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Species distribution and antifungal susceptibility of *Candida* species in blood culture isolated from a Tertiary care Hospital in Odisha: our experience

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Abstract: Blood stream infections caused by species of Candida may occur both in the community and in the hospital admitted patients and are the cause of significant morbidity ,mortality and health care cost.Difficulty in detection of fungus in clinical specimen is an important cause of less studies on fungal blood stream infections than bacterial.The present study was done with the aim of to determine the species distribution of Candida in blood stream infections and antifungal susceptibility patterns of different species of candida isolated from blood samples collected from patients admitted in different wards of the SUM hospital, Odisha and in patients attending OPD of the same hospital during the study period(2 years from January 2020 to December 2021). Species-level identification and antifungal susceptibility of these fungal isolates were performed using a fully automated VITEK 2 machine.Of the 2746 blood samples obtained from OPD, IPD and ICU during the last 2 years, a total of 166 (6.04%) blood samples were positive for yeast. 115 (69.27%) were from the intensive care unit (ICU), 43 (25.9%) from other wards and 8 (4.81%) were from OPD. Most common species isolated was *Candida auris* in patients with different clinical diagnosis, C.auris 66 (40.74%), C.parapsilosis29 (18%), C.tropicalis20 (12.34%), C. albicans 18 (11.11%), C. dobushaemulonii 13 (802%), C.glabrata 6 (3.70%), C.pelliculosa 3 (1.85%), C.famata2 (1.23%), C.lipolytica2 (1.23%), C.gullienmondii 2 (1.23%), C.kefyr 1 (0.6%). Male affected 61.44% and female affected 38.55%. Automated culture system (VITEK) must include C.auris. Additionally, hospitals should update their own protocols for antifungal drug use by evaluating yeast species growing in their own units. Consultation with an infectious disease specialist should be part of the protocol when treating candidaemia.

Key words: candidaemia, Blood stream infections, Fungemia, nosocromial, emerging fungal pathogens.

INTRODUCTION:

Blood stream infections constitute one of the most serious situations among infectious diseases, caused by viruses, parasites, bacteria and fungi, posing a threat to public health globally.(1,4,16). Studies on bacterial blood stream infection are common in comparison to fungus. Although fungal blood stream infections are cause of significant rate of mortality and increased health care cost(9),they have received less attention epidemiologically as, detection of fungal infection in clinical specimens is difficult and awareness is less.(3) There is also limited studies on fungal blood stream infection demonstrating clinical importance, epidemiological shifts in their aetiologies and drug resistance.(8) Fungemia (fungus in blood)usually occurs in immune-compromised patients, patients suffering from cancer, on chemotherapy, with serious or terminal illness(10).Recent studies have suggested species of *Candida* as “emerging” fungal pathogens both in the community and in hospitals of all sizes(8-10% of all nosocromial blood stream infection), in all age groups

throughout the world depending on underlying disease, country, hospital, type of ward and length of hospitalization.(10)

Aetiological Shift and Clinical Significance of Candida BSIs:

Blood stream infections caused by *Candida* account for more than 90% of fungal BSIs and previous studies revealed the range between 0.3 and 5 per 1000 hospital admitted patients globally.(10)It is important to note that they are also associated with high mortalities, between 35% and 71% and may increase when empirical antifungal therapy is delayed and there is inappropriate initial treatment.(11,12) Intrinsic resistance of *Candida* species to echinocandins is responsible for lower susceptibility to fluconazole, so they may be cross-resistant to other azoles.(13) Therefore, antifungal susceptibility testing is crucial for the management of patients suffering from invasive *Candida* infection and species distribution models (SDMs) are being used as an emerging tool in the study of fungi.(14)

Aims: To determine the species distribution of *Candida* in blood stream infections and antifungal susceptibility patterns of different species of candida isolated from blood samples collected from patients admitted in different wards of the SUM hospital, Odisha and in patients attending OPD of the same hospital.

MATERIALS AND METHODS:

This is a retrospective observational study conducted in the Microbiology Department of IMS and SUM Hospitals for 2 years from January 2020 to December 2021. *Candida* species distribution and its antifungal susceptibility testing were performed using blood samples from OPD patients and hospitalized patients (ICU, IPD). Data on risk factors were obtained from hospital electronic medical records. During this study, blood samples for culture were taken from two sites on the patient's periphery by a nurse or hospital-trained phlebotomist patients following the step-by-step process and procedures outlined in CLSI (Clinical and Laboratory Standards Institute) guidelines . Prior to taking blood samples, the skin was disinfected by with 70% isopropyl alcohol, followed by with a 2% iodine tincture. The antecubital fossa was the preferred sampling site and sterile needles and syringes were used. If only a peripheral site was available and the patient had a central venous catheter, a second blood culture sample was obtained from the central venous catheter.15 mL blood samples collected into individual blood culture bottles (BACT/ALERT FN PLUS) for pediatric and adult and incubated in continuous monitoring automated blood culture system (BACT/ALERT 3D | bioMerieux). Subculture done from positive flagged bottles on 5%heep blood agar (SBA).Gram staining was performed .Those strains determined to be yeast by conventional methods (direct microscopy, Gram staining) were inoculated onto Sabouraud dextrose agar (SDA) medium and left in an incubator for 24 hours.Species-level identification and antifungal susceptibility of these fungal isolates were performed using a fully automated VITEK 2 machine.

Exclusion Criteria:

Infusions of blood <5 mL into blood culture bottles,multiple samples from the same patient and low discrimination by VITEK2 were excluded to avoid false negative results.

Results: Of the 2746 blood samples obtained from OPD, IPD and ICU during the last 2 years, a total of 166 (6.04%) blood samples were positive for yeast. Cryptococcus laurentii were only four. 115 (69.27%) were from the intensive care unit (ICU), 43 (25.9%) from other wards and 8 (4.81%) were from OPD (**Fig1**).So, cases from ICU were much more,may be due to crtically ill,immunocompromised state. Male affected 61.44% and female affected 38.55%.(**Fig 2**)Over two years, among Candida strains growing in blood cultures, the most common strains were C.auris 66 (40.74%), C.parapsilosis29 (18%), C.tropicalis20 (12.34%), C. albicans 18 (11.11%), C. dobushaemulonii

13(8.02%), C.glabrata 6 (3.70%), C.pelliculosa 3 (1.85%), C.famata2 (1.23%), C.lipolytica2 (1.23%), C.gullienmondii 2 (1.23%), C.kefyr 1 (0.6%) **(Figure 3).** Looking at the target age, 15 (9%) were 0 to 14 years old, 93 (56%) were 15 to 60 years old, and (35%) were 58to >60 years old **(Fig. 4).** VITEK 2 did not give sensitivity report of multidrug resistant *Candida auris* .Antifungal susceptibility of *Candida* species detected showed that the species *Candida parapsilosis* was100% sensitive to caspofungin,flucytosin and micafungin but susceptibility was less to amphotericinB (93.10%),fluconazole (82.75%),voriconazole(62%).The sensitivity of the species *Candida albicans* was most sensitive to flucytosine and micafungin(89%),whereas sensitivity to caspofungin was 83% and sensitivity to amphotericin B and fluconazole were 77.77%,to voriconazole 44%.Species of *Candida tropicalis* were 85% sensitive to amphotericinB,70% to caspofungin ,flucytosine and micafungin but 75% to fluconazole and 30% to vorikonazole.Species of *Candida glabrata* were 66% sensitive to amphotericin B,flucytosine and micafungin but 33% to caspofungin and 16% to fluconazole and voriconazole.The members of the species Candida dobushaemulonii were completely resistant to amphotericinB,caspofungin,micafungin and sensitive to flucytosine 84.6% and to vorikonazole 38.4%.**(Fig 5)**.Most common species isolated was *Candida auris* in patients with different diagnosis.Of which Type 2 DM 9 (13.63%).), CKD 8 (12%), burns 7 (10.60%), pneumonia 6 (9.09%), post-Covid pneumonia, sepsis, CVA (4 each), 6.06%. Coronary artery disease, midline, postoperative fever (3 each), 4.54% tuberculosis pleural effusion, pulmonary tuberculosis, liver disease, CA (2 each), 3.03% COPD, thalassemia, encephalitis, epilepsy, fever of unknown cause, cellulitis, surgery Poisoning (1 each), 1.51%.**(Fig 6).**Area distribution of Candida auris was as follows 47 (71.2%) were from the ICU, 14 (21.2%) from another ward, and 5 (7.57%) from the OPD. Looking at the age of involvement, it was 39 years old (59%). Between the ages of 15 and 60, 24 (36.36%) were aged ≥60 and only 3 (4.54%) were aged 0 to 4.*Candida parapsilosis* was detected in patients with the following diagnoses: Postoperative fever (6, 55.17%) Type 2 DM (5, 17.24%) CVA (4, 13.8%) %) CKD (3, 10.34%) CA colon (2, 6.9%) Burn, ARDS, thalassemia, cellulitis, midline, traffic accident, pneumonia, surgical poisoning, seizure disorder (1.3, 44%) .Diagnosis of patients with *Candida tropicalis* were pneumonia (4.20%) type 2 DM (3,15%) burns, CVA (2,10% each) sepsis, acute kidney injury, chronic kidney disease, pleural effusion, thrombocytopenia, fever of unknown origin, ALL, central line, encephalitis (1.5% each) .The species Candida albicans was diagnosed in patients with burn (4,22%),central line(3,16%),road traffic accident,post operative fever,seizure disorder (each2,11%),pneumonia,liver disease,type 2 DM,CKD,CA colon(each1,5.55%).The species *Candida dobushaemulonii* was detected in patients with diagnosis of CKD, central line, CA (2, 15.4% each), CVA, pleural effusion, postoperative fever,post COVID pneumonia,burn,CVA,aplastic anaemia,nephritic syndrome (1,7.7% each).The species *Candida glabrata* was detected in patients with AKI with metabolic acidosis (2, 33.33%),fever of unknown origin ,liver disease,CKD,adrenal CA(1,16.7% each).There were three premature infants with *Candida pelliculosa* blood infection, two premature infants with *Candida lipolytica*, and one premature infant with *Candidagullienmondii* blood infection. There was one patient with AML and one patient with coronary artery disease had *Candida famata* blood infection.One patient with pulmonary tuberculosis had *Candida gullienmondii* blood infection, and one CVA patient suffered *Candida kefyr* blood infection.

Limitations:

It is a single center study,so,collaborative multicenter studies are needed to confirm whether *C. auris* is currently the dominant species in India.VITEK is not updated to give drug sensitivity report of *Candida auris.*

Discussion:

This study described *Candida auris* as the most common identified species.The increased application of antifungal agents for prophylactic or empirical treatment may contribute to the cause of, emergence of drug resistant fungal pathogens like multidrug-resistant *Candida auris* (24,25). It is observed that burn patients, immunocompromised persons with comorbidities like malignancies(ALL,AML,NHL,CA ovary,CA stomach,CA colon,CA bladder,adrenal CA), type 2 diabetes mellitus,thalassemia,nutropaenia,pulmonary tuberculosis,tubercular pleural effution,HBV, pneumonia,post covid pneumonia,seizure disorder,chronic

kidney disease,chronic liver disease,cellulitis,encephalitis,meningitis,sepsis,hypokalemia,aplastic anaemia,superior mesenteric artery thrombosis,metabolic acidosis,congenital bronchial atresia, and haematological aberrations,persons in whom central venous catheters have been placed,and those in intensive care are at risk.Furthermore, total parenteral nutrition, long term empiric antimicrobial use, being a preterm infant, being a neonate with low birth weight,road traffic accident,cerebrovascular accident,pyrexia of unknown origin, having abdominal surgery also predispose to *Candida* blood stream infection.Our study reflects the changing epidemiology of candidemia in India. Unlike previous large studies of *Candida* infections from India, our study showed that *C. auris* was the most common *Candida* species in patients admitted to our center with candidemia showing increase of non albicans.

Conclusions:

Antimicrobial stewardship programme and robust infection control practices should be strictly implemented. It may not be practical to use MALDI-TOF in every laboratory,it is not really possible in developing countries like India, but at least we should have our updated automated culture system (VITEK). It must include *C.auris*. Additionally, hospitals should update their own protocols for antifungal drug use by evaluating yeast species in clinical samples growing in their own units and identify. This will reliably improve morbidity and mortality in patients suffering from fungemia. It is clear from previous research that appropriate initial antifungal treatment to patients with severe fungal infections can significantly reduce mortality. Consultation with an infectious disease specialist should be part of the protocol when treating candidaemia.

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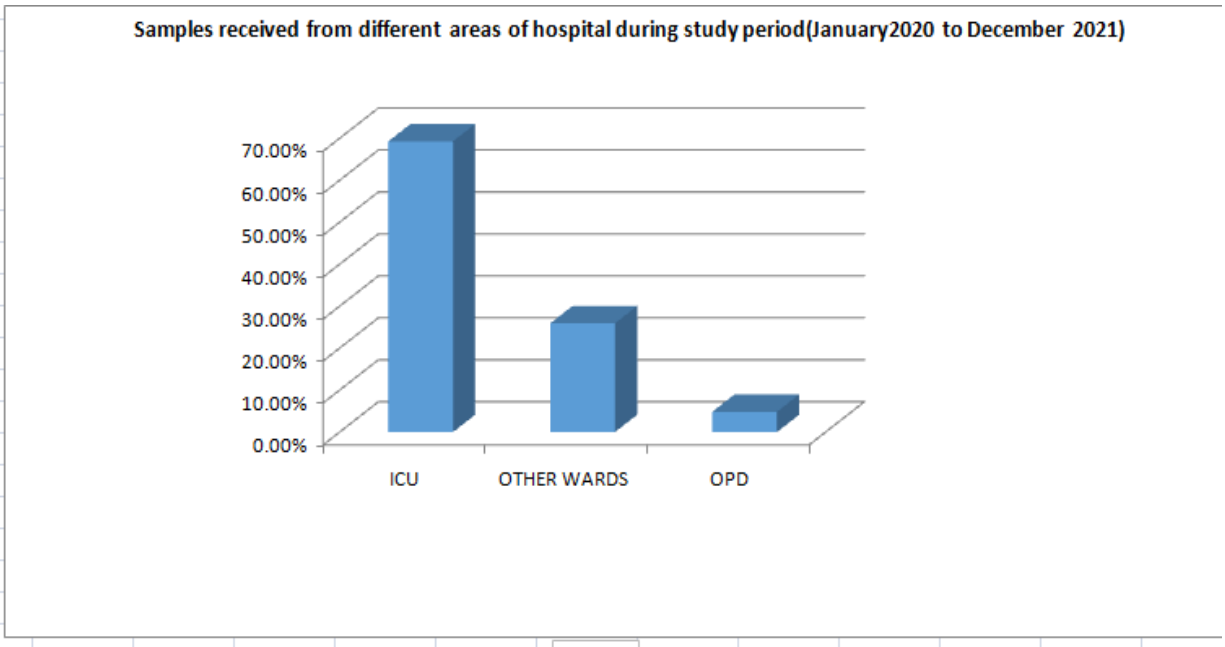

Fig 1

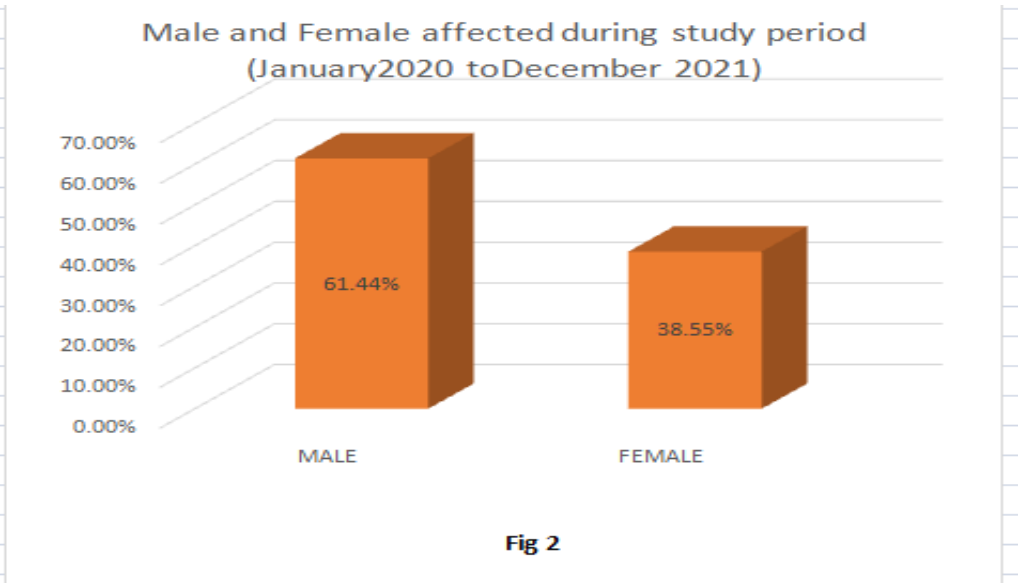

Fig 2

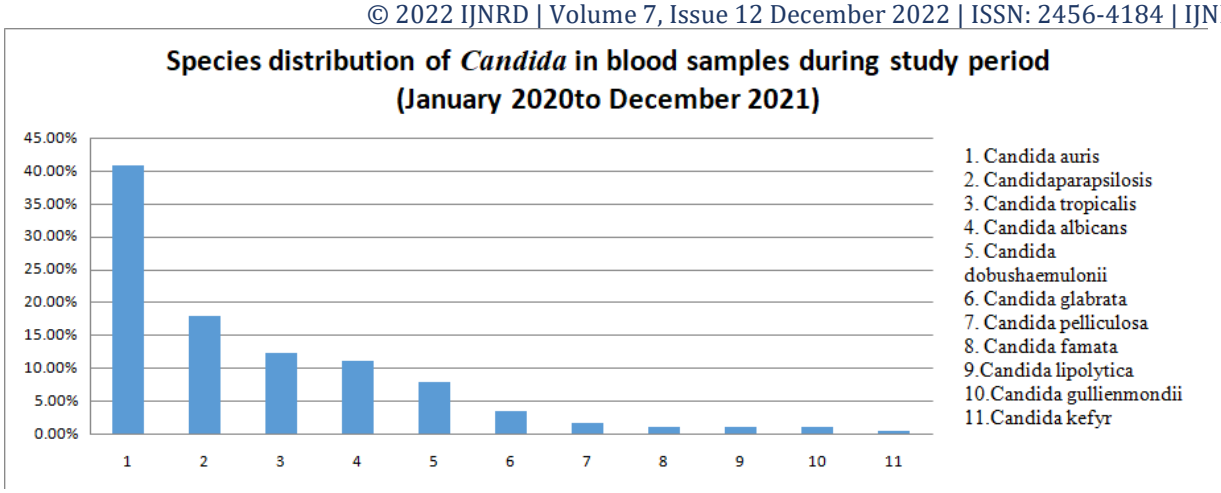
Species distribution of Candida in blood samples during study period
(January 2020to December 2021)
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
1
2
3
4
5
6
7
8
9
10
11
1. Candida auris
2. Candidaparapsilosis
3. Candida tropicalis
4. Candida albicans
5. Candida dobushaemulonii
6. Candida glabrata
7. Candida pelliculosa
8. Candida famata
9.Candida lipolytica
10.Candida gullienmondii
11.Candida kefyr

Fig 3

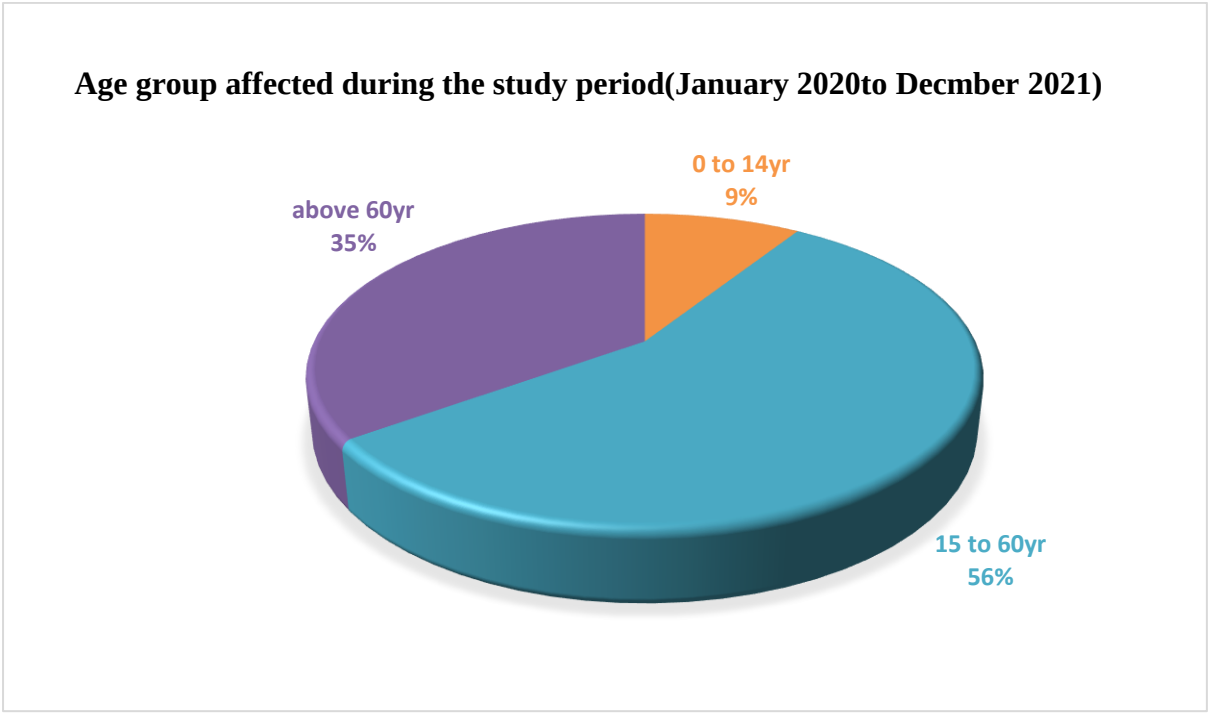
Age group affected during the study period(January 2020to Decmber 2021)
0 to 14yr
9%
above 60yr
35%
15 to 60yr
56%

Fig 4

Antifungal sensitivity of Candida species

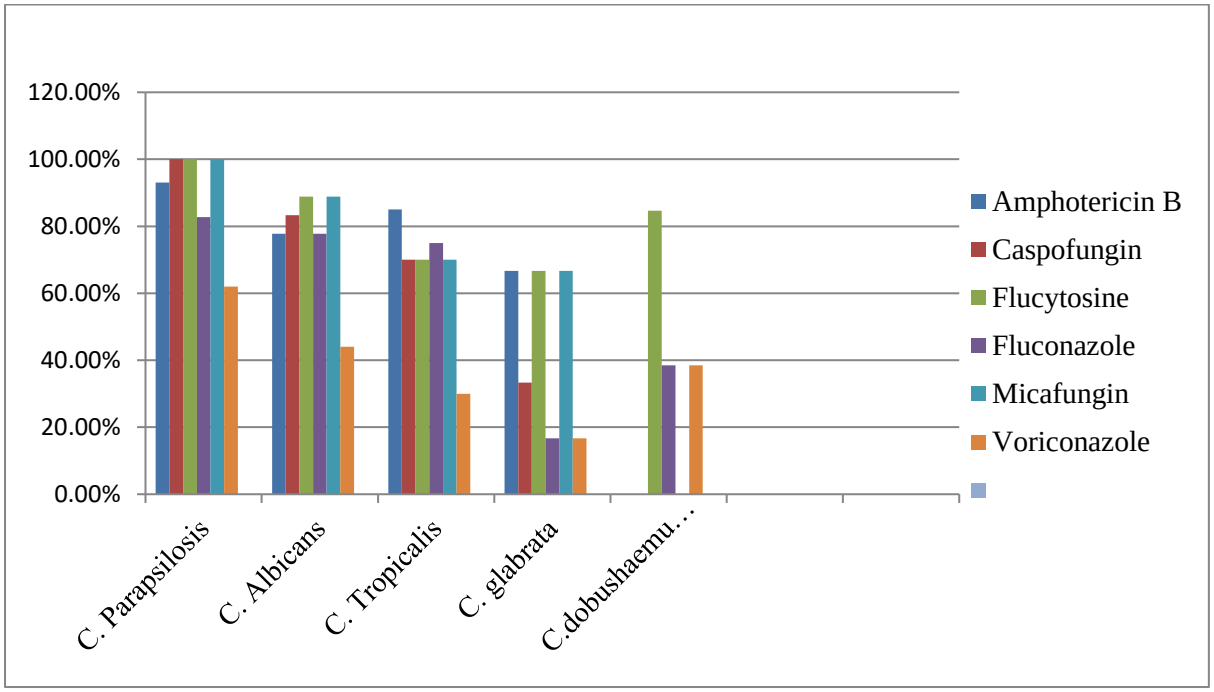

Fig 5

Diagnosis of patients detected with *Candida auris* infection

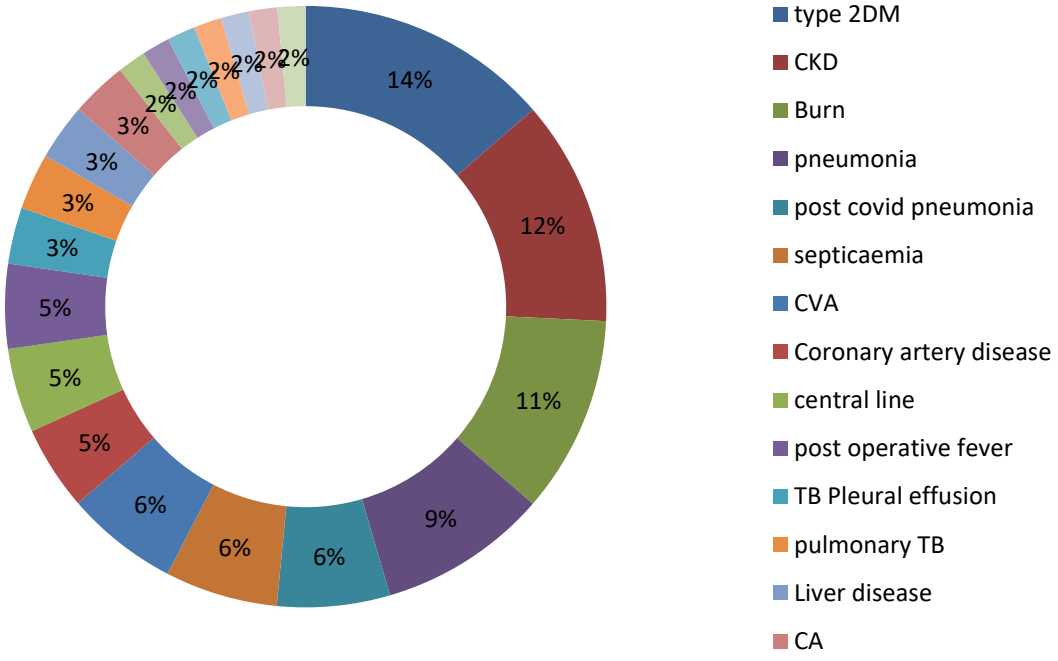

Fig 6

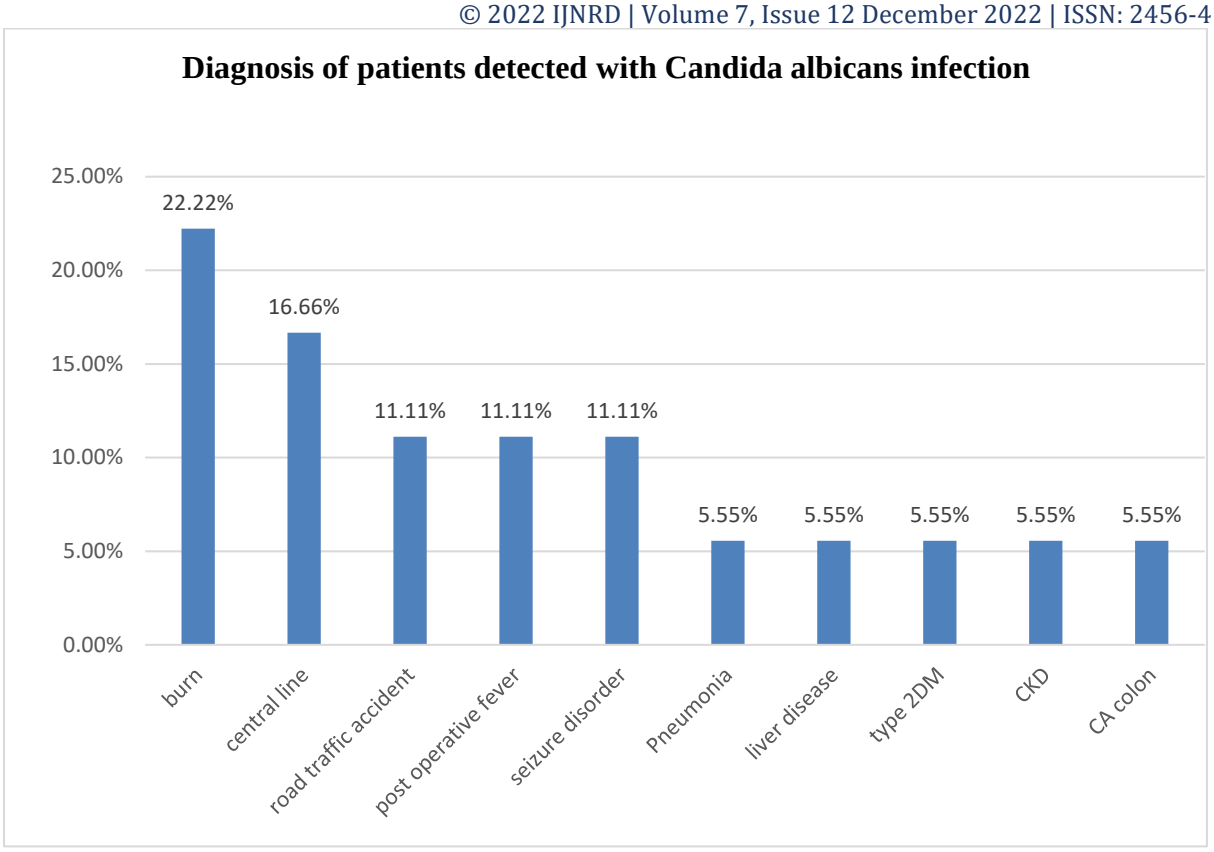
Diagnosis of patients detected with Candida albicans infection
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
22.22%
16.66%
11.11%
11.11%
11.11%
5.55%
5.55%
5.55%
5.55%
5.55%
burn
central line
road traffic accident
post operative fever
seizure disorder
Pneumonia
liver disease
type 2DM
CKD
CA colon

Fig 7