Determination of incidence of different *Candida spp.* in clinical specimens and characterisation of *Candida species* isolates

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Abstract

Aims & Objectives:

1. To isolate and characterize the Candida species from various clinical samples.
2. To determine the spectrum of Candidiasis.
3. To determine the predisposing factors for Candidiasis.

Method: This Prospective Analytical study was conducted in the Department of Microbiology of a Teaching Tertiary care Rural hospital of Western India over a period of 1½ years after obtaining Ethical clearance. A total of 1438 samples submitted to department of microbiology for routine diagnostic work up were assessed. A detailed review of history & clinical findings was undertaken. Fifty isolates which fulfilled the diagnostic criteria for Candidiasis were selected, included in the study and subjected to a battery of microbiological investigations aimed at detecting, isolating, identifying and characterizing Candida sp.so as to determine the spectrum of Candidiasis.

Results: The incidence rate of Candidiasis in clinical specimens came out to be 35 per thousand samples per year. The species wise distribution of Candida isolates was C.albicans(42%), C.tropicalis(50%), C.glabrata(4%), C.parapsilosis(2%) & C.krusei(2%).

Conclusion: Results indicate a significant shift in the species causing invasive Candidiasis away from C.albicans to more resistant Non-albicans species which has important clinical implications.

Keywords: Candidiasis, Characterisation, Shift, Non-albicans sp., Germ tube test, Chlamydospore, Sugar assimilation test.

Introduction

Candida species are components of normal microbial flora of human body inhabiting mouth, intestines and vagina[1,2]. When immunological defence mechanisms are compromised it causes infection in the sites where it is colonised and also elsewhere in the body.

Candida is an opportunistic endogenous infection. The factors predisposing to opportunistic infections act either by altering the balance of normal microbial flora of the body or by lowering the host resistance[1,2,3,4].

The dramatic surge in incidence of candida spp.is due to increasing population of terminally ill, debilitated, immunocompromised patients, increasing use of advanced therapeutic modalities for advanced life support; indwelling devices; widespread use of broad spectrum antibiotics; long term use of immunosuppressive agents; increasing incidence of HIV infection; high prevalence of Candida hand carriage in health care workers and ability of Candida sp.to survive on environmental surfaces[3,5,6,7,8].

Candida sp. had become 4thleading cause of blood stream infection & 6th most common nosocomial pathogen. It is associated with prolonged hospitalization, increased cost of treatment & delays recovery requiring extra resources for investigations, management & nursing care. Candidemia is associated with increase in attributable mortality.[1,3,6,7,8]

Candida species are important nosocomial pathogens in critically ill and immunocompromised patients and there has been an important shift in the species causing invasive candidiasis away from Candida albicans to more resistant non-albicans variety which is associated with higher mortality and significant morbidity. These infections are often severe, rapidly progressive, difficult to diagnose and refractory to therapy[2,4,5].

As a result of introduction of fluconazole prophylaxis non-albicans Candida has now emerged as a significant pathogen[4,9].

Knowledge of patterns of genital Candida species level identification is important for management as species other than Candida albicans often fail first line treatment. Definite microbiological diagnosis be made for women with recurrent symptoms or those failing initial treatment to guide appropriate therapy. Early detection and initiation of appropriate therapy may alter the course of these infections and improve the prognosis[10,11].

In the light of above facts present study was undertaken to determine the incidence of Candida infection among patients of a tertiary care rural teaching hospital of western India and to characterise species of Candida isolates from various clinical specimens so as to determine the species distribution and to detect any shift in incidence from C.albicans to Non-albicans species.

Material and Methods

This Prospective analytical study was carried out at Department of Microbiology of a Rural Tertiary care Teaching Hospital of Western India over a period of 1½ years after obtaining ethical clearance.

Sample size consisted of 50 consecutive isolates from the clinical samples submitted to Dept. of Microbiology for routine diagnostic workup. A total of 1438 samples were processed. Different samples included Urine, Blood, Sputum, Pus, Body fluids collected under aseptic precautions were processed by a battery of microbiological investigations aimed at detecting, isolating, identifying and characterizing the Candida sp. so as to determine the spectrum of Candidiasis.

Processing of the samples using Standard Microbiological techniques[1,12,13,14,15,16,17,18,19,20]:
The following protocol was instituted:

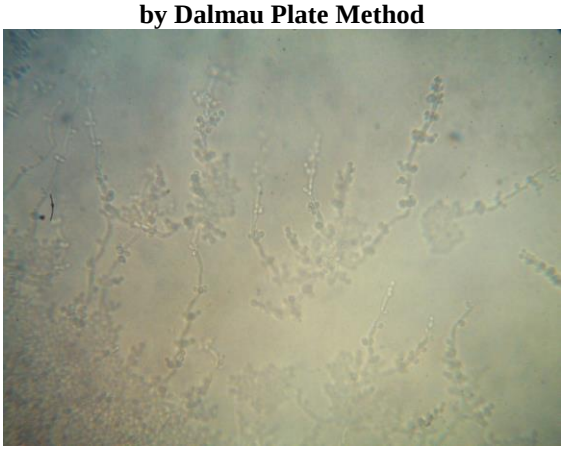
1. Direct Microscopy: KOH-Wet-Mount Normal Saline preparation
2. Gram staining of primary smear.
3. Culture on Blood agar
4. Gram staining of culture – smear
5. Subculture on Sabouraud's Dextrose Agar (SDA) Slants (Containing Cycloheximide and Chloramphenicol)

Incubated at 35ºC and examined twice a week for growth of cream coloured smooth pasty colonies S/o Candida growth.

Slants were incubated for 1 week and discarded if no growth occurred by then.

1. LPCB mount prepared from the colonies to examine yeast cells and pseudohyphae.
2. Germ Tube Test (Reynolds Braude Phenomenon)
3. Chlamydospore formation on Corn-meal agar (Dalmau Technique)
4. Sugar Assimilation Test
5. Culture on Tetrazolium Reduction Medium (Chromogenic medium)

Photo 1: C. albicans-Corn Meal Agar Morphology by Dalmau Plate Method



Production of terminal, thick walled large chlamydospore and presence of blastoconidia that are arranged in dense clusters, evenly distributed along the pseudohyphae.

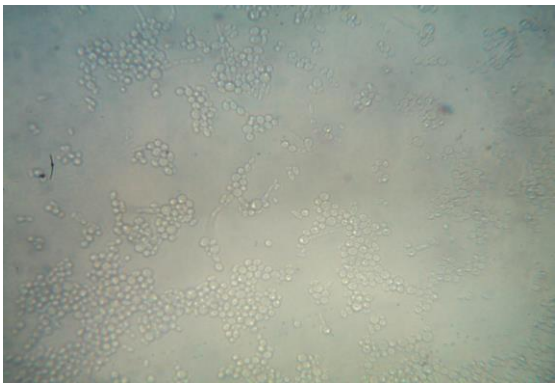
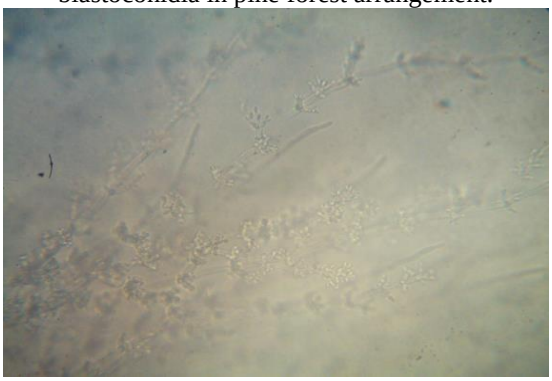


Photo 2: C.tropicalis: Corn Meal Agar Morphology by Dalmau Plate Method produces pseudohyphae with blastoconidia in pine forest arrangement.



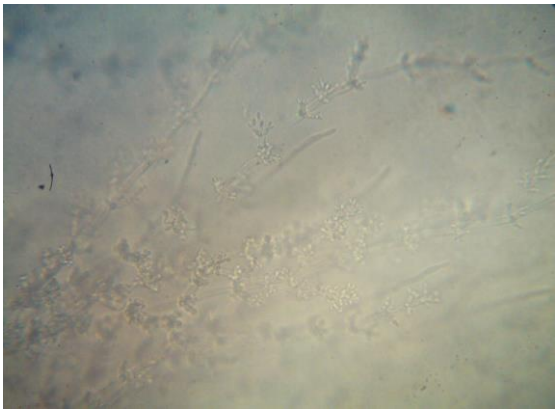


Photo 3: C. parapsilosis –Corn Meal Agar Morphology by Dalmau Plate Method: Curved hyphae (Sage brush pattern)

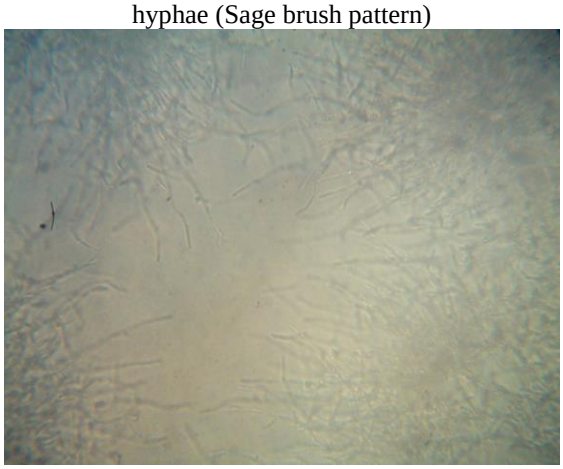


Photo 4: C. Krusei- Corn Meal Agar Morphology Elongated yeasts, abundant pseudohyphae (crossed match stick like app.)

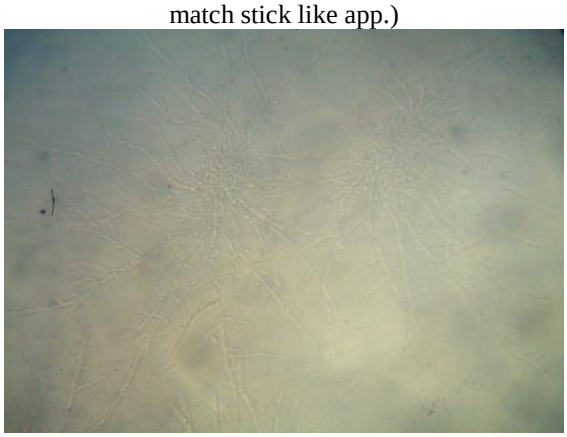


Photo 5: C. glabrata-Cornmeal Agar Morphology: Yeast forms only no – pseudohyphae

Sugar Assimilation Test (Auxanographic plate method Haley and Standard modification[1,12,13,14]
Sugar discs used were as follows:

1. Dextrose
2. Maltose
3. Galactose
4. Sucrose
5. Xylose
6. Trehalose
7. Cellobiose
8. Lactose
9. Raffinose

Table 1: Sugar Assimilation Pattern of Candida isolates

	Dext.	Mal	Gal	Suc	Xyl	Tre	Cel	Lact	Raff
1. C.albicans	+	+	+	+	+	+	+	+	-
2. C.tropicalis	+	+	+	+	+	+	+	-	-
3. C.parapsilosis	+	+	+	+	+	+	-	-	-
4. C.glabrata	+	-	-	-	+	+	-	-	-
5. C.krusei	+	-	-	-	+	-	-	-	-

Dext.-Dextrose Mal- Maltose Gal-Galactose Suc-Sucrose Xyl-Xylose
Tre-Trehalose Cel-Celobiose Lact-Lactose Raff- Raffinose

Photo 6: Sugar Assimilation Pattern of Candida albicans

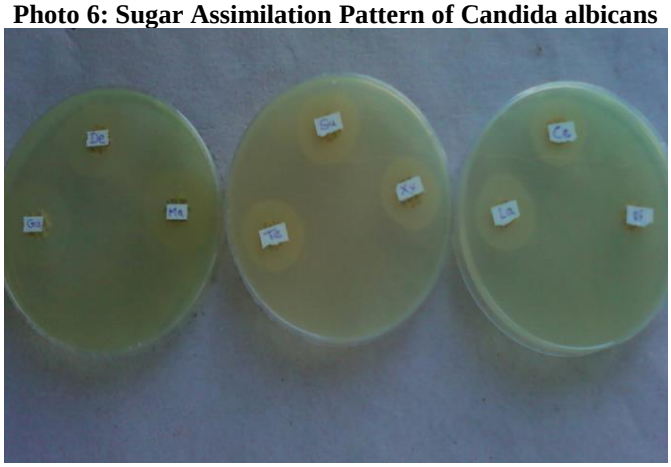


Photo 7: Sugar Assimilation Pattern of Candida tropicalis

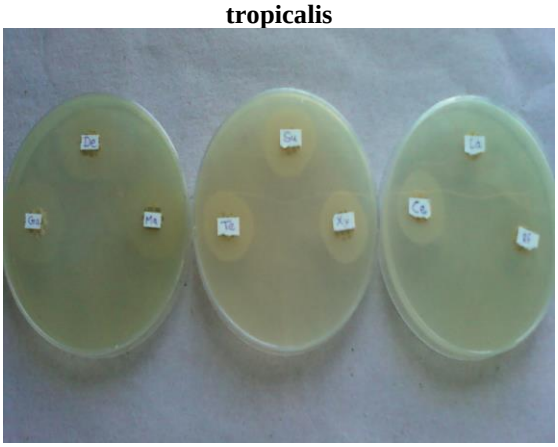


Photo 8: Sugar Assimilation Pattern of Candida parapsilosis

Photo 9: Sugar Assimilation pattern of Candida glabrata

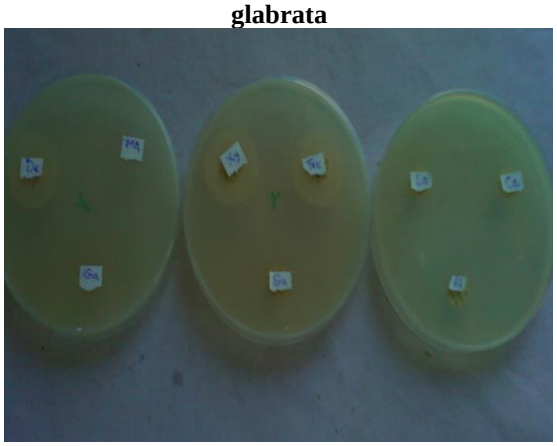


Photo 10: Sugar Assimilation Pattern of Candida krusei

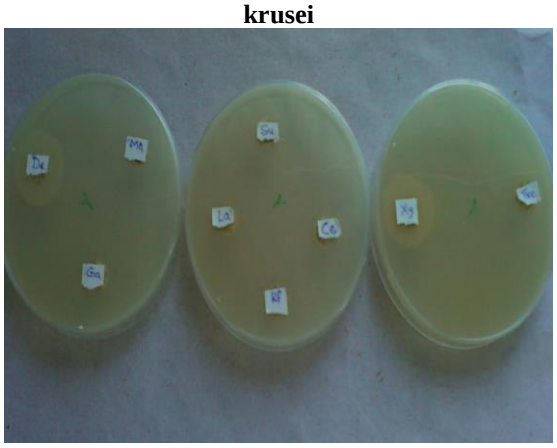


Photo 2: Differentiation of Candida species on Tetrazolium Reduction medium

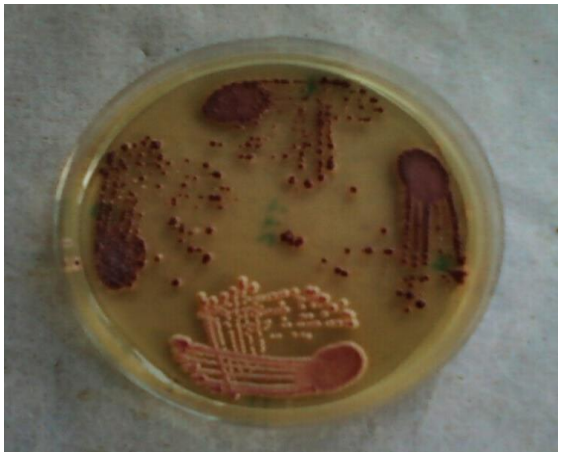


Table 2: Differentiation of Candida species on Tetrazolium Reduction medium

Candida Species	Colour of colonies
C.albicans	Pale pink
C.tropicalis	maroon
C.parapsilosis	Rose-pink
C.glabrata	Pale pink
C.krusei	Pink and dry

Results and Observations

In this prospective analytical study a total of 1438 clinical samples submitted to Department of Microbiology for routine diagnostic workup were processed.

Candida isolates selected from 50 cases fulfilling the diagnostic criteria for Candidiasis were included in the study and were further processed for species determination.

The incidence rate of Candidiasis in Clinical specimens submitted to the department of microbiology for routine diagnostic work up had come out to be 35 per thousand samples processed per year.

Out of fifty isolates number of Candida albicans isolated was 21(42%) whereas Non albicans species constituted 58% of Candida isolates with C.tropicalis being the commonest isolate comprising half of the total isolates. Other species included C.glabrata(4%), C.krusei(2%) and C.parapsilosis(2%).

In the present study it is observed that infection due to Candida non-albicans is more common than Candida albicans indicating a shift of trend from C.albicans to non-albicans which is arising as a emerging infection.

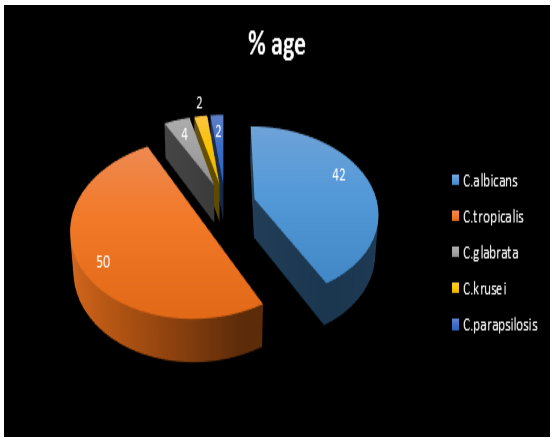


Fig. 1: Species wise distribution of candida isolates

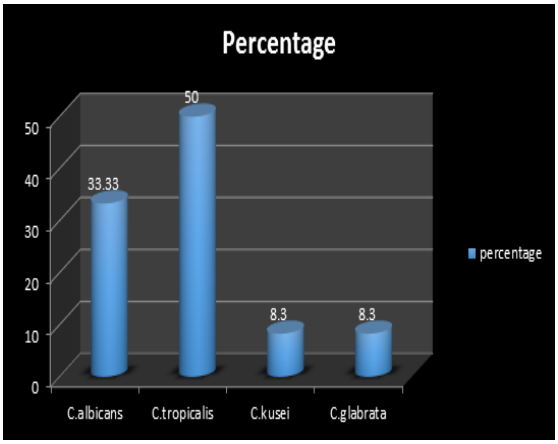


Fig. 2: Species wise distribution of Candida sp. isolated from blood

Out of 50 candida isolates 12(24%) were isolated from blood specimens. Candida tropicalis was the predominant organism constituting half of the total isolates from blood followed by C.albicans(33.33%), C.krusei(8.3%) and C.glabrata(8.3%).

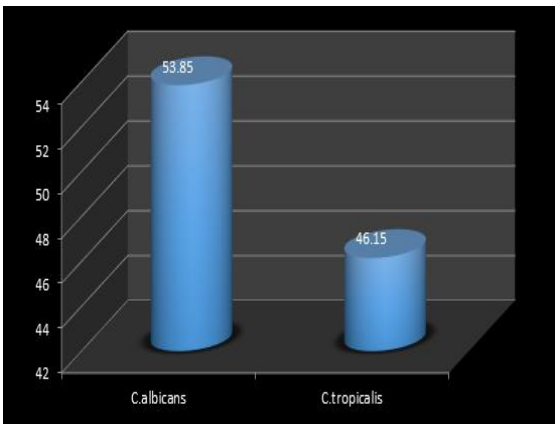


Fig. 3: Species wise distribution of Candida species isolated from sputum

Out of 50 isolates, thirteen (26%) were isolated from sputum. Candida albicans was the predominant

organism constituting 53.85% of the sputum isolates followed by Candida albicans(46.15%)

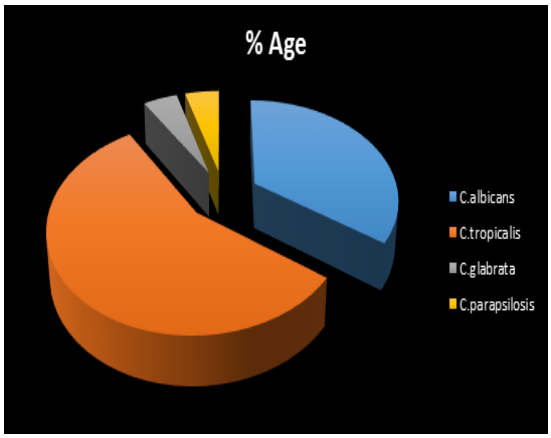


Fig. 4: Species wise distribution of Candida sp. isolated from urine

In the present study the largest number of Candida isolates were from urine (46%), C.tropicalis was the predominant organism (56.52%) followed by C.albicans (34.8%), C.glabrata (4.5%) and C.parapsilosis (4.5%).

Predisposing factors present in patients included in the study

Table 3: Predisposing factors present in patients under study

Predisposing Factor	Numbers(n=50)
Infants	10
Age more than 50yrs.	17
Diabetes mellitus	10
Indwelling devices	50
Steroids	13
Surgery	3
Antibiotic usage	46
Mech, Ventilation	6
Prolonged hospitalisation	32
ARF/CRF	4
Hemodialysis	4

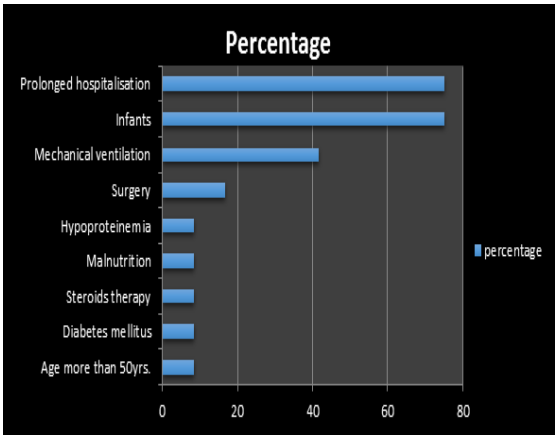


Fig. 5: Percentage distribution of predisposing factors present in patients under study

Out of fifty patients 17(34%) were more than 50yrs.of age and 10 were less than 1yr.of age. The most common predisposing factor were indwelling devices and antibiotic usage followed by prolonged hospitalization(64%). Other associated risk factors were Diabetes mellitus(20%), Steroids(26%), Surgery(6%), Mechanical ventilation(12%), ARF/CRF(8%) and Hemodialysis(8%).

There were multiple risk factors present in all the cases. Other predisposing factors present were preterm babies, premature low birth weight babies, babies with congenital anomalies, endocrinopathies other than DM, malnutrition, hypoproteinemia, neoplasm, renal calculi, trauma, wounds, neutropenia and cytotoxic drugs.

Table 4: Predisposing factors in patients yielding candida isolates in sputum

Predisposing Factor	Numbers(n=13)
Age more than 50yrs.	5
Diabetes mellitus	1
Indwelling devices	13
Steroids	6
Antibiotic Usage	13
Mechanical Ventilation	4
Prolonged Hospitalisation	7
Pulmonary Tuberculosis	6
COPD	4
Smoking	5

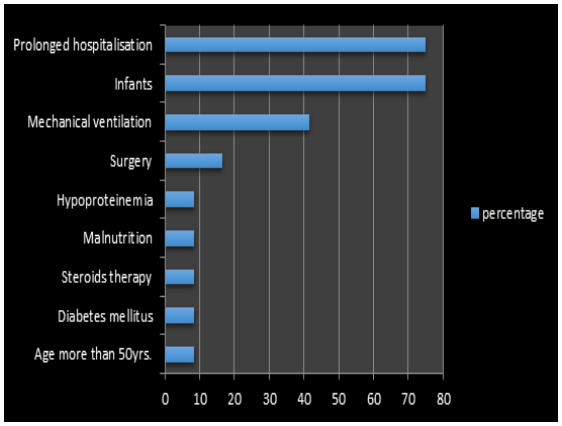


Fig. 6: Percentage distribution of predisposing factors in patients yielding candida isolates in sputum

Table 5: Predisposing factors in cases yielding candida isolates in urine

Predisposing factors	Number of patients
Age more than 50yrs	8
Diabetes mellitus	7
Prolonged hospitalisation	7
Steroid	3
Indwelling devices	23
Urinary Cathetres	3
Urinary tract pathology	6
Antibiotic usage	20
Malignancy	1

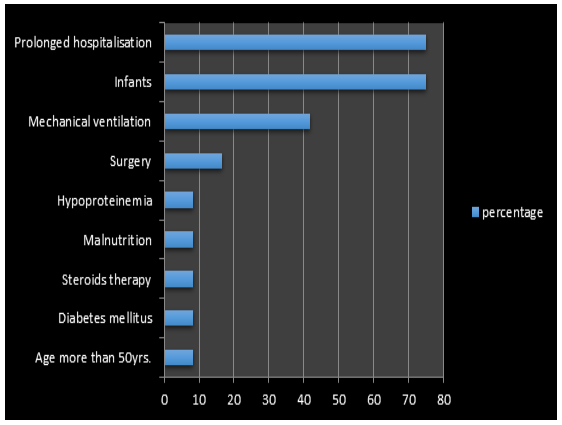


Fig. 7: Percentage distribution of predisposing factors in cases yielding Candida isolates in urine

Table 6: Predisposing factors in cases of candidemia

Predisposing factors	Number of patients
Infants	9
Age more than 50yrs.	1
Diabetes mellitus	1
Prolonged hospitalisation	9
Steroid	1
Indwelling devices	12
Antibiotic usage	10
Surgery	2
Malnutrition	1
Hypoproteinemia	1
Mechanical ventilation	5

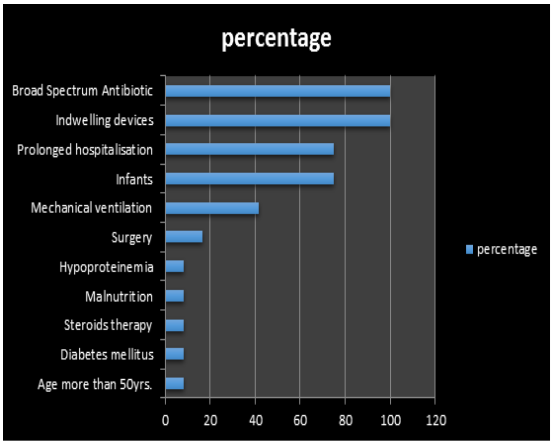


Fig. 8: Percentage distribution of predisposing factors in candidemia cases

Table 7: Sample wise distribution of candida isolates

Samples	C.albicans	C.tropicalis	C.krusei	C.glabrata	C.parapsilosis	Total
Urine	8	13	-	1	1	23
Blood	4	6	1	1	-	12
Sputum	7	6	-	-	-	13
Pus	2	-	-	-	-	2
Total	21	25	1	2	1	50

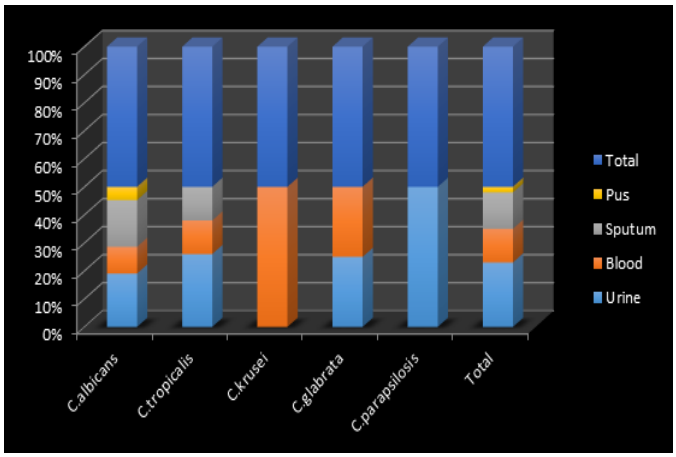


Fig. 9: Sample wise distribution of different candida isolates

Discussion

This prospective study was carried out at Department of Microbiolgy of a Rural tertiary care Teaching Hospital of Western India from January2010 to August 2011. A total of 1438 samples submitted to Department of Microbiology for routine diagnostic workup were processed. Candida isolates from fifty cases which fulfilled the diagnostic criteria for Candidiasis were selected and included in the study and further processed by a battery of microbiological investigations aimed at detecting, isolating, identifying and characterizing the Candida sp. so as to determine the spectrum of Candidiasis.

Sample wise distribution: The present study had a sample size of Fifty with sample wise distribution of Candida isolates as: Urine-46%(23), Blood-24%(12), Sputum-26%(13) and Pus-4%(2). A predominance of Urinary isolates was observed.

Capoor MR et al in a study carried out in 2005 at Safdarjung Hospital, New Delhi also observed a predominance of Urinary isolates(62.7%) followed by Blood isolates.

Agarwal S et al in a study conducted at a Pediatric tertiary care hospital recovered 169 isolates from Blood(58.5%), Urine(26%), tracheal aspirate(10%) and CSF(0.6%).

Age and sex wise distribution of cases: In the present study the age range of the cases was from 1day to 85yrs. The median age being 32yrs. Maximum number of patients belonged to age group 0-10yrs (28%) majority of them being infants, followed by age groups 31-40(16%), 51-60(14%) and 61-70(14%)yrs.

This observation is in accordance with studies conducted by Shivprakasha S et al, Bannerjee U et al and Capoor MR et al.

In the present study there was predominance of males (62%) over females (38%) Male:Female ratio being 1.63:1.

This observation is in accordance with studies conducted by Bannerjee U et al, Capoor MR et al and Shivprakasha S et al.

Predisposing Factors observed: In the present study the important associated predisposing factors were persistent use of Broad spectrum antibiotics, presence of indwelling devices, prolonged hospitalisation, Steroids, Diabetes mellitus, Mechanical ventilation and extremes of age.

All of our patients had history of administration of Broad spectrum antibiotics for variable periods, 92% of our patients were having indwelling devices (intravenous cannulae, Central venous catheter, Urinary catheter, Ryle's Tube, Endotracheal Tube), 64% had history of prolonged hospitalisation, half of the patients were in extremes of age, 26% were on steroids, 20% were diabetic, 12% were on mechanical ventilation, 8% were having Acute or chronic renal failure,8% were on hemodialysis and 6% had undergone major surgery.

These findings are in accordance with various Indian studies conducted by Capoor MR et al, Chakrabarti et al, Saha et al, Sahni et al, Chowta MN et al, Banerjee U et al, Goel N et al, Adhikari R et al, Rani R et al, Arora D et al and international studies like that of Nur Yapar et al, Benzamin DK et al and Dimopoulos G et al.

Hospital Location wise distribution of Candida isolates: In the present study it was observed that maximum number of isolates were from patients admitted in different intensive care units of the hospital(56%) viz MICU(32%), NICU(16%), PICU(6%) and SICU(2%). Twenty two isolates were from wards out of which eight were from patients transferred to wards from different ICUs.

The above observation is in accordance with the studies conducted by Capoor MR et al, Shivprakasha S et al, Sahni V et al and NurYapar et al where maximum number of isolates where obtained from ICUs.

Species wise distribution of Candida isolates:

In this study out of 50 Candida isolates following 5 species were isolated: *C.albicans, C.tropicalis, C.parapsilosis, C.glabrata* and *C.krusei*.

This finding is in accordance with the studies conducted by Petmey J et al, Ariane BN et al,

Mokaddas EM et al, Jha BK et al and Kothari A et al who also isolated these five species.

The species wise percentage distribution of Candida isolates in this study is:

C.albicans(42%), C.tropicalis(50%), C.glabrata(4%), C.krusei(2%), C.parapsilosis(2%).

In this study Non albicans Candida species had outnumbered *C.albicans* with 58% of the total isolates belonging to Non albicans species, *C.tropicalis* being the most frequently encountered isolate.

This observation is in accordance with various Indian studies conducted by Shivprakasha et al, Chakrabarti et al, Kothari et al, Jain M et al, Rani R et al, Adhikary R et al, Goel N et al, Saha R et al, Banerjee U et al, Sahni V et al and Agarwal J et al which showed a predominance of Non-albicans species with *C.tropicalis* emerging as most frequent isolate.

In the studies conducted by Narain S et al, Ariane BN et al, Capoor MR et al, Mokkadas EM et al, Lee JS et al and Cisterna R et al although C.albicans was found to be the predominant species but a significant shift towards Non albicans species was reported.

One of the probable reasons behind emergence of Candida spp.other than *C.albicans* apart from the associated risk factors and underlying conditions was the widespread use of Fluconazole as empirical therapy or prophylaxis which would select the yeast species intrinsically resistant or less sensitive to Fluconazole such as *C.krusei*, *C.glabrata* or *C.tropicalis*. The selection of such innately resistant species creates problems and complicates the management of Candidiasis leading to treatment failures.

The studies conducted by Capoor MR et al, Mokaddas EM et al, Shivprakasha S et al, Lee JS et al and Cisterna R et al showed a significantly high isolation rates of *C.parapsilosis*.

A five year study(2001-2005) by Banerjee U et al showed an increase in isolation rate of *C.parapsilosis* from 15% to 34% as a result it became most predominant species in 2005.

C.parapsilosis is known to cause fungaemia among hospitalised patients and is known to form biofilm in glycosylated solutions and adhere to plastic materials such as catheters, gastric probes and parenteral nutrition tubes.

Conclusion

This study has reported a significant shift in incidence of invasive Candidiasis from C.albicans to Non albicans species. Non-albicans Candida spp. has emerged as an important opportunistic pathogen and assuming an increasing role in nosocomial infections.

This shift is alarming as the infections caused by non-albicans species are often severe, rapidly progressive, refractory to therapy and associated with higher mortality and significant morbidity. The innate and intrinsic resistance of Non-albicans sp. to the commonly used antifungal agents create problems and complicate the management of the patients. This leads to prolonged hospitalisation, increased costs of treatment and delays recovery requiring extra resources for investigations, management and nursing care. Continued surveillance of invasive Candidiasis will be important to track trends of this serious infection and to document changes in its epidemiological features. More active screening in high risk groups should be done to avoid diagnostic delay.

Early detection of the pathogen and institution of appropriate timely therapy alters the course of infection and improves the prognosis thus benefitting the patient.

Conflict of Interest: None

Source of Support: Nil

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