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PREVALENCE OF DRUG RESISTANT CANDIDA SPECIES IN A TERTIARY CARE SETTING

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Abstract

Background: With the rise in patients who are elderly, immunocompromised, getting aggressive and protracted antimicrobial cancer chemotherapy, or having invasive surgery and organ transplantation, candidiasis has become a concerning opportunistic disease. There have been many instances of Candida infections in India during the past few decades. The most prevalent and aggressive pathogenic species in the genus Candida is thought to be Candida albicans.

Aim : The aim of this study was to isolate and identify Candida species from clinical samples and to determine the prevalence of drug resistance in Candida species in a tertiary care setting.

Methodology: This cross-sectional study was conducted in a tertiary care hospital between February 2024 and July 2024. The study included a total of 100 Candida isolates from individuals with a clinical suspicion of fungal infections. Samples were prepared using a standard microbiological technique, and antifungal susceptibility testing was carried out in accordance with CLSI guidelines.

Results: Among 100 samples, Candida albicans (44) were found predominantly found with increased resistance to itraconazole**.**

Conclusion: In conclusion, long-term itraconazole prophylaxis in patients is associated with reduction in susceptibility to itraconazole.

KEYWORDS: andida species; Candida albicans; Fungal infections; Antifungal resistance.

Introduction:

Candida species are the most common cause of fungal infection. One of the leading causes of mortality and morbidity. Its prevalence is rising quickly among high-risk groups, including elderly patients, organ transplant recipients, and immunocompromised people receiving long-term antibiotic medication. Patients who have received a renal transplant are more likely to develop invasive candidiasis. It is more challenging to treat candiduria.[1]Although Candida albicans is the most commonly isolated species from clinical samples isolation of other species of candida include Candida parapsilosis, Candida krusei, Candida tropicalis, and Candida glabrata.[2]

Candida are yeast-like fungi that typically reside in mouth, throat, intestine, genital area, and urinary tract.[3] Blood culture that tests positive for Candida species is an indication of candidemia.[4]

Candidemia is a potentially fatal fungal infection that has a 38% fatality rate and can lengthen hospital stays by up to 30 days.[5] Candida species infections are one of the four most frequent causes of bloodstream infections, catheter-associated UTIs, and hospital-acquired infections.[6]

Some people had candidemia as a result of an infected indwelling catheter, whereas others developed candidemia as a symptom of invasive candidiasis.[7] The appearance of non-albicans Candida (NAC) species, a strain that poses a risk

of rising mortality and resistance to antifungal medications, has altered the spectrum of Candidaemia.[8] An antifungal medication is always necessary to treat candidemia.[9] Numerous studies have observed the high mortality rates linked to candidemia and have demonstrated that patients who did not receive antifungal medication had the highest mortality rates.[10,11]

Through mechanisms like the expression of efflux pumps, which decrease drug accumulation, changes in the structure or concentration of antifungal target proteins, and modifications to the composition of membrane sterols, pathogenic Candida species are becoming resistant to antifungal agents, particularly triazole compounds.[12]

This study emphasizes the need for a better knowledge of how candida infections arise and how they become resistant to traditional antifungal medications. Henceforth, the aim of this study was to isolate and identify Candida species from clinical samples and to determine the prevalence of drug resistance in Candida species in a tertiary care setting.

Aim & objectives:

To isolate and identify Candida species from clinical samples and to determine the prevalence of drug resistance in Candida species in a tertiary care setting.

Methodology :

For six months, from February 2024 to July 2024, this cross-sectional study was carried out in the microbiology department of Vinayaka Mission's KirupanandaVariyar Medical College and Hospital in Salem. One hundred people make up the study sample. Patients of all age groups with isolates of Candida species were included in the study, while patients without candidal infection were excluded in the study.

Clinical specimens such as nasopharyngeal swabs, blood, pus, urine, vaginal swabs, skin scrapings, respiratory secretions like sputum, endotracheal secretions, throat swabs, and other body fluids were collected in sterile containers with strict aseptic precautions in accordance with CLSI criteria.

Initially the isolates were identified using standard microbiological methods, and the species of Candida were identified using Sabouraud's dextrose agar (SDA), which was incubated at 25° and 37°C as milky white, pasty colonies. [1] The colonies cultivated on Sabouraud's dextrose agar were subjected to Gram staining. There were visible gram-positive budding yeast cells with pseudohyphae. After growth, the species was isolated using tests for fermentation and carbohydrate assimilation (done for speciation), growth at 45° C (positive for Candida albicans).

CHROM agar, and germ tube testing were done to identify the species. The Corn Meal Agar (CMA) test used for identification of Candida albicans. This medium is enriched with cornmeal infusion and agar, which supports fungal growth and helps in the production of chlamydospores.

CHROM agar test is a microbiological method that uses chromogenic agar to identify and differentiate microorganisms based on the color of their colonies. This medium contains chromogens — colorless molecules that react with specific enzymes produced by microorganisms. When the enzyme cleaves the chromogen, it releases a colored compound, allowing for easy visual identification. For example, CHROM agar Candida is commonly used to differentiate Candida species, with each species producing colonies of distinct colors.

Germ Tube Test is a rapid diagnostic method used to identify *Candida albicans* . These species are known to form germ tubes — long tube-like projections when incubated in a protein-rich medium like human or sheep serum at 37°C for 2-4 hours.

The Kirby Bauer disc diffusion method was used to test for antifungal susceptibility on Muller Hinton agar with 2% glucose using commercially available discs, such as amphotericin B (20µg), flucanazole (10µg), itraconazole (10µg), voriconazole (1µg), ketoconazole (10µg), clotrimozole (10µg), and caspofungin (5µg). A 0.5 Mc Farland standard is prepared by comparing the turbidity of a test suspension to a barium sulfate suspension of known concentration. The mixture was incubated at 45° C for the entire night with each disk spaced 20 mm apart. After that, the results were assessed using the CLSI criteria. Carbohydrate fermentation and assimilation test was done with sugars such as glucose, sucrose , maltose, lactose and galactose.

Results :

Table 1. Total Candida species collected

Candida species	Total Count
Candida albicans	44
Candida krusei	40

Candida tropicalis	16
Total	100

Table 1 presents the distribution of Candida species collected in the study. The table lists three specific species: Candida albicans, Candida krusei, and Candida tropicalis, along with the number of samples identified for each. Candida albicans was the most frequently isolated species, accounting for 44 samples. Candida krusei followed closely with 40 samples. Candida tropicalis was the least common, with 16 samples. The total number of samples collected across all species was 100. This table provides a snapshot of the prevalence of different Candida species within the sample population.

Table 2. Candida albicans (n = 44)

Parameter	Value
Total Isolates	44
Itraconazole Resistant	36
Percentage Resistant	81%

Table 2 provides data specific to the 44 isolates of Candida albicans identified in the study. Among these isolates 36 were found to be resistant to Itraconazole, a commonly used antifungal agent. This corresponds to a resistance rate of 81%, indicating a high prevalence of antifungal resistance within this species. The table highlights the potential challenge of managing Candida albicans infections due to significant drug resistance.

Table 3. Candida krusei (n = 40)

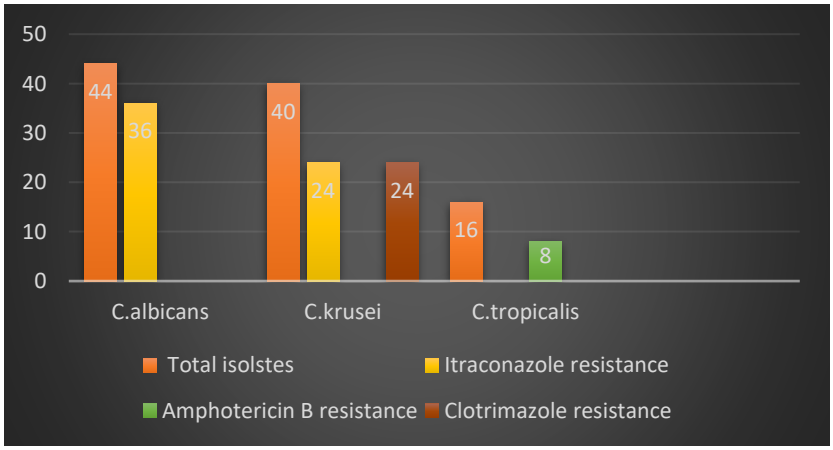
Parameter	Value
Total Isolates	40
Itraconazole Resistant	24
Clotrimazole Resistant	24
Itraconazole Resistance Percentage	60%

Table 3 summarizes the antifungal resistance profile of 40 isolates of Candida krusei. The data indicate that, 24 isolates (60%) were resistant to Itraconazole. The same number, 24 isolates, also showed resistance to Clotrimazole. These findings suggest a notable level of resistance to both antifungal agents in Candida krusei.

Table 4. Candida tropicalis (n = 16)

Parameter	Value
Total Isolates	16
Amphotericin B Resistant	8
Amphotericin B Resistance %	50%
Itraconazole Resistant	0
Itraconazole Resistance %	0%

Table 4 presents the antifungal resistance profile of 16 isolates of Candida tropicalis. The results show that, 8 isolates (50%) were resistant to Amphotericin B. No resistance was observed against Itraconazole, with a 0% resistance rate. This indicates a moderate level of resistance to Amphotericin B, while Itraconazole appears to be fully effective against Candida tropicalis in this sample.



Graph 1: Antibiotic Resistance in Candida species

Graph 1 shows the comparison of antifungal resistance among three Candida species. C. albicans shows the highest number of isolates (≈44) with significant Itraconazole resistance (≈36), but no Amphotericin B resistance. Candida krusei has around 40 isolates with moderate Itraconazole resistance (≈24) and no Amphotericin B resistance. Candida tropicalis has the fewest isolates (≈16) but shows Amphotericin B resistance in about half of them (≈8), with no Itraconazole resistance. Overall, Itraconazole resistance is seen in Candida albicans and Candida krusei, while Amphotericin B resistance is only observed in Candida tropicalis.

Discussion :

A rising issue that needs to be addressed is the immunocompromised infections caused by Candida species particularly by the non-albicans species, have significantly grown over the past three decades.[13] Both host vulnerability factors and fungal virulence are responsible for this higher incidence. Immunocompetent people are more susceptible to candidiasis due to prolonged use of antibiotics, which eventually changes the normal flora, corticosteroid use, surgeries, malnutrition, and hormonal imbalance.[14] Long-term usage of commonly prescribed antifungal medications changes the incidence of Candida spp.[15]

In our study, Candida albicans was the most frequently isolated species, accounting for 44 samples, followed by Candida krusei reported for 40 samples and the least was Candida tropicalis for 16 samples. Our study results were contradictory to the study done by Anam et al Candida tropicalis was the most isolated species in all samples, followed by *Candida albicans*.[16]

Candida albicans was the most often isolated pathogen in the study by Siddiqui et al., and Candida parapsilosis was shown to be more prevalent in their investigation.[17] This is consistent with a research by Capoor et al[18] that found that patients in intensive care units had a high isolation incidence of Candida tropicalis.

However, in a research by Roy et al,[19] C. glabrata accounted for 32% of the isolated non-albicans Candida, followed by C. tropicalis at 30%. The majority of invasive fungal infection patients had lung infections, sepsis, and urinary tract infections when they first arrived. In their study of invasive fungal infection patients, Pagano et al. found that the respiratory tract was the most often affected system.[20] On the other hand, Pahwa et al. 2014, found that the majority of samples were blood, then urine.[21]

Among 44 isolates of C.albicans, 36 (81%) showed resistane to itraconazole whereas among the Candida.krusei isolates, 24 (60%) were equally resistant to itraconazole and cotrimazole. But the isolates of Candida.tropicalis showed 50% resistant to the amphotericin B.

Nystatin, Amphotericin B, and Miconazole demonstrated 100% sensitivity among the nine Candida albicans investigated by Choudhary et al, followed by ketoconazole (88.88%), itraconazole (88.88%), and fluconazole (88.88%).[22] This is related to the research by Capoor et al,[18] in which isolates of Candida showed sensitivity to amphotericin B. On the other hand, all of the Candida isolates in the Bhattacharjee et al. research were responsive to fluconazole.[23] Similar results were also reported by Rajeevan et al., who found that the highest sensitivity was to 100% Nystatin and 100% Amphotericin B.[24]

Since itraconazole is the most widely used azole for treating widespread candidiasis, including candidemia, resistance to it is extremely concerning. It is more affordable than other antifungal medications and comes in both oral and intravenous formulations with great absorption. Despite being effective against the majority of Candida species strains, amphotericin B is not the first medication of choice for treating candidemia due to its nephrotoxicity. There are numerous hypothesized causes of azole resistance. Some of the mechanisms for azole resistance in Candida species include altered drug efflux, decreased intracellular accumulation of fluconazole as a result of altered CDR genes, and enhanced expression of the ATP-binding cassette transporter gene. Increased drug resistance and the gradual replacement of albicans species with nonalbicans drug-resistant strains as the primary etiologic agent of infection are both potential outcomes of extensive itraconazole use.

Conclusion:

In the present study, Candida albicans found to be predominant species with increased resistance to itraconazole. An increase in the pattern of antifungal resistance highlights the importance of using antifungal prophylaxis. In order to begin the precise treatment for immunocompromised individuals as soon as feasible, antifungal susceptibility patterns will assist clinicians in early diagnosis and identification of the causative agent. This is essential for tracking the emergence of resistance and helping medical practitioners establish protocols for the responsible use of these medications.

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