International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 55-62
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Changing Trends in Distribution and Antifungal Susceptibility Pattern of Candida Species Isolated from Various Clinical Samples at A Tertiary Care Hospital, Jaipur

Jogender[1], Anjali Kulshrestha[*2], Jitender[3], Jitesh[4], Suman Rishi[5]

[1]Demonstrator, Dept of Microbiology, NIMS, Jaipur.
[2]Assistant Professor, Dept of Microbiology, PMCH, Udaipur.
[3]Demonstrator, Dept of Microbiology, SHKM, Nalhar, Nuh.
[4]Consultant, Oro -Dental surgery, Nirmal Clinic, Jaipur.
[5]Ex-HOD, Dept of Microbiology, NIMS, Jaipur.

Received: 10 Oct 2018 / Accepted: 8 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: anjalikulshrestha2185@gmail.com

Abstract

The changing epidemiology from *Candida albicans* to *Non-albicans Candida* along with increasing antifungal resistance is a matter of great concern in health care settings. The studies on *Candida* have largely been carried out by the morphological identification but to the best of my knowledge very little work has been done in Rajasthan to find out antifungal susceptibility pattern of *Candida* species. Keeping in view the above facts, this study was undertaken for identification and evaluation of antifungal susceptibility pattern of *Candida* species isolated from various clinical samples in a tertiary care hospital, Jaipur. A total of 51 candida species were isolated during a period from Jan 2017- May 2017 and identified by standard microbiological procedures. Antifungal susceptibility testing was carried out on the basis of CLSI M44-A guidelines. Out of 51 Candida isolates, 23 were candida albicans and 28 were Non-albicans candida. Among Non – Candida albicans, 17 isolates were C.tropicalis, 9 and 2 isolates were C. krusei and C. glabrata respectively. Candidiasis was more commonly found in female patients and in 20-39 years of age group. Overall, antifungal susceptibility of Candida species to Fluconazole was 57%, Voriconazole 76%, 88% to Amphotericin B and 94 % to Nystatin. Therefore, The changing trends in epidemiology of candidiasis, necessitates the speciation of candida species which inturn facilitate the development of effective measures to prevent and control transmission of resistant pathogen.

Keywords
Changing trends, candida species, candidiasis, antifungal susceptibility.

*****

INTRODUCTION

The incidence and prevalence of fungal infection due to Candida species are increasing significantly in the recent few decades, so contributing to morbidity and mortality[1]. This increase is mainly due to expanding population of immunocompromised patients that use total parenteral nutrition and intravenous catheters; increasing use of prolonged antibiotic therapy; in organ transplant recipients and also a rise in use of invasive procedures for diagnosis and treatment[2,3].

Candida spp, belonging to yeast like fungi are the normal commensal microflora of skin, mucous membrane of oral cavity, gastrointestinal, genitourinary and respiratory tract[4]. They become pathogenic particularly when the host defense mechanism is lowered and causes an opportunistic infection[5,6].

Genus Candida includes > 500 species, in which only 20 species are recovered from human samples[7]. Among these 20 Candida species, *Candida albicans* is generally considered as major pathogen. But recently published epidemiological data highlights an increase of Non-albicans candida species such as *C. tropicalis, C. parapsilosis, C. glabrata, C. dubliniensis and C. krusei*[8].

In addition, the numbers of new Candida species isolated from clinical samples are increasing continuously every year, which were previously considered "non-pathogenic". The major reason for this could be the use of various commercially available identification methods by clinical microbiology laboratories worldwide to supplement the conventional methods of identification, also a rapid increase in number of immunocompromised patients worldwide in view of the HIV epidemic, organ transplantation and malignancies[9].

Candidiasis is the commonest fungal disease, usually endogenous in origin. All Candida species causes diseases ranging from simple mucosal colonization, superficial infections such as oral thrush to invasive fungal diseases, yet they show difference in disease severity and susceptibility to different antifungal agents[10].

Fluconazole is a triazole most effective and frequently prescribed antifungal drug for treatment of candidiasis, as it has an excellent patient tolerance and minimal side effects. A growing worldwide increase in use of this drug for treatment of candidiasis, in turn lead to drug resistance and also one of the principal causes of recent increase in prevalence of non- candida albicans candidiasis[11].

The changing trends of candidiasis necessitates the speciation of candida species which inturn facilitate the development of effective measures to prevent and control transmission of resistant pathogen. Hence the present study was carried out to detect the clinical distribution of candida species in various clinical specimens along with their antifungal susceptibility pattern.

MATERIAL AND METHOD:

Source of material - The present study was conducted in the department of Microbiology, NIMS Medical College and Hospital, Jaipur (Rajasthan) from January 2017 to May 2017.

Inclusion criteria

- All the *Candida* species isolated from various clinical samples will be included in the study.

Exclusion criteria

- All other fungal isolates except *Candida* species were excluded from the study.

Processing of Samples

During this period all clinical samples suspected of fungal infection, which were received from different Wards, ICUs and OPDs of the hospital and were submitted to microbiology laboratory with all aseptic precautions and processed in the following manner -

1. Direct examination of sample[7]- Direct microscopic examination by 10% KOH mount and Gram staining reveals presence of oval budding yeast cells with / without pseudohyphae.

2. Isolation and Identification of Candida species[7] - This was done by standard conventional method ie inoculation of the sample on to Blood agar and SDA, both were incubated at 37°C for 48-72 hrs. All the samples which showed growth were identified by colony characteristics and by gram staining. Once the conformation of colonies was done, they were further speciated by germ tube test, chlamydospore formation on Corn Meal agar, sugar fermentation and assimilation test.

3. **Antifungal susceptibility pattern[12,13]**- This was done by agar disc diffusion method as recommended by CLSI (M-44A) guidelines. Mueller - Hinton agar with 2% glucose and 0.5ug/ml methylene blue and antifungal agents like Fluconazole, Voriconazole, Nystatin and Amphotericin B were used.

RESULT:

In the present study, a total of 51 (4.5%) Candida species were isolated from 1113 clinical specimens (Fig-1). Maximum Candida were isolated from patients in the age group of 20-39 yrs (33.3%) followed by extremes of age ie 60-79 yrs (23%) and <19 yrs (22%) (Fig-2). Candidiasis was more common in female patients ie 28 (55%) than in males' patients ie 23(45%). Female: Male ratio was 1.2:1 (fig 3).

Out of 51 Candida isolates, most common isolated species was non - Candida albicans ie 28 (55%) followed by Candida albicans ie 23 (45%) (Fig 4). Out of 28 Non-albicans candida, C.tropicalis was most frequently isolated (33%) followed by C.krusei (18%) and C.glabrata (4%) .

Majority of Candida species were isolated from urine samples (51%) followed by sputum (22%), HVS (10%), ear swab (8%), 2% each from foleys catheter, blood, stool, ET secretion and throat swab (Fig 5). Among the isolates derived from urine, C.tropicalis (42%) was predominant followed by C.albicans (27%) and C.krusei (23%), where as C.albicans(82%) was most frequently isolated from sputum sample followed by C.krusei (18%) (Fig 6).

Overall, antifungal susceptibility of Candida species to Fluconazole was 57%, Voriconazole - 76%, 88% to Amphotericin B and 94 % to Nystatin. Hence among the 4 antifungal drugs highest susceptibility was observed for Nystatin and highest level of resistance to Fluconazole 43%. Candida albicans was 100% sensitive to Nystatin and Amphotericin B, 87% and 78 % sensitive to Fluconazole and Voriconazole respectively, where as C.tropicalis was 88%, 82%, 65% and 59% sensitive to Nystatin, Amphotericin B, Voriconazole and Fluconazole. 100 % resistance was seen in C. glabrata against Fluconazole, followed by 100% sensitivity to Nystatin and Voriconazole. In case of C.krusei, 89% resistance was reported for fluconazole, followed by 11%, 22% and 33% resistance to Nystatin, Amphotericin B and voriconazole respectively. (graph 1,2,3,4).

Figure 1: Isolation rate of Candida species from various clinical samples

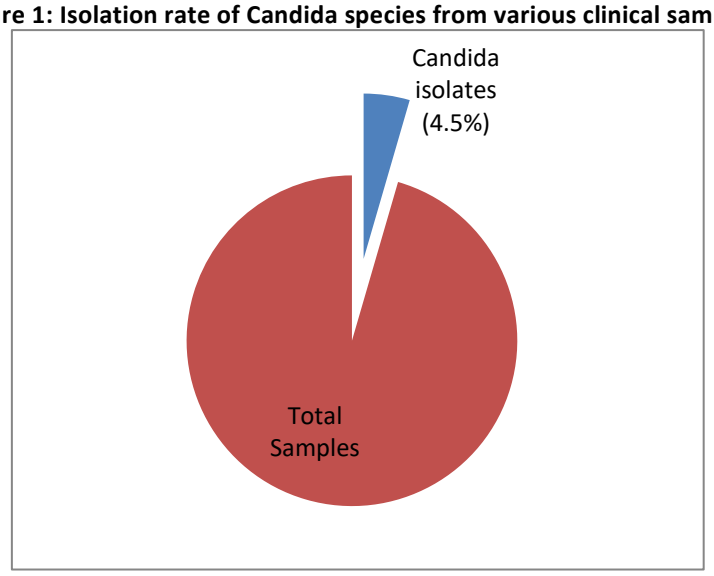


Figure 2: Age wise distribution of patients with Candida infection

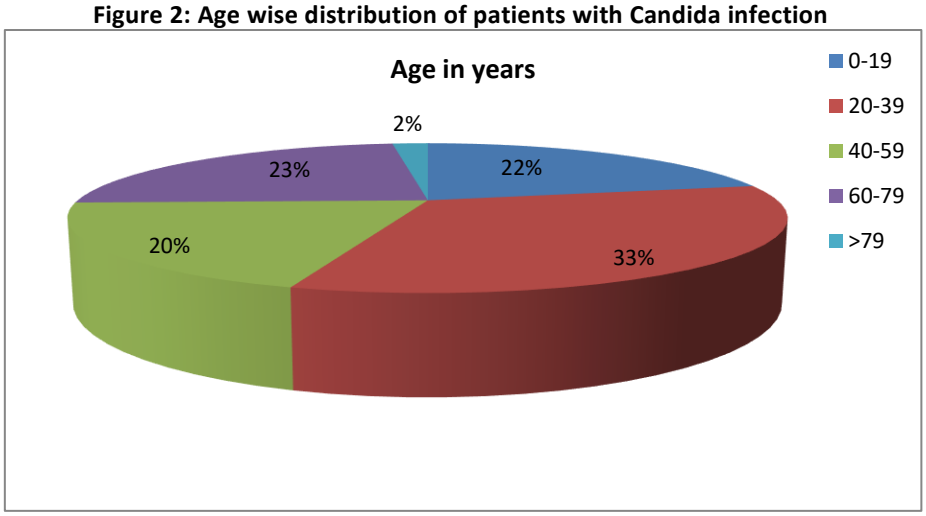


Figure 3: Sex wise distribution of patients with Candida infection

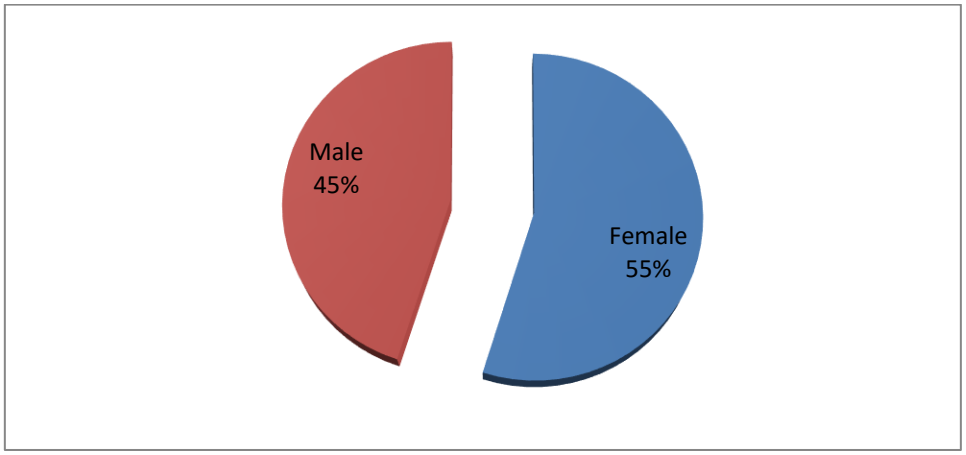


Fig 4: Distribution of Candida species

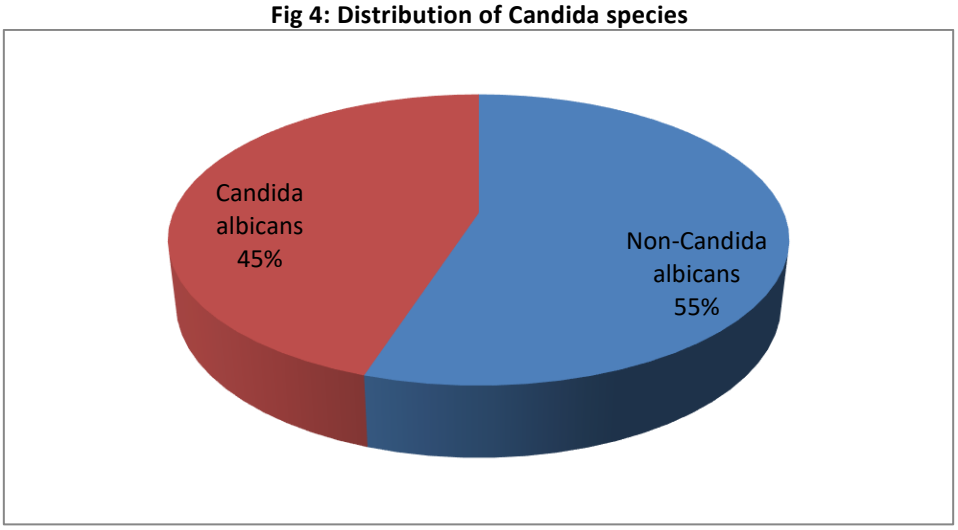


Figure 5: Specimen wise distribution of Candida Species

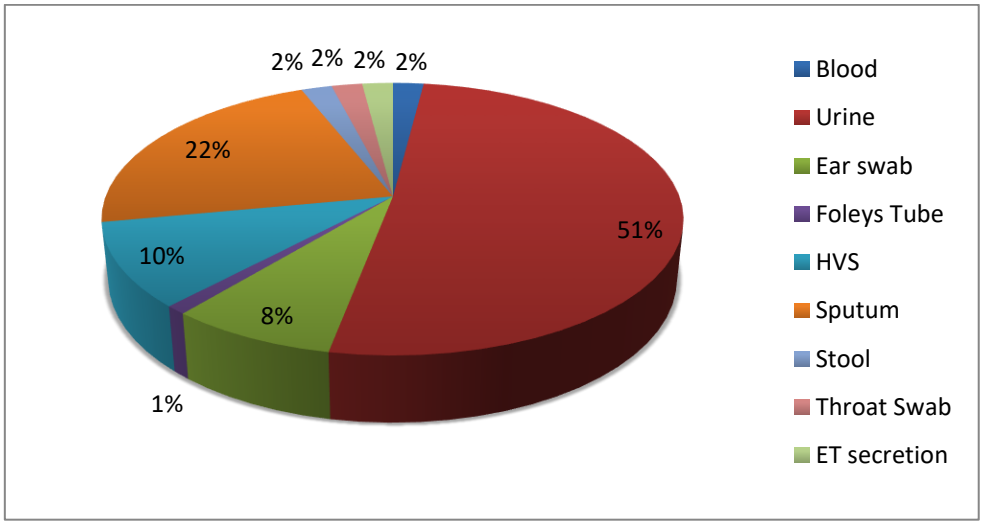
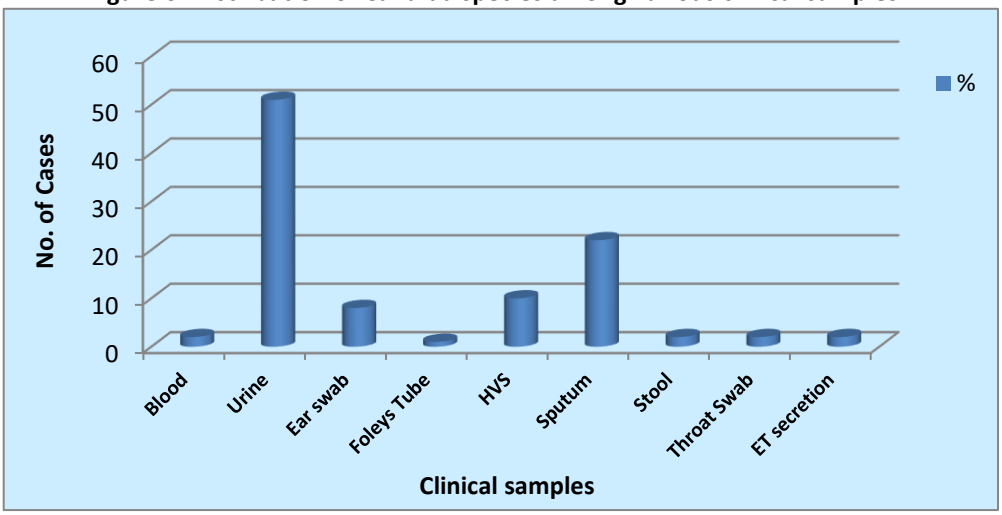
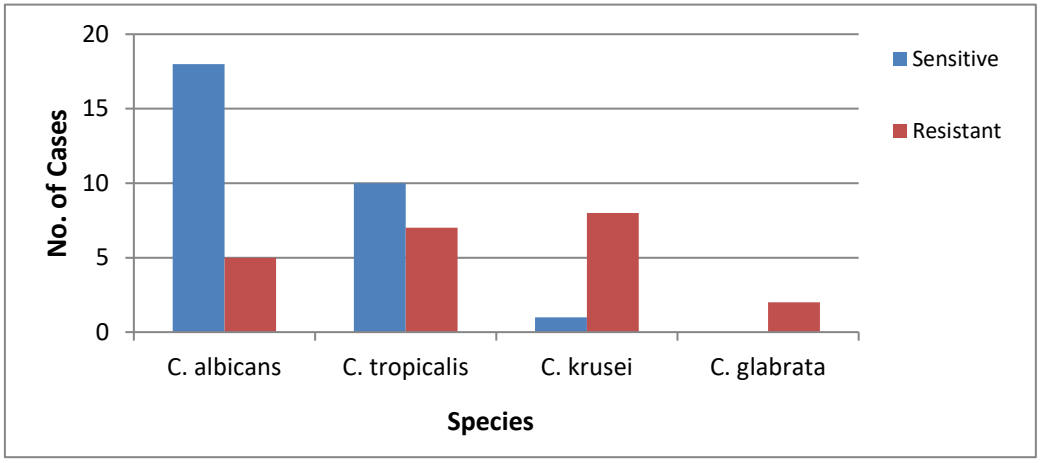


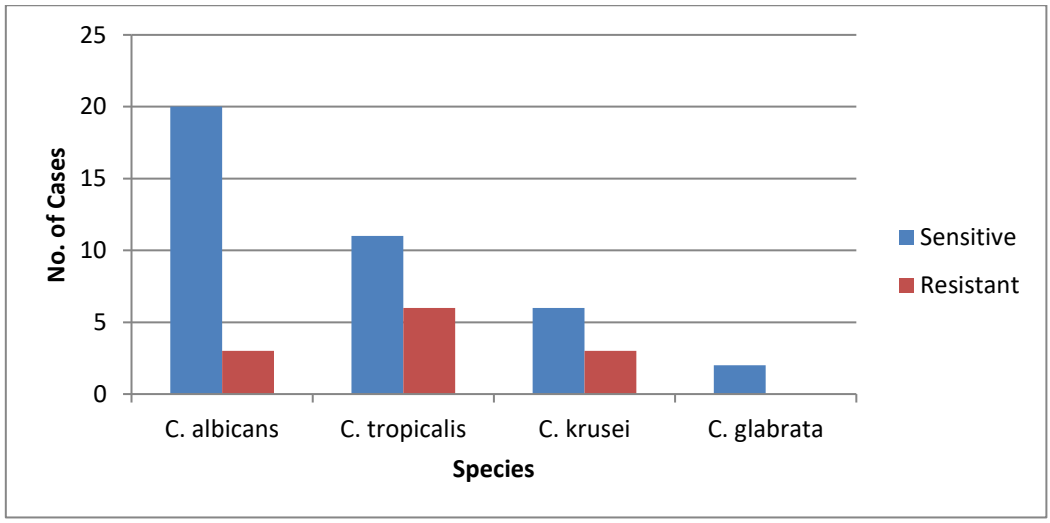
Figure 6: Distribution of Candida species among various clinical samples



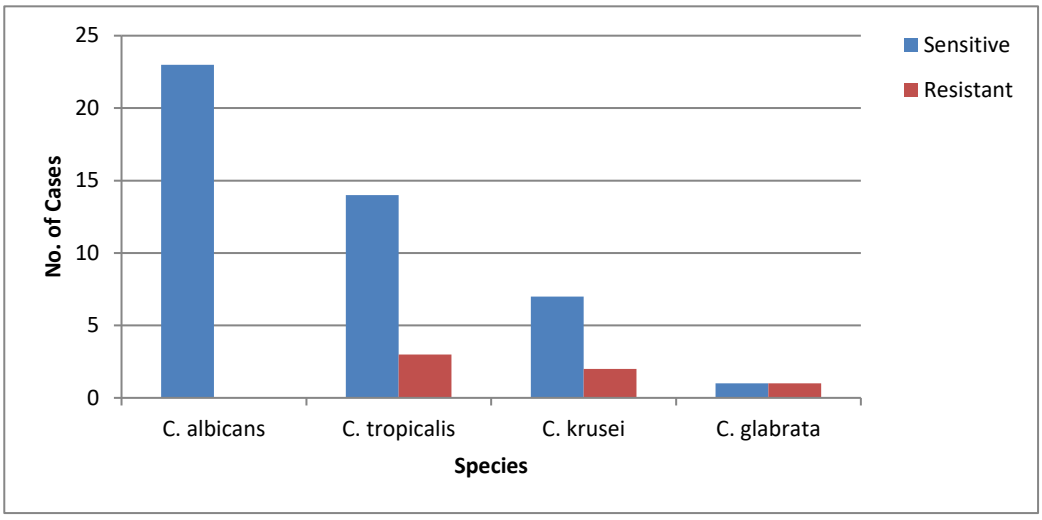
Graph 1: Antifungal susceptibility of Candida isolates to Fluconazole



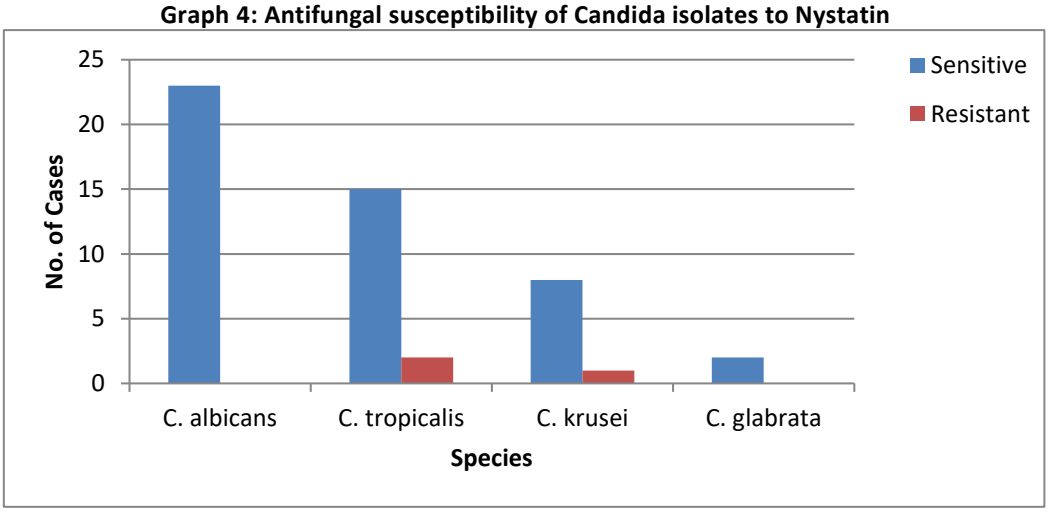
Graph 2: Antifungal susceptibility of Candida isolates to Voriconazole



Graph 3: Antifungal susceptibility of Candida isolates to Amphotericin B



Graph 4: Antifungal susceptibility of Candida isolates to Nystatin



DISCUSSION:

Over the past two decades, a significant increase in the incidence of mycosis in general and Candidiasis in particular is reported in various studies[14]. Also, in recent years non- Candida albicans, emerged as a major pathogen causing serious diseases in humans as they are resistant to commonly used antifungal agents[15]. To tackle this grave situation, the present study was under taken to provide a detailed analysis of the distribution and antifungal susceptibility of 51 isolates of Candida species in various clinical samples and in patients of different age group and gender.

In the present study maximum number of Candida species were isolated from urine samples followed by sputum samples. This is similar to the study conducted by Sukumaran et. al.[16] and Agarwal et. al.[17], in which more no of Candida isolates were found in urine samples. Similarly, a study conducted by Sundar Khadka et. al.[18] isolated maximum number of Candida species from urine (48%) and sputum (42%) samples. These studies indicate increased incidence and isolation of Candida species in Urinary tract and respiratory tract infection.

In the present study Non-albicans candida were isolated at a higher rate (55%) than C.albicans (45%),which was in agreement with the studies conducted by Ragini et al.[19],Chakrabarthi et al.[9] and Agarwal et al.[21] These studies indicate that Non-albicans candida are emerging as a major pathogen and they are treat for future. This change in pattern has been partly attributed to increased immune suppression resulting in higher number of susceptibilities in immunocompromised patients and also to the prophylactic use of antifungal agents in critically ill patients. Hospitalization (especially in

ICU), placement of central venous catheters and the other indwelling devices, previous antimicrobial therapies have played significant role in this changing pattern of Candidiasis.

Among the non- Candida albicans, C.tropicalis(33%) was most frequently isolated followed by C.krusei(18%) and C.glabrata(4%).These findings are comparable to the studies conducted by different researchers like Divya Dadhich et al.[22] and L. Sumitra deviet al.[23] while a study carried out by Mokadas et al.[24] reported C.parasilosis as the most common Non-albicans candida species. In our study the most common isolate in Urine and blood samples was C. tropicalis which correlates well with the study of L. Sumitra Devi et al.[23] C. albicans was the most common isolate in Sputum which is in accordance with the studies of B. Madhumati et al.[25]

In the present study, it was found that Candidiasis can occur at all ages. The youngest in our study was one-year old baby while the eldest was 85 years. The mean age was found to be 43 years. The highest incidence was seen in the age group of 20-39 years. This is similar to the study conducted by Lata R Patel et al, Dalal et al.[100], Jayalaxmi et al[101] showing maximum cases in the age group of 21-40 years.

Comparatively Candida isolation was more in female patients (55%) than in male patients (45%) in our study. Similar finding was also reported by Megha Pawar et al and Sujatha R et al with female predominance. The possible reason is that Candiduria and genital Candidiasis is more common in females during reproductive age group.

In this study, Candida species were more susceptible to Nystatin, followed by Amphotericin B (88%), Voriconazole (76%) and Fluconazole (57%). Hence Nystatin and Amphotericin B emerged as most efficacious drug for the treatment of Candidiasis and also 43% Candida isolates demonstrated resistance to fluconazole which is similar to study done by Deorukhkar et al. Highest resistance to fluconazole was reported by Non – Candida albicans.

CONCLUSION:

To conclude, the present study highlights the predominance of Non- Candida albicans in various clinical specimens. Among commonly used antifungal drugs Nystatin, Amphotericin B, Voriconazole showed high rates of sensitivities while Fluconazole was least effective for candidiasis. This study in our set up will help in recognizing the emerging candida species along with their increasing drug resistance. Therefore, the changing trends of candidiasis, necessitates the speciation of candida species along with their antifungal susceptibility pattern, this will enable the clinicians to choose appropriate antifungal agents, which will inturn decrease the patient's morbidity and mortality.

REFERENCES

1. Fridkin SK. The changing face of fungal infections in health care settings. Clin Infect Dis 2005; 41:1455-1460.
2. Akins RA. An update on antifungal targets and mechanisms of resistance in *Candida* albicans. Med Mycology. 2005; 43:285-318.
3. Akins RA. An update on antifungal targets and mechanisms of resistance in *Candida* albicans. Med Mycology. 2005; 43:285-318.
4. ShaoL. C., ShengC. Q., ZhangW. N. (2007). [Recent advances in the study of antifungal lead compounds with new chemical scaffolds]. *Yao XueXue Bao*42, 1129–1136.
5. Dignani MC, Solomkin JS, Anaissie E. Candida. In: Anaissie E, McGinnis MR, Pfaller MA, editors. Med mycology. 1st. ed. Philadelphia: Churchill Livingstone, 2003; p. 195-239.
6. Colombo AL, Guimarães T. Epidemiologia das infecçõeshematogênicas por Candida spp. Rev Soc Bras Med Trop 2003; 36:599-60
7. Jagdish Chander. *Text Book of Medical Mycology*. 3rd edition. 2009.
8. PfallerM. A., DiekemaD. J., ProcopG. W., RinaldiM. G. (2007). Multicenter comparison of the VITEK 2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against *Candida* spp. *J Clin Microbiol*45, 3522–3528.
9. Chakrabarti A: Microbiology of systemic fungal infections. *J Postgrad Med* 2005 Vol 51 suppl 1.
10. EggimannP., GarbinoJ., PittetD. (2003). Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis*3, 685–702.
11. Giolo MP, Svidzinski TIE. Fisiopatogenia, epidemiologia e diagnóstico laboratorial da candidemia. J Bras Patol Med Lab 2010; 46:225-234.
12. Clinical Laboratory Standards Institute (CLSI). Method for Antifungal disk diffusion Susceptibility testing of Yeasts: Approved Guidelines, second edition. CLSI document M44-A2 (ISBN 1-56238-703-0). Clinical Laboratory Standard Institute, Wayne: Pennsylvania; 2009.
13. Susceptibility testing of yeasts [internet]; 2011
14. Deorukhkar SC. Changing Trends in Epidemiology of Candidaisis and Role of Non-albicans candida Species. Adv Tech Clin Microbiol. 2016, 1:1.
15. Chakrabarthi A, Ghosh A, Batra R, et al. Antifungal susceptibility on Non-albicans candida and distribution of species isolated from Candidaemia cases over a 5-year period. Indian J Med Res. 1996; 104:171–6.
16. Sukumaran J, Sundaram JM, Sivan RR. Changing trend in the clinical distribution of *Candida* species in a

tertiary care hospital. J NTR Univ Health Sci 2012; 1:222-6
17. Agarwal S, Manchanda V, Verma N, Bhalla P. Yeast identification in routine clinical microbiology laboratory and its clinical relevance. Indian J Med Microbiol 2011; 29:172-7.
18. Sundar Khadka, Jeevan Bahadur Sherchand, Bharat Mani Pokhrel, Keshab Parajuli, Shyam Kumar Mishra, Sangita Sharma, Niranjan Shah, Hari Prasad Kattel, Subhash Dhital, Sulochana Khatiwada, Narayan Parajuli, Manoj Pradhan and Basista Prasad Rijal2Isolation, speciation and antifungal susceptibility testing of *Candida* isolates from various clinical specimens at a tertiary care hospital, Nepal*BMC Res Notes (2017) 10:218*
19. Ragini AK, Sandhya B, Gayatridevi, Indumal. Characterization and antifungal susceptibility testing for Candida in tertiary care hospital. J Health Sci Res. 2011;2(2):1–12.
20. Jones JM. Laboratory diagnosis of invasive candidiasis. Clinical microbiology.1990;3:32-45.
21. Agarwal J, Seema B, Mallik GK, Jain A. Trends in neonatal septicaemia: emergence of Non-albicans candida. Indian Pediatrics. 2004; 41:712–6.
22. L. Sumitra Devi, Megha Maheshwari. Speciation of *Candida* species isolated from clinical samples by using chrom agar and conventional methods. *International Journal of Scientific and Research publications*. March 2014, volume 4, issue 3.
23. Dadhich D, Saxena N, Chand AE, Soni G, Morya S. Detection of *Candida* Species by Hichrom Agar and Their Antimycotic Sensitivity in Hadoti Region. *Int J Sci Stud* 2016;4(4):23-26.
24. Mokaddas EM, Al- Sweih NA, Khan ZU. The species distribution and antifungal susceptibility of candida blood stream isolates in Kuwait. J Med Microbiology,2007;56:255-9.
25. Madhumati and R. Rajendran,:Evaluation of Chrom Agar in Speciation of *Candida* Species from Various Clinical Sample in a Tertiary Care Hospital. *Int.j.Microbiol.App.Sci*(2015) 4(9): 463-472