Original Research Article

A study on the susceptibility of the human immunodeficiency virus-infected individuals' isolation of Candida to antifungal drugs.

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ABSTRACT

Background: On a worldwide scale there is an increase in fungal infection resistance, which is crucial for people with compromised immune systems. One of the most frequent causes of fungal infections in these individuals is candida fungus infection, which can have fatal consequences. This study aimed to investigate the medication sensitivity profile of Candida in HIV seropositive patients.
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: 6.5% of the population was found to contain one or more Candida species. The most prevalent isolate was discovered to be 20 Candida tropicalis (45.45%), followed by 9 Candida albicans (20.45%), 7 Candida glabrata (15.9%), 4 Candida parapsilosis, and 4 Candida krusei. Antifungal susceptibility test performed by disk diffusion method revealed resistance to Amphotericin B in Candida tropicalis (25%), Candida albicans (33.33%) and Candida glabrata (28.5%).
Conclusion: The clinician can select the most efficient antifungal drug with the help of a quicker identification of the Candida species in immunocompromised patients, resulting in lower treatment costs and shorter hospital stays.

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

1. INTRODUCTION

The spread of persistent fungal infections and proliferation in a large population of immunocompromised individuals and/or those hospitalised with serious underlying diseases has been facilitated by advances in diagnostic methods and therapy choices. Candida spp. is the main cause of illness and mortality in these risk categories. The prevalence of candida infections has increased globally in a variety of therapeutic settings [1].
Candidiasis is a primary or secondary infection caused by a member of the genus Candida and makes for 66–80% of all fungal diseases. The clinical spectrum of human infections caused by Candida species spans the superficial skin infection, mucous membrane infection, life-threatening candidemia, and infections contracted in hospitals.[2]
Severe illnesses include candidemia, generalised infections, CNS infections, endophthalmitis, and osteomyelitis are all examples of invasive candidiasis. The most prevalent and devastating clinical manifestation of invasive candidiasis is candidemia, a Candida species bloodstream infection, which contributes to a significant amount of mortality as well as morbidity in patients in hospitals [3].

The most prevalent nosocomial bloodstream infection in the United States and Europe is candidemia [4]. Similar to this, 10-15% of nosocomial urinary tract infections (UTIs) are caused by Candida spp.

HIV/AIDS, underlying cancers, invasive procedures, the use of broad-spectrum antibiotics, parenteral nutrition, intravascular catheter use, long-term hospitalisation, and immunosuppressive medications are the most frequent triggering factors of invasive candidiasis.

Despite being a natural component of the oral, gastrointestinal, and genitourinary tract flora in humans, the Candida spp. can cause clinical infections in weak or immunocompromised hosts. Out of the more than 150 diverse group species that make up the genus Candida, about 20 distinct Candida species are hazardous to humans.

Despite its declining shares, Candida albicans continues to be a significant cause of candidiasis. In recent years, non-albicans candida epidemiology has increased.[2] More than 90% of invasive infections are thought to be caused by five species of Candida: C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, and C. krusei. However, the relative distribution of these species depends on the geographical location, patient population, risk factors, local hospital-related factors, and the types of antifungal agents used [5].

The main factor contributing to the colonisation of non-albicans Candida (NAC) species and the rise in antifungal medication resistance is the widespread use of antifungals for prophylaxis [6]. Non-alcicans species of Candida, such as C.glabrata, C.krusei, C.tropicalis, and C.parapsilosis, are becoming more frequently the cause of fungemia[7-8].

This article aims to provide a comprehensive overview of Candida infections in immunocompromised patients in this region. We will review the existing literature on Candida infections in India, as well as specific studies conducted in the Madhya Pradesh region.

We will also discuss the risk factors, clinical manifestations, and diagnostic methods for Candida infections, and highlight the challenges associated with managing these infections in resource-limited settings. Our findings will contribute to a better understanding of Candida infections in this region and inform the development of effective prevention and treatment strategies.

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

Children who were having examination for candida infection and immunocompromised condition as well as patients of both genders who were prepared to give written informed consent were also included 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.

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.
3. Excluded patients included individuals with bacterial infections, the ones on antifungal therapy, as well as those with superficial fungal infections.

2.7 Specimen Collection

Standard Microbiological Practises were followed in the collection and processing of the samples. To look for cells that resembled budding yeast, Sabourad's Dextrose Agar culture, 10% KOH, and Gram stain were utilised. The acquired Candida isolates were subsequently analysed using the germ tube test, chlamydospore formation on maize meal agar, sugar fermentation, and sugar assimilation assays.

2.8 Antifungal Susceptibility

Antifungal suscepectibility test will be done using the National Committee for Clinical Laboratory Standards 2011, method for antifungal disc diffusion susceptibility for yeast with approved guidline M44-A.

2.9 Transport

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

2.10 Sample processing:

The samples were processed in a BSL-II laboratory using the appropriate aseptic procedures and PPE. Each sample's quality was assessed visually, much like a sputum sample. A new sample was taken if there was additional saliva in the original sample.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.

2.11 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 specimen was incubated at 25°C after being injected on SDA gradients. Daily observations of the slopes were made for two to three weeks. Based on these traits and the Gramme stain, colonies were located. Following confirmation of the colonies, speciation was carried out via the following techniques:

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 as shown in figure 1.

Candida species were isolated from patients as shown in figure 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%).

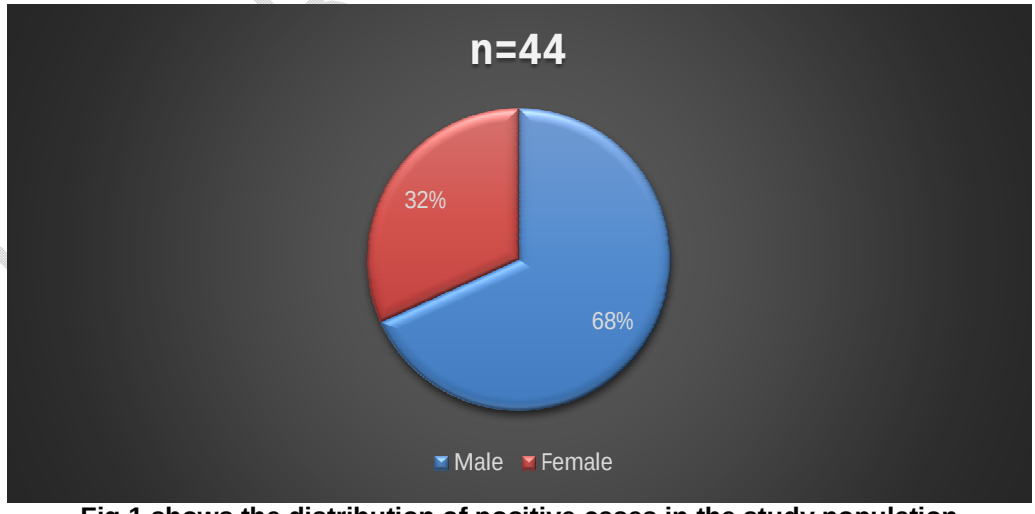

Fig 1 shows the distribution of positive cases in the study population

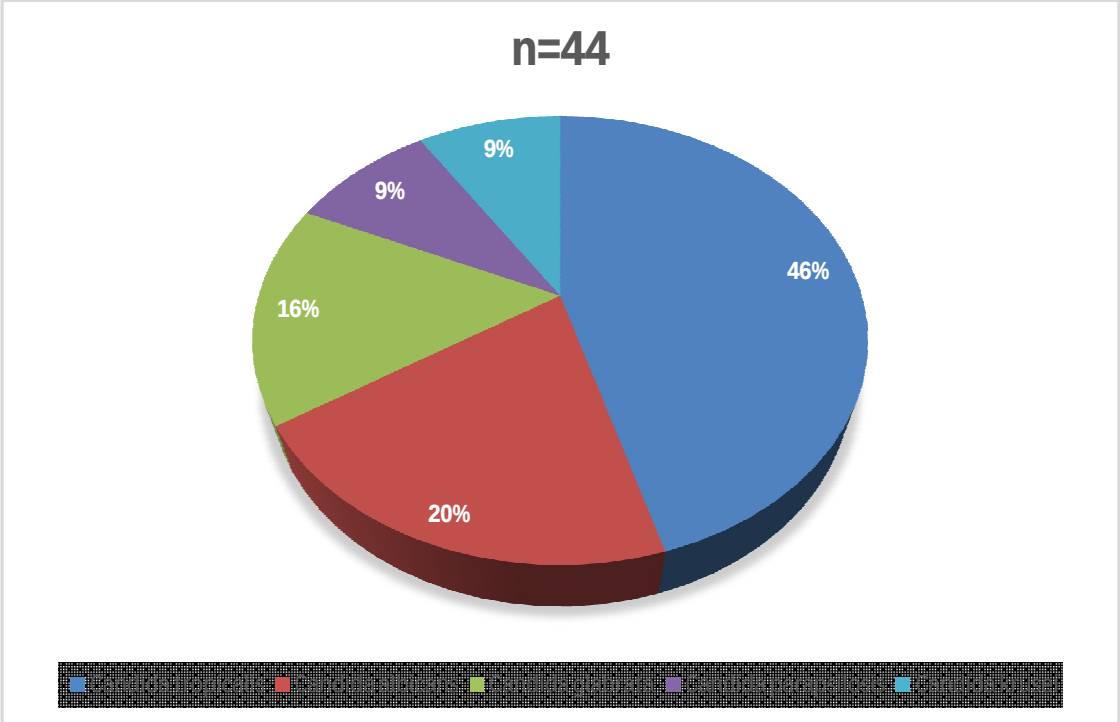

Figure 2 shows the prevalence of Candida species among (immunocompromised) patients (n=44)

	Amphotericin B			
Candida Spp.	**No (%)**	**S (Zone≥15mm) (%)**	**SDD/I (Zone 13-14mm) (%)**	**R (Zone≤12mm) %**
C. tropicalis	20(45.45)	13(65)	2(10)	5(25)
C. albicans	9(20.45)	6(66.6)	0(0)	3(33.33)
C. glabrata	7(15.9	3(42.9)	2(28.6)	2(28.6)
C. parapsilosis	4(9.09	3(75)	0(0)	1(25)
C. krusei	4(9.09)	3(75)	0(0)	1(25)
Total	44	28(63.6)	4(9.9)	12(27.27)

Table 1. Susceptibility of *Candida* species to antifungal drugs - Amphotericin B, (S – Sensitive, SDD – Susceptible Dose Dependent, I– Intermediate, R – Resistant)

	Fluconazole			
Candida Spp	**No (%)**	**S (Zone≥15mm) (%)**	**SDD/I (Zone 13-14mm)**	**R (Zone≤12mm) %**

			(%)	
C. tropicalis	20(45.45)	20(100)	0(0)	0(0)
C. albicans	9(20.45)	9(100)	0(0)	0(0)
C. glabrata	7(15.9)	0(0)	0(0)	7(100) †
C. parapsilosis	4(9.09)	4(100)	0(0)	0(0)
C. krusei	4(9.09)	0(0)	4(100)	0(0)
Total	44	33(75)	4(9.09)	7(15.9)

Table 2. Susceptibility of *Candida* species to antifungal drugs – Fluconazole

Itraconazole

Candida Spp	**No (%)**	**S (Zone≥15mm) (%)**	**SDD/I (Zone 13-14mm) (%)**	**R (Zone≤12mm) %**
C. tropicalis	20(45.45)	20(100)	0(0)	0(0)
C. albicans	9(20.45)	9(100)	0(0)	0(0)
C. glabrata	7(15.9)	7(100)	0(0)	0(0)
C. parapsilosis	4(9.09)	4(100)	0(0)	0(0)
C. krusei	4(9.09)	4(100)	0(0)	0(0)
Total	44	44(100)	0(0)	0(0)

Table 3. Susceptibility of *Candida* species to antifungal drugs – Itraconazole

Voriconazol

Candida Spp	**No (%)**	**S (Zone≥15mm) (%)**	**SDD/I (Zone 13-14mm) (%)**	**R (Zone≤12mm) %**
C. tropicalis	20(45.45)	20(100)	0(0)	0(0)
C. albicans	9(20.45)	9(100)	0(0)	0(0)
C. glabrata	7(15.9)	7(100)	0(0)	0(0)
C. parapsilosis	4(9.09)	4(100)	0(0)	0(0)
C. krusei	4(9.09)	4(100)	0(0)	0(0)
Total	44	44(100)	0(0)	0(0)

Table 4. Susceptibility of *Candida* species to antifungal drugs – Voriconazol

Disc diffusion screening for antifungal susceptibility found that Candida tropicalis (25%), Candida albicans (33.33%), and Candida glabrata (28.9%) were resistant to amphotericin B. Both Candida glabrata (28.5%) and Candida tropicalis (10%) exhibited intermediate susceptibility to Amphotericin B (Table 1). Fluconazole-induced intrinsic resistant phenotypes in Candida glabrata (100%), and Candida krusei (100%), were found to be susceptible-dose dependent as. shown in table 1 & 2. Itraconazole and voriconazole sensitivity was discovered in all isolates (table 3&4).

Amphotericin B was effective against 63.6% of Candida species, 9.9% only somewhat or moderately, and 27.27% were resistant.

Fluconazole was effective against 75% of Candida species, 9.09% of them only partially (C. krusei), and 15.9% of them (C. glabrata) were resistant to it. Itraconazole was shown to be fully susceptible to all isolated Candida species. None found any resistant isolates. It was

discovered that all isolated Candida spp. were completely sensitive to voriconazole. There was none shows inherent resistance.

Discussion

Candidemia has been shown to raise morbidity and mortality rates, particularly in people with impaired immune systems.

Candida nonalbicans has replaced Candida albicans as the predominant species causing candidemia in recent years. The two most prevalent isolates of candidemia in Southern India are Candida tropicalis and Candida parapsilosis [9].

According to reports, the most prevalent co-morbidities among people with candidemia are diabetes mellitus and cancer. Major risk factors for candidemia include increased use of corticosteroids and antibiotics, protracted hospital stays, neutropenia, cancer treatment, AIDS, intravascular catheterization, and other immunosuppressive diseases. Patients who are not immunocompromised are increasingly more likely to get candidiasis in intensive care and critical care units.

The majority of the isolates tested positive for antifungal susceptibility to all four medications, including Amphotericin B (63.6%), Fluconazole (75%), Itraconazole (100%) and Voriconazole (100%) in line with prior investigations [10-13].

However, it was discovered that Candida glabrata was completely resistant (100%) due to innate resistance to Fluconazole itself [10-11]. Few isolates demonstrated intermediate sensitivity (9.9%) and resistance (27.2%) to amphotericin B. The resistance profile of C. tropicalis to itraconazole (26.2%), ketoconazole (24.6%), and fluconazole (37.7%) is revealed by Yesudhason B et al. [14].

The rising number of patients who are immunocompromised and the evolving epidemiology and etiological agents that cause persistent fungal infections present a substantial challenge in the management and treatment of the disease [15].

4. CONCLUSION

Faster identification of the Candida species in immunocompromised patients can aid the doctor in choosing the most effective antifungal medication, ultimately lowering treatment costs and shortening hospital stays. To determine the true incidence of the disease and compare the microbiological patterns, which will aid in a better understanding of the issue in our population, large-scale surveys of candidemia are required in some communities at risk.

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.

REFERENCES

1. Bassetti, M, Peghin, M, and Timsit J. F. The current treatment landscape: candidiasis. Journal of Antimicrobial Chemotherapy. 2016; 71(suppl_2), ii13-ii22.

2. Badiee P, Kordbacheh P, Alborzi A, Zeini, F, Mirhendy, H and Mahmoody M. Fungal infections in solid organ recipients. Experimental and clinical transplantation: official journal of the Middle East Society for Organ Transplantation 2005; 3(2), 385-389.
3. Ben-Ami R. Treatment of Invasive Candidiasis: A Narrative Review. Journal of Fungi (Basel). 2018; 4(3): 97.
4. Falagas M. E Roussos N and Vardakas K. Z. Relative frequency of albicans and the various non-albicans Candida spp among candidemia isolates from inpatients in various parts of the world: a systematic review. International Journal of Infectious Diseases. 2010; 14(11), e954-e966.
5. Spampinato C and Leonardi D. Candida infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. Biomed Research International. 2013; 2013:204237.
6. Kluwer, M. W. (2017). Medical microbiology. Indian Journal of Medical Microbiology, 35(1).
7. Wise GJ, Silver DA. Fungal Infections of the Genitourinary System. Journal of Urology. 1993; 149:1377-88.
8. Girmenia C, Martino P. Fluconazole and the changing epidemiology of candidemia. Clin Infect Dis. 1998 Jul;27(1):232-4.
9. Thomas M, Oberoi A and Dewan E. Species distributionand antifungal susceptibility of candidemia at a multispecialty center in North India. CHRISMED Journal of Health and Research. 2016; 3(1): 33-36.
10. Gade N, Neral A, Monga N, Aradhey P, Singh R, Barapatre R, Sherwani N and Joshi SG. Antifungal Susceptibility Pattern of Clinical Isolates of Candida from a Tertiary Care Hospital in Chhattisgarh, India. Saudi Journal of Pathology and Microbiology. 2019; 4(12): 906-913.
11. Kumudhavalli KS. A Study on Invasive Fungal Infections among Immunocompromised Patients in a Tertiary Care Hospital (Doctoral dissertation) Madras Medical College, Chennai: TN MGR Medical University; 2013.
12. Tan TY, Hsu LY, Alejandria MM, Chaiwarith R, Chinniah T, Chayakulkeeree M, Choudhury S, Chen YH, Shin JH, Kiratisin P, Mendoza M, Prabhu K, Supparatpinyo K, Tan AL, Phan XT, Tran TT, Nguyen GB, Doan MP, Huynh VA, Nguyen SM, Tran TB and Van Pham H. Antifungal susceptibility of invasive Candida bloodstream isolates from the Asia-Pacific region. Medical Mycology. 2016; 54(5): 471-477.
13. Sabhapandit D, Lyngdoh WV, Bora I, Prasad A, Debnath K and Elantamilan D. Prevalence of non-albicans candidemia in a tertiary care hospital in Northeast India. International Journal of Medical Science and Public Health. 2017; 6(11):1620-1625.
14. Yesudhason BL and Mohanra MK. Candida tropicalis as a predominant isolate from clinical specimens and its antifungal susceptibility pattern in a tertiary care hospital in Southern India. Journal of Clinical Diagnostic Research. 2015; 9(7): DC14-16.
15. Low CY and Rotstein C. Emerging fungal infections in immunocompromised patients. F1000 Medicine Reports. 2011; 3:14. From: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155160/

Abbreviation

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

HIV	Human Immunodeficiency Virus
I	Intermediate
ICU	Intensive Care Unit
KOH	Potassium Hydroxide
S	Sensitive
SDA	Sabouraud Dextrose Agar
SDD	Susceptible Dose Dependent
R	Resistance
†	Indicates intrinsic resistance.