Original Research Article

A study of the susceptibility of *Candida* species isolated from Human immunodeficiency virus infected patients to antifungal drugs.

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ABSTRACT

Background: On a worldwide scale there is an increase inresistancein 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)antimicrobial sensitivity profile of Candida in HIV seropositive patients.
Method: The study includeda total of674*Candida* isolates. *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: Six and a half percent(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,thereby 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 itsproliferation in a large population of immunocompromised individuals and/or those hospitalised with serious underlying diseases have 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 accounts 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 hospitalization, and immunosuppressive medications are the most frequent triggering factors of invasive candidiasis[5].
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[6].
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 [7].
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 [8]. Non-alcicans species of Candida, such as *C.glabrata, C.krusei, C.tropicalis,*and *C.parapsilosis*, are becoming more frequently the cause of fungemia[9-10].
Thisstudyaims to provide a comprehensive overview of *Candida* infections in immunocompromised patients in Madhya Pradesh region.

2. MATERIAL AND METHODS

ThestudywasconductedintheDepartmentofMicrobiologyatIndexMedical College Hospitaland Research Centre,Indore(M.P.)

2.1 Studydesign
A cross-sectional study was conducted on hospitalized patients.

2.2 Samplesize

Anumberof 674samples,whichincludedOral swabs, ear swabs, vaginal swabs, stool, urine, CSF, sputum, blood, pusandnail scrapings.

2.3 Studyduration:
Twoyears(July2020 –July2022)which includedsixMonths ofdataanalysis.

2.4 Studypopulation

Children who were having examination for*Candida* infection and immunocompromised condition as well as patients of both genders who were able 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 gave 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's Collection

Standard microbiological practices were followed in the collection and processing of the samples. To look for cells that resembled budding yeast, Sabouraud'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.

2.8Transport

Thespecimensweretransportedinsealedcontainers,packedand insulatedtransportcontainer.

2.9Sampleprocessing:

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.

2.10 Antifungal Susceptibility

Antifungal suscepectibility test was done using the National Committee for Clinical Laboratory Standardsmethod for antifungal disc diffusion susceptibility for yeast with approved guidline M44-A(2011).

2.11 Mycological examinations:

1. **Direct examination:**

   a) Wet Mount- The specimen is placed on the glass slide for direct microscopic observation, 20% KOH (potassium hydroxide) is added, and a cover slip is placed on the specimen. The slide is then slightly warmed and the cover slip is gently pressed over the specimen to release any trapped air. An examination was carried out, first at low power and then at high power.

   In the instance of a nail specimen, 40% KOH is put to a tiny test tube along with a nail clipping, which is then incubated for an overnight period in order to dissolve the

nail's keratin. Next, a microscope was used to examine the specimen. The yeast cells and pseudohyphae were tried to be identified.

b) Gram's stain- Smears are created using the clinical sample on a clean glass slide, and the glass slide is simply heated over a flame to fix them. The smear was then stained using the Gram's Method, examined with an oil immersion objective, and checked for the presence of gram-positive oval yeast cells that were at the budding stage as well as pseudohyphae.

2.Culture

For Culture Sabouraud'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

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- A single colony was gently touched with a loop, then the colony was emulsified in 0.5 ml of human serum. The colony was then cultured at 37°C for 2 to 4 hours while being checked every half-hour by taking a loopful of the suspension and examining it under a high-power aobjective. The yeast cells' lengthy, tube-like extensions known as germ tubes were observed. At the point of attachment to the cell (Drumstick appearance), there was no constriction. In C. albicans, germ tube development begins within 4 hours.

b. Corn meal agar inoculation- Small portion of yeast colony was inoculated in to the depth of media with the straight wire at 45-degree angle, and cover slip applied so as to cover a part of streaked media to create partial anaerobic environment and incubated 5°C for 48 to 72 hours. Then plate was directly examined under the microscope for Chlamydospore and pseudohyphae.

c. Sugar Fermentation- The Durham's tube was submerged in each of the four carbohydrate broths, which included 2% each of dextrose, sucrose, lactose, and maltose along with 1% peptone and 0.5% sodium chloride for gas detection.

Each carbohydrate broth was inoculated with yeast colonies, cultured at 25°C for a week, and checked for acid (pink colour) and gas at intervals of 48–72 hours.

3. RESULTS

Intensive care units (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.Thirty (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%).

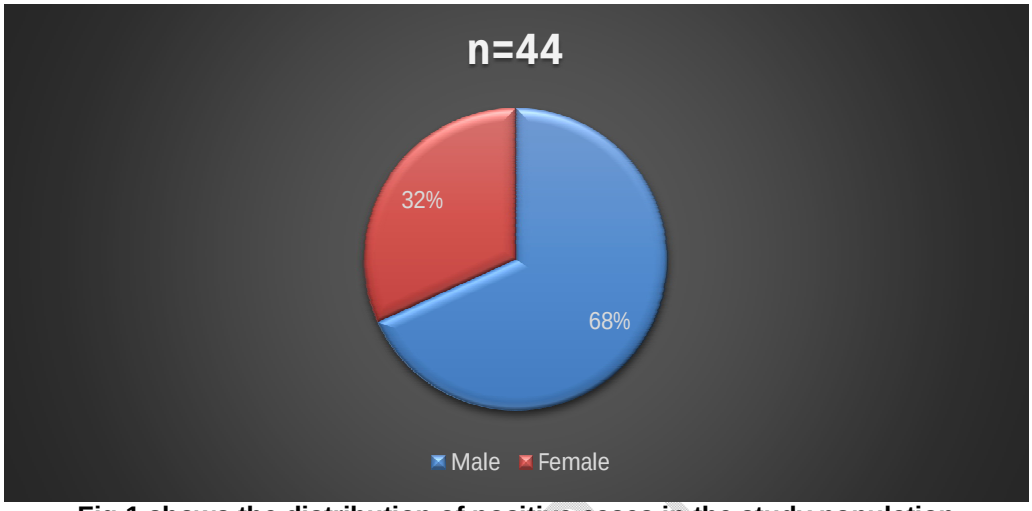

Fig 1 shows the distribution of positive cases in the study population

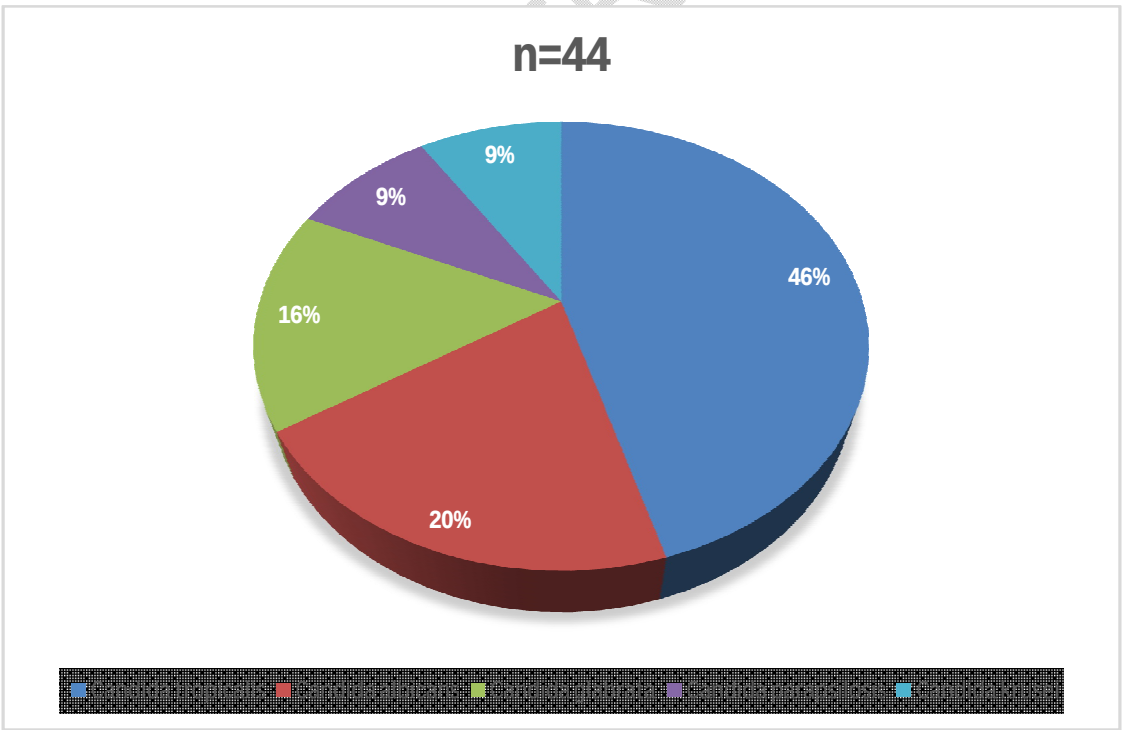

Figure 2 shows the prevalence of Candida species among (immunocompromised) patients (n=44)

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.

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

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

		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 3. Susceptibility of *Candida* species to antifungal drugs – Intraconazole

		Itraconazole		
Candida Spp	**No (%)**	**S (Zone≥15mm)**	**SDD/I**	**R**

		(%)	(Zone 13-14mm) (%)	(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

	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)

Discussion

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

*(Candida)*Non-albicans*Candida* 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* [11].

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[12].

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 [13-16].

In our study, it was found that Candida glabrata had intrinsic resistance to fluconazole, making it totally resistant (100%) to the drug[13-14]. 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. [17]

The rising number of patients who are immunocompromised and the evolving incease in the epidemiology and etiological agents (that cause)of persistent fungal infections present a substantial challenge in the management and treatment of the disease [18].

4. CONCLUSION

Faster identification of the *Candida* species in immunocompromised patients can aid the doctor in choosing the most effective antifungal medication,thereby 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)there is an urgent need ofa better understanding of the issue in our population,so large-scale surveys of candidiasis 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.

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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.