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Molecular Expression Patterns of Virulence Factor Genes in Candida Isolates from Bloodstream Infections at A Tertiary Care Hospital

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ABSTRACT

Introduction: The most prevalent fungal infection caused by Candida species which causes significant morbidity, mortality rates, and medical expenses in critically ill individuals is Candida species. Better management of Candidemia is an important phenomenon to understand the molecular epidemiology of gene expression of virulence factors and susceptibility pattern antifungal drugs. of Candida species. In this study, the distribution of Candida species, susceptibility pattern, and identification of virulence genes were performed at King George's Medical University, India.

Methods: Total of 237 Candida isolates were isolated from blood samples from tertiary care hospital during 2021 to 2022. Candida species were identified using blood culture methods, including staining, germ tube test, CHROMagar, and Corn Meal Agar, confirmed by MALDI-TOF MS. PCR was done for species identification, and virulence genes were detected.

Results: Out of 237 Candida species, 26 *Candida parapsilosis* (26.1%), 26 *Candida tropicalis* (26.1%) (34),32 *Candida albican* (13.5%), 25 *Candida auris* (10.5%), 37 *Candida utilis* (15.6%), 12 *Candida glabrata* (5.06%), 2 *Candida krusei* (0.84%) and other species like *Candida rugosa*, *Candida orthopsilosis*, *kodamaeaohmeria*, *Candida lustaniae*, *Candida guillermondii* were 1(0.42%). Higher MIC values were noted for Fluconazole and Voriconazole, with *Candida auris* exhibiting resistance to Amphotericin B and Fluconazole. *C. albicans* displayed a higher frequency of virulence genes (Als1 (, HWP1, SAP) compared to non- albicans Candida.

Conclusion: This present study emphasizes the significance of identifying various Candida isolates and their patterns of susceptibility, aiding clinicians in targeting antifungal treatments and reducing morbidity and mortality in Tertiary Care Unit patients.

Keywords: *Blood, Candida, Diagnosis, Virulence genes, PCR.*

1. INTRODUCTION

Candidemia, a systemic fungal infection caused by various species of Candida with a significant threat to hospitalized patients in India ranging from 2.2% to 17.2% and emerged as a vital concern in the healthcare settings (1). Initially, patients with compromised immune systems, such as in intensive care units and those who have undergone complex medical procedures. Candida species cause blood stream infections and also disseminates candidiasis. In the United states Candida

species considered 4th rank among blood stream infection pathogen and 7th in Europe, despite advances in diagnosis and treatment. Candidemia rates ranges from 6-18% and a increase in non- albicans Candida (NAC) species from blood infection in India (1). The genus Candida includes more than 17 species can infect humans, *C. albicans, C.tropicalis, C. parapsilosis C.krusei and C.glabrata* are responsible for more than 90% of invasive cases. The key challenges in dealing candidemia are the diverse range of Candida species (2).

Based on recent investigation there are 6.9 occurrence of candidemia for 1000 patients in ICU & 7.5% of these patients are treated with antifungal treatment (3). Mortality is increased by 20-49% as a result of candidemia (4). The attributable death rate may increase 49% and the crude mortality rate is known to approach 60% in cases of nosocomial candidiasis (1)

Among NAC species mostly *C.tropicalis, C.glabrata, C.parapsilosis* and *C.krusei*. *C.albicans* is the most commonly isolated species from blood samples (3). Thus the diversity of virulence and resistance to antifungal treatments observed among different Candida species add complexity to the management of candidemia, presenting a challenging and dynamic task for healthcare providers.

Global estimates indicate that invasive candidiasis affects more than a quarter of a million people each year, with candidemia incidence rates ranging from 2–14 per 100 000 persons in population-based studies (4). Candidaemia is the fourth most prevalent nosocomial bloodstream infection increases morbidity and mortality rate in severely ill patients. Patients at high risk include immunosuppressed, undergone complex abdominal surgery and those admitted to ICUs. Despite being widely studied, emergence of new fungal pathogens and rising antifungal resistance stress the need of continuous monitoring of the disease (5).

Diagnosing of candidemia accurately and promptly is crucial for effective management whereas blood culture widely used and has the high rate of potential false negatives results with n extensive turnaround time (6). Microorganisms can be quickly and accurately identified because of sophisticated techniques like matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and polymerase chain reaction (PCR) (7).

The molecular gene analysis, particularly sequencing of specific regions for 28sRNA like ITS1 and ITS4, has proven invaluable in understanding the genetic diversity of Candida populations (8). These genes identify different Candida strains and enable the construction of phylogenetic trees, offering insights into the evolutionary relationships between various species and genes like ALS1, HWP1, and SAP providing pathogenic potential of different Candida strains (9). ALS1, encodes protein for the adhesion of Candida to host cells, facilitating tissue invasion. HWP1 supports invasion by promoting hyphal growth and biofilm formation and SAP encodes enzymes that aid in evading host immune defenses and tissue damage (10).

The application of multiplex PCR has shown promise in the field of diagnostics. Several DNA targets can be amplified and detected simultaneously in a single reaction according to this molecular approach (11). Multiplex PCR markedly shortens the diagnostic time and boosts the sensitivity and specificity of Candida detection, showcasing its effectiveness in expedited and accurate identification of the pathogen.

In the present study, we aimed to identify Candida isolates from blood samples using routine conventional methods and high throughput molecular techniques, to determine their molecular epidemiology or diversity, virulence gene, and antifungal susceptibility patterns to facilitate the tracking of Candida strains in healthcare facilities.

2. MATERIAL & METHODS

Clinical Isolates:

The study was carried out at the Microbiology Department, Mycology section at King George's Medical University, India from July 2021 to June 2022. Total 237 candida species were isolated blood samples from patients with febrile neutropenia, not respond broad range antibiotics in hospitalized patients. Patients who were enrolled gave written consent to participate in study. This study was approved by KGMU's ethical committee. (reference no. 117th ECM II B-Ph.D./PI). (Figure 4).

Algorithm of suspected cases of septicaemia

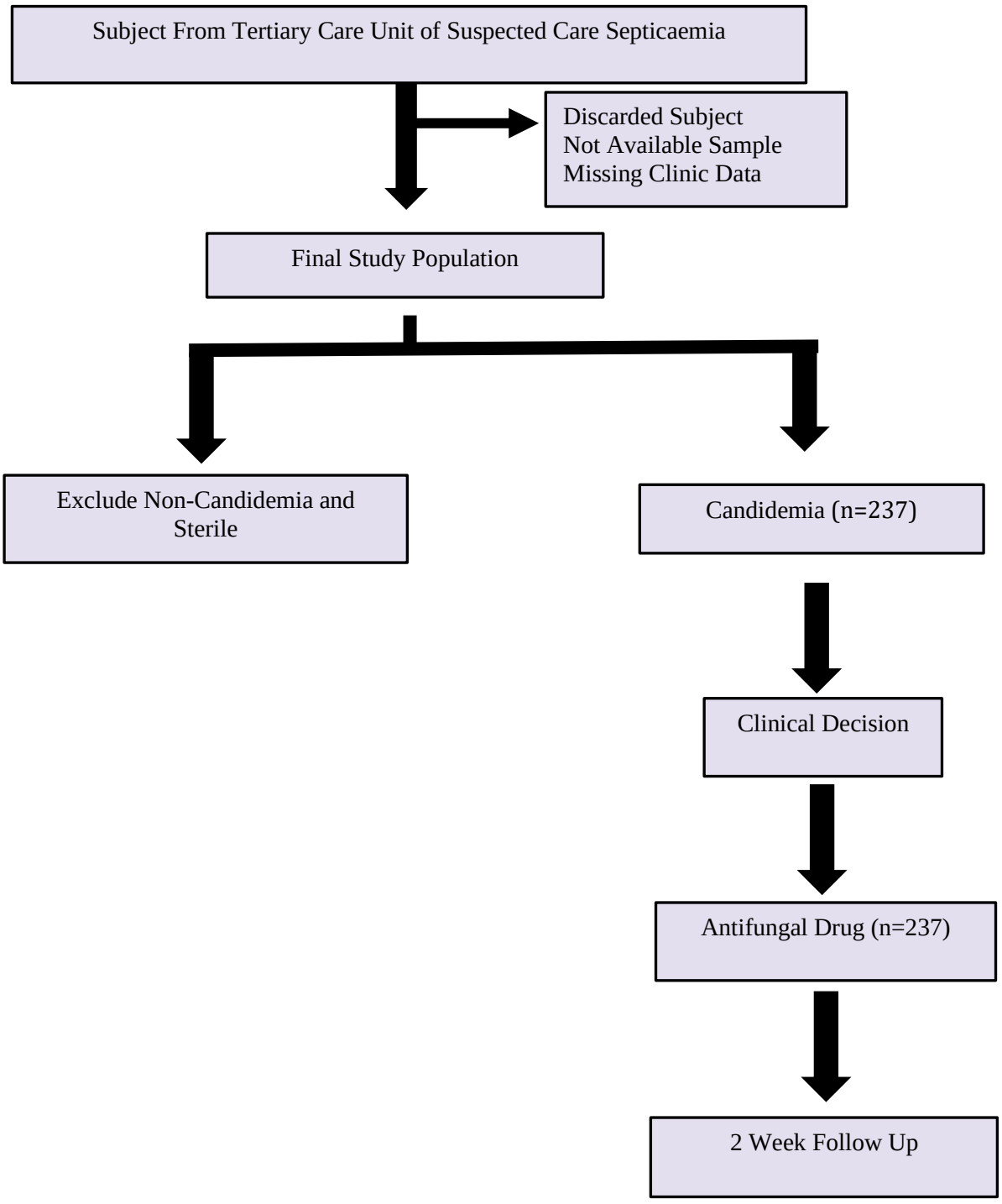

Figure4: Flow Chart of the study, consecutive subjects with suspected septicemia who fulfilled the inclusion & exclusion criteria. Subjects were removed from the analysis when blood samples were not available, positive for bacterial infection, sterile or there was insufficient clinical data to perform the analysis. The final study population included in the conventional method analysis comprised 237 with candidemia which were treated with antifungal drugs i.e. AmpB, FLU, VOR, CAS.

Phenotypic Identification of Isolates:

Candida isolates were identified through various standard mycological techniques including Gram's stain, colony morphology on 5% sheep blood agar plates (BIOMERIUX) at 37°C overnight, test using a germ tube, morphological traits

on Cornmeal agar, and color production on chromogenic medium (CHROMagar, Himedia, INDIA) were used phenotypically (7). To summarise, for the germ tube test, the Candida solution was mixed with pooled human serum and incubated at 37 °C for two hours. Germ tube formation was then observed under a 100X microscope. On a cornmeal medium enriched with Tween 80, specific morphological traits including chlamydospores, blastospores, pseudohyphae, and yeast cell arrangement were seen. The strains were preserved in 15 % glycerol broth at -80°C for future research.

Automated method identification of yeast: Yeast species identification was also by MALDI-TOF MS assays (BrukerDaltonik GmbH, Bremen, and Germany).

Antifungal Susceptibility:

Antifungal susceptibility was performed by broth microdilution method, according to the mentioned guidelines in the Clinical and Laboratory Standards Institute (CLSI) document M 38-A2 (8). The antifungal susceptibility drugs used were voriconazole, amphotericin B, fluconazole, and echinocandins (caspofungin,). *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were used in the study as quality control strains.

3. MOLECULAR IDENTIFICATION OF CANDIDA FROM CULTURE

DNA extraction:

The manufacturer-recommended protocol from ZYMO Research was used for DNA extraction in a Biosafety Cabinet 2 (BSC2) in Telstar, India. The extracted DNA was stored at -20°C freezer ((VESTFROST SOLUTIONS) and its concentration was measured using the Bio Spectrometer (Eppendorf, Germany) at 260-280 nm.

1st Reaction (Genus specific)

The primer ITS was used for targeting internal transcribed sequence, primer forward ITS1 and reverse ITS-4 (Eurofins Genomics India Pvt. Ltd.) were applied, which amplify the highly conserved variable ITS1 and ITS2 region. Using a Bio-Rad C1000 touch heat cycler, PCR was performed in a total volume of 25 µl using a 2 x PCR master mix (Thermo Scientific, Wilmington, DE). The conditions for the PCR are as follows: 5 minutes of initial denaturation at 95 °C; 45 seconds of denaturation at 94 °C; 45 seconds of annealing at 50 °C; and 45 seconds of extension at 72 °C.

2nd Reaction (Species-specific)

Positive samples from the ITS region of the first reaction were further analyzed for species identification using specific primers targeting sequences of various Candida species. These primers was includes sequence 5’ to 3’ CALB½, CGL½, CTR½, CPAR 3/2 and CKR 2/3 for species identification (9). PCR was employed, with the ITS PCR product diluted at a 2:100 ratio and used as the DNA template. The PCR reaction was conducted in a 30 µl volume using Thermo Scientific's 2X Master Mix. The PCR conditions were as follows: five minutes of initial denaturation at 95 °C; 45s of subsequent denaturation at 95 °C; 45s of annealing at 67–58 °C; ten cycles of 45s at 95 °C, 45s at 58 °C, and 45s at 72 °C; and twenty cycles of final extension at 72 °C for five minutes (9). These reactions were conducted in a controlled environment using the ISOCIDETM facility (ESCO PCR Cabinet, Singapore). This approach allowed for efficient and accurate species identification of Candida isolates from the samples.

Virulence gene expression of Candida species:

Extracted Genomic DNA was used for PCR. Specific primers were used for Als1, HWP1 and SAP4 genes for identification of virulence factors. PCR was carried out on a Bio-Rad C1000 touch heat cycler with a total capacity of 25 l utilizing a 2 x PCR master mix (Thermo Scientific, Wilmington, DE). The five minutes of initial denaturation at 95 °C, the minute of subsequent denaturation at 95 °C, the minute of annealing at 55 °C, and the minute of extension at 72 °C are the conditions for the PCR. Next are 32 cycles and a 10-minute final extension at 72 °C (10).

Gel Electrophoresis:

Amplified DNA was electrophoresed in 1.5% or 2% agarose gel.100bp DNA ladder (Next Gen, Genetix, India) in a separate well. Run electrophoresis with 70-80 volts for 30 minutes. Transfer the gel to a UV trans-illuminator to visualize the band's size of the PCR product matched with the target base pair and visualized on ultraviolet (10).

Statistical Analysis:

A variable of interest for our study are age, gender, location, outcome and neutropenia count for two above categories are reported as frequency (percentage). Chi-square tests were used to compare such categorical variables age, gender, location, outcome, and neutropenia count between groups. As the table shows only neutropenia count has been statistically connected with both patient groups with a p-value of 0.03 (<0.05), whereas other variables like age, gender, location, and outcome are not statistically significant associated with the groups as a p-value (>0.05). As a result, we can draw the conclusion that the infection is the cause of the fever and rising neutropenia count. Statistical analysis was performed with SPSS R27 software.

4. RESULTS

Total 237 blood samples were positive for culture, showing white cream pastry colonies and seen budding yeast-like cells under microscope. A total of 237 patients were infected with candidemia during their stay, and we recorded their informed consent from different wards in a hospital with the most stays in 69; NICU, 39; CCM,28; MICU, 18; surgical ward, 15; PICU,10 ;RICU, 7 BICU, 28 Trauma,7; Obs & Gynae, 7; Neurology ward and 9 other IPD Patients. Sex wise distribution was 53.5% (n= 127) and 46.41% (n=110) of Candida isolates (n=237) in male and female respectively. 32 isolates of these 237 isolates positive for *Candida albican* and 205 had non-albicans candida. Total 237 patient records that were split into two categories: candidemia and non-albican candida. Chi-square tests were used to compare categorical variables such age, gender, location, outcome, and neutropenia count between groups. Table shows, only neutopenia count has been statistically connected with both patient groups with a p-value of 0.03 (<0.05), whereas other variables like age, gender, location, and outcome are not statistically significant associated with the groups as a p-value (>0.05). As a result, we can draw the conclusion that the infection is the cause of the fever and rising neutropenia count (Table 1).

Table1: Demographic and clinical characteristics of patients positive for Candida infection in Blood

Demographic Characterstics	Candida albican (n=32)	Non albican candida(n=205)	Df	P-value
Age 0-10	9(28.12%)	86(41.95)	2	0.7584
11-20	2(6.25%)	11(5.37)		
21 onwards	21(65.62%)	108(52.68)		
Gender			1	1
Male	17(53.12%)	110(53.66)		
Female	15(46.88%)	95(46.34)		
Location			1	0.507
Rural	16(50%)	119(58.66)		
Urban	16(50%)	86(41.95)		
Symptoms			2	0.03
Fever	6(18.75)	66(32.20)		
Neutropenia	0	18(8.78)		
Fever/Neutropenia	26(81.25)	121(59.02)		
Outcome Discharge	21(65.62)	125(60.96)	1	0.331
Death	11(34.78)	80((39.02)		

Microscopy and colony morphology was performed for genus identification. Species was identified by conventional procedures of diagnostics in laboratories. Budding yeast was seen by gram staining. Species identification of Candida was performed by germ tube test, dalamau technique, CCA, and sugar assimilation.Total 237 Candida isolates species were confirmed by conventional and automated method (Maldi-Tof).

Blood culture was positive for C. parapsilosis (26.1%), C. tropicalis (26.1%), C. albican (13.5%), C. auris (10.5%), C. utilis (15.6%), C. glabrata (5.06%), C. krusei (0.84%) and other species like Candida rugosa, Candida orthopsilosis, kodamaeaohmeria, Candida lustaniae, Candida guillermondii were 1(0.42%). C. parapsilosis (26.1%) and C. tropicalis (26.1%) were the most common species in blood found in present study. These species were identified by automated method i.e. Maldi-Tof. While producing color on Candida Chromagar, the species of Candida were determined in 24-48 hours. It required 48–72 hours to identify other non-albican candida species using sugar assimilation and CMA. After 24 hours, MIC values were analyzed. Conventional methods provide final results at least 5-7 days after a positive blood cultures.

C. parapsilosis was sensitive to 61(98.3%) to AmpB (MIC range - ≤ 1µg/ml), 62 (100%) Caspofungin (MIC range – ≤1µg/ml) and resistant towards 10(16.12%) fluconazole (MIC range - >8µg/ml), 4 (6.45%) Voriconazole (MIC range –– ≥4µg/ml). *C. tropicalis* shows resistant towards 10(16.12%) fluconazoles (MIC range - >64µg/ml) and 2 (3.22%) voriconazole (MIC range - >16µg/ml). *C. utilis* was sensitive to 34 (91.89%) AmpB (MIC range - ≤ 1µg/ml), 37 (100%) Caspofungin(MIC range – 0.125µg/ml), 34 (91.89%) voriconazole (MIC range –– ≤ 0.125µg/ml) and resistant towards 4(10.81%) fluconazole (MIC range - >32µg/ml). Multidrug resistant *C. auris* isolates shows resistance to 25 (100%) Amphotericin B (MIC range >= 2µg/ml), 25 (100%) Fluconazole (MIC range - >64µg/ml) and sensitive to 25 (100%) caspofungin (MIC range <= 1µg/ml). *C. albicans* shows resistant towards 3 (9.37%) fluconazoles (MIC range - >64µg/ml), 2 (6.25%) voriconazole (MIC range - >16µg/ml) and 1(3.12%)amphoterecin B (MIC range – >2µg/ml). *C. glabrata* was sensitive to 12 (100%) AmpB (MIC range -≤ 1µg/ml), 12(100%) caspofungin (MIC range – ≤1µg/ml), 12(100%) voriconazole (MIC range –– ≤0.25µg/ml) and resistant towards 2 (16.66%) fluconazole (MIC range - >8µg/ml) (Table 2).

Table2: Distribution of antifungal susceptibility pattern of *Candida albicans* and Non albican Candida

Species/Drug	No.	FLU			AMP			VOR			CAS	
		R	I	S	R	I	S	R	I	S	R	S
C. tropicalis	62	10	1	51	3	-	59	2	1	59	-	62
C. auris	25	25	-	-	25	-	-	-	-	-	-	25
C. albican	32	3	-	26	1	-	31	2	1	29	-	32
C. parasiplosis	62	10	-	52	1	-	61	4	2	56	-	62
C. krusei	2	2	-	-	-	-	2	-	-	1	-	2
C. glabrata	12	2	-	10	-	-	12	-	-	12	-	12
C. utilis	37	4	-	33	3	-	34	3	-	34	-	37
C. orthopsilosis	1	-	-	1	-	-	1	-	-	1	-	1
Kodamaea ohmeri	1	-	-	1	-	-	1	-	-	1	-	1
C. lusitaniae	1	-	-	1	-	--	1	-	-	1	-	1
C. guilliermondii	1	-	-	1	-	-	1	-	-	1	-	1
C. rugosa	1	1	-	-	-	-	1	-	-	1	-	1
Total	**237**	**57**	**1**	**176**	**33**		**204**	**11**	**4**	**196**	**-**	**237**

FLU: Fluconazole, AMP: Amphoterecin B, VOR: Voriconazole, CAS: Caspofungin ,R: Resistance; I: Intermediate; S:Susceptibility

PCR was performed for species identification of isolates from blood to minimize the time which is taken in conventional methods. PCR is considered as a fast and robust technique but techniques show limitations for species identification. Five most pathogenic species were further processed for molecular identification and virulence gene estimation.*C. tropicalis* (62), *C. albican* (32) *C.parasilopsis* (62), *C. glabrata* (12) and *C. krusei* (2). PCR and other molecular techniques were also used to confirm these isolates. The highly variable ITS1 and ITS2 sequences around the 5.8S-coding sequence, which is the target Candida species gene, are situated between the Large Sub Unit-coding sequence (LSU) and the Small Sub Unit-coding

sequence (SSU) of the ribosomal unit. These sequences were identified using ITS 1 and ITS 2 primers. A single band of approximately 600 bp was found for Candida tropicalis, 600 bp for Candida albican, 600 bp for Candida glabrata, 600 bp for Candida krusei, and 600 bp for the above species (Figure 1). PCR was performed in which species genes single band of around for *C. tropicalis* (357 bp), *C. albican* (272bp) *C. parasilopsis* (297pb), *C. glabrata* (423 bp) and *C. krusei* (362bp) (Figure 2).

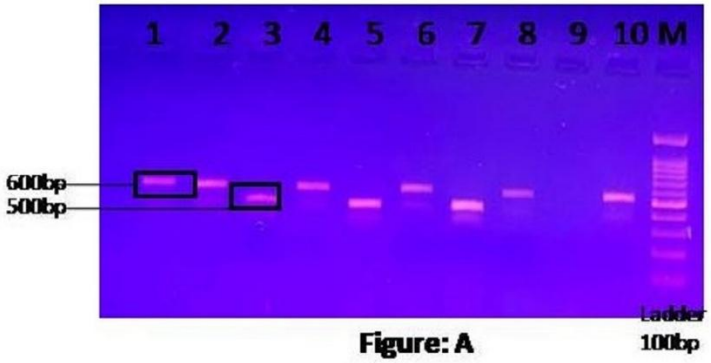

Figure 1 : Gel Electrophoresis of PCR for some Candida isolates: Lane M showing DNA ladder 100 bp. Lane 1,2,4,6,8 showing positive for 600bp for genus Candida (Yeast). Lane 3, 5, 7 showing positive for 500bp for genus Candida (Yeast). Lane 9 showing negative control and lane 10 showing 600bp for ATCC 90028 *Candida albican.*

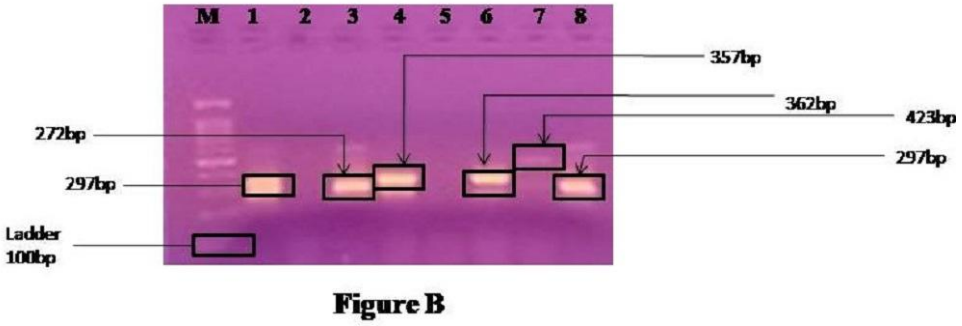

Figure 2: Gel Electrophoresis of species specific PCR for Candida: Lane M showing DNA ladder 100 bp. Lane 1 shows positive for *C. parapsilosis* (297bp) and *C. tropicalis* 357bp.Lane 2 shows negative control. Lane 3 shows 272bp for *C. albican*. Lane 4 shows 357bp for *C. tropicalis*. Lane 5 was negative. Lane 6 shows 362bp for *C. krusei*. Lane 7 shows 423bp for *C. glabrata*. Lane 8 shows 297bp for *C. parapsilosis*.

Table 3: List of Primers used in this study:

Primer Name	Sequence (5' → 3')	Reference
ITS 1-F	TCCGTAGGTGAACCTGCGG	(9)
ITS4-R	TCCTCCGCTTATTGATATGC	
Species specific Primer		
CALB ½ F	TTTATCAACTTGTCACACACCAGA	
CALB ½ R	ATCCCGCCTTACCACTACCG	
CGL ½ F	TTATCACACGACTCGACACT	

CGL ½ R	CCCACATACTGATATGGCCTACAA	
CTR ½ F	CAATCCTACCGCCAGAGGTTAT	
CTR ½ R	TGGCCACTAGCAAAATAAGCGT	
CPAR 3/2 F	GCCAGAGATTAAACTCAACCAA	
CPAR 3/2 R	CCTATCCATTAGTTTATACTCCGC	
CKR2/3 F	ACTACACTGCGTGAGCGGAA	
CKR 2/3 R	ACTACACTGCGTGAGCGGAA	
Primers for Virulence Genes		
Als1-F	ACCAGAAGAAACAGCAGGTG	(10)
Als1-R	GACTAGTGAACCAACAAATACCAG	
HWP1-F	ATGACTCCAGCTGGTTC	
HWP1-R	TAGATCAAGAATGCAGC	
SAP4- F	GAGTGTTCTTGCTTTCGCTTTA	
SAP4-R	TTGCCCACATCATTTCTACC	

The distribution of virulence gene was screened during this present study. Als1, HWP1 and SAP1 gene were visualised at 319bp, 503bp and 201bp respectively (Figure: 3).The frequency of virulence Als1 gene was present in *C. albican* (27/32; 84.3%) *C. tropicalis* (6/62; 9.6%) and *C.glabrata* (6/12; 50%). The Als1 gene was not detected in *C. parapsilosis* and *C. krusei.* These genes were visualised on gel electrophoresis to show the HWP1 and SAP1 genes were present in *C. albican* (25/32; 78.1%), and *C. glabrata* (7/12; 58.3%). HWP1 gene was not showing presence of *C. tropicalis*. This study also focuses on the frequency of SAP1 gene as follows: *C. albican* (18/32; 56.25%), *C. tropicalis* (2/62; 50%) and *C. glabrata* (6/12; 50%). Als1, HWP1 and SAP1 genes were not seen in existence in *C. parapsilosis* and *C. krusei*.

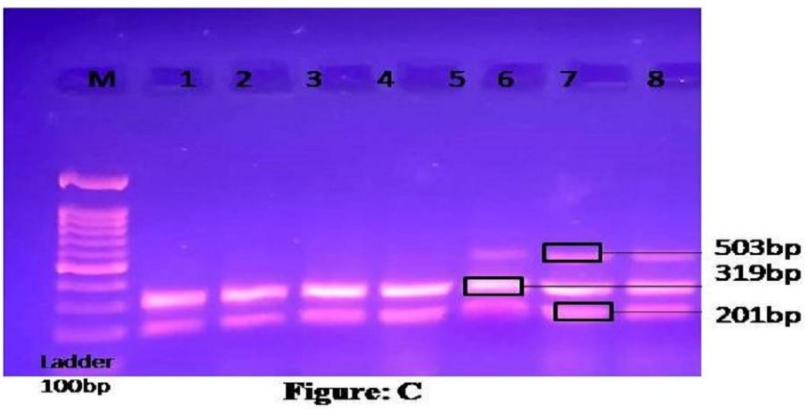

Figure 3: Distribution of virulence genes of Candida species.Lane M showing DNA ladder 100 bp. Lane 1,2,3,4 showing positive als1 gene for 319bp and 201bp for SAP4.. Lane 5,6,7 showing positive als1 gene for 319bp and SAP4 gene for 201bp and 503bp for HWP1 gene.

5. DISCUSSION

The present study provides a molecular-based identification of virulence genes in Candida isolates from bloodstream infections in a tertiary care hospital. The findings offer critical insights into the distribution of Candida species, their antimicrobial susceptibility profiles, and the prevalence of key virulence genes, which have significant implications for clinical management and treatment strategies for candidemia.

In present scenario, bloodstream infections caused by Candida species have emerged as a significant public health concern, particularly in tertiary care settings where patients are often struggling with severe underlying conditions and compromised immune systems (14). Candida species are opportunistic fungal pathogens that possess various virulence factors enabling them to establish infections in humans (12). In tertiary care facilities, Candida infections are frequently occurring in hospital-acquired infections. Infections with Candida spp., particularly non-albicans Candida (NAC), have increased in the last several years. Accurate laboratory diagnosis is essential for species identification and timely initiation of appropriate treatment (1,15).

The study identified 237 Candida isolates from bloodstream infections, with a predominance of non-albicans Candida species (86.5%), while Candida albicans accounted for only 13.5% of the isolates. This shift towards non-albicans Candida species aligns with global trends and underscores the need for precise identification and targeted antifungal therapy. Notably, *C. parapsilosis* and *C. tropicalis* were the more common species, each comprising 26.1% of the isolates. The high prevalence of these species necessitates heightened vigilance and specific management protocols in clinical settings. In our study, a total of 2.04% of Candida were isolated from total blood culture. The prevalence and incidence of candidemia in India vary widely. The incidence and prevalence of candidemia differ greatly in India. Bhattacharjee P reported incidence of Candida species was 4.03% in West Bengal (1). One study by Sahin et. al. reported an incidence rate of 6.9% for Candida species in New Delhi (16) Similar studies also reported a 1.31% incidence rate of Candidemia by Kaur H *et. al.* (17). In South India, children with hematological malignancies had an incidence of 5.7% and other reported candida species variation reported a prevalence rate of 6% and an incidence rate of 1.61%. (1) These studies demonstrate the notable regional variations in India's candidemia rates.

The antifungal susceptibility testing revealed significant variability among the Candida species. Notably, Candida auris exhibited multidrug resistance, being resistant to Amphotericin B, Fluconazole, and Voriconazole, but sensitive to Caspofungin. The emergence of multidrug-resistant C. auris is particularly concerning due to its potential to cause outbreaks and its limited treatment options. Candida parapsilosis and Candida tropicalis also showed considerable resistance to Fluconazole, a commonly used antifungal agent. These findings highlight the importance of antifungal susceptibility testing for guiding appropriate therapy and mitigating the risk of treatment failure. Antifungal susceptibility tests revealed that every one of the 34 C. albicans isolates was fluconazole (FLC) susceptible. On the other hand, 44.12% of the cases had resistance to Amp B, 52.94% to 5FC, 8.82% to VRC, and 17.65% to ITC. Resistance to AMP, FLC, 5FC, VRC, and ITC was noted in 30.56%, 61.11%, 33.33%, 19.44%, and 38.89% of cases for the 36 NAC isolates, respectively(1,3).Total 74% for FLU, 86% for AmpB, 82.7% for VOR and 100% for CAS of Candida isolates was susceptible towards antifungal. Candida species shows resistance against fluconazole were 10 (16.12%) in *C. parapsilosis*, 3 (9.37%), *C. albican*, 25(100%) *C. auris*, 2(16.66%) glabrata and 2(100%) krusei. Related number present across the world *C. tropicalis* (2.6 -14.3%), *C. albican* (1.4- 5.2%), *C. parapsilosis* (2.7%- 10.5%), *C. glabrata* (1.5% -93.8%) (8,9,10,11,12). Other international studies focused on resistance of drugs in our study mainly itraconazole (4.7%), voriconazole (1.2- 5.9%) and echinocandins (0.3-2.2%) (4).

The molecular identification of Candida species using PCR provided rapid and accurate results, with species-specific bands observed for Candida tropicalis, Candida albicans, Candida parapsilosis, Candida glabrata, and Candida krusei. The application of PCR in routine diagnostics could significantly reduce the time required for species identification, thereby facilitating timely and appropriate treatment interventions.

The study further examined the expression of key virulence genes—Als1, HWP1, and SAP1—across different Candida species. The Als1 gene, associated with adhesion and biofilm formation, was predominantly detected in Candida albicans (84.3%), followed by Candida glabrata (50%) and Candida tropicalis (9.6%). The high prevalence of Als1 in C. albicans underscores its role in the pathogenicity of this species. In contrast, C. parapsilosis and C. krusei did not exhibit Als1 expression, suggesting different mechanisms of virulence.

Similarly, the HWP1 gene, which is involved in host cell adhesion, was present in Candida albicans (78.1%) and Candida glabrata (58.3%), but absent in Candida tropicalis, C. parapsilosis, and C. krusei. This differential expression pattern indicates species-specific variations in virulence strategies(18,19) The SAP1 gene, linked to proteolytic activity, was also detected predominantly in Candida albicans (56.25%) and Candida glabrata (50%), with lower prevalence in Candida tropicalis (3.2%). (20)

The findings of this study have several clinical and epidemiological implications. The predominance of non-albicans Candida species and the emergence of multidrug-resistant strains highlight the need for continuous surveillance and tailored antifungal stewardship programs. The differential expression of virulence genes among Candida species necessitates a deeper understanding of their pathogenic mechanisms to develop targeted therapeutic interventions.

Moreover, the rapid and accurate identification of Candida species using molecular methods can significantly improve patient outcomes by enabling timely and appropriate antifungal therapy. The integration of molecular diagnostics with routine laboratory practices could enhance the detection and management of candidemia, particularly in critical care settings where timely intervention is crucial.

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6. CONCLUSION

The study provides valuable insights into the virulence factor gene expression patterns of Candida isolates from bloodstream infections in a tertiary care hospital in Northern India. The diverse expression profiles observed among different Candida species highlight the complexity of candidemia and emphasize the need for personalized therapeutic interventions. Continuous research, surveillance, and implementation of infection control measures are essential to mitigate the impact of Candida bloodstream infections and improve patient outcomes. Furthermore, the study findings serve as a foundation for future studies aimed at developing innovative antifungal strategies, ultimately enhancing the management of candidemia and reducing associated morbidity and mortality rates.

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