International Journal of Research and Review
www.ijrrjournal.com E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Isolation, Identification and Anti-Fungal Susceptibility Testing of Candida Species from Various Clinical Specimens of ICU Patients in a Tertiary Care Hospital of Bhagalpur, Bihar

Mukesh Azad[1], Suprakash Das[1], Amit Kumar[2]

[1]Senior Resident, Dept of Microbiology, AIIMS, Patna.
[2]Associate Professor and H.O.D, Dept of Microbiology, JLNMCH, Bhagalpur.

Corresponding Author: Suprakash Das

ABSTRACT

Background: C*andida* spp. are the normal flora of human skin and mucosa and there is an increasing incidence of candida infections among hospitalized patients mostly the immune-suppressed individuals. *Candida albicans* predominantly cause candidiasis responsible for about 60-80% of infections. Emergence of non-albicans species in increasing numbers as potential pathogens has been noted during last decades. Species level identification of *Candida* spp. is clinically important due to the fact that they differ in virulence and antifungal susceptibility. Rapid identification of *Candida* spp. can also help with early management of antifungal therapy. Not much study have been done about the *C. albicans* and NAC infections from Bihar, so we aimed this study to know the prevalence and anti-fungal susceptibility of fungal isolates from ICU patients.

Methods: This prospective analytical study was conducted in the department of microbiology of Jawahar Lal Nehru Medical College and Hospital, Bhagalpur during the period of June 2017 to June 2018 to evaluate anti-fungal susceptibility of fungal isolates from 100 patients admitted in different ICUs.

Results: A total of100 samples were received from different ICUs during the study period, of which 25 (25%) were positive for fungal growth. The prevalence of *Candida* and NAC isolates are 20 % and 5 % respectively.

Conclusion: We conclude that the Candida spp. are an important opportunistic pathogen in ICU settings over the 1 year study period and constant monitoring of the changing epidemiology and resistance pattern of Candida species is needed to guide the clinicians.

Key Words: Candida, Non-albicans candida (NAC), ICUs, Fluconazole, Amphotericin-B.

INTRODUCTION

Candida spp. are human commensal that belong to the category of yeast like fungus and can behave like opportunistic pathogen if there are lowering of local or systemic host resistance. [1] C*andida* spp. are the normal flora of human skin and mucosa, but have been reported more frequently as pathogen due to risk factors such as excessive consumption of a broad spectrum antibiotic, underlying malignant diseases, HIV infection, organ transplantation, prolonged hospital stay, and exposure to invasive procedures. [2,3] *Candida albicans* predominantly cause candidiasis, responsible for about 60-80% of infections. Emergence of non-albicans species in increasing numbers as potential pathogens has been noted during last decades. [4, 5]

Candida spp. can cause a variety of infections including systemic infections like blood stream infections (BSIs) and disseminated candidiasis. Studies show that *Candida* ranks fourth in the United States and seventh in Europe as a causative agent of Blood Stream Infections. [6, 7] Only few studies from India have reported candidemia rates (6-18%) and increase in isolation of non-albicans *Candida* (NAC) from BSIs. [8-11] Candidemia increases mortality rate by 20-49% [12, 13] and nosocomial candidiasis are associated with crude mortality rate of over 60%, while the attributable mortality rate may be as high as 49%. [14, 15] The genus *Candida* is consists of more than 17 different *Candida* spp. those are responsible for different human infections. However, more than 90% of invasive infections are caused by *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis,* and *Candida krusei*. [16, 17]

Recovery of yeasts from normally sterile body fluids (blood, cerebrospinal fluid, etc), recovery from patients whose defenses were compromised from chronic diseases and repeated recovery from multiple specimens certainly indicates infection with the yeasts. [17] Antifungal agents available for the treatment of systemic and invasive candidiasis are restricted to polyenes, allylamines, azoles, and the recently developed echinocandin class of molecules. Characterization to species level helps to identify those strains which might be intrinsically resistant to some antifungal agents. [18] Incidence of antifungal resistance among *Candida* spp. is increasing over the past decade. [19, 20]

Species level identification of *Candida* spp. is clinically important due to the fact that they differ in virulence and antifungal susceptibility. [21] Rapid identification of *Candida* spp. can also help with early management of antifungal therapy. Not much study have been done about the *C. albicans* and NAC infections from Bihar, so we aimed this study to know the prevalence and anti-fungal susceptibility of fungal isolates from ICU patients.

MATERIALS AND METHODS

Study design- This prospective study was conducted in the department of microbiology of Jawahar Lal Nehru Medical College and Hospital, Bhagalpur during the period of June 2017 to June 2018.

Study Population and sample size- A sample size of 100 was considered for this study and were collected from patients admitted in different ICUs with intravenous therapy, chronic respiratory tract infections including tuberculosis, patients with post-surgery, sepsis, dialysis, PUO on long term therapy. The associated condition like HIV was seen in 18 cases and diabetes mellitus in 25 patients.

Sample- Urine, Blood, Sputum, Pus, Wound swab, Corneal ulcer, Throat swabs and Stool sample from patients with antibiotic associated diarrhea were included in the study. The specimens showing *Candida* isolation in two consecutive samples were included in the study.

Isolation and Identification-

Gram's stain was performed from direct samples and inoculated on Sabouraud dextrose agar, incubated at 37°C for 24 hours. The yeast like colony growth on culture showing budding yeast cells in Gram's staining method were confirmed as Candida by negative urease test.

Germ-tube test was performed on all urease negative yeast isolates for presumptive identification of *C. albicans*. *C. albicans* was further identified and differentiated from *C. dubliniensis* by growth at 45°C and chlamydospore formation on cornmeal agar (incubated at 30°C for 2-5 days and studied microscopically for the presence of pseudohyphae, chlamydospores & blastospores). [22] Acid & gas production in sugar fermentation testsusing Glucose, Maltose, Sucrose and Lactose in 2% concentration with Andred's indicator and Durham's tube were noted. [23]

Inoculated chromogenic HiCrome Candida agar (HiMedia, Mumbai) was studied after 48 - 72 hrs incubation at 30°C and the colour of the growth was used for speciation of Candida spp. according to the

manufacturer instructions. The isolates were tested biochemically by Hi-Candida Identification kits (HiMedia, Mumbai) for further confirmation (Fig- 7 and 8).

Antifungal susceptibility test- It was performed for all the isolates of Candida using disc diffusion method on Mueller Hinton agar supplemented with 2% glucose and 0.5 µg / ml of methylene blue as per CLSI guidelines. [24] The commercially available antifungal discs (HiMedia, Mumbai.) were used and zone of inhibition were measured after 24-48 hours incubation at 37°C. The antifungal discs used were Amphotericin - B (20 µg), Clotrimazole (10 µg), Fluconazole (10 µg), Itraconazole (10 µg), Ketoconazole (10 µg) and Nystatin (100 units). *C.albicans* (ATCC90028) was used as quality control strain.

RESULTS

A total of100 samples were received from different ICUs during the study period, of which 25 (25%) were positive for fungal growth. Among the positive samples, there were 20 *Candida albicans* isolates and 5 non *albicans candida* (NAC) isolates. The prevalence of *Candida* and NAC isolates are 20 % and 5 % respectively. There was a male predominance with a male to female ratio of 15: 10 = 1.5: 1 (Table-1 and Fig. 1, 2). Most of the positive isolates were from patient age group of 51-60 years old (6 isolates, 24%). (Table-3and Fig.-4) Among the NAC isolates the most common was *C. glabrata* (8%). (Table-4 and Fig.-5) Most of the *Candida albicans* isolates (10,50%) were isolated from urine and blood samples which also constitutes major proportion (53%) of the samples. NAC isolates were mostly isolated from blood samples (2, 40%). (Table-1 and Fig.-2) Most of the *Candida albicans* and NAC isolates were from medicine ICUs (32%). (Table-2 and Fig.-3) All of the *Candida albicans* isolates were sensitive to Amphotericin B while 80 % of NAC isolates were sensitive to Amphotericin B. NAC isolates show higher resistance to most of the antifungal agents than *Candida albicans* isolates for example Clotrimazole (60%), Fluconazole (60%), Itraconazole (40%) and Ketoconazole (20%). All *Candida* isolates were sensitive to Nystatin. The NAC isolates were resistant to multiple antifungal agents. (Table-5, 6 and Fig-6)

Table 1: Distribution of specimens and Candida isolates.

Sr. No.	Sample	No.	Male	Female	*C. albicans*	NAC	Total
1	Sputum	20	14	6	3	1	4
2	Urine	28	16	12	5	0	5
3	Blood	25	14	11	5	2	7
4	Pus	15	9	6	3	1	4
5	Wound swab	6	4	2	2	0	2
6	Corneal scrapings	2	2	0	1	1	2
7	Stool	4	3	1	1	0	1
Total		100	62	38	20	5	25

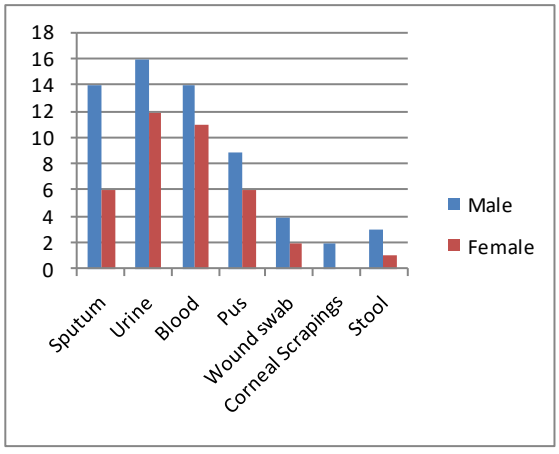

Fig. 1- Sample and Gender distribution of Candida isolates

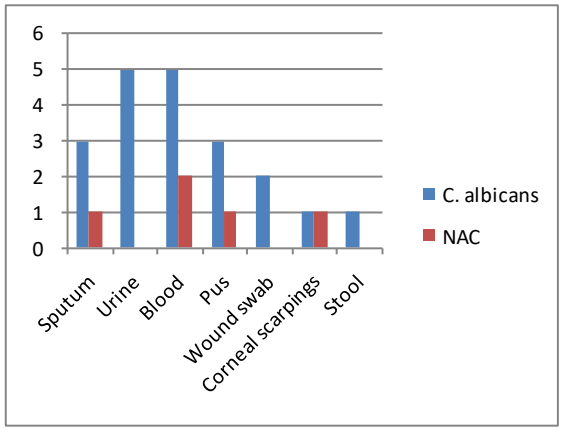

Fig. 2- Distribution of Candida isolates among various samples.

Table 2: Distribution of Candida isolates in different ICUs.

Sr. No.	ICU	*C. albicans* number	NAC	Total
1	Medicine	6	2	8(32%)
2	Surgery	4	1	5 (20 %)
3	Neonatal	3	0	3 (12%)
4	Paediatrics	4	2	6 (24%)
5	Trauma	3	0	3 (12%)

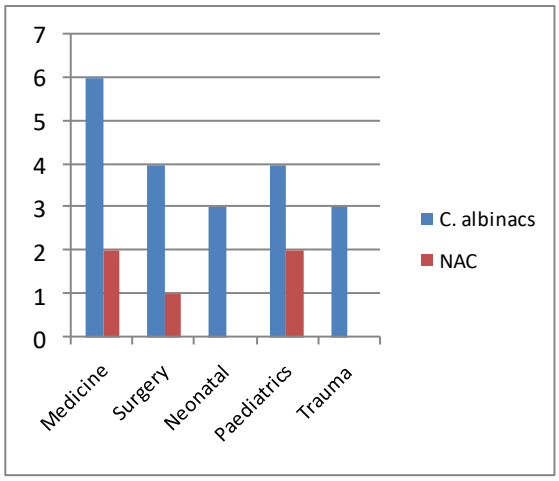

Fig. 3- Distribution of Candida isolates in different ICUs.

Table 3: Age and gender-wise distribution of *C.albicans*and non-albicans*Candida* species

Age	C. albicans		Non-albicans Candida		Total
	Male	Female	Male	Female	
0-10	1	1	1	0	3
11-20	1	2	0	0	3
21-30	2	1	0	0	3
31-40	1	0	0	2	3
41-50	0	0	0	1	1
51-60	3	2	1	0	6
61-70	3	1	0	0	4
71-80	2	0	0	0	2
Total	13	7	2	3	25

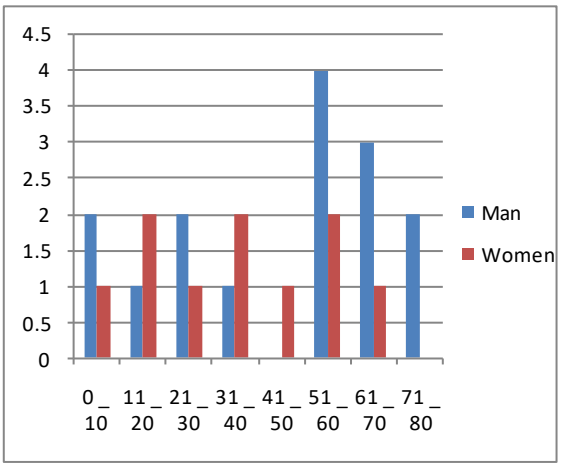

Fig. 4- Age and gender-wise distribution of Candida isolates

Table 4: Species distribution of Candida isolates (n=25)

Candida species	No. of Isolates	Percentage
Candida albicans	20	80
C. tropicalis	1	4
C. glabrata	2	8
C. parapsilosos	1	4
C. krusei	1	4
Total	25	100

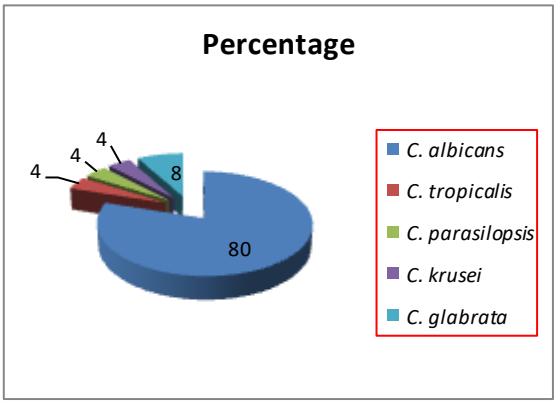

Fig. 5- Species distribution of Candida isolates

Table 5: Antifungal susceptibility pattern of the isolates of Candida (n=25)

Species	Amphotericin B		Clotrimazole		Fluconazole			Itraconazole		Ketoconazole		Nystatin	
	S	R	S	R	S	SDD	R	S	R	S	R	S	R
C.albicans (n=20)	20 (100%)	_	17 (85%)	3 (15%)	18 (90%)	_	2 (10%)	19 (95%)	1 (5%)	20 (100%)	_	20 (100%)	_
C. tropicalis (n=1)	1 (100%)	_	_	1 (100%)	_	_	1 (100%)	_	1 (100%)	1 (100%)	_	1 (100%)	_
C. glabrata (n=2)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	_	1 (50%)	2 (100%)	_	2 (100%)	_	2 (100%)	_
C. parapsilosos (n=1)	1 (100%)	_	1 (100%)	_	_	_	1 (100%)	1 (100%)	_	1 (100%)	_	1 (100%)	_
C. krusei	1 (100%)	_		1 (100%)	_	_	_	_	1 (100%)	_	1 (100%)	1 (100%)	_

S = sensitive, R = resistant, SDD = susceptible dose dependent

Table 6: Resistance pattern of *Candida* species to different antifungal agents

Anti-Fungal	Total number of Resistant Isolates (%)	
	C. albicans	NAC isolates.
Amphotericin B	0 (0%)	1 (20%)
Clotrimazole	3 (15%)	3 (60%)
Fluconazole	2 (10%)	3 (60%)
Itraconazole	1 (5%)	2 (40%)
Ketoconazole	0 (0%)	1 (20%)
Nystatin	0 (0%)	0 (0%)

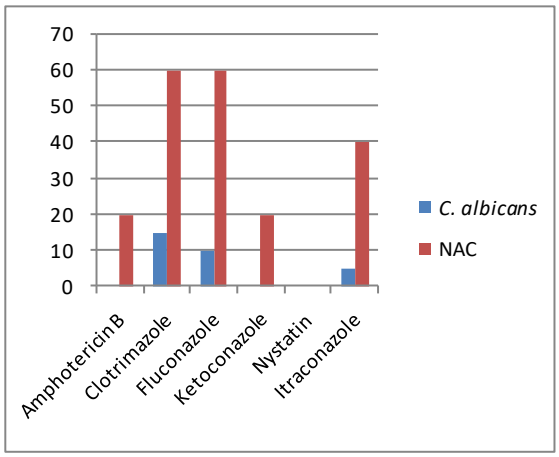

Fig. 6- Resistance pattern of *Candida* species to different antifungal agents

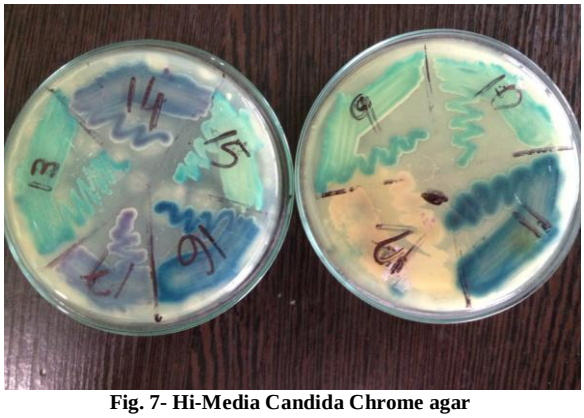

Fig. 7- Hi-Media Candida Chrome agar

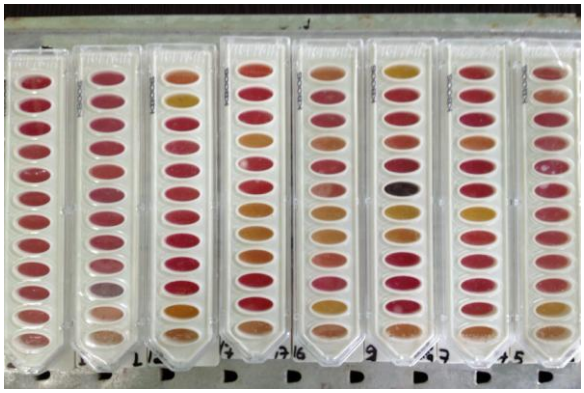

Fig. 8- HiCandida Identification kit

DISCUSSION

Candida spp. belongs to the commensal flora of human body and colonizes the mucosal surfaces soon after birth. There is an increasing incidence of candida infections among hospitalized patients mostly the immune-suppressed, and both host factors and virulence factors of *candida* spp. contribute to this. There are several host factors that predispose to candida infections like prolong use of Corticosteroids or antibiotics, poor nutrition, metabolic derangements and invasive procedures. Due to prolong and extensive use of anti-fungal drugs, there is a change in infection prevalence caused by different candida spp. and it is now important to identify the infective fungi up to species level as different species of candida vary in their virulence and anti-fungal susceptibility. [25-27] *Candida albicans* is predominate species in our study (80%). Studies done by different other authors like Manjunath et al. and Jayalakshmi L et al. has similar findings where *Candida albicans* is the predominate species. [28, 29]

The incidence of non albicans candida (NAC) isolates is on rise and similar results have seen in studies done by various authors. [30, 31] In year 2002, a study done by Kaviarasan et al. showed prevalence of NAC isolates was 39.5% which rise to 73.6% in year 2011 as shown in study by R Adhikary et al. [32, 33] Empirical antifungal therapy depends on the species involved due to higher resistance rates to multiple antifungal agents in non albicans candida (NAC) isolates. This is also evident in our study where the resistance to most of the Azole antifungal agents is much higher in NAC isolates in compare to *Candida albicans* isolates. The higher resistance rates in NAC isolates have also been seen in studies done by Hii et al., Sabhapandit *et al.* and other authors. [34, 35] Since the inception of anti-fungal susceptibility testing by disc diffusion methods by CLSI in 2003 many studies by different authors showed the development of anti-fungal resistance in Candida spp. [36] The rise in resistance among NAC isolates for Fluconazole which is a frequently used Azole anti-fungal is evident from previous studies done by various authors. In year 2007, study by MA Pfaller *et al.* showed resistance rate to Fluconazole was 9.9% which rise in consecutive years the resistance rate rise as high as 32.4% as shown in a study done by Jayalakshmi L et al in year 2014. Our present study shows resistance rate of 60% which may be due to

smaller number of isolates but go with the trend of increasing resistance rates. [37]

In our study *C. glabrata* was most predominate species (40%) among NAC isolates which contrasts from studies done by Sabhapanditet al. and Jayalakshmi L et al. in which *C. tropicalis* was the predominant species and *C. glabrata* was in second position. *C. glabrata* has emerged as an important opportunistic pathogen in last few decades and this is evident by study done by Li et al. and Trick et al. showing an extraordinary increase in its incidence. [38,39] Most of the Candida isolates were isolated from blood and urine samples (53%) which is similar to study done by Jayalakshmi L et al. and Sharma M et al. in which 42% and 43.3% of samples were blood and urine. [29, 40] Most of the isolates (48%) in our study were from patients above 50 years of age and study done by Bhattacharjee et al. also show similar age group contributing major portion (44%) of the isolates. [41]

CONCLUSION

The present study shows that Candida spp. are an important opportunistic pathogen in ICU settings and patients from older age group is particularly vulnerable. The emergence of NAC isolates and the increasing resistance among NAC isolates to multiple anti-fungal drugs is a matter of concern to initiate empirical antifungal therapy. It is now important to identify the isolates to species level so that proper antifungal to which the isolate isn't intrinsically resistant can be initiated as empirical treatment. Constant monitoring of the changing epidemiology and resistance pattern of Candida species is needed to guide the clinicians and make proper antimicrobial policy.

Funding: Self
Conflict of Interest: None declared
Ethical approval: Taken

REFERENCES

1. Anaissie EJ, McGinnis MR, Pfaller MA, eds. Clinical Mycology, 2nd edn. Philadelphia, Churchill Livingstone, 2009.
2. Messer SA, Jones RN, Fritsche TR. International surveillance of Candida spp. and *Aspergillus* spp.: report from the SENTRY antimicrobial surveillance program (2003). J ClinMicrobiol. 2006; 44(5):1782–7.
3. Richardson M, Lass-Flörl C. Changing epidemiology of systemic fungal infections. Clin Microbial Infect. 2008; 14(Suppl 4):5–24.
4. Mokaddas EM, Al-Sweith NA, Khan ZU. Species distribution and antifungal susceptibility of *Candida* bloodstream isolates in Kuwait: a 10-years study. J Med Microbiol. 2007;56:255-9.
5. Srinivasan L, Kenneth J. Antibiotic susceptibility of *Candida* isolates in a tertiary care hospital in Southern India. Ind J Med Microbiol. 2006;24:1-8.
6. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004; 39(3):309-17.
7. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidaemia in Swiss tertiary care hospitals: secular trends, 1991-2000. Clin Infect Dis. 2004; 38(3):311-20.
8. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care centre of north India: 5-year study. Infection. 2007; 35(4):256-9.
9. Kothari A, Sagar V. Epidemiology of *Candida* bloodstream infections in a tertiary care institute in India. Indian J Med Microbiol. 2009;
10. Shivaprakasha S, Radhakrishnan K, Karim PM. *Candida* spp. other than *Candida albicans*: a major cause of fungaemia in a tertiary care centre. Indian J Med Microbiol. 2007; 25(4):405-7.
11. Chakrabarti A, Chatterjee SS, Rao KL, Zameer MM, Shivaprakash MR, Singhi S, et al. Recent experience with fungaemia: change in species distribution and azole resistance. Scand J Infect Dis. 2009; 41(4):275-84.
12. Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis. 2003; 37(9):1172-7.

13. Arendrup MC, Sulim S, Holm A, Nielsen L, Nielsen SD, Knudsen JD, et al. Diagnostic issues, clinical characteristics, and outcomes for patients with fungemia. J ClinMicrobiol. 2011; 49(9):3300-8.
14. Lark RL, Chenoweth C, Saint S, Zemencuk JK, Lipsky BA, Plorde JJ. Four year prospective evaluation of nosocomial bacteremia: epidemiology, microbiology, and patient outcome. DiagnMicrobiol Infect Dis. 2000; 38(3):131-40.
15. Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidaemia, revisited. Clin Infect Dis. 2003; 37(9):1172-7.
16. Fidel PL Jr, Vazquez JA, Sobel JD. Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. ClinMicrobiol Re. 1999; 12(1):80-96.
17. Koneman E, Allen S, Janda W; Koneman's Colour Atlas and Textbook of diagnostic Microbiology. 5th edition, Lippincott-Raven publishers, 1997: 1043-1047.
18. Golia S, Reddy KM, Karjigi KS, Hittinahalli V. Speciation of *Candida* using chromogenic and cornmeal agar with determination of fluconazole sensitivity. Al Ameen J Med Sci. 2013;6(2):163-6.
19. Skrodeniene E, Dambrauskiene A, Vitkauskiene A. Susceptibility of yeast to antifungal agents in Kaunas University of Medicina Hospital. Medicina. 2006; 42(4):294-9.
20. Kothavade RJ, Kura MM, Yaland AG, Panthaki MH. *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. J Med Microbiol. 2010; 59(Pt 8):873-80.
21. Baillie GS, Douglas LJ. Iron-limited biofilms of *Candida albicans* and their susceptibility to amphotericin B. Antimicrob Agents Chemother. 1998; 42(8):2146-9.
22. Chander J; Textbook of Mycology. Chapter 2, 5, 6, 45, 3rd edition, Mehta publishers, 2009: 9-20, 35 – 56, 266-290.
23. Parija SC, Sujatha S, Dhodapkar R; Standard operating procedure manual, Department of Microbiology, JIPMER, Pondicherry, 2011: 37– 42.
24. Clinical Laboratory Standards Institute (CLSI); Method for antifungal disk diffusion susceptibility testing of yeasts. Approved guidelines. 2nd edition, CLSI document - M44– A2, 2009; Volume 29, No.17.
25. Chander J; Textbook of Mycology. 4th edition, New Delhi: Jaypee publishers, 2018: Chapter 20: 410-433.
26. Merz WG, Hay RJ; Topley & Wilson's Medical Microbiology and Microbial infections, Medical Mycology. Chapter - 1, 15, 30, 10th edition, Edward Arnold (Publishers) Ltd., 2005: 11, 256, 579-620.
27. Rippon JW; Candidiasis and the Pathogenic yeasts. In Medical Mycology. 2nd edition, WB Saunders Philadelphia, 1982: 484-531, 723-737.
28. Manjunath V, Vidya GS, Sharma A, Prakash MR, Murugesh N. Speciation of *Candida* by Hi-Chrom agar and sugar assimilation test in both HIV infected and non-infected patients. Int J Biol Med Res. 2012;3(2):1778-82.
29. Jayalakshmi L et al. Isolation, Speciation and Antifungal Susceptibility Testing of Candida from Clinical Specimens at a Tertiary Care Hospital. Sch. J. App. Med. Sci., 2014; 2(6E):3193-3198
30. Wang T Y et al. The clinical outcomes and predictive factors for in-hospital mortality in non-neutropenic patients with candidemia. Med 2016 Jun; 95(23): e3834.
31. VigneshKanna B et al. Isolation and identification of candida species from various clinical samples in a tertiary care hospital.*Int J Res Med Sci. 2017 Aug;5(8):3520-3522*
32. Kaviarasan PK, Thappa DM, Jaisankar TJ, Sujatha S; Candidiasis in HIV infected patients - a clinical and Microbiological study. Natl Med J India, 2002; 15(1): 51-52.
33. Adhikary R, Joshi S; Species distribution and antifungal susceptibility of candidaemia at a multi super speciality center in southern India. I J Med Microbiol., 2011; 29 (3): 309-311.
34. Hii et al. Resistance rates of non-albicans Candida infections in Taiwan after the revision of 2012 Clinical and Laboratory Standards Institute breakpoints. Infection and Drug Resistance 2019:12 235–240
35. Sabhapandit*et al.* Prevalence of non-albicans candidemia in a tertiary care hospital in Northeast India. DOI: 10.5455/ijmsph.2017.0823622092017.
36. Clinical Laboratory Standards Institute (CLSI); Method for antifungal disk

diffusion susceptibility testing of yeasts. Approved guidelines. 2nd edition, CLSI document - M44 A2, 2009; Volume 29, No.17.
37. Moehring R W et al. Challenges in Preparation of Cumulative Antibiogram Reports for Community Hospitals. Journal of Clinical Microbiology Aug 2015, 53 (9). 2977-2982.
38. Li, L., Redding, S. and Dongari-Bagtzoglou, A. (2007) 'Candida glabrata, an Emerging Oral Opportunistic Pathogen', *Journal of Dental Research*, 86(3), pp. 204–215.
39. Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP. Secular trends of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. Clin Infect Dis. 2002; 35(5): 627-30.
40. Sharma M, Pant ND and Pandey P (2016). Prevalence of Non-Albicans Candida among the patients attending a Tertiary Care Hospital in Kathmandu, Nepal. Nepal J. Biotechnology, 4(1): 43-46.
41. Bhattacharjee P. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016; 2(2): 20-27.

How to cite this article: Azad M, Das S, Kumar A. Isolation, identification and anti-fungal susceptibility testing of candida species from various clinical specimens of ICU patients in a tertiary care hospital of Bhagalpur, Bihar. International Journal of Research and Review. 2019; 6(9):115-122.

******