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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF DIFFERENT METHODS FOR SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA IN BLOODSTREAM INFECTIONS IN A TERTIARY CARE HOSPITAL OF ESTERN ODISHA.

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Abstract: ***Background***: Bloodstream infections (BSIs) are always a cause of concern for clinicians and clinical microbiologists. Intravascular catheterization, prolonged antibiotic exposures, long-term bedridden patients, and various immunocompromised conditions increase the chances of opportunistic pathogens and nosocomial agents to predominantly cause BSIs. Among the opportunistic pathogens, the genus Candida is the most common fungal pathogen in Intensive critical care units (ICCUs). Candida is an asexual, diploid fungus that is present on humans and in the environment . Only a few number of Candida species are human pathogen. Candidal infections may vary from superficial to deep mycoses. Candida act as pathogens only when there is an interruption of normal host defences. Candidemia is reported as the fourth common cause of BSIs in the Intensive Care Units (ICUs) and account for 10% of all BSIs . In the last 20 years with the surge of non albicans Candida species, Clinical importance of species level identification is important as they differ in the antifungal susceptibility pattern. The present study was undertaken to determine species of Candida isolated from bloodstream and to know its antifungal susceptibility by VITEK-2 YST (software version 8.01) and then compare the results with various conventional methods namely Germ tube test, Urease test, CHROM agar, Sugar fermentation test, Carbohydrate utilization test, Dalmau technique and disk diffusion method. The study also concentrated on the changes observed in the species distribution, the shift towards non- albicans Candida (NAC) species in our hospital. A total of 14,400 blood samples were received in the Microbiology laboratory during a period of two years from October 2022 to September 2024 for which culture and sensitivity was performed. Out of these 14,400 samples, 4800 (33.33%) yielded significant growth. Out of these, Candida isolates were127 (2.64%),with C.auris being the commonest species among them with a 25% mortality rate.

Keywords: Antifungal susceptibility, Candida species, Blood stream infection.

INTRODUCTION

Infections in the today's world, are one of the most important cause of mortality and morbidity more so in low and lower-middle income countries [1,2] . Global Burden of Diseases Study have also reported that even though the mortality and morbidity due to infections has decreased in the last 30 years, they still remain as an important cause of death [2] and also persist as the most important cause of disability [1,3]. Multiple factors that are responsible for these complications are; rapid urbanization, ageing population, and recently emerging viral and bacterial infections combined with the age-old upstream predisposing factors such as poverty, inequality, and illiteracy [4,5,6]. In India also, infections remain an important cause of morbidity and mortality like the rest of the world [7,8]. The incidence of fungal infections has risen substantially over the past several decades posing a serious threat [9].

Intensive care units (ICUs) have been an important source of infections, worldwide, especially bacterial [10-15]. Studies conducted in Europe and North America have reported that primary (at the time of admission) as well as secondary (nosocomial, ventilator- associated, device-related, and others) infections are common in ICUs. These have been associated with higher age and APACHE-2 scores and high prevalence of comorbid conditions [14]. Extensive as well as inappropriate use of antimicrobial agents and use of immunosuppressant drugs in various diseases has contributed to the increased propensity for opportunistic fungal infections. An important factor leading to opportunistic fungal infections is hospitalization of patients, especially in an intensive care unit (ICU) [16].

The incidence of life threatening fungal infections has increased recently, especially in immunocompromised patients, AIDS, organ transplantation, vascular catheterizations, prolonged administration of broad spectrum antibacterial agents, blood cancer and in

neonates. They are potential threat worldwide due to high rate of morbidity and mortality associated with them [17]. Major fungal genera responsible for causing infection in humans are Candida, Aspergillus and Cryptococcus. Candida is the component of normal flora of human beings [18]. Candida species are ubiquitous yeast like fungi associated with the human beings for a quite long time. There are about 200 species of Candida, dwelling as saprophytes in soil and aquatic environment and also colonizing various animal reservoirs. In approximately 70% of healthy individuals, Candida exists as commensals of the gastrointestinal (GIT) and genitourinary tracts [19]. They are seen in oral cavity, GIT, reproductive tract, etc. And they are the yeast-like organisms most commonly isolated from clinical specimens [18]. Candidiasis is a state of the Candida caused ailments. Some of them are oropharyngeal candidiasis, onychomycosis, vulvovaginitis, candidemia, disseminated infection [20]. Despite the predominance of Candida albicans, non-albicans Candida are emerging as colonizers and cause both superficial and systemic infections [21].

The knowledge of Candida species isolated is important for treatment because some species are intrinsically resistant to antifungal agents. Like Candida krusei has known resistance to fluconazole. Candida auris has emerged as an important nosocomial pathogen which is highly drug resistant [17].

Aim and objective of the study is

1. To know the prevalence rate of Candida infection in ICU patients.
2. To isolate and identify Candida species from blood culture of ICU patients.
3. To compare conventional, commercial, chromogenic and automated methods of identification of Candida species.
4. To study the antifungal susceptibility pattern of various Candida species

MATERIALS AND METHODS

DESIGN OF STUDY

Prospective study

SETTING AND PERIOD OF STUDY

The present study is conducted in the Department of Microbiology, IMS & SUM Hospital, BBSR for a period of 2years (from October 2022-Sept 2024). During this tenure 14,400 patients admitted in different wards and ICU's of this hospital with clinical suspicion of Blood stream infection were included in the study.

MATERIAL

Blood samples were received in the laboratory for culture.

INCLUSION CRITERIA

All blood culture samples having growth of Candida species.

EXCLUSION CRITERIA

Blood culture samples with other etiology.

DATA COLLECTION

All data were entered into Microsoft Excel spread sheet.

ETHICAL COMMITTEE APPROVAL

Institutional Ethical committee approval was obtained before the start of the study and the approval has taken.

COLLECTION OF SAMPLE

Blood samples were collected in a blood culture bottle, preferably before antimicrobial treatments have been started. In patients receiving antibiotics, the timing of collection of blood samples were just before the next dose of the antibiotic. Using a sterile needle and syringe by aseptic precaution about 10 ml (adults) or 2-3 ml (paediatric patients) of venous blood was drawn [22].

Clinical details about the patient were obtained and recorded. History, clinical findings, Co-morbid conditions, Surgical interventions and results of relevant investigations were also recorded. Ethical clearance was obtained.

SPECIMEN PROCESSING

Blood sample from the ICU patients were received in a blood culture bottle, was put in the BacT/Alert automated culture system. When it flagged positive, it was sub cultured on Blood agar and Mac Conkey agar plates... Isolated yeast like colonies were examined by Gram stain. Colonies which showed budding yeast like cells on gram stain, were then processed further for species identification. The colonies were processed both by manual and automated methods. For automated method, VITEK- 2 was used. For manual methods identification was done by germ tube test, Chlamydospore production on corn meal agar, Hichrome agar, and using the HiCandida identification kit. Antifungal susceptibility testing (AFST) was done by disk diffusion method according to CLSI document M44-A2 & MIC by using VITEK-2 [22].

A total of 14,400 patients having clinical suspicion of Blood stream infections were included in the study, out of which 4920 were flagged positive in BacT /Alert automated blood culture method. Out of 4920, only 4800 grew on plate. From 4800 samples, 4666 bacterial isolates (97.20%) were identified by Vitek -2 method. 134 out of 4800 samples showed fungal growth (2.79%), and 127 out of 134 were identified as Candida species in VITEK 2. Other fungal isolates were identified as Kodamaea ohmeri (3 isolates), Cryptococcus laurentii (1 isolate) and Rhodotorula species (3 isolates).

Total samples processed = 14,400Culture negative = 9600 (66.67%)Culture positive = 4800 (33.33%)Total bacterial isolates = 4666 (97.20%)Total fungal isolates = 134 (2.79%)Total Candida isolates = 127 (2.64%)Other fungal isolates = 7 (0.145%)These 127 Candida isolates were taken for detailed work-up.

RESULTS

Table 1 : Association of candida isolates with demographic profiles &co-morbidities.

Parameters	Identified variables	N(%)
Age	1 month-20 year	10 (7.8%)
	21-40 year	25 (19.6%)
	41-60 year	36 (28.3%)
	60-80 ear	50 (39.3%)
	> 80 year	6 (4.7%)
Gender	Male	66 (52%)
	Female	61 (48%)
Place of isolation	ICU	101 (80%)
	MICU	46 (36.2%)
	SICU	13 (10.25%)
	OTHER ICUS	42 (36.2%)
	IPD	26 (20%)
Associated risk factor	Diabetes	51 (30%)
	Hypertension	49 (28%)
	CKD	24 (14%)
	Malignancy	16 (9%)
	COPD	15 (9%)
	Stroke	12 (7%)
	Hypothyroidism	6 (3%)

Table-2 :Mortality in relation to age group.

AGE GROUP	MORTALITY / TOTAL CASES
UPTO 18 YR	0 / 10
19 TO 40 YR	2 / 25
41 TO 59 YR	8 / 36
60 TO 70 YR	15 / 36
71 TO 80 YR	5 / 14
>81 YR	2/ 6
TOTAL	32 / 127 (25%)

The table given shows total mortality of approximately 25% and out of which maximum mortality was observed in the age group of 60-70 years (42%), followed by 71-80 years (36%). No mortality was observed in age group to 0-18 years.

Table 3: Chi square test between age and mortality rate.

CHI SQUARE TEST	MORTALITY	TOTAL CASES	Marginal Row Totals
LESS THAN 40 YR	2 (7.45) [3.98]	35 (29.55) [1]	37
MORE THAN 41 YR	30 (24.55) [1.21]	92 (97.45) [0.3]	122
Marginal Column Totals	32	127	159 (Grand Total)

The p-value is .010787. Significant at $p < .05$.

Table 4 : Comparison of prevalent candida auris in relation to the wards and mortality rates.

AREA	NO of C .auris cases	No of mortality	cause
BICU	5	0	
CCU	1	0	
GICU	1	1	MODS
HDU	1	0	
HICU	3	0	
MED WARD	2	0	
MICU	19	10	SEPSIS MODS
NSICU	6	3	MODS
PSW	3	1	SEPSIS
SICU	3	1	MENINGITIS
SURG WARD	1	0	
TOTAL	45	16	

Maximum isolates of *Candida auris* were isolated from the Medicine ICU (MICU) and maximum mortality rate was also seen in MICU due to Multi-organ dysfunction syndrome (MODS) and sepsis.

Table 5: Chi-square test between mortality rates of c. auris and c. parapsilosis.

Chi square test	Mortality	Total cases	Marginal Row Totals
C. auris	16 (13.42) [0.5]	45 (47.58) [0.14]	61
C. parapsilosis	6 (8.58) [0.78]	33 (30.42) [0.22]	39
Marginal Column Totals	22	78	100 (Grand Total)

The p-value is .223271. The result is not significant at $p < .05$

The table above depicts the mortality rates due to three Candida species and the p-value on doing Chi-square test was not significant.

Table 6 : Chi-square test between mortality rates of c. auris, c. parapsilosis and c. tropicalis.

CHI SQUARE TEST	MORTALITY	TOTAL CASES	Row Totals
C. auris	16 (12.20) [1.18]	45 (48.80) [0.30]	61
C. parapsilosis	6 (7.80) [0.42]	33 (31.20) [0.10]	39
C. tropicalis	3 (5.00) [0.80]	22 (20.00) [0.20]	25
Column Totals	25	100	**125 (Grand Total)**

The p-value is 223271. The result is not significant at $p < .05$.

The table above depicts the mortality rates due to three Candida species and the p-value on doing Chi-square test was not significant.

Table 7: Percentage of various candida species.

Candida species	Total isolates	Percentage
Candida auris	45	35.%
Candida parapsilosis	33	26%
Candida tropicalis	22	17%
Candida albicans	13	10%
Candida glabrata	5	4%
Candida utilis	2	1.5%
Candida famata	2	1.5%
Candida guilliermondii	1	1%
Candida haemulonii	1	1%
Candida krusei	1	1%
Candida pelliculosa	1	1%
Candida rugosa	1	1%
Total	**127**	**100%**

Table 8: Comparison of results of vitek-2 and various conventional methods for candida species identification.

VITEK-2	Total by Vitek -2	Germ tube	Chrom agar	Dalmau technique	Sugars fermented	HiCandida identification	Species of Candida	Total by manual methods
C. albicans	13	formed	Light green	Chlamydospores formed	Glu, Mal	Mal, Gal, Xyl, Tre utilized	*C. albicans*	13
C. parapsilosis	33	No	Light pink	Pseudohyphae, giant cells	Glu	Xyl	*C. parapsilosis*	33
C. tropicalis	22	No	Metallic blue	Branching pseudohyphae, blastoconidia Branching	Glu, Mal, Suc	Mal, Suc, Gal, Cel, Xyl, Tre	*C. tropicalis*	22
C. glabrata	5	No	White-mauve	BYC	Glu	Tre	*C. glabrata*	5
C. krusei	1	No	Purple, fuzzy	Cross-match stick appearance	Glu	No	*C. krusei*	1

C. guilliermondii	1	No	Light pink	Blastoconidia chains, scanty pseudohyphae, blastoconidia	Glu, Suc	Mel, Suc, Gal, Cel, Xyl, Dul, Raf, Tre	C. guilliermondii	1
C. rugosa	1	No	Light blue, rough	BYC	No	Xyl	C. rugosa	1
C. famata	1	No	White	BYC	Glu, Mal, Suc	Mel, Suc, Gal, Cel, Xyl, Dul, Raf, Tre	C. famata	1
C. auris	45	formed	35-White, 10-Light pink	BYC	Glu, Suc	available	Candida species	0
C. pelliculosa	1	No	Light pink	BYC	Glu, Mal, Suc	Not available	Candida species	0
C. utilis	2	No	Light pink	BYC	Glu, Suc	Not available	Candida species	0
C. haemulonii	1	No	Light pink	BYC	Glu, Suc	Not available 78	Candida species	0
Confirmed species (Total)	**127**	**13**	**42**	**71**	—	—	**78**	—

Various types of methods used are automated (Vitek-2), Conventional (Germ tube and Sugar fermentation), Chromogenic (CHROM agar) and Commercial (HiCandida identification kit). The results have been compared in the table given above.

So as we can observe from the table given above, only 78 out of 127 *Candida* species identified by Vitek-2 can be correctly identified by manual methods. The isolate of *C. auris, C. pelliculosa, C. utilis* and *C. haemulonii* were not confirmed by manual (conventional, chromogenic and commercial) methods. The percentage of Concordance between two (automated and manual) methods is 61.4%.

Table -9 : Sensitivity percentage of candida species by vitek-2 & disc diffusion method.

	FLU	VOR	CAS	MICA	AMPB	FLUCY	KETO	ITR
C.albican	100%	100%	100%	100%	100%	100%	69%	85%
C.glabratta	90%	80%	80%	100%	100%	100%	60%	80%
C. gullerimundi	0%	100%	100%	100%	0%	100%	0%	100%
C. haemulonii	0%	100%	-	-	0%	100%		
C. parapsilosis	71.5%	98.5%	94%	100%	100%	97%	67%	58%
C. pelliculosa	100%	100%	-	-	100%	100%	0%	0%
C. rugosa	100%	100%	100%	100%	100%	100%	100%	100%
C. tropicalis	95.5%	100%	100%	100%	100%	100%	68%	77%
C. utilis	100%	100%	100%	100%	100%	100%	50%	100%

DISCUSSION

Bloodstream infections (BSIs) are always a serious concern for clinicians and clinical microbiologists. Factors like intravascular catheterization, prolonged antibiotic exposures, long-term bedridden patients, and various immunocompromised conditions increase the chances of opportunistic pathogens and nosocomial agents to predominantly cause BSIs. Among the opportunistic pathogens, the genus Candida is the most common fungal pathogen in Intensive critical care units (ICCUs) [23]

In the present study, Candidaemia occurred in all the age groups, ranging from 2 days to 87 years' old. This is concordant with the study conducted by Giri, et al. whose results were similar to this study [24]. In our study, the maximum incidence of candidemia is seen above the age of 60 years (43.8%). In the present study, incidence of candidemia seen in patients above 40 years of age is 71.8% which is contradicting with the study conducted by Shivprakasha S et al where the it was found to be 23.7% [25]. The aging population is especially susceptible to fungal infections mainly due to underlying medical conditions and the associated increase in the rates of hospitalization. In a European study conducted by Aikaterini Flevari et al, in which 28% of candidemic episodes were diagnosed in subjects over the age of 60 years [26].

Whereas in a study conducted by Thomas T and Dias M higher incidence is seen in middle age groups (18-50 years) [27].
The second highest percentage age group is 40-60 years of age (28%), followed by 19- 40 years (20%) and the lowest percentage was seen below 18 years.

In our study, out of 127 Candida isolates, 62 (52%) were from male patients and 61 (48%) from female patients with a male to female ratio of 1.08:1. There was no statistical difference with regards to gender. In a study conducted by Pallavi S Tatte et al, showed male predominance of 64% [28].

In this study, female predominance is observed in the age group of 0-18 years, slightly higher incidence in males in 19-59 years and approximately similar incidence in above 60 years of age.

In our study, maximum number of isolates were reported from the ICU's (80%) than the wards (20%). And maximum number of cases were reported from the Medicine ICU (36.2%). In a similar study conducted by Sahni et al., 100% cases were from the ICU's [29]. In the United States almost 50% of candida infections are from the ICU compared to 21% in Europe and 27% in the UAE [30,31,32].

In the present study, total mortality rate was approximately 25%. Maximum mortality was observed in the age group of 60-70 years (42%), followed by 71-80 years (36%). No mortality was observed in age group to 0-18 years. Chi-square test was done between the mortality rates of less than and above 40 years, it was not significant with a p-value of .010787.

In a similar study conducted by Gandham NR et al, where it was found to be 24% [33]. This is in the range of 10–49% reported in another study [34]. However, this is much lower than a study from Northern part of India (60%) [35].

Maximum mortality in Candida auris was also seen in MICU due to Multi-organ dysfunction syndrome (MODS) and sepsis. Maximum mortality in Candida parapsilosis was seen in MICU due to sepsis. Maximum mortality in Candida tropicalis was seen in MICU, GICU and HICU due to Multi-organ dysfunction syndrome (MODS) and sepsis. Chi-square test done between mortality rates of C. auris and C. parapsilosis and; between mortality rates of C. auris, C. parapsilosis and C. tropicalis that was not significant. This suggests that mortality rate is not significant for any particular species.

In our study, 51 isolates out of 127 were from patients suffering from diabetes mellitus as co-morbid condition. Among various co-morbid conditions; the incidence of DM was found to be 30%, followed by HTN (28%), CKD (14%), malignancy (9%), COPD (9%) and a few others. In a study conducted by; Madumathi et al, Shivanand Dharwad and Saldanha Dominic, Amar C. Sajjan et al, the incidence of diabetes mellitus in Candidiasis was found to be 28%, 32% and 33% respectively [36,37,38]. In a study conducted by Thomas T and Diaz M, the most common risk factors were malignancy followed by malnutrition and diabetes mellitus [27].

The higher incidence of Candida in diabetes mellitus could be due to the fact that it is one of the most common co-morbidity in India. India is termed as the "diabetes capital of the world". This might be due to "Asian Indian Phenotype", which refers to certain unique clinical and biochemical abnormalities in Indians which includes increased insulin resistance, greater abdominal adiposity, lower adiponectin and higher C-Reactive Protein levels [39].

In the present study; out of 4800 positive blood culture samples (blood culture positivity=33.33%), 127 isolates were found to be Candida species with an incidence rate of 2.64%.

The incidence of candidemia among all blood cultures is reported between 1% and 5% in various studies. In a study conducted by Nageswari et al, the incidence was found to be 3.4% with a blood culture positivity of 23.3% which compares well with most studies [33]. In a study conducted by Verma et al., from SGPGI, Lucknow ranked Candida species as 8th among all isolates causing BSI with incidence rate of 1.61% [40]. In a five- year study from All India Institute of Medical Sciences (AIIMS), New Delhi, Xess et al., found a prevalence of 6 % for Candida species in BSI [41]. Sahni et al., from Maulana Azad Medical College, New Delhi found the incidence rate of Candidemia to be 6.9 % [9]. Another New Delhi based study found that the percentage of Candida species among positive blood culture isolates was 18% [42].
The present study showed that among 127 Candida isolates, there is predominance of non-albicans Candida (NAC) species contributing to 90% of isolates and C.

albicans contributing to 10% of the isolate. Similar pattern has been found in the study conducted by Pallavi S. Tatte et al., where prevalence of NAC was found to be 69% [28]. In a study conducted by Gandham NR et al., the prevalence of NAC was found to be 89% [33]. Previously, C. albicans accounted for 70-80% of the Candidal disease. However, in the last 10 years, infections due to non-albicans species account for a majority of invasive candidal infections [34]. More than 90% of invasive infections are attributed to five species: C. tropicalis, C. albicans, C. glabrata, C. parapsilosis, and C. krusei [23]. Studies from Japan also show a low prevalence of C. albicans [43,44] whereas, Brazil, Taiwan, Spain, Denmark and other countries worldwide revealed that C. albicans is the main agent associated with fungemia [45,46,47]. It confirms the variation of Candida species in different geographical regions. In a study conducted by Bhattacharjee P. et al, the prevalence of C. albicans was found to be 48.57%, where it was found to be the predominant species [48]. On the contrary in a study conducted by Osrach et al., C. albicans was the most common species in Switzerland [49]. Majority of the studies indicate a shift in the prevalence of C. albicans to NAC.

In our study twelve species of Candida have been identified by Vitek-2. In the present study, Candida auris (35%) is the most commonly detected species followed by Candida parapsilosis (26%), Candida tropicalis (17%), Candida albicans (10%), Candida glabrata (4%), Candida utilis (1.5%), Candida famata (1.5%) also Candida guilliermondii, Candida haemulonii, Candida krusei, Candida pelliculosa and Candida rugosa 1% each. So, in our study C. auris is the most predominant species (35%). A similar study done by Mathur P. et al., identified C. auris (17.5%) as the second most important species causing candidemia in a tertiary care trauma centre in Delhi India [50]. Candida auris was first reported from Japan in 2009 from the external ear canal of a patient [51]. In India, it has been reported to account for 5.2% of Candidaemia in ICU patients [52,53].

In our study, C. parapsilosis (26%) is the second most common species and C. tropicalis (17%) is the third most predominant species, C. albicans (10%) as fourth and C. glabrata (4%) as fifth predominant species identified by Vitek-2. Jabeen et al. reported that C. albicans (27.9%) was the most commonly detected species in Pakistan in 2016, followed by C. parapsilosis (26.9%) and C. tropicalis (26.0%) [54]. In a study conducted by Jeon JS et al., shows after C. albicans; among NAC the number of specimens with C. glabrata and C. tropicalis were the highest (19%), followed by C. parapsilosis (12.7%) [55]. In a study conducted Bhattacharjee P. et al., after C. albicans; C. tropicalis (24.28%) was the highest; followed by C. haemulonii (8.57%), C. glabrata (5.71%) also C. pelliculosa (2.86%), C. rugosa (2.86%) and C. famata (2.86%) among others [48]. NAC species namely C. tropicalis and C. parapsilosis have emerged as common species in developing countries [56]. In the study by Mathur P. et al., C. tropicalis was the most prevalent species (39%) causing Candidaemia followed by C. auris, C. albicans and C. parapsilosis [50]. The study conducted by Gandham NR shows C. tropicalis was the most common isolate, followed by C. parapsilosis accounting 62% also C. albicans and C. krusei were isolated in 10% each along with isolates of C. haemulonii, C. famata and C. rugosa. Some studies shows Candida tropicalis as the predominant isolate [57,58,59], some studies had Candida parapsilosis complex as the common pathogen [60] and some observed Candida glabrata as the common pathogen [61,62]. In study by Pallavi S. Tatte, C. albicans (31.25%), C. tropicalis (25%), C. glabrata (20.84%), C. krusei (16.66%), C. parapsilosis (4.17%) and C. guilliermondii (2.08%) were major isolates [28]. In a study conducted by J. Meletiadis, two isolates of C. utilis were found among 584 isolates [63]. All the isolates identified in our study have been found to cause candidemia in various studies.

In our study, all 13 isolates which have been identified as C. albicans by Vitek-2 produced germ tube and no germ tube was produced in all other isolates. Thus, it shows a sensitivity of 100% for C. albicans. In a study conducted by SOUZA M.N. et. Al., the results of germ tube test sensitivity of 89.3% were observed in case of C. albicans [64].

In the present study, all the 127 isolates of Candida were subjected to testing in chromogenic media. The 13 isolates of C. albicans (light green), 22 isolates of C. tropicalis (metallic blue), 5 isolates of C. glabrata (white-mauve), 1 isolate of C. krusei (purple, fuzzy) and 1 isolate of C. rugosa (light blue, rough) were identified in the CHROM agar with a 100% sensitivity. Other isolates such as 33 isolates of C. parapsilosis, 10 isolates of C. auris, 2 isolates of C. utilis, 1 isolates of C. guilliermondii, 1 isolate of C. haemulonii and 1 isolate of C. pelliculosa appeared to be light pink in color; also 35 isolates of C. auris and 2 isolates of C. famata were white in color. So they cannot be identified only on the basis of chromogenic media.

The unique light green color production by C. albicans and metallic blue color by C. tropicalis is similar in the study done by Vidya Nerukar et al., and in that study also the isolates of C. albicans and C. tropicalis species were identified with a sensitivity of 100% and 90.7% respectively [65]. 100% sensitivity for C. krusei by chrome agar have been in various studies [66,67,68]. Certain reports suggest Chrom agar's utility in reliably identifying C. glabrata, while others have discussed issues with colour intensity of C. glabrata strains [69,70]. Findings in Vidya Nerukar et al., study corroborate with the C. glabrata group, as characteristic mauve glossy colonies were observed only in 14 of the 20 C. glabrata isolates [65]. Odds et al., have also reported pink to purple coloured colonies of C. pelliculosa on Chrom agar [71]. C. rugosa isolates light blue coloured rough

colonies on Chrom agar, a finding also described by Paritpokee et al., [67]. In the study conducted by A Birinci et. Al., light pink (pale pink) color colonies of C. guilliermondii and C. parapsilosis which were indistinguishable from each other were observed [72]. Various studies have varied results but our study is comparable with some of the studies.

Sugar fermentation test using glucose, maltose, sucrose, lactose was performed on all the 127 isolates of Candida as identified by VITEK-2. All the isolates of C. albicans were glucose and maltose positive. C. parapsilosis, C. glabrata and C. krusei were only glucose positive. C. tropicalis, C. pelliculosa and C. famata were glucose, maltose and sucrose positive. C. auris, C. guilliermondii, C. utilis and C. haemulonii were glucose and sucrose positive. C. rugosa did not ferment any sugar. All the species were 100% sensitive but due to the same type of reaction given by other species also, alone it cannot be used to identify the Candida species. But in conjunction with various other tests it can be able to identify some of the Candida species. As suggested by a study conducted by Charles, et al., Chrom agar has higher sensitivity than sugar fermentation for all four common Candida species C. parapsilosis, C. glabrata, C. tropicalis and C. albicans [73]. In a study conducted by Sidhartha Giri et al., the sensitivity of sugar fermentation was found to be 94.8% [74].

For the isolates of C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. guilliermondii, C. famata, C. rugosa and C. krusei the sensitivity was found to be 100% in comparison to Vitek-2. Other species identified in our study cannot be identified by this study due to the lack of reference literature. In a study conducted by Mustafa et al., the sensitivity of various species identified by the kit are; C. albicans 92.3%, C. glabrata 88.09%, C. krusei 87.8%, C. parapsilosis 90% and C. tropicalis 84.7% [75].

Out of 127 Candida isolates identified by Vitek-2, only 78 isolates can be confirmed by the cumulative interpretation of various conventional, chromogenic and commercial media. The percentage of Concordance between the automated and manual methods is found to be 61.4%. In a study conducted by Vidya Nerurkar et al., reliable identification of 73.7% of isolates was possible using Germ tube and Chrom agar tests only as 75.2% of isolates are constitute by C. albicans and C. tropicalis [65]. This is not the case with our study as 35% of isolates were of C. auris which cannot be identified by manual methods. In the study conducted by Vidya Nerurkar et al., 100% of the isolates were identified by using Germ tube and Chrom agar tests along with API ID 32C [65]. In a study conducted by Bhaskaran et al., the sensitivity and specificity in comparison to Multiplex PCR was found to be; Vitek-2 as 100%, germ tube (88% and 100%), HiCrome agar (91.25% and 100%), and Corn meal agar (83.75% and 100%) [76].Thus, from our study it is seen that if manual methods are performed alone their sensitivity and specificity are low but if the results are made in conjunction it increases the sensitivity and specificity of the results and leads to accurate identification except in case of C. auris, C. pelliculosa, C. utilis and C. haemulonii. But Vitek-2 is better as it can identify C. pelliculosa, C. utilis, and C. auris (most cases) correctly.

CONCLUSION

This study gives us the knowledge of prevalence of various Candida species in bloodstream infections and its antifungal sensitivity testing.

It has given us the the knowledge about shift in the pattern of species causing candidemia from Candida albicans to non-albicans Candida (NAC), among which Candida auris being the most prevalent species (35%) is a major concern. Because it is a multidrug resistant species with serious complications. The species is frequently misidentified as Candida haemulonii, Candida famata, Candida haemulonii, Candida duobushaemulonii, non-pigmented Rhodotorula glutinis or as several other Candida species by common commercial identification systems making the things more difficult. Above all for its antifungal susceptibility testing; even as per CDC, to date, there are no established minimum inhibitory concentrations (MICs) breakpoints for susceptibility testing of C. auris. The most reliable way to identify C. auris is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

From our study it can be concluded that in our hospital setting, Voriconazole, Micafungin and Amphotericin B can be used as empirical drugs because of higher sensitivity than other drugs. For Fluconazole, Ketoconazole and Itraconazole prior antifungal testing is highly recommended.

Also since, C. auris can colonize the hospitalised patients for many months, persist in the environment, and withstand some commonly used disinfectants in healthcare facilities and can easily be transmitted rendering tremendous implications from infection control standpoint. Thus good hospital infection control practices are also highly recommended by the help of this study.

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