Original Article

IDENTIFICATION, SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN ON URINARY ISOLATES OF *CANDIDA SPECIES* ISOLATED FROM HOSPITALISED PATIENTS IN A TERTIARY CARE HOSPITAL - A PROSPECTIVE STUDY.

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ABSTRACT

Aim: The aim is to speciate *Candida* isolated from the urine samples of hospitalized patients, to correlate risk factors associated and to find out the Antifungal susceptibility of the *Candida species* isolated.

Methods: This is a single centre, cross – sectional study with study group of 100 hospitalized patients with candiduria having a colony count of more than 10 ^{4}/ml of urine. Patients satisfying the inclusion criteria were interviewed by structured questionnaire and their hospital records were used to know about the history, risk factors, duration of Candiduria and treatment details.

Results: A total of 100 patients were included in the study, Males constituted 54% and females 46% of the total population, with asymptomatic presentation 46%, symptomatic 25% and unconsciousness (patients unable to voice their symptoms), 29%. Fever (17%) and dysuria (13%) were the common symptoms in patients with candiduria. *C.albicans* constituted 14% and *non-albicans Candida spp.* constituted 86% of the total isolates obtained. Study showed Hi-Chrom agar was the best method of speciation, compared to Sugar Assimilation Test (SAT) using Rapid identification kit. Hi-chrom agar showed 100% sensitivity while SAT showed only 86% of sensitivity. Resistance to fluconazole was seen in 34% of isolates, with *C.tropicalis* being the most resistant (14%).72% isolates were susceptible to itraconazole. 90% of the isolates were susceptible to amphotericin B.

Conclusion: The increase in rates of resistance particularly among the *non-albicans Candida spp*. emphasizes the need for speciation and antifungal susceptibility testing. Hence the present study was conducted to speciate *Candida* isolated from the urine samples of hospitalized patients, to correlate risk factors associated and to find out the Antifungal susceptibility of the *Candida species* isolated.

Keywords: Candiduria, Speciation**,** Hi-Chromagar, SAT, Risk factors, Antifungal susceptibility.

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INTRODUCTION:

Candida species can cause wide spectrum of clinical diseases ranging from superficial infections of skin, nails and mucosal surfaces to deep seated infections involving various internal organs and disseminated disease causing significant morbidity and mortality. It can cause lower urinary tract infections and renal infection in hospitalised patients commonly. Epidemiological surveillance indicates that *Candida* species are the most common pathogens causing nosocomial blood stream and urinary tract infection[1].

Candiduria is defined as the presence of *Candida spp.* in urine and represents colonization or infection. It should never be ignored, as it may be one of the early indications of disseminated and invasive candidiasis, especially in critically ill patients[2,3].The common risk factors for candiduria include indwelling urinary tract devices, prior surgical procedures, recent use of antibiotics, advanced age, female sex, Diabetes mellitus, immunosuppressive therapy and prolonged hospital stay[4].

Over the last decade, however, there has been an increase in the incidence of candidiasis caused by other *Candida* species, such as *C. glabrata, C. krusei, C. tropicalis and C.parapsilosis*[5].*Candida albicans* accounts for 40-60% yeasts isolated in developed countries, whereas Indian reports show an increased predominance of *non-albicans Candida spp*.

*C.glabrata*is less susceptible and *Candida krusei*is intrinsically resistant to Fluconazole. *C. tropicalis* has the highest adherence rate to inanimate materials such as urinary and vascular catheters, and is often involved in biofilm formation, that is more resistant to antifungal agents. Resistance to azoles in *C. Tropicalis* and *C.albicans* has also been increasingly reported[6,7,8].

So it is important to know the *Candida* species causing UTI before initiating the treatment as *non- albicans Candida* species are on the rise in the hospital environment and majority are inherently resistant to treatment with Fluconazole[9].

As the conventional identification of *Candida* take several days, employing chromogenic media may help to reduce the time of isolation and identification by 48 - 72 hrs. This will help the clinician in optimizing the selection of antifungal agents and provide a more rational and customized therapy[10].

Considering the above facts, the present study was conducted to speciate *Candida* isolated from the urine samples of hospitalized patients, to correlate risk factors associated and to find out the

Antifungal susceptibility of the *Candida species* isolated.

MATERIALS AND METHODS:

This is a single centre, cross – sectional study, carried out over a period of 1 year after the approval of Institutional Ethical Committee. The Study group included 100 hospitalized patients with candiduria having a colony count of more than 10^4/ml of urine.

Inclusion Criteria:

Hospitalized Patients with urine colony count of any *Candida species* more than or equal to 10^4/ml.

Patients older than 12 yrs of age.

Both males and females were included.

Exclusion Criteria:

Candiduria with colony counts less than 10^4/ml of urine.

Patients less than 12 yrs of age.

Outpatients.

Patients satisfying the inclusion criteria were included in the study and after getting informed consent, they were assigned serial numbers. They were interviewed by structured questionnaire and their hospital records were used to know about the history, risk factors, duration of Candiduria and treatment details.

Specimen Collection:

Specimens collected were mid-stream urine specimens, and catheter collections. They were immediately transported to laboratory without any delay, as urine is an excellent culture medium for other microorganisms to grow. Delay of more than 1-2 hr in transportation if unavoidable, it should be stored in refrigerator at 4°C or transported in a container with 1.8% boric acid.

Specimen Processing:

Direct Microscopical Examination:

Microscopic visualization of uncentrifuged urine was done. One to three *Candida* per high power field was found to be equivalent to colony count of approximately 15000/ml with 80% accuracy. A wet mount of uncentrifuged urine was done. A small volume of urine was applied to a glass microscope slide, allowed to air dry, stained with Gram's stain, and examined microscopically for presence of grampositive budding yeast cells with or without pseudohyphae.

Culture:

The uncentrifuged mid-stream urine was cultured on blood agar, Mac Conkeyagar, Cystine lactose electrolyte deficient (CLED) agar and Sabouraud's Dextrose Agar (SDA) for primary isolation of *Candida spp*.

Examination of Culture: On SDA:

Colonies were cream colored, pasty and smooth. A wet mount, lacto phenol cotton blue (LPCB) mount and Gram's stain were

done from the culture isolates to confirm it as *Candida species.*

Species Identification:

The various *Candida spp.* were identified based on the following tests

Germ Tube Test:

Isolates producing germ tubes were presumptively identified as *C.albicans or C.dubliniensis.*

Growth at 45°C:

Isolates of *C.albicans* grow, while *C.dubliniensis*do not grow at 45°C.

Candida Hi - Chrom Agar:

The various species of *Candida* were identified by their colony colour, size, texture, and presence of color diffusion into the surrounding agar presumptively in 48hrs.

Sugar Assimilation Test:

Sugar assimilation test (SAT) was done using rapid Hi-Candida identification kit (KB006) from Hi-media Mumbai. KB006 kit is a standardized colorimetric identification system utilizing twelve conventional biochemical tests**.**

Table 1: Various species of *Candida on Chrom Agar*:

Candida species	**Colour on HI-CHROM agar**
C. albicans	Light green colour colonies
C. parapsilosis	Cream coloured colonies.
C. tropicalis	Steel blue colonies with a pink halo
C. krusei	Pale pink, dry rough colonies with spreading, pale edges.
C. glabrata	Pink to Purple colonies.

Antifungal Susceptibility Testing:

Antifungal susceptibility testing for *Candida* isolates was done by, Disc diffusion method, as per CLSI Guidelines on Antifungal Susceptibility testing in M-44A document

RESULTS:

Basic Demographic Data:

In about 100 patients, Males contributed 54% and females contributed 46% with majority of the study population above 60years age group (28%), followed by (21%) patients in 20-29 & 40-49 years age group. Table 2 showed that females were predominated in the age group of 20-29 yrs (28.2%), where as males predominated after 60 yrs (31.4%).

Table 2: Age distribution of the study population

Age (years)	**Total number of cases n=100 no (%)**	**Male n=54 no(%)**	**Female n=46 no(%)**
<20	4(4%)	3(5.5%)	1(2.1%)
20-29	21(21%)	8(14.8%)	13(28.2%)
30-39	7(7%)	2(3.7%)	5(10.8%)
40-49	21(21%)	11(20.37%)	10(2.1%)
50-59	19(19%)	13(24%)	6(13.04%)

>60	28(28%)	17(31.4%)	11(23.91%)
TOTAL	**100(100%)**	**54(100%)**	**46(100%)**

Based on Symptoms:

Most of the patients had asymptomatic candiduria (46%), followed by symptomatic patients (25%) and unconscious patients who were unable to tell the symptoms (29%). Symptomatic and unconscious patients were common in the age group of > 60yrs and asymptomatic patients were common in the age group of 40-49yrs and 50-59yrs.Among the patients with candiduria, fever (17%) and dysuria (13%) were the predominant symptoms. Symptoms of frequency, urgency and lower abdominal pain were present in 9%, 8% and 5% of patients respectively. Dysuria, increased frequency and lower abdominal pain were predominant in males than in females (Table 3).

Table 3: Various symptoms in patients with candiduria

Symptoms	Male n=54	PERCENT	Female n=46	No (%)	TOTAL n=100	No (%)
Fever	13	(24.07%)	4	8.6%	17	17%
Dysuria	10	(18.51%)	3	6.5%	13	13%
Frequency	7	(12.96%)	2	4.3%	9	9%
Urgency	6	((11.11%)	2	4.3%	8	8%
Lower abdominal pain	3	(5.5%)	2	4.3%	5	5%
Incontinence	4	(7.4%)	1	2.1%	5	5%

Urine Sample:

73% of urine samples were catheterized urine samples followed by 27% of midstream urine samples. 74.07% of samples from males and 71.1% of samples from females were catheterized urine samples.

Patient Distribution:

Patients from medical ward had Diabetes mellitus as a major risk factor and patients from urology and nephrology had CKD and other diseases of urinary tract as the major risk factors. ICU, Surgery, Orthopaedics and neurosurgery patients had antibiotic use and catheterization as major risk factor. In addition, immune suppressives were a major risk factor for neurosurgery patients. (Table 4)

Table4: Ward distribution in patients with candiduria

Ward	**Number of patients(n)**	**Risk factors associated & no (%)**
Medicine	36	Diabetes mellitus-32(88.8%) Antibiotics-26(72.22%) Catheterization-23(63.88%) Unconsciousness-14(38.88%) Imunosuppresives-10(27.7%)
Nephrology	19	CKD and other diseases of urinary tract-19(100%) Hemodialysis-10(52.6%) Antibiotics-14(73.68%) Transplant-4(21.05%) Immunosuppresives-4(21.05%)
Urology	14	CKD and other diseases of urinary tract-14(100%) Catheterization-14(100%) Antibiotics-12(85.71%)
ICU(Intensive Care Unit)	12	Antibiotics-12(100%) Catheterization-12(100%) Unconsciousness-12(100%) Diabetes mellitus-6(50%)
Surgery	9	Antibiotics-8(88.88%) Catheterization-8(88.88%) Diabetes mellitus-2(22.22%)
Orthopaedics	6	Catherization-6(100%) Antibiotics-6(100%) Diabetes mellitus-2(33.33%)
Neurosurgery	4	Catheterization-4(100%) Antibiotics-4(100%) Immunosuppresives-3(75%) Unconsciousness-3(75%) Diabetes mellitus-2(50%)

Risk Factor Assessment:

Major risk factors included antibiotics use in 82%, catheterization of urinary tract in 67%, followed by Diabetes in 44% and Chronic Kidney Disease in 33% of the patients with candiduria (Table 5). *Non-albicans Candida spp* was more common in catheterized patients (70.9%) and unconscious patients (30.23%).

Table 5: Risk factors in *candiduria* due to C.*albicans* and *non albicans candida species.*

Risk factors	**No. of patients N (%)**	**Candiduria due to *C.albicans* N=14**		**Candiduria due to *nonalbicans Candida species* N=86**	
		TOTAL	**(%)**	**TOTAL**	**(%)**
Prolonged antibiotics	82(82%)	9	64.28	73	84.88
Catheterization	67(67%)	6	42.9	61	70.9
Diabetes mellitus	44(44%)	6	42.9	38	44.1
Chronic kidney disease and diseases of urinary tract	33(33%)	1	7.14	32	37.20
Unconsciousness	29(29%)	3	21.42	26	30.23
Immunosuppressives	17(17%)	2	14.28	15	17.44
ICU stay	12(12%)	2	14.28	10	11.62
Hemodialysis	10(10%)	3	21.42	7	8.1
Renal transplant	4(4%)	0	7.1	4	4.6

Species Identification:

Of the 100 *Candida* isolates, 62 were *C.tropicalis*, 14 isolates were *C.albicans*, followed by 10 isolates of *C.glabrata*. 9 isolates of *C.krusei and* 5 isolates of *C.parapsilosis*. *C.tropicalis 62%* was the predominant isolate among the *non-albicans Candida* species (Table 6).

C.albicans was more common in midstream urine (37%) sample than from catheterized urine samples. *C.tropicalis* was more common in catheterized urine samples (72.6%) than from midstream urine samples (33.33%).

Table 6: Distribution of *candida* isolates among catheterised and midstream urine samples

SPECIES	**Total no of isolates, n=100 No(%)**	**No. of isolates in catheterized urine samples, n=73 No(%)**	**No. of isolates in midstream urine, n=27 No(%)**
C.albicans	14	4 (5.4%)	10(37%)
C.tropicalis	62	53 (72.6%)	9(33.33%)
C.glabrata	10	6 (8.2%)	4(14.8%)
C.krusei	9	7 (9.5%)	2(7.4%)
C.parapsilosis	5	3 (4.1%)	2(7.4%)
Total	**100 (100%)**	**73 (100%)**	**29(100%)**

Hi-Chrom Agar Vs Sugar Assimilation Kit:

Hi-Chrom agar was the best method of *Candida* speciation as it identified all *Candidaspecies* from the 100 isolated samples with a sensitivity of 100%. The ability of sugar assimilation kit using rapid HiCandida identification kit (KB006) to

identify the different *Candida* species from the 100 isolated samples was only 86% and rest was unidentifiable by this method. This shows Hi-Chrom agar is more sensitive for *Candida* speciation (Table7).

Table 7: Comparison of methods of *candida* speciation

SPECIES	Total no of isolates n=100	Method used			
		Hi-Chrom agar		Sugar assimilation	
		n	%	n	%
C.albicans	14	14	14	12	12
C.tropicalis	62	62	62	58	58
C.glabrata	10	10	10	6	6
C.krusei	9	9	9	6	6
C.parapsilosis	5	5	5	4	4
Total	**100**	**100**	**100%**	**86**	**86%**

Antifungal Susceptibility by Disk Diffusion Method Fluconazole:

55(55%) *Candida* isolates were sensitive and 34(34%) isolates were resistant. The overall susceptibility rate of fluconazole for *C.albicans* was 71.4% and *C.tropicalis* was 67.7%. Susceptible dose dependent for *C.albicans* 21.4% *and C.tropicalis* 9.6%. Resistance for *C.albicans* was 7.1% and *C.tropicalis* 22.5%. Resistance for *C.albicans* was 7.1% and *C.tropicalis* 22.5%. In our study *C.glabrata* and *C.krusei* showed resistance to fluconazole(Table 8).

Table 8: Antifungal susceptibility to fluconazole

SPECIES	No of isolates	Susceptible n(%)<8µg/ml		Susceptible Dose Dependent n(%) 16-32µg/ml		Resistant n (%)>64µg/ml	
		n	%	n	%	n	%
C.albicans	14	10	71.4	3	21.4	1	7.1
C.tropicalis	62	42	67.7	6	9.67	14	22.5
C.glabrata	10	-	-	-	-	10	100
C.krusei	9	-	-	-	-	9	100
C.parapsilosis	5	3	60	2	40	-	
Total	**100**	**55**	**55**	**11**	**11**	**34**	**34**

Itraconazole:

72% Candida isolates were sensitive and 18% isolates were resistant. The overall susceptibility rate for itraconazole was, for *C.albicans* 57.1%, for *C.glabrata* 60%, for *C.tropicalis* 77.4% and for *C.krusei* 55.5%. Susceptible dose dependent for *C.albicans* was 21.4% and *C.glabrata* 20%. Resistance for *C.albicans* was 21.4%, for *C.glabrata* 20%, for *C.tropicalis* 16.1% and for *C.krusei* 44.4% (Table 9).

Table 9: Antifungal susceptibility to itraconazole

Species	No of isolates n=100	Susceptible n(%) 0.25µg/ml		Susceptible dose dependent n(%) 0.25-0.50µg/ml		Resistant n(%) >1µg/ml	
		n	%	n	%	n	%
C.albicans	14	8	57.1	3	21.4	3	21.4
C.tropicalis	62	48	77.4	4	6.4	10	16.1
C.glabrata	10	6	60	2	20	2	20
C.krusei	9	5	55.5	-	-	4	44.4
C.parapsilosis	5	5	100	-	-	-	-
Total	**100**	**72**	**72**	**9**	**9**	**19**	**19**

Amphotericin B: Antifungal susceptibility testing to amphotericin B shows minimal resistance pattern and high susceptibility rate to different *Candida* species. 90% of the Candida isolates were sensitive. Susceptibility rate for *C.albicans* was 85.7%, for *C.glabrata* was 100%, *C.tropicalis* was 93.4% and for *C.krusei* was 66.6%. Resistance for *C.albicans* was 14.2%, for *C.glabrata* 0%, for *C.tropicalis* 6.4% and for *C.krusei* 33.33% (Table 10).

Table 10: Antifungal susceptibility to amphotericin b

Species	Number of isolates n=100	Susceptible		Resistant	
		N (%)	%	N (%)	%
C.albicans	14	12(85.7)	85.7	2(14.2)	14.2
C.tropicalis	62	58(93.54)	93.54	4(6.4)	6.4
C.glabrata	10	10(100)	100	-	-
C.krusei	9	6(66.6)	66.6	3(33.33)	33.33
C.parapsilosis	5	4(80)	80	1(20)	20
TOTAL	**100**	**90**	**90**	**10**	**10**

DISCUSSION:

A significant rise in prevalence of Urinary Tract Infections (UTI) due to *Candida species* has occurred over the last decade with the upsurge of *non albicans Candida species.* Clinical importance of species level identification is important as they differ in expression of virulence factors and antifungal susceptibility pattern. The correct identification of *Candida* species is of great importance, as it presents prognostic and therapeutical significance, allowing an early and appropriate antifungal therapy.

Current study was undertaken to speciate the *Candida* isolated from urine samples of hospitalized patients and to find their antifungal susceptibility pattern. The study also concentrated on the changes observed in species distribution and the surge of *non-albicans Candida spp.* in our hospital. In this study, males contributed to 54% of the study population. This was similar to the results of study by Arlene O.Cantillep et al[11]. Although there is an increased risk

in female gender, the other associated risk factors like Diabetes and chronic kidney disease were common in males in our study. There was predominance of patients in the age group of >60 yrs contributing to 28% of total patients, followed by 21% in 40-49 yrs. This correlated well with the study done by S.Krcmery et al, were the mean age was 62.4 yrs [12].

An important observation was that about 46%of patients with candiduria were asymptomatic. It is an important complicating factor in defining candiduria. Many patients on long term urinary catheterization cannot vocalize on symptoms of dysuria or increased frequency.Asymptomatic patients were common in the age groups 40-49 and 50-59 yrs. According to a study done by Mauricio Carvalho et al in 2001, only 13% of patients had symptoms suggesting UTI. Current study showed symptomatic candiduria in 25% of patients. Symptoms of UTI were predominant in the age group of > 60 yrs and were more common in males.In the present study, about 29% of patients were unconscious and on prolonged catheterization. So these patients could not be categorized either as symptomatic or asymptomatic. Fever was the most common presenting symptom in 17% of patients with candiduria followed by dysuria in 13%.This was lesser than the results of the study by Paul A Tambyah et al[13], where in fever was present in 17.7% patients and dysuria in 6% of catheterized patients with UTI. Tambyah et al's study population was that of both bacterial and fungal infections in catheterized patients were as the current study included only patients with candiduria. Catheterization also makes the patient asymptomatic, as the presence of a catheter in the urethra prevents continuous exposure of urethral mucosa to organisms in infected urine thereby preventing infectious urethritis that produces dysuria, urgency in infected non catheterized patients.

Among the type of urine samples obtained, catheterized samples contributed to a total of 73% and midstream urine samples in 27% of patients. This was comparable to the study by Cl´audia CasteloBrancoArtiaga Kobayashi et al[14]and study by Arlene O.Cantillep et al[11]where in 84.4% and 89% of patients were catheterized. Catheterization was described by many authors as the most important risk factor for Candiduria. Most of the patients in our study belonged to medical ward, contributing to 36% of patients, followed by nephrology 19%. This was similar to the study by Stephen P storfer[15]. The risk factors were also found to vary in different wards. Diabetes mellitus was the most common risk factor

in patients from medical wards, CKD and other diseases of urinary tract were common in nephrology and urology. ICU and surgery ward patients showed prolonged antibiotic use and catheterization as the common risk factors[11].

In the present study prolonged antibiotic use (82%) and catheterization (67%) were the most common risk factor associated with candiduria. This was lower than the results by Uma Chaudary et al[16], who showed antibiotics as a risk factor in 99.6% of patients, catheterization in 90%, because the study population in her study included critically ill patients with candiduria rather than the hospitalized patients in our study. Antibiotics alter the normal flora of the genito-urinary tract, thus making way for colonization by *Candida species*. The surface of catheters also help in colonization with *non-albicans Candida spp.*

Diabetes mellitus (44%) was most common disease associated, followed by CKD (33%). This was slightly higher than the study by Cl´audia Castelo Branco et al[14], who showed 26.7% of the patients, had Diabetes probably because; Indians are more prone to Diabetes.India is termed as the "Diabetes capital of the world". Immuno suppressives like steroids were also important risk factors, as they alter the natural immunity to *Candida spp*. These drugs were used as a therapy for transplant recipients, neurosurgery patients and for patients with auto immune diseases. Unconsciousness was another risk factor, as most of the patients were on prolonged indwelling catheter and antibiotic therapy.

The present study showed predominance of *non-albicans Candida spp.* contributing to 86% of isolates and *C.albicans*contributing only 14% of the isolates. This was comparable to the results obtained by Manisha jain et al[17], who showed *non-albicans Candida spp*. as predominant isolate in 71.4% from urine isolates.

The species distribution was as follows, *C.tropicalis* 62%, *C.albicans* 14%, *C.glabrata 10% C.krusei* 9% and *C.parapsilosis* 5% which is comparable to the results by Manisha Jain et al[17] from north India. Her study showed 52.9% of isolates as *C.tropicalis* and 29.8% as *C.albicans*. In contrast, the studies by Elza Helena Da Silva et al[18]and N.Febre et al[19] from Brazil showed *C.albicans* as predominant species contributing to about 56% and 46.15% respectively. *Non-albicans Candida spp* were common in both catheterized and mid-stream urine samples. This is an important observation as 26.5% of catheter associated infections are due to fungi[20]. Biofilm formation

of the *C. tropicalis* strain on the catheter surface may contribute to the colonization in patients with urinary catheter. Biofilms of *C.tropicalis*, with an extensive, hexosamine-rich matrix, were poorly penetrated by antifungal agents, whereas biofilms of *C.albicans*, with a less-extensive glucose rich matrix, were more readily penetrated by drugs[21]. The exact reason for the increase in *non-albicans Candida spp.* is incompletely understood. *C.albicans* was found in 37% of the midstream samples but it contributed to only in 5.4% in catheterized patients.*C.albicans* was more common in midstream urine samples than catheterized patients.

Hi-Chrom agar was used for *Candida* speciation in our study. The methods of identification of *Candida* by corn meal agar and sugar assimilation tests are very time consuming; On CMA it takes around (24hr-72hr) and in case of sugar assimilation test it may take around (72hrs-2weeks). Besides, these procedures are labour intensive and take a longer time to determine the diagnosis. Several chromogenicsubstrate containing culture media has been developed. The advantages are, it should support the growth of yeast but not of bacteria. It should facilitate the recognition of specimen containing mixture of yeast species and exposure of fungi to the different indicator substances should not affect the viabilities for subsequent subculture. CHROM agar is a chromogenic substrate containing culture medium which fulfils this entire requirement. CHROM agar *Candida* is a differential culture medium being widely used to differentiate *Candida*species. These chromogenic media yield colonies of different colours secondary to chromogenic substance that react with the enzymes secreted by the organisms[22]. The major advantage of CHROM agar is the ability to detect mixed cultures of yeast in clinical specimens[23].

In the present study, *C.albicans* was identified as light green colour colonies in CHROM agar and C.*glabrata*produced pink to purple colour colonies as compared to studies done by Baradkar et al[24]. *C.parapsilosis* produced cream coloured colonies and*C.tropicalis* produced steel blue colour colonies as mentioned in Hi-media.

Study done by Yucesoy et al[26], revealed that all *C.krusei* isolates produced rough, fuzzy spreading big pink colonies on CHROM agar. Our study showed colonies of *C.krusei* to be large, fuzzy, rough and pink. Rapid identification of *C.krusei* with chromogenic media is important because it exhibits innate resistance to flucanazole.

In this study, sugar assimilation test was done using Rapid identification kit from Hi-media Mumbai [KB006 HiCandida Identification Kit]. KB006 is a standardized test system that can be used for identification and differentitiation of *Candida* species. Each KB006 Kit is a standardized colorimetric identification system utilizing twelve (Urease, Melibiose, Lactose, Maltose, Sucrose, Galactose, Cellobiose, Inositol, Xylose, Dulcitol, Raffinose, Trehalose) conventional biochemical tests. The test is based on the principle of pH changes which are indicated by a spontaneous colour change in the media. *Candida* species isolated by using rapid identification kit was *C.albicans* 12% (12cases), *C.glabrata* 6% (6cases), *C.tropicalis* 58%(58 cases), *C.krusei* 6% (6cases) and *C.parapsilosis* 4% (4 cases) with a total sensitivity of 86% which was similar to study done by AnjanaGopi et al[25].

In this study we observed that Hi-Chrom agar was the best method of speciation as it identified all the *Candida* species with 98% sensitivity and 100% specificity, which was similar to studies done by Yucesoy et al which showed 97% sensitivity and 100% specificity and Wilinger et al showed 98.8% sensitivity and 100% specificity for all *Candida* species as compared to sugar assimilation test using rapid identification kit[26,27].In vitro antifungal susceptibility testing is becoming important because of the emergence of new *non-albicans Candida* species and increased intrinsic and acquired resistance to azoles and amphotericin B.Soagar based antifungal susceptibility testing is an alternative to the microdilution method. It is easy to perform and inexpensive for routine laboratories. CLSI M44-A disc diffusion testing with glucose methylene blue Muller Hinton Agar is a very convenient method for antifungal susceptibility testing [28].In the current study, the overall susceptibility rate of fluconazole for *C.albicans* was 71.4% and *C.tropicalis* was 67.7%. Susceptible dose dependent for *C.albicans* 21.4% *and C.tropicalis* 9.6%.Resistance for *C.albicans* was 7.1% and *C.tropicalis* 22.5%. In our study *C.glabrata* and *C.krusei* showed resistance to fluconazole, as similar to study done by Sobel et al[29].*C.krusei and C.glabrata* is known for its intrinsic resistance to fluconazole[30]. The overall susceptibility rate for itraconazole was, for *C.albicans* 57.1%, for *C.glabrata* 60%, for *C.tropicalis* 77.4% and for *C.krusei* 55.5%. Susceptible dose dependent for *C.albicans* was 21.4% and *C.glabrata* 20%. Resistance for *C.albicans* was

21.4%, for *C.glabrata* 20%, for *C.tropicalis* 16.1% and for *C.krusei* 44.4%. Antifungal susceptibility testing to amphotericin B shows minimal resistance pattern and high susceptibility rate to different *Candida* species. Susceptibility rate for *C.albicans* was 85.7%, for *C.glabrata* was 100%, *C.tropicalis*was 93.4% and for *C.krusei* was 66.6%. Resistance for *C.albicans* was 14.2%, for *C.glabrata* 0%, for *C.tropicalis*6.4% and for *C.krusei* 33.33%. Our finding shows an overall susceptibility rate of amphotericin B to be 90% which correlate well with the study done by Saldanha et al and Noake et al were *Candida* species were more susceptible to amphotericin B (92%)[31,32].

CONCLUSION:

Candiduria should never be ignored as it can be the only indication of systemic or invasive candidiasis. Our study showed a predominance of *non-albicans Candida spp.* of about 86%. *C.tropicalis*(62%) was the most common isolate obtained followed by *C.albicans* (14%), C.glabrata (10%), *C.krusei* (9%) and *C.parapsilosis* (5%). Indwelling urinary catheter was an important associated risk factor for *non-albicans* candiduria. Multiple risk factors like long term antibiotic therapy, prolonged catheterization and Diabetes mellitus were present in many patients.

Hi-Chrom agar takes only 48 hrs for species identification, and is a comfortable alternative to conventional methods, that take 96-120 hrs. Hi-Chrom agar is superior to other conventional methods available for rapid detection of Candida species. Further the increasing rates of resistance particularly among the *non-albicans Candida spp*. emphasizes the need for speciation and antifungal susceptibility testing a routine in all microbiology laboratories due to alarming increase of resistant fungal infections.

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