Prevalence of Non-*albicans Candida* and its Antifungal Susceptibility at a Tertiary Care Hospital, Jaipur

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ABSTRACT

Introduction: Candidiasis is the commonest fungal disease found in humans affecting mucosa, skin, nails and internal organs. It is caused by various species of yeast like fungi belonging to the genus *Candida* . *Candida albicans* used to be the predominant cause of candidiasis. A shift toward non-*albicans Candida* (NAC) species has been recently observed. These NAC species demonstrate reduced susceptibility to commonly used antifungal drugs. This study was undertaken to find out the prevalence of Non albicans candida species among various clinical isolates and to investigate the susceptibility pattern of these species to the common antifungal agents.

Material and Methods: Different clinical Specimens were collected from patients visiting to a tertiary care centre, Jaipur from Jan 2019 to Dec 2019. They were subjected to direct microscopy in 10% KOH mount, culture in Sabouraud's dextrose agar for fungal growth. The growth of *Candida* spp. on sabouraud dextrose agar was confirmed by Gram staining(where gram-positive budding yeast cells were observed), Germ tube test and CHROM Agar morphology. The species identification as well as antifungal susceptibility testing were performed with VITEK 2 compact automated system using VITEK-2 cards for identification of yeast and yeast-like organisms (ID-YST card). Antifungal susceptibility testing was carried out with VITEK 2 fungal susceptibility card (YST -08 kit).

Result : 200 different clinical isolates of Candida were isolated from different clinical samples (116 urine, 37 blood, 17 swabs, 11 E.T secretion, 9 Vaginal swabs, 6 sputum and 4 others). The maximal number of patients positive for *Candida* spp were in the age group of 19-60 years (%) followed by >60 years age group (%). The sex distribution showed a female preponderance with 55% females and 45% males. Out of 200 *candida* spp. isolated, 74%(n=148) belonged to the Non *Albicans Candida* spp. (NAC), while 26%(n=52) species were of *Candida albicans*. 32.69%(n=129) were from Intensive Care Units (ICUs) followed by Wards 27.50%(n=55) ; and OPDs 8%(n=16) .

Conclusion: Species-level identification of Candida and their antifungal sensitivity testing should be performed to achieve better clinical result and to select an appropriate and effective antifungal therapy .

Keywords: Non-*albicans Candida*, Antifungal Susceptibility

INTRODUCTION

Candida species is a normal commensal flora of the oral cavity, gastrointestinal tract and vagina of a human body. In healthy individuals these inhabit mucosal surfaces in an asymptomatic manner, but can become a significant cause of disabling and lethal infection.[1]

Fungi were once considered as non-pathogenic or less virulent but in the last decade, the incidence of mycotic infections has increased progressively. They are now considered as a primary cause of morbidity and mortality in severely ill and immunocompromised patients. Ranging from mucocutaneous overgrowth to bloodstream infections, they are responsible for various clinical manifestations.[2]

As per the Nosocomial Infections Surveillance (NIS) study of the Centre for Disease Control (CDC) *Candida albicans* and its related species have been labelled as the 6th most common cause of nosocomial infections.[3]

It is the third leading cause of central-line associated bloodstream infections and the second leading cause of catheter-associated urinary tract infections in the US.[4]

There are more than 17 different *Candida* species that are known to be the aetiological agents of human infection and majority of the invasive infections are caused by *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, Candida glabrata, and C.krusei.*[5]

The antifungal susceptibility pattern of the drugs used commonly among the types of *Candida* species these days show significant variation. The drug resistance scenario has been increasing due to excessive use of random antifungal agents in the last decade.[6] Several studies have been conducted till now which show the emergence of drug resistant *Candida* species in global scenario.[7,8]

Candida auris, a type of yeast which causes severe illness in hospitalized patients has been reported by healthcare facilities of several countries in the last few years. This yeast can cause serious invasive infections by entering the bloodstream and spreading throughout the body. Studies show that most often this yeast is misidentified. It does not respond to commonly used antifungal drugs, making infections difficult to treat.[9]

This yeast can cause serious invasive infections by entering

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the bloodstream and spreading throughout the body. Studies show that this yeast often is misidentified and does not respond to commonly used antifungal drugs, making infections difficult to treat.[9] Candidiasis with its various manifestations is responsible for the dilemma in both diagnosis and treatment. This makes it a major contributor to the actual causes of death in the patient.

Most of the conventional methods are tedious and time consuming to perform and there is a lack of support of reliable mycological investigations. Thus, it is increasingly important that the isolation, identification and susceptibility testing of *Candida* species in clinical specimens is done by the inclusion of rapid and reliable methods so that an early diagnosis can be established and effective treatment can be initiated.

Hence a periodic review of the institutional prevalence and susceptibility patterns is a valuable guide for establishing a treatment protocol.

MATERIAL AND METHODS

All the isolates of *Candida* obtained during one year period; Jan 2019-Dec 2019, from different clinical specimens submitted to the Department of microbiology, MGMCH were included for the study.

The different clinical samples included: blood, urine, sputum, E.T secretion, pus swab, high vaginal swabs and others which include bronchioalveolar lavage and sterile body fluids.

Procedure

The samples were subjected to:

(i) Direct microscopy - KOH mount. A drop of 10% KOH is poured on the specimen with a cover slip on it and observed for the presence of any budding yeast cells.

(ii) Gram's stain – prepared from the samples were screened for the presence of gram positive budding yeast cells.

(iii) Culture - Samples were inoculated on Sabaroud dextrose agar (SDA) with Chloramphenicol (for the isolation of pathogenic fungi simultaneously avoiding bacterial contamination) were incubated at 37°C aerobically for 24 - 48 hrs.

Any visible growth seen on SDA slope was processed further for identification of species. Suspected colonies of *Candida* will appear as small creamy to white coloured colonies. From the isolated colony, macroscopic examination, Gram staining, germ tube test was performed.

(iv) Lacto Phenol Cotton Blue Mount (LPCB) was prepared to study morphological features of fungal isolates.

(v) Germ Tube Test- Germ tubes are seen as long tube-like projections extending from the yeast cells, without any constrictions at the point of attachment to the yeast. It is formed within 2 hrs of incubation in *C.albicans and C.dublinensis* and not in any other spp. of this genus.

(vi) CHROM Agar- CHROM Agar is a rapid plate-based test for the simultaneous isolation and identification of various species of *Candida*.

Yeast isolates sub-cultured on chromogenic medium were incubated overnight at 35ºC for presumptive identification made by colour and morphology of the colonies as per the colour code which is provided with the chromogenic media.[10]

(vii) Vitek-2 cards were used for identification of yeast and yeast like organisms (ID-YST) and the antifungal susceptibility testing was performed with AST-YST 08 kits. Standard operative procedures as described by the manufacturer were followed.

RESULT

A total of 200 *Candida* species were isolated from various clinical samples between January 2019 to December 2019. Majority of *candida* spp. were isolated from Urine (n=116; 58%) followed by Blood (n=37; 18.5%) and swabs (n=17; 8.5%). The distribution of *Candida* spp. from various clinical specimens is shown in Table-1.

Out of 200 *Candida* spp. isolated, it was found that a majority of the isolates 74%(n=148) belonged to the Non-*albicans Candida* spp. (NAC), while 26%(n=52) species were of *Candida albicans* [Figure-3].

	Urine (N=116)	Blood (N=37)	Swab (N=17)	Vaginal Swab(N=9)	E.T Secretion (N=11)	Sputum (N=6)	Others (N=4)	Total
C.albicans	28	3	8	6	4	3	-	52
C.tropicalis	40	13	4	1	2	3	2	65
C.famata	18	5	-	1	3	-	1	28
C.glabrata	11	2	-	1	-	-	-	13
C.auris	7	6	-	-	-	-	-	13
C.parapsilosis	3	4	2	-	-	-	1	10
C.lusitaniae	2	2	-	-	-	-	-	4
C.kefyr	2	-	2	-	-	-	-	4
C.cieferri	1	-	1	-	2		-	4
C.krusei	1	1	-	-	-	-	-	2
C.catenulata	2	-	-	-	-	-	-	2
C.utilis	-	1	-	-	-	-	-	1
C.duboshaemulonii	1	-	-	-	-	-	-	1
Total	116	37	17	9	11	6	4	200

Table-1: Distribution of Candida species in different clinical isolates.

Among the NAC spp. most common was *C.tropicalis* (n=65;32.5%) followed by *C.famata* (n=28; 14%), *C.glabrata* (n=14;7%), *C.auris* (n=13; 6.50%) and *C.parapsilosis* (n=10; 5%). [Table-2]. The maximum number of *Candida* positive isolates were isolated from Intensive Care Units (ICUs) (n=129; 32.69%), followed by Wards (n=55; 27.50%) and OPDs (n=16; 8%) [Table-5].

The maximal number of patients positive for *Candida* spp. were in the age group of 19-60 years (65%) followed by the age group of >60 years (28.5%).[Table-4]

The gender distribution showed a female preponderance with 55% females and 45% males.[Figure-2]

All yeast isolates sub-cultured on chromogenic medium CHROM agar grew well and developed distinctive coloured colonies after overnight incubation. Presumptive identification was made by colour and morphology of the colonies as per manufacturer's instructions.

C. albicans produced blue-green colonies, *C. tropicalis* produced dark blue-blue grey colonies, *C. parapsilosis* produced creamish colour to pink, *C. krusei* produced purple, *C. glabrata* produced pink to mauve. [Figure-1]

Species	Total	Percentage
C.albicans	52	26%
C.tropicalis	65	32.50%
C.famata	28	14%
C.glabrata	14	7%
C.auris	13	6.50%
C.parapsilosis	10	5%
C.lusitaniae	4	2%
C.kefyr	4	2%
C.cieferri	4	2%
C.krusei	2	1%
C.catenulata	2	1%
C.duboshaemulonii	1	0.50%
C.utilis	1	0.50%
Total	200	100%

Table-2: Species of candida isolated

	Non-*albicans Candida* spp. (N=101)	*C.albicans* (N=52)
Fluconazole		
S	91(90%)	51(98%)
R	10(9.9%)	1(1.9%)
Voriconazole		
S	98(97%)	51(98%)
R	3 (2.97%)	1(1.9%)
Amp. B		
S	99(98%)	52(100%)
R	2(1.98%)	0%
Caspofungin		
S	96(95%)	52(100%)
R	5(4.95%)	0%
Flucytosine		
S	98(97%)	52(100%)
R	3(2.97%)	0%

Table-3: Susceptibility of Candida species to antifungals

Candida species differ in their susceptibility to antifungal agents as confirmed by Vitek-2. The Vitek-2 system used does not provide the antifungal susceptibility of *C.famata, C.auris* and *C.catenulata*. Therefore, the susceptibility of these species has not been included.

In our study, resistance rates for Fuconazole (FLU), Voriconazole (VOR), Amphotericin B (AMP), Caspofungin (CAS) and Flucytosine (FCy) in Non-*albicans Candida*

Age	*C.albicans*	NAC	Percentage
<3 yrs	1	3	2%
4-18 yrs	1	8	4.5%
19-60	31	99	65%
>60	19	38	28.5%

Age-wise distribution of *Candida spp.*

Table-4: Distribution of Candida species among different age groups

Patient Location	Total	Percentage
OPD	16	8%
WARD	55	27.50%
ICU	129	64.50%

OPD: Out patient department, IPD: In-patient department, ICU: Intensive care unit

Table-5: Department wise distribution of *Candida species*

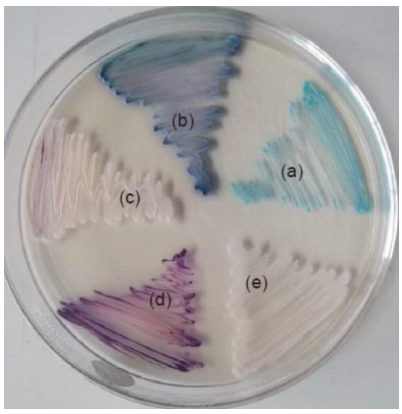

Figure-1: Colonies of different species of Candida on CHROM agar.
(a) *C.albicans* (b) *C.tropicalis* (c) *C.krusei* (d) *C.glabrata* (e) *C.parapsilosis*

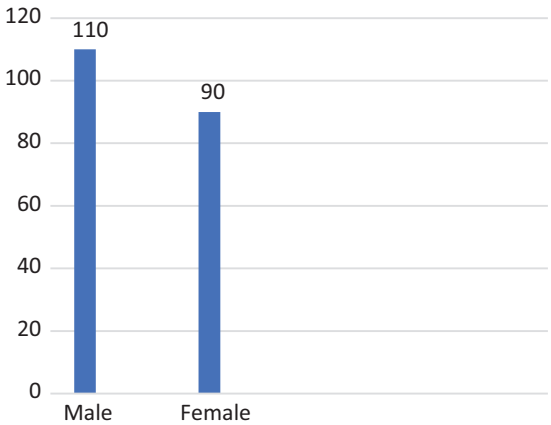

Figure-2: Gender wise distribution of *Candida* spp.

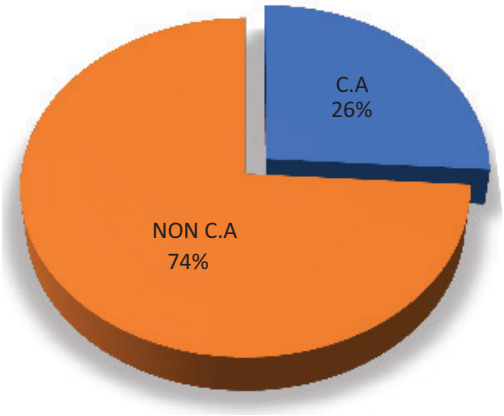

Figure-3: *Candida* species.

spp were 9.9%, 2.97%, 1.98%, 4.95%. 2.97% respectively. In *Candida albicans* the resistance rates were 1.9% and 1.9% in Fluconazole and Voriconazole respectively whereas no resistance in AMP, CAS and FCy. So, we see here that resistance to all the Antifungal drugs tested was more common in NAC spp. as compared to *C.albicans*. For instance, resistance to the azole group of antifungal was common in NAC spp. (6.53%) which in *C.albicans* group is (0.65%) [Table-3].

DISCUSSION

The increasing incidence of hospitalization and increasing use of antimicrobial agents accompanied by better adaptation of microorganisms to the hospital environment have combination led to increase in health care associated infections (HCAIs). Due to their versatility of adapting to a variety of different habitats including various medical devices, incidence of *Candida* spp. as one of the leading causes of HCAIs has increased during the past years.[11]

Here we have used chromogenic medium, HiMedia CHROM agar for speciation of *Candida* species. It offers a rapid, convenient and reliable method for identification of clinically important *Candida* species. It is based on the reactions between the specific enzymes of the different species and the chromogenic substances imparting different colour to the *Candida* species.[10]

For identifying species of *Candida* and for their antibiotic susceptibility we have used Vitek-2 also. It is a rapid technique, clinically useful for determining susceptibility of *Candida* spp. and other yeast Species.[12]

The maximum number of *Candida* positive isolates isolated from Intensive Care Units followed by wards and OPDs can be explained on the basis of the fact that the use of invasive devices are common in the ICUs and the immune status of the patients are also compromised to the maximum as compared to that of the patients of other wards and the OPDs.

In this study *Non albicans Candida* species had predominance over *C. albicans* which is in line with the published report from different parts of the world.[13,14]

C. tropicalis was the most predominant species in all the clinical samples followed by *C.albicans* supported by most of the Indian studies which also show *C. tropicalis* as the predominant isolates.[15,16]

The *Candida* isolates belonging to this group often demonstrate reduced susceptibility to fluconazole either innate or acquired or both. Hence, this epidemiological shift is a major concern.This can be related to widespread empirical use of azole group of antifungal agent like fluconazole which are the most commonly used antifungal agents for prophylaxis and treatment of mycoses. These drugs are safe and effective also for the treatment of all clinical types of candidiasis.[12,17]

Antifungal resistance has increased recently which was rarely documented once. The problem is augmented by aggressive immunosuppression (acquired or induced), an ageing population, and the emergence of virulent and intrinsically resistant organisms.[18]

Resistance to Fluconazole in *Candida* isolates is of great concern as it is the most common azole drug used in the treatment of disseminated candidiasis and candidemia. Voriconazole, Amphotericin B, and Flucytosine are shown to have good efficacy. This is supported by many Indian studies that demonstrate very high resistance to FLU for all *Candida* isolates although AMP susceptibility is high.[19]

In this present study, Caspofungin shows 100% sensitivity pattern to all the *C.albicans* spp and 95% sensitivity to NAC species,.

We also isolated a large number of *Candida auris*. It appears to typically produce a light pink coloured colony on chromogenic agar medium. Amongst the NAC spp it is an emerging yeast pathogen of global significance being mostly resistant to most of the antifungal drugs as shown in clinical studies.[20] It is isolated increasingly from diverse clinical specimens which in our study was predominantly from Blood and urine.

To identify this pathogen is quite challenging because of the inability of the conventional methods to identify *C.auris* accurately. This calls for a greater understanding of the epidemiology, risk factors for acquiring its infection and management strategies.[21]

Therefore, the task of identification and isolation of *Candida* species has now become very important.

CONCLUSION

This study emphasizes the requirement of rapid and precise identification methods of *Candida* isolates up to species level. Prior knowledge of species distribution in clinical isolates and drug sensitivity pattern among the species will help in identifying the intrinsically resistant *candida* species. This will help the clinician to choose early effective empiric antifungal therapy thus giving a better clinical outcome by decreasing patients' morbidity and mortality.

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