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 included 674 Candida isolates in total. Candida was isolated from clinical samples using wet mount, gram stain, and SDA culture. Germ tube test, cornmeal agar morphology, sugar assimilation, fermentation tests, and BACT/ALERT 3D were used to further speciate the organisms.

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].

2. MATERIAL AND METHODS

The study will be 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

Patients of all age groups and both genders who are undergoing evaluation for immunocompromised status and candida infection and are willing to give written informed consent will be enrolled in the study.

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.
4. **2.6 Exclusion criteria**

1. Patients visiting with signs and symptoms of fungus other than Candida species.
2. Patients who have not given their consent.

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

The specimens will be transported in sealed containers, packed and insulated transport container.

Sample processing:

The specimens will be processed in BSL-II laboratory with proper aseptic precautions and personal protective equipment. Visually the quality of sample will be judged like sputum sample. If contains more saliva, a fresh sample will be 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.

2.8 Mycological examinations:

1. **Direct examination:**

a) Wet Mount

b) Gram's stain

2.9 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 regularly daily from 2 days to 3 weeks.

Colonies were identified by the colony characters and by gram's stain. Once the colonies were confirmed, speciation was done by following methods.

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

3. RESULTS

ICU patients provided a total of 674 samples, of which 44 (6.5%) tested positive for the isolation of Candida spp. and 93.5% tested negative. 30 (68.2%) of the 44 samples (which were all positive) were from men, while 14 (31.8%) were from women. Patients with weakened immune systems made up all positive cases in total.

Candidemia was more frequently discovered in people between the ages of 51 and 60 as shown in table 1.

Table 1: The age distribution of the study population

Age Group	Number of Patients (%)
<10	0
11-20	2 (4.5%)
21-30	2 (4.5%)
31-40	4 (9%)
41-50	6 (13.63%)
51-60	16 (36.36%)
61-70	10 (22.72%)
71-80	4 (9%)
81-90	0
Total	44

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 among immunocompromised patients (n=44)

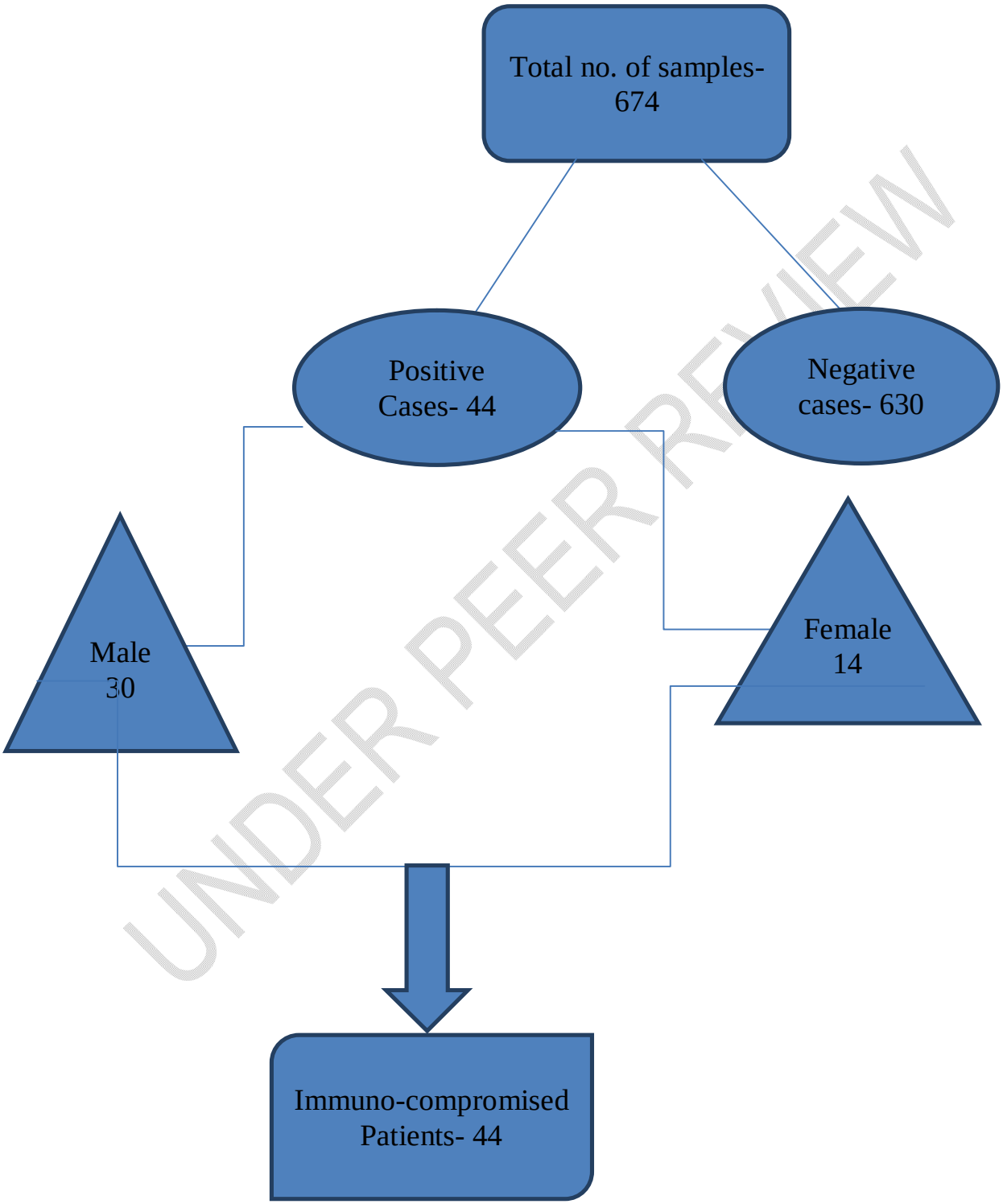
Age Group	Immuno-compromising Condition	Gender		
		Male	Female	Total
31-40	Leukaemia	6	2	8
51-60	Diabetes mellitus	12	6	18
61-70	Chronic kidney disease (On Dialysis)	8	6	14
61-70	AIDS	4	0	4
Total		30	14	44

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	167.5 (24.8%)
2.	Chronic Kidney disease (CKD)	134.6 (19.9%)
3.	Malignancy (Lymphoma/ tumor/ leukaemia)	123.5 (18.32%)
4.	Congestive heart failure	63.6 (9.4%)
5.	Myocardial infarction	62.2 (9.2%)
6.	Vascular disease	52.3 (7.7%)
7.	Chronic liver disease	25.8 (3.8%)
8.	Chronic respiratory illness	22.1 (3.2%)
9.	Connective tissue disease	9.5 (1.4%)
10.	AIDS	7.2 (1.06%)
11.	Chronic neurological disorder	5.3 (0.7%)
	Total	674 (100%)

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



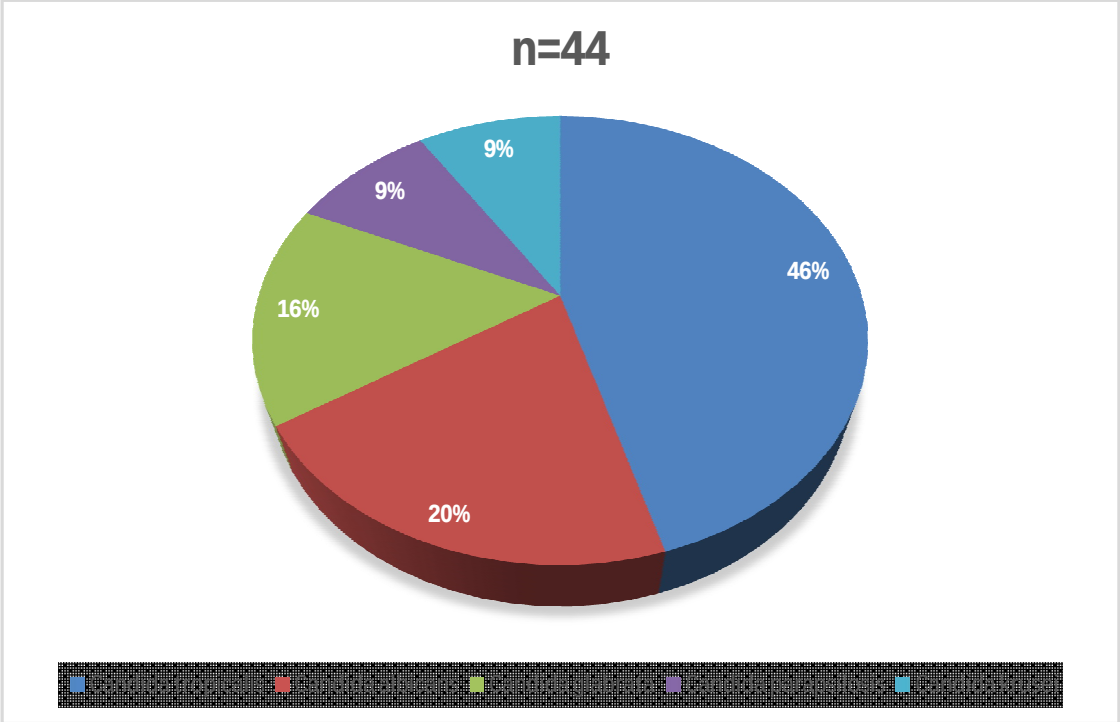


Figure 2. shows the prevalence of Candida species among (immunocompromised) patients (n=44)

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 Dimopoulus G et 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. Michalopuolos 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