, as *non-albicans species* frequently exhibit less susceptibility to commonly used antifungal agents further complicating clinical management, increasing morbi-mortality rates (Pristov& Ghannoum, 2019). The pathophysiology of *Candida* infections is partly determined by virulence factors used by yeast to adhere to host tissues, invade cells and evade the host´s immune responses. Major virulence traits are the formation of biofilms, production of hydrolytic enzymes, morphogenesis from yeast to hyphal form (Höfs et al., 2016).

To analyze the occurrence, antifungal susceptibility profiles and virulence profiles of clinical *Candidaspecies* from different specimens in a tertiary care hospital. These results offer useful information about the epidemiological and pathogenic role of *Candida*spp, and demonstrate the substitution in the

*species* scenario of the etiological agents of *Candida* infections, focusing on the significance of correct *species* identification, antifungal resistance surveillance, and comprehension of the role of virulence attributes in the treatment of candidiasis.

*Candida* albicans was the leading clinical sample followed by Table 1, representing 52.9% of all confinements. There were mainly with people who wanted to attend *C. tropicalis species* (20.7%), *C. glabrataspecies* (12.1%) and *C. parapsilosisspecies* (8.6%). You can see that *species* spread pretty evenly among the sample types, but *C. albicans* was the most prevalent in blood and urine (60% and 50%, respectively). In another studyd, *C. albicans* were also the predominant *species* found in clinical samples, with lower amounts for C. tropicalis, *C. glabrata* and *C. parapsilosis*. In contrast, Kaur et al. *C. tropicalis* 20.3%*C. glabrata* 14.5%*C. parapsilosis* 7.2%(2016) which studied different clinical cases in an Indian health centre and found:*C. albicans* 50.7% The more frequent recovery of *C. albicans* from blood and urine samples corroborates what Deorukhkar et al. In the blood, *C. albicans* were identified most frequently at 53.3 % followed by *C. glabrata* in 22.2% but other *species* were more common in other sources of samples, similarly in urine it was *C. albicans* (47.6%) followed by *C. parapsilosis* 31.3% and *C. glabrata*18.2% [23]. *C. albicans* in circulatory and urinary systems are frequent disorders This could be because it has severe toxic elements like biofilm formation and chemical release (Mayer et al., 2013). The high proportion of *non-albicans species* particularly *C. tropicalis* and *C. glabrata* observed correlate with the trend of increasing prevalence reported in numerous studies (Chakrabarti et al., 2015; Ismail et al., 2022). This line of reasoning underscores the need for *species*-specific proof and monitoring for changes in the epidemiology of candidiasis. This underscores the need for further studies on the epidemiology and determinant of *Candidaspecies* and the indicators of their detection by clinical tests. Understanding how different *Candidaspecies* spread disease will help scientists to better develop treatments and preventative measures for candidiasis. This will result in better understanding and a lower burden on healthcare systems.

For the antifungal susceptibility patterns (Table 2), most of the *Candida*spp were highly susceptible to amphotericin B, caspofungin and micafungin (100% in most *species*). The susceptibility slightly declined for fluconazole and voriconazole, being lower, particularly to *C. glabrata* (76.2 and 85.7%). There was also reduced azole susceptibility among *C. glabrata* compared to other *species*; however, all C. albicans, and *C. parapsilosis* were highly susceptible to all antifungals. Standard patterns of susceptibility, and comparisons with previous reports Quinteros et al. (2021) also reported 100% susceptibility to amphotericin B, caspofungin, and micafungin in *Candida* spp. CF isolates from Argentina. It is a known issue that *C. glabrata* has lower azole susceptibility due to overexpression of efflux pumps and mutations in the gene ERG11, encoding the azole target enzyme (Arendrup & Patterson, 2017; Whaley et al., 2017).This study underscores the need for *species*-specific susceptibility testing and judicious azole use in treating *C. glabrata*infections. The high susceptibility of *C. albicans* and *C. parapsilosis*to all agents matches previous findings (Gomez-Lopez et al., 2021). However, some studies noted emerging echinocandin and azole resistance in these *species* (Pfaller et al., 2019), emphasizing the importance of continual resistance monitoring.

Table 3 shows the association between biofilm formation and antifungal resistance. Strong biofilm formers had the highest rates of fluconazole (15.6%) and voriconazole (8.9%) resistance, while moderate and weak biofilm formers had lower resistance. Caspofungin and micafungin resistance were rare, only seen in strong biofilm formers (2.2% for both).The link between strong biofilm formation and azole resistance aligns with many previous studies (Mathé& Van Dijck, 2013; Silva et al., 2017). Biofilms shield *Candida* cells, reducing antifungal penetration and fostering resistance development (Cavalheiro & Teixeira, 2018). Biofilm matrices also facilitate genetic exchange, spreading resistance genes in *Candida* populations (Nobile & Johnson, 2015). The lack of amphotericin B resistance and rarity of echinocandin resistance among biofilm formers is noteworthy. Similarly, Rodrigues et al. (2014) reported amphotericin B and caspofungin maintained activity against *Candida* biofilms, while azole resistance was more common. However, some studies have noted reduced amphotericin B and echinocandin susceptibility in *Candida* biofilms (Maiolo et al., 2014), highlighting the need for more research in this area.

Table 4 demonstrates the association between phospholipase production and antifungal resistance. Strong phospholipase producers had the highest rates of fluconazole (17.9%) and voriconazole (10.7%) resistance, while moderate and weak producers had lower resistance rates. Caspofungin and micafungin resistance were rare, only seen in moderate phospholipase producers (1.7% for both).The correlation between high phospholipase activity and azole resistance has been reported before (Ying et al., 2016; Rossoni et al., 2019). Phospholipases are enzymes that help *Candida* invade by degrading host cell membranes (Mohan das & Ballal, 2008). Phospholipase secretion is linked to greater virulence and antifungal resistance, especially to azoles (Rossoni et al., 2015). The absence of amphotericin B resistance and rarity of echinocandin resistance among phospholipase producers matches Saikat et al.'s (2018) finding that phospholipase production wasn't significantly associated with resistance to those drug classes. However, more research is needed to clarify the relationship between phospholipase activity

and amphotericin B and echinocandin resistance in *Candida*.

Table 5 shows the distribution of virulence factors (biofilm formation and phospholipase production) across different clinical specimens. Moderate biofilm formation was most common for all specimen types, ranging from 40-44.4%. Strong biofilm formation was also notable, especially in blood and urine samples (26.8-30%). Phospholipase production showed a similar trend, with moderate levels being most frequent (33.3-35.7%). Wound swabs had the highest rate of strong phospholipase production at 50%. Overall, while moderate biofilm formation and phospholipase production predominated across all specimens, strong levels were also seen, suggesting virulence may vary by specimen type. Further analysis could explore correlations between these virulence factors and clinical outcomes.

The relatively consistent distribution of virulence factors across clinical specimens suggests that the site of infection or specimen type may not strongly influence virulence factor expression. However, some studies have noted variations in virulence factor expression by clinical source. Rajendran et al. (2016) found *Candida* bloodstream isolates had higher biofilm formation and phospholipase activity than isolates from other specimens. Dabiri et al. (2018) reported higher proteinase activity in *Candida* isolates from urine compared to other sources. The consistency in virulence factor distribution across specimens in this study may be due to the similar proportions of *Candidaspecies* isolated from each specimen type. Since different *Candidaspecies* are known to vary in their virulence factor expression (Saikat et al., 2018; Khan et al., 2020), the homogeneity in *species* distribution may have contributed to the consistent virulence factor expression observed.

This study provides valuable insights into the distribution, antifungal susceptibility, and virulence factors of *Candidaspecies* from various clinical specimens in a tertiary care hospital. The findings highlight the predominance of *C. albicans*, the rising prevalence of *non-albicans species* like *C. tropicalis* and *C. glabrata*, and the association between virulence factors (biofilm formation, phospholipase production) and antifungal resistance, especially to azoles. The relatively consistent distribution of virulence factors across specimens suggests infection site or specimen type may not strongly influence virulence factor expression. However, more research is needed to elucidate the relationship between virulence factor expression and clinical outcomes, and to develop targeted therapies addressing specific virulence mechanisms of different *Candidaspecies*.The study also emphasizes the importance of ongoing surveillance of antifungal resistance patterns and prudent use of antifungal drugs, particularly given the emerging resistance among *non-albicans Candidaspecies*. Implementing effective infection control measures and developing new antifungal agents targeting specific virulence factors could help improve the management of *candidiasis* and reduce the burden of this significant opportunistic fungal infection.

Limitations of this study include its single-center design and the relatively small sample size, which may limit the generalizability of the findings. Additionally, the cross-sectional nature of the study does not allow for the assessment of temporal changes in *Candida* epidemiology and antifungal resistance patterns. Furthermore, the study did not investigate the molecular mechanisms underlying the observed associations between virulence factors and antifungal resistance.

Future research should focus on conducting multi-center, longitudinal studies with larger sample sizes to validate the findings of this study and to provide a more comprehensive understanding of the epidemiology and antifungal resistance patterns of *Candidaspecies*. Investigating the molecular basis of virulence factor expression and antifungal resistance in *Candidaspecies* using advanced techniques such as whole-genome sequencing and transcriptomics will provide valuable insights for the development of targeted therapeutic strategies. Additionally, exploring the impact of virulence factors on clinical outcomes and treatment response will help guide clinical decision-making and improve patient care.

## CONCLUSION

This study highlights the predominance of *C. albicans* and the increasing prevalence of *non-albicans Candidaspecies* among clinical isolates from a tertiary care hospital. The association between virulence factors (biofilm formation & Coagulase activity) and antifungal resistance, particularly to azoles, emphasizes the importance of considering these factors in the management of candidiasis. The relatively consistent distribution of virulence factors across different clinical specimens suggests that their expression may not be strongly influenced by the site of infection. Continuous surveillance of antifungal resistance patterns and the development of targeted therapeutic strategies addressing specific virulence mechanisms are crucial for improving patient outcomes.

## REFERENCES

1. Pappas, P. G., Lionakis, M. S., Arendrup, M. C., Ostrosky-Zeichner, L., & Kullberg, B. J. (2018). Invasive *candidiasis*. Nature Reviews Disease Primers, 4, 18026. https://doi.org/10.1038/nrdp.2018.26
2. Pristov, K. E., & Ghannoum, M. A. (2019). Resistance of *Candida* to azoles and echinocandins worldwide. Clinical Microbiology and Infection, 25(7), 792-798. https://doi.org/10.1016/j.cmi.2019.03.028
3. Höfs, S., Mogavero, S., & Hube, B. (2016). Interaction of *Candida* albicans with host cells: Virulence factors, host defense, escape strategies, and the microbiota.

Journal of Microbiology, 54(3), 149-169. https://doi.org/10.1007/s12275-016-5514-0
4. Rajendran, R., Sherry, L., Nile, C. J., Sherriff, A., Johnson, E. M., Hanson, M. F., Williams, C., Munro, C. A., Jones, B. J., & Ramage, G. (2016). Biofilm formation is a risk factor for mortality in patients with *Candida* albicans bloodstream infection-Scotland, 2012-2013. Clinical Microbiology and Infection, 22(1), 87-93. https://doi.org/10.1016/j.cmi.2015.09.018
5. Cavalheiro, M., & Teixeira, M. C. (2018). *Candida* biofilms: Threats, challenges, and promising strategies. Frontiers in Medicine, 5, 28. https://doi.org/10.3389/fmed.2018.00028
6. Richardson, J. P., Moyes, D. L., Ho, J., &Naglik, J. R. (2019). *Candida* innate immunity at the mucosa. Seminars in Cell & Developmental Biology, 89, 58-70. https://doi.org/10.1016/j.semcdb.2018.02.026
7. Saikat, A., Guha, P., Das, S., Naha, A., Banerjee, S., & Das, P. (2018). *Candidaspecies* isolated from various clinical samples of ICU patients and their antifungal susceptibility patterns in a tertiary care hospital in Kolkata, India. Cureus, 10(3), e2363. https://doi.org/10.7759/cureus.2363
8. Abdelmalek, R., Amor, G. M., Aloulou, H., Guedes, M., Allal, F., Mansour, H. B., Kallel, K., Daroui, R., Bouallegui, Y., Zili, B., Ranque, S., Kallel, L., & Chaker, E. (2022). Virulence determinants, antifungal susceptibility profiles and genetic variability among *Candida* albicans and *non-albicans Candida* isolates from hospital patients in Tunisia. Antibiotics, 11(3), 409. https://doi.org/10.3390/antibiotics11030409
9. Desai, J. V. (2018). *Candida* albicans hyphae: From growth initiation to invasion. Journal of Fungi, 4(1), 10. https://doi.org/10.3390/jof4010010
10. Khan, M. A., Dar, A. A., Gull, A. R., Hussain, S., Shafi, H., Maroof, P., Ahmed, F., Qadri, R. A., & Manzoor, N. (2020). Differential virulence attributes and clinical outcomes of *Candidaspecies* isolated from various clinical conditions and specimens. Brazilian Journal of Microbiology, 51(4), 2119-2129. https://doi.org/10.1007/s42770-020-00352-8
11. Vila, T., Sultan, A. S., Montelongo-Jauregui, D., & Jabra-Rizk, M. A. (2020). Oral *candidiasis*: A disease of opportunity. Journal of Fungi, 6(1), 15. https://doi.org/10.3390/jof6010015
12. Seifi Z, Zarei Mahmoudabadi A, Zarrin M. Extracellular enzymes and susceptibility to fluconazole in *Candida* strains isolated from patients with vaginitis and healthy individuals. Jundishapur J Microbiol. 2015 Mar 21;8(3):e20162. doi: 10.5812/jjm.20162. PMID: 25861438; PMCID: PMC4386072.
13. Dabiri, S., Shams-Ghahfarokhi, M., & Razzaghi-Abyaneh, M. (2018). Comparative analysis of proteinase, phospholipase, hydrophobicity and biofilm forming ability in *Candidaspecies* isolated from clinical specimens. Journal de MycologieMédicale, 28(3), 437-442. https://doi.org/10.1016/j.mycmed.2018.04.009
14. Yenisehirli, G., Bulut, N., &Onuk, E. E. (2021). Virulence factors and antifungal susceptibility patterns of various *Candidaspecies* isolated from bloodstream infections. European Journal of Clinical Microbiology & Infectious Diseases, 40(8), 1679-1687. https://doi.org/10.1007/s10096-021-04198-2
15. Kaur, R., Dhakad, M. S., Goyal, R., & Kumar, R. (2016). Emergence of *non-albicans Candidaspecies* and antifungal resistance in intensive care unit patients. Asian Pacific Journal of Tropical Biomedicine, 6(5), 455-460. https://doi.org/10.1016/j.apjtb.2015.12.019
16. Deorukhkar, S. C., Saini, S., & Mathew, S. (2014). *Non-albicans Candida* infection: An emerging threat. Interdisciplinary Perspectives on Infectious Diseases, 2014, 615958. https://doi.org/10.1155/2014/615958
17. Mayer, F. L., Wilson, D., & Hube, B. (2013). *Candida* albicans pathogenicity mechanisms. Virulence, 4(2), 119-128. https://doi.org/10.4161/viru.22913
18. Chakrabarti, A., Sood, P., Rudramurthy, S. M., Chen, S., Kaur, H., Capoor, M., Chhina, D., Rao, R., Eshwara, V. K., Xess, I., Kindo, A. J., Umabala, P., Savio, J., Patel, A., Ray, U., Mohan, S., Iyer, R., Chander, J., Arora, A., ... Patel, M. R. (2015). Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Medicine, 41(2), 285-295. https://doi.org/10.1007/s00134-014-3603-2
19. Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant *Candida*: Epidemiology, molecular mechanisms, and treatment. The Journal of Infectious Diseases, 216(suppl_3), S445-S451. https://doi.org/10.1093/infdis/jix131
20. Whaley, S. G., Berkow, E. L., Rybak, J. M., Nishimoto, A. T., Barker, K. S., & Rogers, P. D. (2017). Azole antifungal resistance in *Candida* albicans and emerging *non-albicans Candidaspecies*. Frontiers in Microbiology, 7, 2173. https://doi.org/10.3389/fmicb.2016.02173
21. Gomez-Lopez, A., Cendejas-Bueno, E., Colom-Valiente, M. F., Cuenca-Estrella, M., & FUNGEMYCA Study Group. (2021). In vitro antifungal susceptibility of *Candida* spp. strains isolated from candidemia according to the revised Clinical and Laboratory Standards Institute breakpoint. Antibiotics, 10(7), 839. https://doi.org/10.3390/antibiotics10070839
22. Quinteros, M. J., Calvano, M. P., Revale, S., Videla, J. D., Canteros, C. E., & Soria, M. A. (2021). Antifungal susceptibility of clinical isolates of *Candidaspecies* over the last 10 years in Argentina. Journal of Fungi, 7(8), 605. https://doi.org/10.3390/jof7080605
23. Mathé, L., & Van Dijck, P. (2013). Recent insights into *Candida* albicans biofilm resistance mechanisms. Current Genetics, 59(4), 251-264. https://doi.org/10.1007/s00294-013-0400-3
24. Nobile, C. J., & Johnson, A. D. (2015). *Candida* albicans biofilms and human disease. Annual Review of Microbiology, 69, 71-92. https://doi.org/10.1146/annurev-micro-091014-104330
25. Rodrigues, C. F., Silva, S., & Henriques, M. (2014). *Candida* glabrata: A review of its features and resistance. European Journal of Clinical Microbiology & Infectious Diseases, 33(5), 673-688. https://doi.org/10.1007/s10096-013-2009-3
26. Maiolo, E. M., FurustrandTafin, U., Borens, O., &Trampuz, A. (2014). Activities of fluconazole, caspofungin, anidulafungin, and amphotericin B on planktonic and biofilm *Candidaspecies* determined by microcalorimetry. Antimicrobial Agents and Chemotherapy, 58(5), 2709-2717. https://doi.org/10.1128/AAC.00057-14
27. Ying, C., Zhang, H., Tang, Z., Chen, H., Gao, J., & Yue, C. (2016). Antifungal susceptibility and molecular typing of 115 *Candida* albicans isolates obtained from vulvovaginal *candidiasis* patients in 3

Shanghai maternity hospitals. Medical Mycology, 54(4), 394-399. https://doi.org/10.1093/mmy/myv082
28. Rossoni, R. D., Fuchs, B. B., de Barros, P. P., Velloso, M. D. S., Jorge, A. O. C., Junqueira, J. C., & Mylonakis, E. (2019). *Candida* tropicalis-associated arthritis: A systematic review. Journal of Fungi, 5(4), 96. https://doi.org/10.3390/jof5040096
29. Mohan das, V., & Ballal, M. (2008). Proteinase and phospholipase activity as virulence factors in *Candidaspecies* isolated from blood. RevistaIberoamericana de Micología, 25(4), 208-210. https://doi.org/10.1016/S1130-1406(08)70050-0
30. Pandey, N., Gupta, M. K., & Tilak, R. (2020). Extracellular hydrolytic enzyme activities of different *Candida* spp. isolated from patients with vulvovaginal *candidiasis*. Journal of Medical Mycology, 30(1), 100909. https://doi.org/10.1016/j.mycmed.2019.100909
31. Naglik, J. R., Gaffen, S. L., & Hube, B. (2019). *Candida*lysin: Discovery and function in *Candida* albicans infections. Current Opinion in Microbiology, 52, 100-109. https://doi.org/10.1016/j.mib.2019.06.002
32. Pfaller, M. A., Diekema, D. J., Turnidge, J. D., Castanheira, M., & Jones, R. N. (2019). Twenty years of the SENTRY Antifungal Surveillance Program: Results for *Candidaspecies* from 1997-2016. Open Forum Infectious Diseases, 6(Suppl 1), S79-S94. https://doi.org/10.1093/ofid/ofy358
33. Ismail, M. M., Yahia, M. A., & Mahmoud, H. S. (2022). Molecular identification of *Candidaspecies* isolated from different clinical specimens and their biofilm formation, proteinase and phospholipase activity. Al-Azhar Medical Journal, 51(1), 209-220. https://doi.org/10.21608/amj.2022.190899
34. Castanheira, M., Deshpande, L. M., Davis, A. P., Rhomberg, P. R., & Pfaller, M. A. (2021). Monitoring antifungal resistance in a global collection of invasive yeasts and molds: Application of CLSI epidemiological cutoff values and whole-genome sequencing analysis for detection of azole resistance in *Candida* albicans. Antimicrobial Agents and Chemotherapy, 65(6), e02437-20. https://doi.org/10.1128/AAC.02437-20
35. Rossoni, R. D., Barbosa, J. O., Vilela, S. F. G., dos Santos, J. D., de Barros, P. P., Prata, M. C. A., Anbinder, A. L., Fuchs, B. B., Jorge, A. O. C., Mylonakis, E., & Junqueira, J. C. (2015). Competitive interactions between *C. albicans*, *C. glabrata*and C. krusei during biofilm formation and development of experimental *candidiasis*. PLoS One, 10(7), e0131700. https://doi.org/10.1371/journal.pone.0131700
36. Lohse, M. B., Gulati, M., Johnson, A. D., & Nobile, C. J. (2018). Development and regulation of single- and multi-*speciesCandida* albicans biofilms. Nature Reviews Microbiology, 16(1), 19-31. https://doi.org/10.1038/nrmicro.2017.107