

Original Research Article

Prevalence of Candida Species Isolated from Immuno-compromised Patients

ABSTRACT

Background: Resistance to fungal infections is increasing worldwide, which is highly relevant in immunosuppressed individuals. Candida fungi infection constitutes one of the most common causes of fungal infections in such patients, and it can lead to complications and death. The current study sought to examine the prevalence and species diversity of Candida in samples received by the Microbiology Laboratory over a two-year period.

Method: The study involved 674 immunocompromised patients. Candida was isolated from clinical samples using wet mount, gram stain, and SDA culture. To further identify the organisms, several tests were conducted, including the germ tube test, cornmeal agar morphology, sugar assimilation, fermentation tests, and BACT/ALERT 3D.

Results: Candida species were found to be present in 6.5% of the population. Candida tropicalis was found to be the most common isolate 20 (45.45%), followed by Candida albicans 9 (20.45%), Candida glabrata 7 (15.9%), Candida parapsilosis 4 (9.09%), and Candida krusei 4 (9.09%). Diabetes, leukaemia, chronic kidney disease, and AIDS were the most common predisposing factors for candidiasis.

Conclusion: Men had a relatively high candidemia prevalence of 68.18%. Uncontrolled diabetes mellitus (DM) and chronic kidney disease (CKD) have been found to be the most common co-morbidities with candidemia in people aged 51 to 70.

Keywords: Candida, Immuno-compromised, Fungal infection, Candidemia, Chromagar

1. INTRODUCTION

The most prevalent fungus that causes fungal diseases is called Candida. Candida is a typical resident of the skin, mucous membranes of the oral cavity, the gastrointestinal tract, the respiratory tract, and the genitourinary tract, and it has the potential to spread to other regions of the body, particularly in those with impaired immune systems [1].

Exposure to more intense management modalities is accessible to individuals admitted to tertiary care hospitals. This, together with an increase in immune-compromised patients, has contributed to an increase in Candida infections, particularly those caused by non-Candida albican [2].

The fourth most frequent cause of blood stream infections is a type of Candida[3]. Many large-scale surveillance programmes of Candida BSIs have accumulated valuable data on trends in resistance, the distribution of species across nations, and types of infections[4]. These research' findings suggest that antifungal susceptibility profiles and the distribution of species varied significantly[5].

C. albicans was the most frequently isolated yeast-like fungus, although over a 4.5-year period, there was a decreasing tendency in the rate of *C. albicans* isolation (6.74%), coupled with rising rates of isolation for *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. rugosa* (totalling 7.1%). Uncommon yeasts, including *C. pelliculosa*, *Pichia* species, and *C. zeylanoides*, were identified during the study's final two years, but they only made up 1.12% of all the isolates found in 2001[6].

Lethal invasive Candidiasis has been reported more frequently over the past three decades as a result of the use of powerful antibiotic, immunosuppressive, and cytotoxic

medications[7]. Long-term uses of antibiotics, immunocompromised state, chemotherapy, and catheterization are risk factors for Candida infection[8].

The advent of newer antifungal drugs and changes in the drug susceptibility of various species of Candida have increased the importance of in vitro susceptibility testing for antifungal medications and aid in their judicious application[9].

The purpose of this study is to determine the prevalence of Candida species isolated from immuno-compromised patients. Specifically, the study aims to identify the most common Candida species and their relative distribution, as well as to investigate the virulence determinants of Candida tropicalis, the dominant isolate found in the study population. The findings of this study can help to guide clinical management of candidemia in immuno-compromised patients and contribute to a better understanding of the epidemiology and pathogenesis of this infection.

2. MATERIAL AND METHODS

The study was conducted in the Department of Microbiology at Index Medical College Hospital and Research Centre, Indore (M.P.)

2.1 Study design

A cross-sectional study was conducted on hospitalized patients.

2.2 Sample size

A number of 674 samples, including Oral swabs, ear swabs, vaginal swabs, stool, urine, CSF, sputum, blood, pus, nail scrapings etc.

2.3 Study duration:

Two years (July 2020 – July 2022) including six months of data analysis.

2.4 Study population

The study enrolled patients of all age groups and both genders, including children, who were undergoing evaluation for immunocompromised status and candida infection and were willing to provide written informed consent.

2.5 Inclusion criteria:

1. All suspected instances of candidiasis, including endocarditis, meningitis, newborn septicemia, vaginitis, skin and nail infections, diarrhoea, urinary tract infection, respiratory tract infection, diabetic and postoperative wound infections.
2. Patients who have given the consent.
3. Patients who were immunocompromised.

2.6 Exclusion criteria

1. Patients visiting with signs and symptoms of fungus other than Candida species.
2. Patients with bacterial infections, patients on antifungal therapy, and those with superficial fungal infections were excluded.
3. Patients who have not given their consent.

2.7 Specimen Collection

Samples were gathered and processed in accordance with Standard Microbiological Practices. Gram stain, 10% KOH, and culture on Sabourad's Dextrose Agar were used to check for cells that resembled budding yeast. The germ tube test, chlamyospore production on maize meal agar, sugar fermentation, and sugar assimilation tests were used to further speculate on the obtained Candida isolates.

2.8 Transport

The specimens were transported in sealed containers, which were packed in an insulated transport container.

2.9 Sample processing

The specimens were processed in a BSL-II laboratory with proper aseptic precautions and personal protective equipment. Visually, the quality of each sample was judged, similar to a sputum sample. If the sample contained more saliva, a fresh sample was collected.

Blood samples were obtained using aseptic techniques in accordance with standard procedure (CDC recommendations). According to the manufacturer's instructions, blood cultures were loaded into the automated system BACT/ALERT 3D.

2.10 Mycological examinations:

1. Direct examination:

- a) Wet Mount
- b) Gram's stain

2. Culture

For Culture Sabourad's dextrose agar (SDA) is used. Contents of Sabourad's dextrose agar:

1. Dextrose - 40 grams
2. Peptone - 10 grams
3. Agar - 20 grams
4. Distilled water – 1000 ml and ph - 5.4

The sample was inoculated on SDA slopes and incubated at 25°C. The slopes were observed daily from 2 days to 3 weeks. Colonies were identified based on their characteristics and Gram stain. Once the colonies were confirmed, speciation was performed using the following methods:

- a. Germ tube
- b. Corn meal agar inoculation
- c. Sugar Fermentation

3. RESULTS

ICU patients provided a total of 674 immunocompromised patientsamples, out of which 44 (6.5%) tested positive for the isolation of Candida spp., while 690 (93.5%) tested negative, indicating a majority of negative results. Of the 44 positive samples, 30 (68.2%) were from men, and 14 (31.8%) were from women. Individuals between the ages of 11 and 30 experienced immunocompromise, which referred to a weakened immune system that made them more susceptible to infections and other health issues, such as genetic disorders,

medications, and chronic illness. Candidemia was found more frequently in individuals aged between 51 and 60, as indicated in Table 1.

Table 1: The age distribution of the study population

AGE GROUP	NO OF PATIENTS POSITIVE (%)	NO OF PATIENTS NEGATIVE (%)	TOTAL NUMBER OF PATIENTS
<10	0	10(100%)	10
11-20	2(4.5%)	18(95.5%)	20
21-30	2(4.5%)	38(95.5%)	40
31-40	4(9%)	40(91%)	44
41-50	6(13.63%)	38(86.4%)	44
51-60	16(36.36%)	28(63.6%)	44
61-70	10(22.72%)	34(77.3%)	44
71-80	4(9%)	40(91%)	44
81-90	0(0)	10(100%)	10
TOTAL	44(6.5%)	630(93.5%)	674

Among the immunocompromised patients, those with diabetes mellitus (DM) and chronic kidney disease receiving dialysis were more prone to candidemia as shown in table 2.

Table 2: Age and gender distribution of immunocompromised patients with Leukaemia, Diabetes Mellitus, and chronic kidney disease on Dialysis (n=44)

Age Group	Immuno-compromising Condition	Gender		
		Male	Female	Total
31-40	Leukaemia	6	2	8
51-60	Diabetes mellitus	12	6	18
61-70	Chronic kidney disease (On Dialysis)	8	6	14
61-70	AIDS	4	0	4
Total		30	14	44

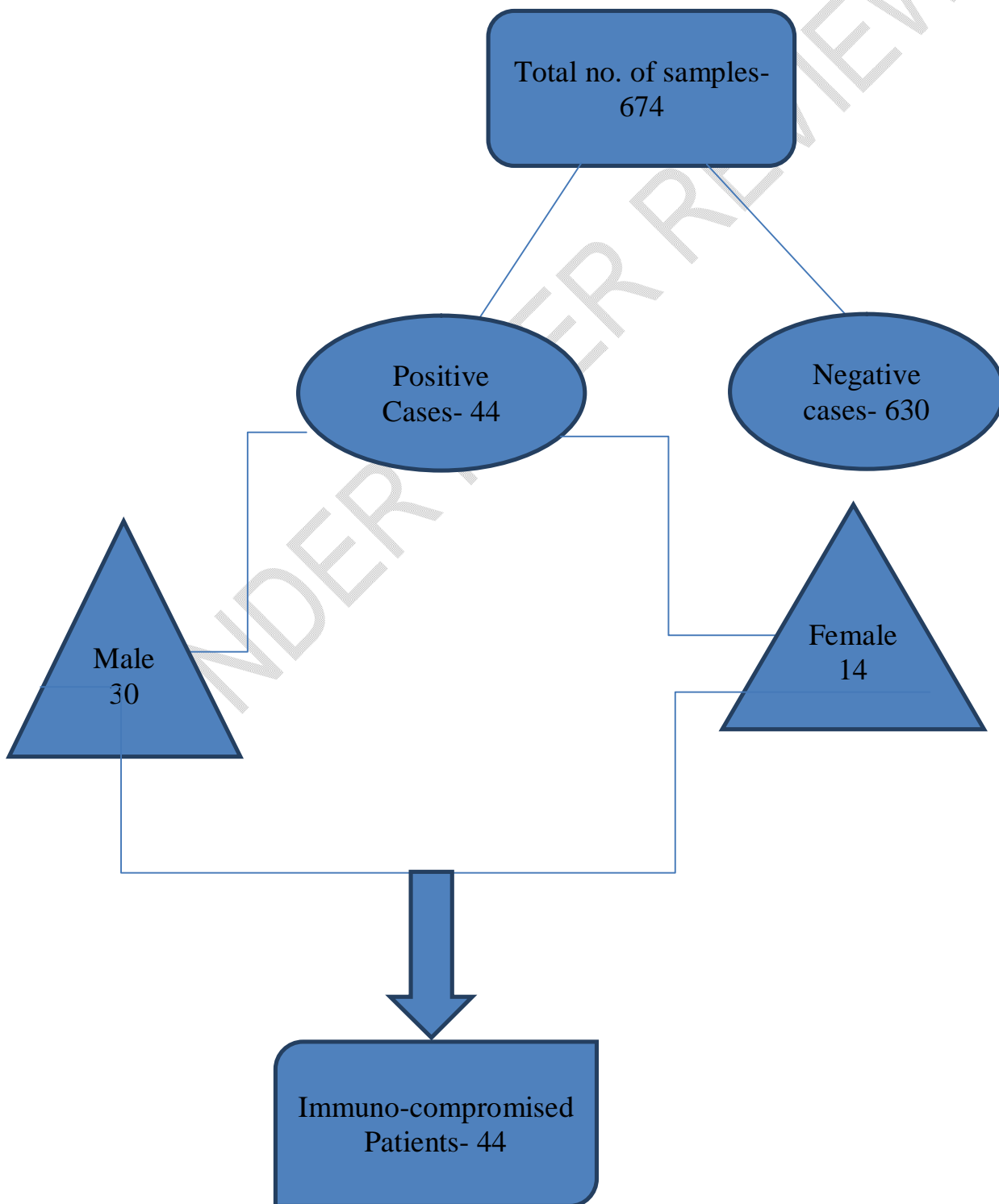
Table 3 lists the risk variables among immunocompromised patients admitted to the ICU whose blood cultures were received at the microbiology lab. DM was identified as the most common risk factor (24.8%), closely followed by chronic kidney disease (CKD; 20%). Candida species were isolated from patients as shown in Fig. 2, with *Candida tropicalis* being the most prevalent isolate 20 (45.45%), followed by *Candida albicans* 9 (20.45%), *Candida glabrata* 7 (15.9%), *Candida parapsilosis* 4 (9.09%), and *Candida krusei* 4 (9.09%).

Table 3: The prevalence of risk factors in immunocompromised patients admitted to the intensive care unit

No.	Risk Factors in Immuno-compromised Patients	Number (%)
1.	Diabetes mellitus	168 (24.8%)
2.	Chronic Kidney disease (CKD)	135 (20%)
3.	Malignancy (Lymphoma/ tumor/ leukaemia)	123(18.32%)
4.	Congestive heart failure	64 (9.5%)
5.	Myocardial infarction	62 (9.2%)

6.	Vascular disease	52 (7.7%)
7.	Chronic liver disease	26 (3.9%)
8.	Chronic respiratory illness	22 (3.3%)
9.	Connective tissue disease	10 (1.5%)
10.	AIDS	7 (1.04%)
11.	Chronic neurological disorder	5 (0.7%)
	Total	674 (100%)

Figure 1. depicts the distribution of positive cases in the study population



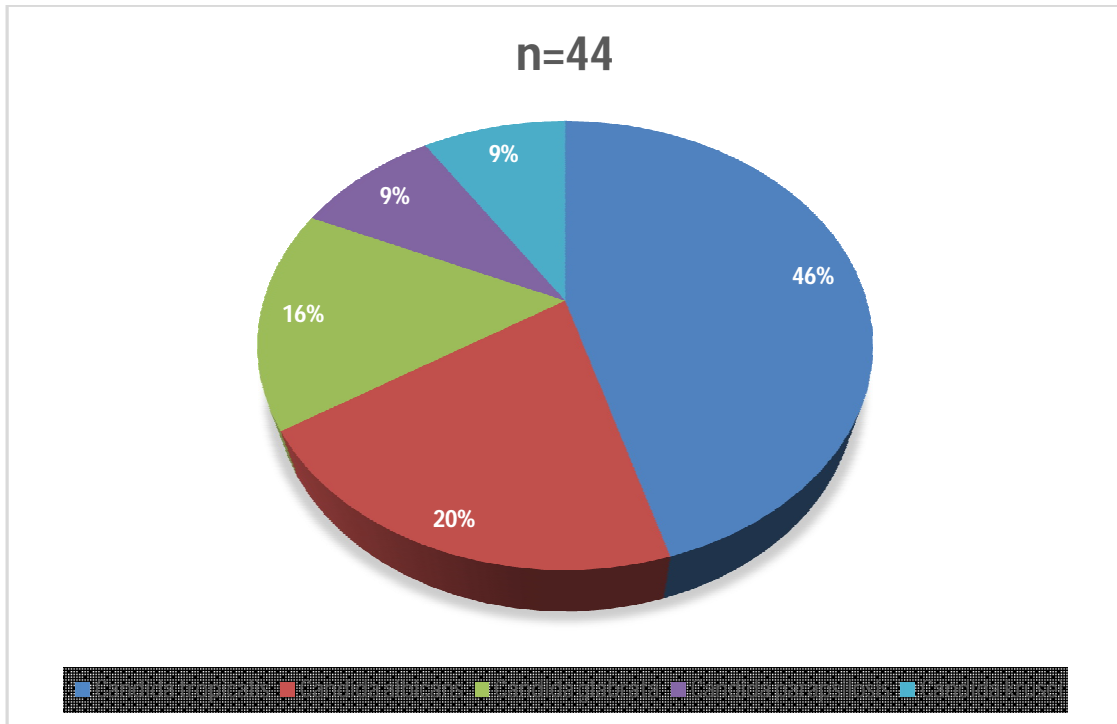


Figure 2. shows the prevalence of Candida species among (immunocompromised) patients (n=44)

4. Discussion

Candidemia is known to cause increased morbidity and mortality rates around the world, particularly in immunocompromised patients.

In recent years, the dominant species causing candidemia has shifted from *Candida albicans* to *Candida nonalbicans*. *Candida tropicalis* and *Candida parapsilosis* are the most common candidemia isolates in Southern India[10]. Cancer and diabetes mellitus have been reported as the most common comorbidities in candidemia patients. Increased use of corticosteroids and antibiotics, prolonged hospital stays, neutropenia, cancer chemotherapy, AIDS, intravascular catheterization, and other immunosuppressive conditions are all major risk factors for candidemia. *Candida* infections are growing increasingly common in intensive care units and critical care units in patients who are not immunocompromised.

Candidemia was ended up finding to be 6.5% in this study, which included 674 cases. Gadham NR et al. and Thomas M et al. observed a prevalence of 14.8% in 225 samples and 7.3% in 1440 samples, respectively, while Tak V et al. found a predominance of 74% over a 4-year period[10-12]

In our study population, men had a higher prevalence of candidemia (68.18%) than women (31.81%) (M:F ratio = 1.8). A comparative study conducted by Bongomin Fet al. found a higher prevalence of candidemia in men in both immunocompromised (M: F= 1.2) and non-immunocompromised (M: F =1.5) patients compared to women [13].

In their 10-year study on candidemia, Alkharashi N et al. found that 53.4% of men were affected compared to 46.6% of women (M: F=1.14), with a mean age group of 49.7±28.1. In our study, the most affected age groups were 51-60 years (40.9%), closely followed by 61-70 years (31.8%) [14].

In our study, the 51–60-year age group consisted of the greatest number of candidemia patients, with diabetes mellitus as a co-morbidity, usually accompanied by patients with chronic kidney disease on dialysis, Leukaemia, and HIV. Michalopoulos et al. confirmed in a univariate regression analysis study that diabetes is strongly associated with total mortality in candidemia, and is a significant predictor in more than 50% of cases[15].

In this study, *Candida* species isolated from blood on repeat samples were recognised pathogens. Among the total study population (n=44), *Candida tropicalis* was found to be the dominant isolate (41%), followed by *Candida albicans* (23%). Similar *Candida non-albicans* trends have been isolated and observed in various studies [11-12] [16-20], whereas other research shows a pervasiveness of *Candida glabrata* and *Candida albicans* [21-24]. Phospholipase occurrence and biofilm emergence have been identified as important virulence determinants in *Candida tropicalis*, both of which contribute to pathogenic organisms in candidemia patient populations [20,25].

The evolving incidence rate and involved in the pathogenesis agents causing invasive fungal infections among an increasing number of immunocompromised patients present an important obstacle in disease management and treatment[26].

Emergence of resistance among isolates notwithstanding these antifungal agents has prompted research into faster detection algorithms and medical interventions.

Rapid and suitable characterization of fungal species, as well as prescriptive antifungal custodianship, could facilitate in disease control and decrease fatality rates [26-29].

4. CONCLUSION

The present research was carried out on clinical specimens, with the vast majority of participants seeming to be males between the ages of 51 and 70. Uncontrolled diabetes mellitus and chronic kidney disease have been identified as raising the occurrences of candidiasis in immunocompromised patients.

We discovered that early detection of causative agents through microscopy and culture can lend a hand minimize mortality and morbidity in people with compromised immune systems.

CONSENT

We included all the age groups and gender after taking written informed consent in our study.

ETHICAL APPROVAL

This study was approved by Independent Ethics Committees (IEC), Index Medical College Hospital & Research Centre (Malwanchal University) vide-MU/Research/EC/Ph.D/2020/53.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
BSL-II	Biosafety Level 2
CSF	Cerebrospinal Fluid
CDC	Centers for Disease Control and Prevention
CKD	Chronic Kidney Disease

DM	Diabetes Mellitus
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
KOH	Potassium Hydroxide
SDA	Sabouraud Dextrose Agar

UNDER PEER REVIEW