

## Original Research Article

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## A study on Proportion, Speciation and Antifungal Resistance Pattern of the *Candida* Isolates in a Tertiary Care Hospital of North Kerala, India

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### ABSTRACT

Candidosis or Candidiasis caused by various species of *Candida* is the commonest fungal disease found in human beings and is among the four most common causes of nosocomial infections. Antifungal resistance poses a major public health concern. Lack of routine diagnostic testing for fungal diseases exacerbates the problem of antifungal drug empiricism. Aim: To study the Proportion, Speciation and Antifungal Resistance Pattern of the *Candida* Isolates in a tertiary care hospital of North Kerala. Materials and Methods: A retrospective study carried out based on review of records of 10803 patients from whom samples of blood, urine, sputum and exudates were collected and processed from January 2016 through December 2016 according to standard protocol. Data Analysis was done by using WHO net antibiotic resistance surveillance software; data was analyzed using EPI INFO 2013 software. Result: Out of the total of 10803 samples of blood, urine, sputum and exudates taken up for the study, culture was positive for growth of microorganisms in 3214 and *Candida* species was isolated in 132, giving an incidence of 4.1 % for Candidosis. The most vulnerable age group was above the age of 50 who constituted 80.3 %. *C. albicans* was the predominant species having recovered from 70(53%). Conclusion: Increasing rates of antimicrobial resistance pose a great problem in treating these infections; therefore early diagnosis and determination of Antifungal Resistance Pattern is mandatory.

#### Keywords

Candidosis,  
Nosocomial  
infections,  
*C. albicans*.

#### Article Info

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### Introduction

Candidosis or candidiasis, caused by various species of *Candida* that are part of the normal flora of the muco-cutaneous membranes of humans, is the commonest fungal disease found in human beings. A variety of factors predispose to candidosis by altering the balance of normal microbial flora or by lowering the host resistance (Chander, 2013) resulting in tissue invasion and life-threatening diseases in patients with decreased cell mediated immunity (Dharwad *et al.*, 2011). The risk factors for candidosis include use of broad-spectrum antimicrobials, chemotherapy, invasive procedures, transplants and prolonged ICU stay. Most of

the invasive infections due to *Candida* species are attributed to *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* (Giri *et al.*, 2012; Ece *et al.*, 2012); however accurate speciation is important for treatment, as not all species respond to the same treatment and due to anti-fungal resistance and emergence of *non- albicans Candida* species (Page *et al.*, 2005). *Candida* species with fluconazole resistance have become more prominent in recent years (Chakrabarti *et al.*, 1996). Early diagnosis and proper treatment is the key for management of candidosis. Hence this study was undertaken to determine the proportion of *Candida*

species prevalent in our centre, to speciate them and to evaluate the resistance pattern to antifungal agents.

## Materials and Methods

After getting approval from Institutional ethics committee, a retrospective study was carried out, based on review of records of 10803 patients from whom samples of blood, urine, sputum and exudates were collected and processed in the diagnostic section of Department of Microbiology, from January 2016 to December 2016, meeting inclusion and exclusion criteria. The basic socio-demographic information and data regarding age, gender, fungal isolates from samples of blood, urine, sputum and exudates and their antifungal resistance pattern were collected using predesigned Performa, according to standard protocol.

Urine, sputum and exudates were collected aseptically in sterile containers and transported without delay. The blood samples were collected aseptically and introduced into the BACTEC blood culture bottles immediately and the bottles shaken well. The samples were immediately placed in BACTEC automated blood culture system.

Urine, sputum and exudates were examined by wet mounts or KOH preparation and Gram stain. The samples were inoculated onto two tubes of Sabourauds Dextrose Agar (SDA) and one was incubated at room temperature and the other at 37<sup>0</sup>C. All BACTEC positive samples were subjected to Gram stain and inoculated onto Blood agar plates and incubated at 37<sup>0</sup>C for 48 hours. Out of a total of 10803 various clinical specimens *Candida* isolates were obtained in 132 of them and their characterization and speciation was done based on cultural characteristics on SDA and CHROM agar, by the production of germ tubes, formation of chlamydoconidia on

cornmeal agar, Gram stain and biochemical reactions for assimilation and fermentation of carbohydrates like glucose, sucrose and maltose (Bailey and Scott's, 2014). Antifungal susceptibility was done to determine the resistance pattern according to the CLSI guidelines by the disc diffusion method and the antifungal discs used are nystatin (100 units), amphotericin B (20µg), fluconazole (25µg), ketoconazole (10µg), voriconazole (1µg), and itraconazole (10µg).

Data Analysis was done by using WHO Net Antibiotic Resistance Surveillance Software; data was entered in Excel and analyzed using EPI INFO 2013 software.

## Results and Discussion

Out of the total of 10803 samples of blood, urine, sputum and exudates taken up for the study, culture was positive for growth of microorganisms in 3214 and *Candida* species was isolated in 132, giving an isolation rate of 4.1 % for Candidosis. Of these the incidence was 59.1% and 40.9% in male and female patients respectively (Figure 1). In relation to age factor, the most vulnerable age group was above 70 years where the incidence was 33.3% and those above the age of 50 constituted 80.3 % (Figure 2).

Out of a total of 132 *Candida* isolates, *C. albicans* was recovered from 70(53%), followed by *C. tropicalis* in 23 (17.4%) *C. parapsilosis* in 13 (9.8%) *C. glabrata* in 11 (8.3%), *C. krusei* in 7(5.3%) of patients (Figure 3).

*C. albicans* was the predominant organism in urine, sputum and exudates followed by *C. tropicalis* while in blood samples there was predominance of *C. parapsilosis* followed by *C. albicans* and *C. glabrata* (Table 1). The antifungal resistance pattern showed that the triazole antifungal agents like voriconazole,

ketoconazole, fluconazole and itraconazole are more resistant to the *Candida* species isolated in this study than nystatin and amphotericin B. Voriconazole and ketoconazole exhibited 9.8% resistance, fluconazole 8.3% and Itraconazole 7.6%; amphotericin B and nystatin showed 3.0% and 2.8 % resistance respectively (Table 2).

Candidosis is an opportunistic fungal infection caused by *Candida* species, a major component of human microbiota, acquired either endogenously or exogenously from sources in the healthcare system (Ahmad *et al.*, 2012). A study by Hidron *et al.* has

reported that infections by *Candida* spp. are the fourth most common cause of device and procedure-associated nosocomial infections – bloodstream infections in patients on central line, catheter-associated urinary tract infections, ventilator-associated pneumonia, and surgical site infections (Hidron *et al.*, 2008). Antimicrobial resistance, a major public health concern, largely arises from excess use of antibiotic and antifungal drugs. Lack of routine diagnostic testing for fungal diseases exacerbates the problem of antimicrobial drug empiricism, both antibiotic and antifungal (Denning *et al.*, 2017)

**Table.1** Distribution of *Candida* species in various clinical specimens

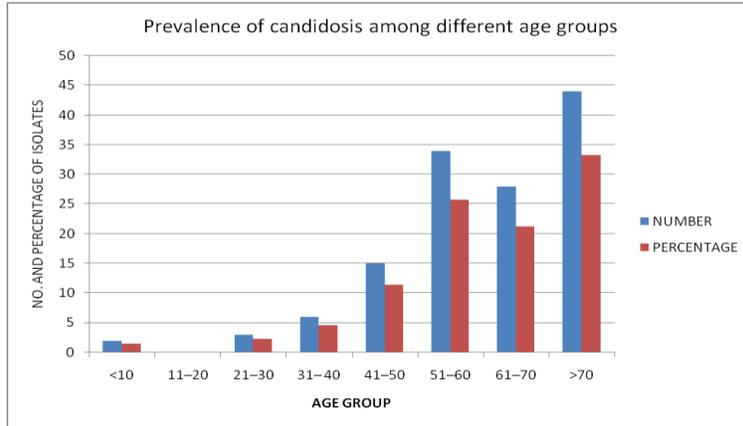
<i>Candida</i> species	Clinical specimen				Total
	Blood	Urine	Sputum	Exudates	
<i>C. albicans</i>	2	30	26	12	70
<i>C. tropicalis</i>	1	13	5	4	23
<i>C. parapsilosis</i>	4	5	2	2	13
<i>C. glabrata</i>	2	6	2	1	11
<i>C. krusei</i>	0	5	0	2	7
<i>Candida spp.</i>	1	3	3	1	8
Total	10	62	38	22	132

**Table.2** Overall Antifungal resistance pattern of *Candida* isolates

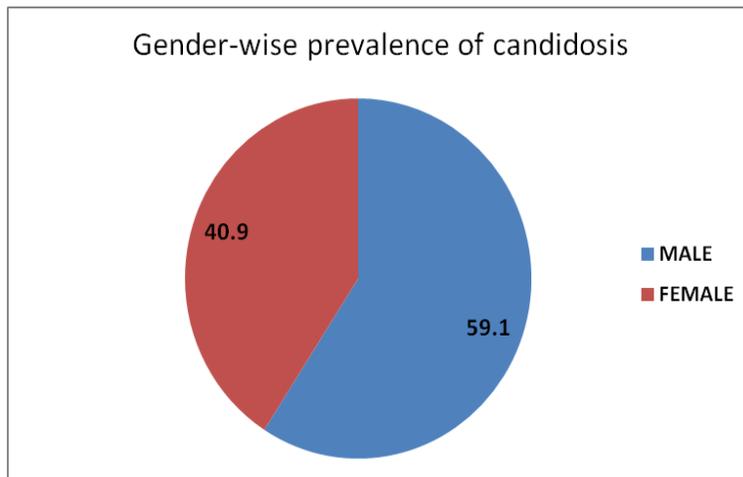
NS= nystatin, AP= amphotericin B, KT= ketoconazole, FLC- fluconazole, VRC= voriconazole, IT= itraconazole

Anti-Fungal Agents	Blood (10)	Urine (62)	Sputum (38)	Exudate (22)	Total (132)	Percentage
NS	0	1	0	2	3	2.8
AP	0	1	1	2	4	3.0
KT	1	6	4	3	13	9.8
FLC	1	5	3	3	11	8.3
VRC	1	6	4	3	13	9.8
IT	1	5	3	2	10	7.6

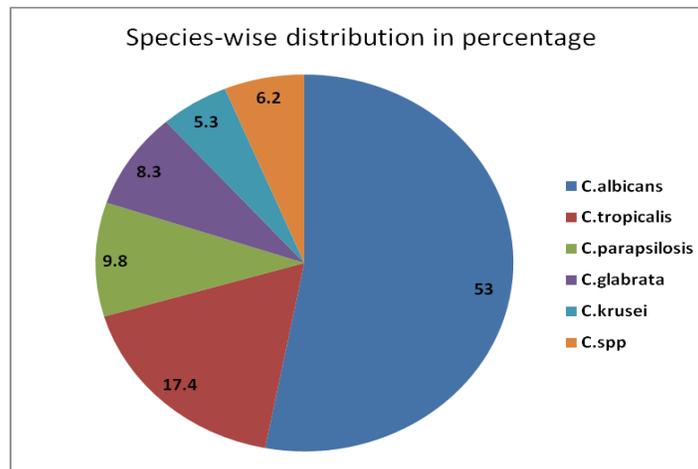
**Figure.1** Age-wise prevalence



**Figure.2** Gender-wise prevalence



**Figure.3** Species-wise distribution



The gender-wise distribution of patients was 54.5% females and 45.5 % males in a study by Guru *et al.*, (2016) and in a similar study by Dharwad *et al.*, (2011) also the incidence of candidiasis was higher in females (64%) than in males (36%). But in our study there is preponderance of male patients over females. Although candidosis can occur at all ages, studies by Dharwad *et al.*, (2016) showed highest incidence of candidosis in the age group of 18 and 45(54%) years followed by the age group of greater than 60 years (22%). In a similar study by Dalal and Kelkar (1980) the highest incidence of candidosis was found to be in the age group of 21–40 years. In our study, the highest incidence was in patients above the age of 50 who constituted 80.3 %.

The risk factors reported by various authors include prolonged antibiotic therapy, catheterization, ICU stay, diabetes, malignancy, cancer chemotherapy, pulmonary diseases and central line and other devices in situ, sepsis, pregnancy, and immunosuppression including HIV (Singhal *et al.*, 2015).

Bloodstream infection and invasive candidiasis are substantially more common than realized and probably result from multiple factors, including unrestrained antibiotic drug use, indwelling devices and increasing populations of immunocompromised patients. Multiple studies have shown the incidence of bloodstream infections with *Candida* spp. to be 1.2–26 cases/100,000 population. (Denning *et al.*, 2017) Distribution of *Candida* species in Candidosis vary from study to study. Although in most studies *C. albicans* remains the predominant species (Pfaller *et al.*, 2007) a shift from *C. albicans* to non-albicans *Candida* too is being reported (Nguyen *et al.*, 1996). In our study the predominant species was *C. albicans* having recovered from 70(53%), followed by *C.*

*tropicalis* in 23 (17.4%). High percentage of resistance against antifungal agent fluconazole by *Candida* species is being reported by various authors. In our study Voriconazole and ketoconazole were more resistant than the other agents tested.

In conclusion Candidosis caused by *Candida* species are on the rise and are among the four most common causes of nosocomial infections. Antifungal resistance poses a major public health concern. Lack of routine diagnostic testing for fungal diseases exacerbates the problem of antifungal drug empiricism. Health care professionals quite often face the challenge of drug resistance in patients on prolonged antibiotic therapy and in such a scenario should raise a high index of suspicion of the possibility of Candidosis and should get the collaboration of Clinical Microbiologist to arrive at a faster diagnosis aiming at improving the patient care.

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