**ORIGINAL RESEARCH**

# Investigation of the Candida Isolation, Speciation, and Identification in a Range of Clinical Samples

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**ABSTRACT**

**Background:** Numerous clinical infections, from mucocutaneous infections to potentially fatal invasive illnesses, are caused by Candida species, and the rise in antifungal medication resistance has raised severe concerns. Over the last ten years, antifungal agent resistance has grown. Therefore, testing for antifungal susceptibility and identifying Candida up to the species level are crucial for managing Candida infections. The study's objectives were to distinguish between different species of Candida and ascertain how susceptible each species was to antifungal medications. **Materials and methods:** The investigation comprised 120 consecutive, non-repetitive Candida isolates from a variety of clinical specimens, including high vaginal swabs, urine, sputum, pus, catheter tips, ear swabs, and stool samples from patients with antibiotic-associated diarrhoea. Direct samples were used to do Gram's stain, which was then inoculated into Sabouraud dextrose agar and incubated for 24 hours at 37°C. The investigation excluded isolates that were identified as fungi other than Candida species. To identify Candida species, a colony on Sabouraud dextrose agar was treated. The isolates underwent urea hydrolysis testing, germ tube testing, Gram's staining, and macroscopic inspection. **Result:** A total of 90 Candida spp. was isolated from various clinical samples. Distribution of samples of Candida isolates were mentioned in Table 2. Candida albicans (49.5%) was the most common species isolated. **Conclusion:** Finding the species that are inherently resistant is aided by the species-level characterisation of Candida. Non-albicans Candida species, such as Candida tropicalis, Candida krusei, Candida glabarata, and Candida dubliniensis, are also increasingly being identified from clinical specimens in addition to Candida albicans. For identifying such species, CHROM agar is an easy, quick, and affordable technique with excellent sensitivity and specificity.

**Keywords:** Candida, Non-albicans candida(NAC), Fluconazole, Amphotericin-B.



## INTRODUCTION

Though it may be linked to both superficial and deep-seated fungal infections, Candida species is a common commensal flora found in the human body that inhabits the skin, mucous membranes, and gastrointestinal system. [1] Numerous virulence mechanisms, including adhesion to host tissues, medical devices, biofilm development, and the production of extracellular hydrolytic enzymes, enable Candida species to transition from commensal to a powerful pathogen. [2] Additionally, non-Albicans Candida (NAC) species are thought to be one of the main pathogens that cause serious infections in humans in recent years. [3]

The susceptibility pattern of the widely used antifungal medications varies significantly depending on the kind of Candida species. The overuse of random antifungal drugs has led to an increase in drug resistance during the last several decades. [4] The widespread rise of drug-resistant Candida species was documented in a number of earlier investigations [5]. Therefore, in vitro susceptibility testing of antifungal medicines has become increasingly significant for employing selective and sensitive drugs due to the change in drug susceptibility pattern of Candida species and the introduction of novel antifungal agents.

As a result, it is now more crucial than ever to isolate, identify, characterise, and test for Candida species in clinical specimens in order to control fungal infections.

The current work demonstrated the susceptibility pattern of Candida isolates from clinical specimens and investigated the characterization of Candida species using agar.

## MATERIALSANDMETHODS

The investigation comprised 120 consecutive, non-repetitive Candida isolates from a variety of clinical specimens, including high vaginal swabs, urine,

sputum, pus, catheter tips, ear swabs, and stool samples from patients with antibiotic-associated diarrhoea.

Direct samples were used to do Gram's stain, which was then inoculated into Sabouraud dextrose agar and incubated for 24 hours at 37°C. The investigation excluded isolates that were identified as fungi other than Candida species. To identify Candida species, a colony on Sabouraud dextrose agar was treated. The isolates underwent urea hydrolysis testing, germ tube testing, Gram's staining, and macroscopic inspection.

Further processing was done on the creamy, pasty, and yeasty colony that had Gram-positive budding yeast-like cells with pseudohyphae and a negative urea hydrolysis test. Both Candida albicans and Candida dubliniensis were discovered as germ tube positives after a test. Chlamydospore development on cornmeal agar and growth at 45°C were used to further identify Candida albicans. To validate the species of Candida, absorption and sugar fermentation tests were performed on each isolate.

In accordance with the manufacturer's instructions, the species were identified by the type and colour of the colonies on CHROMagar media after the Candida spp. were simultaneously inoculated on the medium and cultured for 24 hours at 37°C (Hi-media, India). (Table 1 and Figure 1). A new differential culture medium called CHROMagar is said to make colorimetric presumptive identification easier.

## RESULTS

**Table1: Colour of variousCandida spp.on CHROM agar for identification.10**

| Name | Colour on CHROM agar |
|---|---|
| ***C. albicans*** | Light green |
| ***C. tropicalis*** | Metallicblue |
| ***C. krusei*** | Rosepink |
| ***C. glabrata*** | White |
| ***C. parapsilosis*** | Palecream |
| ***C. dubliniensis*** | Darkgreen |

**Table2: Isolation of Candida spp.from clinical samples.**

| Sample | *C. albicans* | *C. tropicalis* | *C. krusei* | *C. glabrata* | *C. dubliniensis* | *No. of Candida isolates* |
|---|---|---|---|---|---|---|
| **Vaginal swab** | 21 | 4 | 4 | 4 | 0 | 33 |
| **Urine** | 6 | 4 | 3 | 0 | 1 | 14 |
| **Sputum** | 4 | 10 | 4 | 3 | 0 | 21 |
| **Earswab** | 4 | 3 | 0 | 3 | 0 | 10 |
| **Stool** | 3 | 3 | 0 | 0 | 0 | 6 |
| **Pus** | 0 | 0 | 5 | 0 | 0 | 5 |
| **Catheter tip** | 1 | 0 | 0 | 0 | 0 | 1 |
| **Total** | 39 | 24 | 16 | 10 | 1 | 90 |

A total of 90 Candida spp. was isolated from various clinical samples. Distribution of samples of Candida isolates were mentioned in Table 2. Candida albicans (49.5%) was the most common species isolated.

**Table3: Sensitivity and specificity of CHROM agar for speciation of Candida.**

| Candida spp | No. of Candida spp. Identified by conventional method | No. of Candida spp. identified using CHROM agar | Sensitivity of CHROM agar | Specificity of CHROM agar |
|---|---|---|---|---|
| ***C. albicans*** | 37 | 36 | 100% | 95% |
| ***C. tropicalis*** | 21 | 21 | 100% | 100% |
| ***C. krusei*** | 13 | 13 | 100% | 100% |
| ***C. glabarata*** | 7 | 7 | 76% | 100% |
| ***C. dubliniensis*** | 0 | 1 | 97% | 100% |

Among the non-albicans Candida, C.tropicalis(39%), C.krusei(23%), C.glabarata(11%)and C.dubliniensis (2%).Sensitivity and specificity of CHROM agar was 100%forC. Tropicalis and C. krusei. Sensitivity and specificity for C. albicans was 100% and 95% respectively. Sensitivity and specificity for C. glabarata was 76% and 100%, for C. dubliniens is was 97% and 100% (Table 3).

## DISCUSSION

Candida species are part of the human body's commensal flora and quickly colonise the mucosal surfaces after birth. Both host factors and virulence factors of Candida spp. contribute to the rising prevalence of candida infections in hospitalised patients, namely those with compromised immune systems. Candida infections are predisposed to by a number of host variables, including prolonged use of antibiotics or corticosteroids, poor diet, metabolic disorders, and invasive procedures. The incidence of infections caused by various species of Candida has changed as a result of the prolonged and widespread use of antifungal medications. Because the virulence

and antifungal susceptibility of different species of Candida varies, it is now crucial to identify the infectious fungus down to the species level. [7]

The predominant species in our investigation is Candida albicans. Research conducted by many authors, such as Manjunath et al. and Jayalakshmi L., has shown that Candida albicans is the predominant species. [8] Non-Albicans candida (NAC) isolates are becoming more common, and research conducted by different authors have shown comparable findings. [9] A research by Kaviarasan et al. in 2002 revealed that the prevalence of NAC isolates was 39.5%, while a study by R Adhikary in 2011 revealed that this number had increased to 73.6%. [10] Because non-Albicans candida (NAC) isolates have greater rates of resistance to certain antifungal drugs, empirical antifungal treatment is species-specific. This is also clear from our investigation, which found that NAC isolates were much more resistant to the majority of Azole antifungal drugs than isolates of Candida albicans. Studies by Hii, Sabhapandit et al., and other authors have also shown that NAC isolates had increased resistance rates. [11] Numerous research conducted by many authors since CLSI introduced disc diffusion techniques for assessing antifungal susceptibility in 2003 have shown the emergence of antifungal resistance in Candida spp. [12] Previous research by different authors has shown that NAC isolates are becoming more resistant to fluconazole, a commonly used Azole antifungal. According to a 2007 research by MA Pfaller, the resistance rate to fluconazole was 9.9%. Over the following years, the rate increased to 32.4%, as shown by a 2014 study by Jayalakshmi L et al. Our current study's 60% resistance rate may be the result of fewer isolates, but it follows the general pattern of rising resistance rates. [13]

In contrast to research by Sabhapandite et al. and Jayalakshmi L et al., where C. tropicalis was the main species and C. glabrata came in second, our investigation found that C. glabrata was the most prevalent species (40%) among NAC isolates. According to a research by Li et al. and Trick, which shows an unusual rise in its occurrence, C. glabrata has become a significant opportunistic pathogen in recent decades. [14] Similar to studies by Jayalakshmi L et al. and Sharma M., where 42% and 43.3% of samples were blood and urine, the majority of Candida isolates (53%) were isolated from blood and urine samples.

[15] Patients over 50 accounted for the majority of isolates (48%) in our investigation, and a study by Bhattacharjee et al. found that a comparable age group contributed a significant amount (44%) of the isolates. [16]

## CONCLUSION

According to the current research, elderly patients are more susceptible to Candida spp., a significant opportunistic pathogen in intensive care unit settings. It is concerning to start empirical antifungal treatment because of the rise of NAC isolates and their growing resistance to several antifungal medications. In order to start an empirical therapy with an appropriate antifungal to which the isolate is not inherently resistant, it is now crucial to identify the isolates down to the species level. To direct doctors and create appropriate antibiotic policies, it is necessary to continuously study the evolving epidemiology and resistance pattern of Candida species.

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