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Original Research Article

Comparative Evaluation of Multiplex Polymerase Chain Reaction (PCR) and Culture Methods for Rapid Diagnosis of Septicemia

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Abstract: ***Background*:** Infections of the bloodstream and sepsis are significant causes of morbidity and mortality, especially when complicated by multidrug resistance. Molecular diagnosis represents a tool for rapid identification of causative organisms with subsequent optimization of antimicrobial therapy; however, conventional blood culture still plays a critical role as a reference method for microbial identification and susceptibility testing. ***Methods*:** The study included 110 bloodstream isolates obtained from patients with suspected bacteremia and sepsis. Conventional blood culture was used for organism identification, incubation time analysis, and antibiogram testing, while multiplex PCR was used to screen for the most prevalent resistance determinants, namely NDM, OXA-48, KPC, VIM, mecA, and VanA/VanB genes. Bloodstream and non-bloodstream isolates from selected cases of sepsis were compared. ***Results*:** *Klebsiella pneumoniae*: 27 (24.5%), *Staphylococcus aureus*13 (11.8%), *Escherichia coli*8 (7.3%), *Enterococcus faecium* (7; 6.4%), *Acinetobacter baumannii* (8;7.3%), *Enterococcus faecalis* (6; 5.5%), and *Pseudomonas aeruginosa* (6; 5.5%) were the most prevalent species isolated from blood. The majority of cultures became positive within 20 hours from seeding, with 24 out of 97 (24.7%) positive between 0 and 10 hours and 55 out of 97 (56.7%) positive between 11 and 20 hours. The most prevalent resistance determinants were NDM, NDM+OXA-48, mecA, and VanA/VanB. Susceptibility testing revealed that carbapenems were largely ineffective against *K.pneumoniae*, while aztreonam/avibactam, tigecycline, fosfomycin, linezolid, vancomycin, and teicoplanin showed good activity against several strains of bacteria**.** ***Conclusion*:** The present findings demonstrate the potential utility of multiplex PCR as a rapid assay for detection of multidrug-resistant bloodstream pathogens and early optimization of antimicrobial treatment. At the same time, conventional blood culture remains an indispensable tool for reliable bacterial identification and phenotypic resistance testing.

Keywords: Primary Pathogens, Clinical Conditions, Resistance Determinants / Genes, Diagnostic Techniques, Key Antimicrobial Agents.

INTRODUCTION

Septicemia/sepsis constitutes a serious global health burden and manifests as a systemic infection that frequently leads to multi-organ failure and septic shock. Bacterial infections most often cause it, but it can also result from fungal, viral, or parasitic infections [1, 2].There is no single causative organism; any microbe that produces inflammatory or febrile products may cause it. In septicemia, pathogens enter the bloodstream, dysregulating the immune response and triggering a cytokine storm (excessive cytokine release), leading to widespread inflammation and vasodilation, followed by hypotension, tissue hypoxia, and organ dysfunction due to increased vascular permeability [3]. Despite the improvements in critical care management, the mortality rates remain high. The rapid progression of hemodynamic instability amplifies clinical urgency, thus requiring prompt therapeutic intervention to reduce mortality. Early recognition and treatment are critical for improving outcomes, as sepsis progresses rapidly and can be fatal within hours if left untreated or delayed [4, 5].

Given these challenges, timely administration of appropriate antimicrobial therapy is essential to improve prognosis, especially in cases of Multi drug-resistant bacterial infections. However, due to the high

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turnaround time and intrinsic sensitivity limitations, the standard reliance on blood culture-based diagnostics impedes the transition from empirical to targeted therapy. Additionally, patients receiving extensive broad-spectrum antibiotics have substantially lower blood culture diagnostic sensitivity, thus masking the causative pathogen [6].

To address these clinical shortcomings, emerging molecular techniques, such as, probe-based multiplex real-time PCR, are being evaluated for their potential to enable rapid and direct pathogen identification and detection of antibiotic-resistant genes in clinical samples. This can facilitate an expedited transition to targeted antimicrobial treatments. By combining molecular diagnostic methods with routine clinical procedures, the duration of inappropriate therapy and subsequent emergence of antibiotic-resistant strains can be minimized [7].

Compared to conventional culture methods, molecular approaches show improved performance in detecting polymicrobial infections and are less affected by prior exposure to a broad range of antibiotics. A Kenyan pediatric assay detected two *Salmonella-Klebsiella* co-infections plus 3 additional organisms missed by culture [8]. Nonetheless, these molecular assays have certain technical issues, such as the possibility of misinterpreting commensal organisms as pathogens, restricted pathogen panels and inadequate detection of specific resistance mechanisms, such as AmpC production.

Conventional blood culture is still regarded as the reference method for microbiological diagnosis because it enables organism isolation, microbiological confirmation, and antimicrobial susceptibility testing [9]. Blood culture still has a number of benefits that support its continued use as the gold standard for diagnosing bloodstream infections. According to Clinical and Laboratory Standards Institute recommendations, it permits thorough phenotypic antimicrobial susceptibility testing (AST), which determines minimum inhibitory doses and assigns resistance phenotypes [10].

Multiplex PCR panels, which predict resistance from a small number of preset genetic markers and, as new reviews emphasize, are not a conclusive demonstration of AST, cannot replace this level of phenotypic information. A recoverable microbial isolate from positive blood-culture bottles can be used for additional confirmatory research, such as PCR-based detection of carbapenem-resistance genes (blaKPC, blaNDM), biochemically profiled (e.g., VITEK2), characterized by MALDI-TOF mass spectrometry, and archived for outbreak investigation. Blood-culture results give combined information on the causal pathogen and in vitro antibiotic sensitivity, which is then used to adapt empirical therapy. In addition to organism identification, the culture yields information on resistance patterns, which supports targeted de-escalation [11, 12]. Blood-culture workflows are cost-effective, rely on laboratory infrastructure that is already present in nearly all hospital settings, and benefit from decades of standardization, internal quality-control programs, and clinician familiarity.

However, its performance is limited by low sensitivity, prolonged time to recovery of organisms, vulnerability to prior antibiotic exposure, and contamination-related interpretive challenges. In several comparative studies, blood culture failed to identify pathogens in a substantial proportion of clinically suspected sepsis episodes, and microbiologic isolation was achieved in only 56.7% of cases of severe sepsis or septic shock in a multicenter intensive care cohort. As a result, reliance on blood culture alone often delays the transition from empirical to targeted therapy, especially in patients already receiving broad-spectrum antibiotics, in whom diagnostic sensitivity may be substantially reduced [13].

In adult and pediatric cohorts, multiplex PCR has often demonstrated higher positivity rates or shorter turnaround times than blood culture, including detection rates of 47.9% vs 34.2% in nosocomial sepsis and 14.6% vs 10.3% in children with suspected sepsis, with markedly higher positivity in antibiotic-exposed pediatric samples [14, 15]. More recent direct-from-blood assays have likewise reported higher diagnostic yield than concomitant blood cultures in intensive care unit patients, although confirmatory studies remain necessary. Pediatric findings suggest meaningful adjunctive value, with high sensitivity and specificity after exclusion of contaminants and substantially shorter time to results. A Vietnamese comparative study found that combining blood culture with multiplex real-time PCR improved diagnostic performance, with the combined approach reaching 83.3% sensitivity, 100% specificity, and an AUC of 0.95 [9]. Nevertheless, the clinical role of multiplex PCR remains unsettled. The 2021 South African evaluation similarly found that adding multiplex PCR to blood culture increased bloodstream infection detection from 36% to 64% [12]. Moreover, assay performance appears to depend strongly on clinical context. A neonatal early-onset sepsis study reported very low sensitivity and no improvement in diagnostic accuracy or antibiotic decision-making, indicating that molecular testing cannot be generalized across all sepsis populations or screening settings(14). Similarly, studies of whole-blood multiplex real-time PCR in intensive care patients have shown widely variable accuracy, ranging from poor sensitivity in direct whole-blood testing to stronger performance when PCR is applied to positive blood culture bottles or focused target panels.

Thus, agreement between molecular and culture-based methods is often only moderate or poor, and stand-alone interpretation is limited.

Multiplex PCR offers the potential to shorten diagnostic turnaround time, improve pathogen detection in selected settings, and support earlier targeted antimicrobial therapy. While imperfect, blood culture is essential for isolate recovery, phenotypic resistance profiling, and antimicrobial stewardship guidance, and they define the baseline against which any molecular adjunct, including multiplex PCR, must be measured.

Current evidences suggest that multiplex PCR is best viewed as a complementary rather than a replacement tool for blood culture in sepsis diagnosis [16].

Thus, a further comparative evaluation of multiplex PCR and blood culture in patients with sepsis is needed.

MATERIAL & METHODS

Study Design

Between January 2025 and December 2025, a prospective study was conducted at Peerless Hospital and BK Roy research Centre in Kolkata, West Bengal, India using 'single centre' approach. Those who were admitted to the intensive care unit, general ward, and neonatal unit; underwent screening procedures, including those with blood culture positive results. During enrollment, clinical and demographic information was presented. In a subgroup consisting of 10 patients with suspected sepsis, the same organism was obtained from Blood culture and one more clinical specimen. The resistant profile tests were performed for concordance of organism, identity, and isolate characteristics.

Sample Collection

Blood samples were collected aseptically and processed using the BD BACTEC FX40 system, which is a continuous monitoring automatic platform commonly used for Blood culture, incubation and detection of microbial growth.Gram staining and subculture on regular media were used to isolate pure colonies from the bottles which flagged positive, followed by organism identification and antimicrobial susceptibility testing (AST), using the VITEK COMPACT PRO system. The susceptibility result was verified by the Kirby-Bauer disk diffusion method and interpreted according to CLSI criteria, as done in similar bloodstream infection studies using automated AST with supplementary disc diffusion confirmation.

Molecular Biological Test

Total nucleic acid was extracted from positive blood culture broth using a silicon membrane spin-column method in the matched sample subgroup, extraction was also performed from paired non-Blood specimen, including urine, bronchoalveolar lavage fluid (BAL fluid) endotracheal aspirate and sputum. Multiplex real Time PCR was then carried out, for rapid detection of selected pathogen and resistant determinants directly from extracted nucleic acid.

The molecular panel included assays for *Staphylococcus aureus* with mecA/mecC genes for differentiation of MRSA from MSSA, Enterococcus species with VanA/VanB for detection of VRE, and carbapenemase targets including blaKPC, blaNDM, blaOXA-48-like, blaVIM, blaIMP for characterization of carbapenem-resistant Gram-negative isolates.

Antimicrobial Susceptibility Testing

Conventional phenotypic identification and susceptibility testing were used as the reference standard for comparison with PCR findings consistent with published evaluation of molecular testing from positive blood culture.

RESULT

Demographic Distribution of Blood Culture Isolates

The analysis of clinical isolates total of 110 were performed on patients suspected with blood infections. The patients consisted of both male and female with a broad age range from 6 days (neonate) to 94 years. The distribution of isolates across age groups showed a clear predominance among older patients. The highest number of isolates were recorded in the 61–70-year age group, with 35 isolates (31.8%), followed by the 71–80-year group, with 31 isolates (28.2%). Together, these two age groups accounted for 66 isolates (60.0%) of the total. The 51–60-year age group contributed 17 isolates (15.5%), while 10 isolates (9.1%) were obtained from patients aged 81–90 years. The remaining isolates were distributed among the 41–50-year (n=8; 7.3%), 0–10-year (n=4; 3.6%), 31–40-year (n=2; 1.8%), 91–100-year (n=2; 1.8%), and 11–20-year (n=1; 0.9%) age groups. No isolates were recorded in the 21–30-year age group [fig 1].

Of the 110 blood culture isolates analysed, 65 (59.1%) were obtained from male patients and 45 (40.9%) from female patients. *Klebsiella pneumoniae* was the most frequently isolated organism among males, accounting for 16 isolates (24.6%), followed by *Staphylococcus aureus* with 11 isolates (16.9%). Among female patients, *K. pneumoniae* and *Staphylococcus haemolyticus* were the predominant isolates, with 9 isolates each (20.0%) [fig 2].

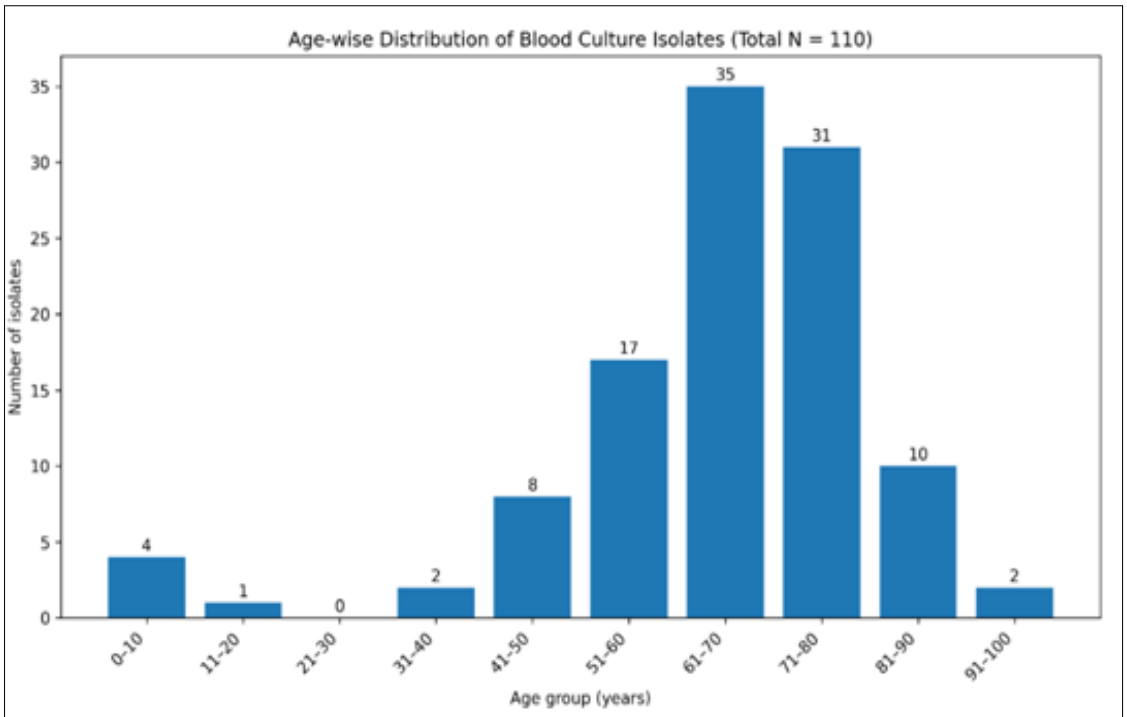

Fig 1- age wise organism distribution graph

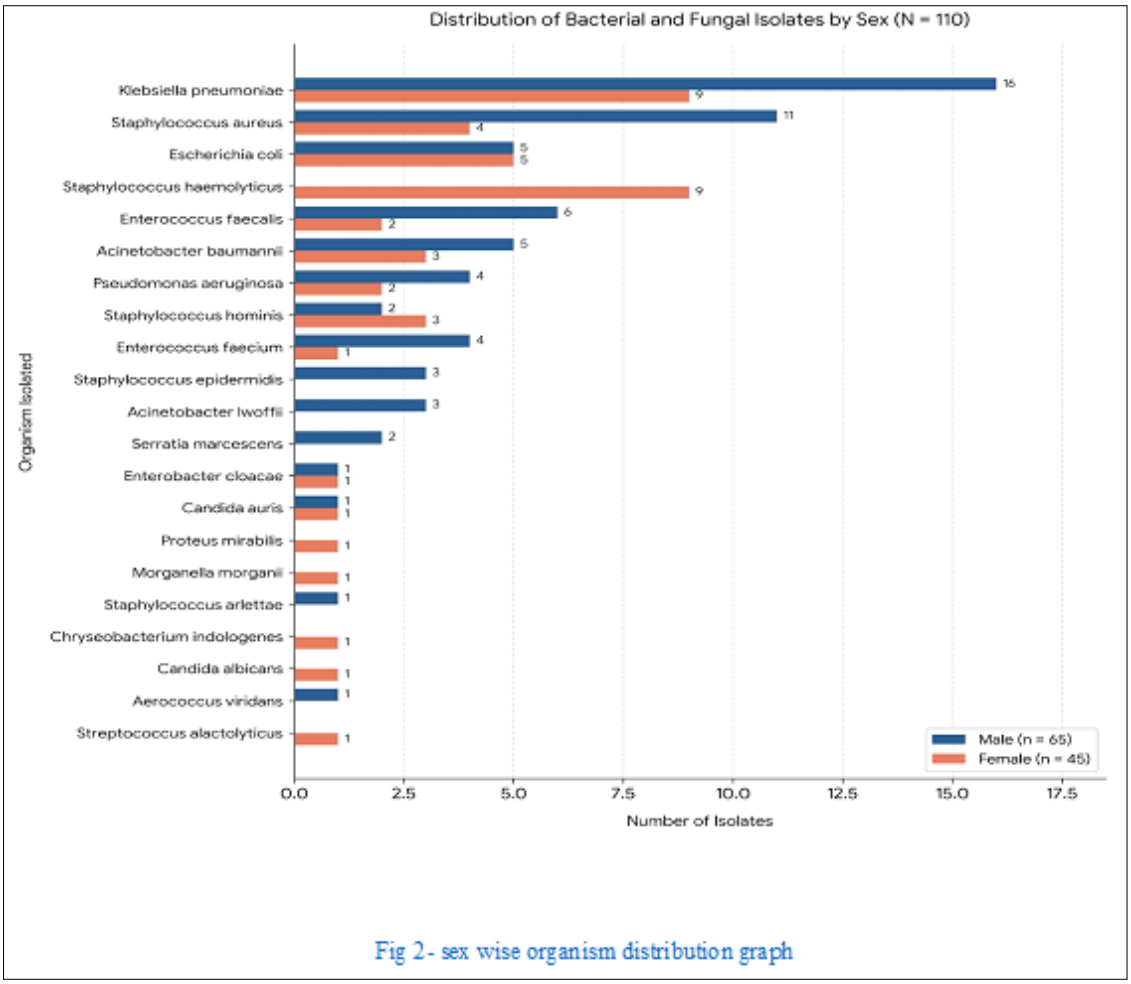

Fig 2- sex wise organism distribution graph

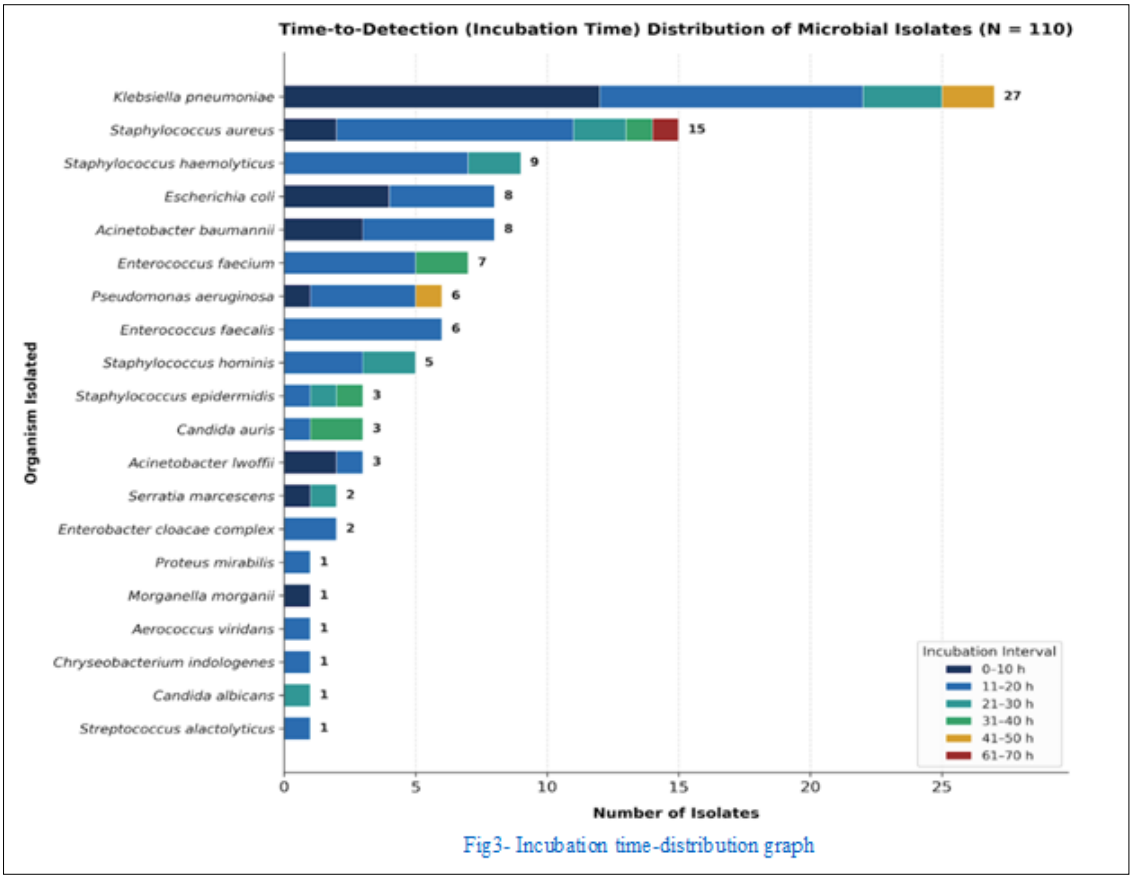

Fig3- Incubation time-distribution graph

INCUBATION PERIOD ANALYSIS

Most bloodstream isolates were detected within the first 20 h of incubation. The 11–20 h interval yielded the highest number of isolates (60/110, 54.55%), followed by the 0–10 h interval (27/110, 24.55%). Altogether, 87 isolates (79.09%) were recovered within 20 h. Subsequent intervals showed a progressive decline, with 10 isolates (9.09%) at 21–30 h, 7 (6.36%) at 31–40 h, 3 (2.73%) at 41–50 h, none at 51–60 h, and 1 isolate (0.91%) at 61–70 h. *Klebsiella pneumoniae* was the predominant pathogen across the early time windows, followed by Staphylococcus aureus and other clinically significant organisms. Delayed positivity beyond 30 h was infrequent and was mainly associated with *Candida auris*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [fig 3].

DISTRIBUTION OF RESISTANT GENES

Targeted multiplex PCR (mPCR) performed on flagged positive blood cultures successfully identified key resistance determinant genes in 88 isolates. NDM was the commonest determinant, present in 22 isolates (25.0%), followed by NDM+OXA-48 co-producers in 19 (21.6%). MecA-mediated methicillin resistance was identified in 19 isolates (21.6%), while VRE with VanA/VanB genes accounted for 8 isolates (9.1%). OXA-48 alone was found in 4 isolates (4.5%), and rare NDM+KPC and NDM+VIM co-producers were each detected in 1 isolate (1.1%). Organism-wise, all *A. baumannii* (6/6) and *A.lwoffii* (3/3) isolates carried NDM, and *K. pneumoniae* showed mixed NDM, OXA-48, and NDM+OXA-48 patterns. Among Gram-positive organisms, *S. aureus* included both MSSA and MRSA, while *S. haemolyticus* and *S. hominis* were mostly mecA-positive. *Enterococci* showed a mixture of VRE and susceptible phenotypes. [Table1]

Table 1: Resistant Gene Distribution

Organism	Total Isolates	Resistant Genes / Profile	No. Of Isolates
Acinetobacter Baumannii	6	Ndm	6
Acinetobacter Lwoffii	3	Ndm	3
Enterobacter Cloacae	1	Vre (Van A / Van B)	1
Enterococcus Faecalis	1	Vre (Van A / Van B)	1
Enterococcus Faecium	6	Vre (Van A / Van B)	6
Escherichia Coli	6	Ndm	5
		Ndm + Oxa 48 Cp Producer	1
Klebsiella Pneumoniae	26	Ndm + Oxa 48 Cp Producer	17

Organism	Total Isolates	Resistant Genes / Profile	No. Of Isolates
		Ndm	5
		Oxa 48	4
Morganella Morganii	1	Ndm	1
Pseudomonas Aeruginosa	4	Ndm	2
		Ndm + Kpc Co-Producer	1
		Ndm + Vim Co-Producer	1
Serratia Marcescens	1	Ndm + Oxa 48 Cp Producer	1
Staphylococcus Arlettae	1	Mec A / Methicillin Resistant (Mrcons)	1
Staphylococcus Aureus	15	Methicillin Sensitive (Mssa / Mscons)	11
		Mec A / Methicillin Resistant (Mrsa)	4
Staphylococcus Epidermidis	3	Methicillin Sensitive (Msse)	2
		Mec A / Methicillin Resistant (Mrse)	1
Staphylococcus Haemolyticus	9	Mec A / Methicillin Resistant (Mrcons / Mrsh)	9
Staphylococcus Hominis	5	Mec A / Methicillin Resistant (Mrcons)	4
		Methicillin Sensitive (Mscons)	1

Resistance determinants were more frequent in older age groups, particularly among patients aged 61–80 years. NDM-positive isolates were highest in the 71–80-year group, followed by 61–70 years and 51–60 years. NDM + OXA-48 co-producers also clustered in older adults, while VRE and mecA-mediated methicillin resistance showed a similar age-associated distribution. These findings indicate that the burden of molecular resistance increased with advancing age in this sepsis cohort [Table 2].

Table 2: Age-wise resistant gene distribution

Resistant Genes / Profile	Age Group (Yrs)	Female	Male	Total
Ndm	0–10	0	1	1
	11–20	0	1	1
	31–40	1	0	1
	41–50	1	1	2
	51–60	1	3	4
	61–70	3	1	4
	71–80	1	6	7
	81–90	0	2	2
Ndm + Oxa 48 Cp Producer	31–40	0	1	1
	41–50	1	1	2
	51–60	0	2	2
	61–70	4	2	6
	71–80	4	1	5
	81–90	0	3	3
Ndm + Kpc Co-Producer	71–80	0	1	1
Ndm + Vim Co-Producer	51–60	1	0	1
Oxa 48	51–60	1	1	2
	61–70	1	0	1
	71–80	0	1	1
Vre (Vana / Vanb)	41–50	1	0	1
	51–60	1	0	1
	61–70	2	1	3
	81–90	1	2	3
Mec A / Methicillin Resistant	0–10	1	2	3
	41–50	1	1	2
	51–60	4	1	5
	61–70	0	2	2
	71–80	4	2	6
	91–100	1	0	1
Methicillin Sensitive	41–50	0	1	1
	51–60	0	1	1
	61–70	2	7	9
	71–80	0	2	2
	81–90	0	1	1

While in general, males presented with a higher overall burden of NDM and NDM + OXA-48 resistance determinants, females had slightly more mecA positive isolates and VRE in this dataset. Rare co-producers were sex-specific, as NDM + KPC was found in a male and NDM + VIM occurred in a female. The differences observed may suggest an association between sex and overall profile of resistance genes, which would be different for various resistance mechanisms [Table 3].

SEX	FEMALE	MALE	TOTAL ISOLATES	PERCENTAGE (%)
NDM	7	15	22	25.00%
NDM + OXA 48 CP PRODUCER	9	10	19	21.59%
MEC A / METHICILLIN RESISTANT (MRSA / MRSE / MRCONS / MRSH)	11	8	19	21.59%
METHICILLIN SENSITIVE (MSSA / MSSE / MSCONS)	2	12	14	15.91%
VRE (VANA / VANB)	5	3	8	9.09%
OXA 48	2	2	4	4.55%
NDM + KPC CO-PRODUCER	0	1	1	1.14%
NDM + VIM CO-PRODUCER	1	0	1	1.14%
TOTAL	37	51	88	100.00%

Table 3: Sex-wise resistant gene distribution

ANTIBIOTIC SENSITIVITY PATTERN

The antibiogram revealed limited effectiveness of carbapenems against Gram-negative isolates. Among 27,*K. pneumoniae* strains tested, only one (3.7%) was susceptible to imipenem and one to meropenem. In contrast, aztreonam-avibactam was active against 18 isolates (66.7%), tigecycline against 19 (70.4%), and fosfomycin against 10 (37.0%). For the eight E. coli isolates examined, tigecycline showed full susceptibility (100%), while fosfomycin and chloramphenicol were effective in 75.0% of cases. *A. baumannii* exhibited low to moderate susceptibility, with trimethoprim being the most active agent at 50.0%.

Among Gram-positive isolates, *S. aureus* remained highly susceptible to chloramphenicol, daptomycin, linezolid, teicoplanin, tigecycline, and vancomycin, with susceptibility rates ranging from 86.7% to 93.3%. All tested *Staphylococcus haemolyticus* isolates were fully sensitive to daptomycin, linezolid, nitrofurantoin, and vancomycin. *Enterococcus faecalis* demonstrated complete resistance to all beta-lactams except penicillin's, showing 100% susceptibility to chloramphenicol and tigecycline, and 83.3% susceptibility to amoxicillin, ampicillin, benzylpenicillin, daptomycin, linezolid, imipenem, teicoplanin, and vancomycin.

The following table summarizes the sensitivity % [Table 4].

Table 4: Antibiotic sensitivity pattern

Organism	Total Isolate (N)	Antibiotic	No. Sensitive (N)	Sensitivity (%)
Klebsiella Pneumoniae	27	Tigecycline	18	66.7%
		CEFTAZIDIME-AVIBACTAM	6	22.2%
		CHLORAMPHENICOL	7	25.9%
		DOXYCYCLINE	5	18.5%
		FOSFOMYCIN	9	33.3%
		TRIMETHOPRIM	4	14.8%
		GENTAMICIN	6	22.2%
		DORIPENEM	1	3.7%
		ERTAPENEM	1	3.7%
		IMIPENEM	1	3.7%
		MEROPENEM	1	3.7%
		MINOCYCLINE	5	18.5%
		AMIKACIN	3	11.1%
		CEFEPIME-ENMETAZOBACTAM	1	3.7%
Staphylococcus Hominis	5	Amoxicillin	2	40%
		AZITHROMYCIN	2	40%
		BENZYLPENICILLIN	1	20%
		CEFAZOLIN	2	40%
		CEFOXITIN	2	40%
		CEFTRIAXONE	2	40%
		CHLORAMPHENICOL	3	60%
		CIPROFLOXACIN	4	80%
		CLINDAMYCIN	5	100%
		DAPTOMYCIN	5	100%
		DOXYCYCLINE	5	100%
		ERYTHROMYCIN	1	20%
		GENTAMICIN	5	100%
		IMIPENEM	2	40%
		LEVOFLOXACIN	3	60%
		LINEZOLID	5	100%
		NITROFURANTOIN	5	100%
		OXACILLIN	2	40%
		RIFAMPICIN	5	100%
		TAZOBACTAM	2	40%
		TEICOPLANIN	4	80%
		TETRACYCLINE	3	60%
		TIGECYCLINE	5	100%
		TRIMETHOPRIM	5	100%
		VANCOMYCIN	5	100%
Pseudomonas Aeruginosa	6	Fosfomycin	1	16.7%
		AMIKACIN	2	33.3%
		AZTREONAM	2	33.3%
		CEFEPIME	2	33.3%
		SULBACTAM	1	16.7%
		CEFTAZIDIME	1	16.7%
		CEFTAZIDIME-AVIBACTAM	1	16.7%
		CIPROFLOXACIN	1	16.7%
		DORIPENEM	2	33.3%
		GENTAMICIN	2	33.3%
		IMIPENEM	2	33.3%
		LEVOFLOXACIN	2	33.3%
		MEROPENEM	2	33.3%
		TAZOBACTAM	2	33.3%
		POLYMYXIN B	1	16.7%
		TOBRAMYCIN	1	16.7%

Organism	Total Isolate (N)	Antibiotic	No. Sensitive (N)	Sensitivity (%)
Escherichia Coli	8	Amikacin	3	37.5%
		CEFTAZIDIME-AVIBACTAM	1	12.5%
		CHLORAMPHENICOL	6	75%
		DORIPENEM	2	25%
		DOXYCYCLINE	5	62.5%
		ERTAPENEM	2	25%
		FOSFOMYCIN	5	62.5%
		GENTAMICIN	5	62.5%
		IMIPENEM	2	25%
		MEROPENEM	2	25%
		MINOCYCLINE	5	62.5%
		PLAZOMICIN	1	12.5%
		SULBACTAM	1	12.5%
		TAZOBACTAM	5	25%
		TIGECYCLINE	6	75%
		TRIMETHOPRIM	2	25%
Acinetobacter Baumannii	8	Amikacin	3	37.5%
		AZTREONAM	1	12.5%
		CEFEPIME	2	25%
		CEFOPERAZONE	1	12.5%
		CEFTAZIDIME	2	25%
		CEFTRIAXONE	2	25%
		CIPROFLOXACIN	3	37.5%
		DORIPENEM	2	25%
		DOXYCYCLINE	3	37.5%
		GENTAMICIN	3	37.5%
		IMIPENEM	2	25%
		LEVOFLOXACIN	3	37.5%
		MEROPENEM	2	25%
		MINOCYCLINE	3	37.5%
		SULBACTAM	3	37.5%
		TAZOBACTAM	3	37.5%
		TIGECYCLINE	4	50%
		TRIMETHOPRIM	5	62.5%
Enterococcus Faecalis	6	Amoxicillin	5	83.3%
		AMPICILLIN	1	16.7%
		AZITHROMYCIN	1	16.7%
		BENZYLPENICILLIN	5	83.3%
		CHLORAMPHENICOL	6	100%
		CIPROFLOXACIN	2	33.3%
		DAPTOMYCIN	5	83.3%
		DOXYCYCLINE	2	33.3%
		ERYTHROMYCIN	1	16.7%
		FOSFOMYCIN	1	16.7%
		GENTAMICIN	4	66.7%
		IMIPENEM	5	83.3%
		LEVOFLOXACIN	4	66.7%
		LINEZOLID	5	83.3%
		NITROFURANTOIN	4	66.7%
		RIFAMPICIN	2	33.3%
		TAZOBACTAM	5	83.3%
		TEICOPLANIN	5	83.3%
		TETRACYCLINE	1	16.7%
		TIGECYCLINE	6	100%
		TRIMETHOPRIM	2	33.3%
		VANCOMYCIN	5	83.3%

Organism	Total Isolate (N)	Antibiotic	No. Sensitive (N)	Sensitivity (%)
Enterococcus Faecium	7	Chloramphenicol	6	85.7%
		DOXYCYCLINE	3	42.9%
		FOSFOMYCIN	1	14.3%
		GENTAMICIN	1	14.3%
		LINEZOLID	6	85.7%
		TIGECYCLINE	5	71.4%
Staphylococcus Aureus	13	Amoxicillin	9	69.2%
		AZITHROMYCIN	5	38.5%
		CEFAZOLIN	9	69.2%
		CEFOXITIN	8	61.5%
		CEFTRIAXONE	8	61.5%
		CHLORAMPHENICOL	13	100%
		CIPROFLOXACIN	4	30.8%
		CLINDAMYCIN	8	61.5%
		DAPTOMYCIN	12	92.3%
		DOXYCYCLINE	11	84.6%
		ERYTHROMYCIN	5	38.5%
		FOSFOMYCIN	11	84.6%
		GENTAMICIN	9	69.2%
		IMIPENEM	9	69.2%
		LEVOFLOXACIN	7	53.8%
		LINEZOLID	13	100%
		NITROFURANTOIN	13	100%
		OXACILLIN	9	69.2%
		RIFAMPICIN	11	84.6%
		TAZOBACTAM	9	69.2%
		TEICOPLANIN	13	100%
		TETRACYCLINE	13	100%
		TIGECYCLINE	13	100%
		TRIMETHOPRIM	12	92.3%
		VANCOMYCIN	13	100%
Staphylococcus Haemolyticus	9	Chloramphenicol	7	77.8%
		DAPTOMYCIN	9	100%
		DOXYCYCLINE	5	55.6%
		LINEZOLID	8	88.9%
		TIGECYCLINE	7	77.8%
		TEICOPLANIN	7	77.8%
		NITROFURANTOIN	9	100%
		TETRACYCLINE	4	44.4%
		VANCOMYCIN	9	100%
		FOSFOMYCIN	3	33.3%
		CLINDAMYCIN	2	22.2%
		RIFAMPICIN	5	55.6%
		TRIMETHOPRIM	7	77.8%
		GENTAMICIN	3	33.3%
		LEVOFLOXACIN	2	22.2%
		CIPROFLOXACIN	1	11.1%
Staphylococcus Epidermidis, Serratia Marcescens. Candida Auris: 3 Each Enterobacter Cloacae Complex, Acinetobacter Lwoffii: 2 Each Staphylococcus Arlettae, Candida Albicans, Chryseobactam Indologenes, Streptococcus Alactolyticus, Proteus Mirabilis: 1 Each				

CROSS-SITE ORGANISM AND RESISTANCE CONCORDANCE

In 10 paired cases of pneumosepsis and urosepsis, 36 clinically significant isolates were recovered from blood, urine, endotracheal suction, bronchoalveolar lavage fluid, tracheal tube suction, and sputum. Blood was the commonest specimen source, followed by urine and respiratory samples. *Klebsiella*

pneumoniae was the predominant pathogen, followed by *Escherichia coli, Acinetobacter spp.,* and *Pseudomonas aeruginosa*. Several isolates demonstrated multidrug-resistant or pan-drug resistant phenotypes, and *K. pneumoniae* frequently carried NDM and/or OXA-48-like carbapenemase genes.

DISCUSSION

In this study, the microbiological profile was determined in 110 clinical isolates of blood stream infection patients and the antibiotic resistance profile was evaluated in the isolates. The effectiveness of the targeted multiplex PCR (mPCR) was also assessed in the diagnosis of septicemia. A high rate of multidrug resistant (MDR) organisms, especially *carbapenem resistant gram-negative bacilli* were identified and the results indicate that rapid molecular testing for pathogen identification and resistance gene detection is beneficial in reducing time to pathogen identification [13-15].

Demographic Profile and Clinical Relevance

The present study reports a significant predominance of patients aged above 60 years, with the highest proportion of sepsis cases recorded among those aged between 71 and 80 years with the majority of patients being male. This finding aligns with the observation that the occurrence of sepsis is higher among the elderly and males, which might be explained by a higher incidence of concomitant illnesses recorded among the geriatric population and higher likelihood of undergoing procedures among males. The implication of the findings is reflected in the fact that timely recognition and management of sepsis within the first hours are paramount to improving the patient outcome, with the delay being particularly detrimental to the health status of the elderly, thus underscoring the need of rapid diagnostic tools allowing for the optimization of antimicrobial therapy in accordance with the standard-of-care guidelines for managing sepsis [17].

The microbiological profile of the cohort was dominated by multidrug-resistant Gram-negative bacilli, especially *Klebsiella pneumoniae* and *Escherichia coli*, with additional contributions from *Staphylococcus aureus*, coagulase-negative *staphylococci*, *enterococci*, and occasional fungal isolates. Taken together, these findings suggest that bloodstream infection in this population was strongly influenced by age-related vulnerability, male predominance, comorbidity burden, and healthcare-associated antimicrobial resistance.

Microbial Spectrum

The most common microbe was *Klebsiella pneumoniae*, which has been found to be the main bacterial cause of hospital-acquired bacterial infections in India. The most common Gram-positive bacterium was *Staphylococcus aureus*, whereas coagulase-negative staphylococci, particularly *Staphylococcus haemolyticus*, were present in significant quantities. These results highlight the present diagnostic dilemma of identifying whether contamination or true bacteremia is the cause of the bacteria in a blood culture test. Other isolates that were obtained were *Enterococcus faecium*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. The discovery of *Candida auris* (n=3) and *Candida albicans* (n=1) underscores the growing problem of Multi-Drug-Resistant Organisms (MDROs) in the intensive care unit [18].

INCUBATION PERIOD & TIME TO DETECTION

The investigation of the incubation duration showed that although the majority of positive blood cultures were detected in between 12-18 hours (median 15:43 hours), other organisms, *such S. aureus*, needed longer incubation time (up to 68 hours). Conventional blood culture systems, which normally take 24 to 72 hours for preliminary results and more time for species identification and antibiotic susceptibility testing, are known to have delay in pathogen identification.

Neonatal Sepsis

Rapid diagnostic approaches are important as the delay in diagnosing in early-onset newborn sepsis can greatly increase morbidity and mortality in vulnerable pediatric groups. Neonates as young as 6 days old, were found in our study, from which *Staphylococcus haemolyticus*, *Staphylococcus epidermidis* and *Escherichia coli were* detected. Our pediatric subset showed the clinical and diagnostic value of a direct molecular panel for the rapid diagnosis of clinically relevant Gram-positive and Gram-negativepathogens. For neonatal septicemia, multiplex molecular diagnostics are early, pathogen-specific diagnostic techniques that can assist direct prompt clinical therapies with the right antibiotics [8-16].

Resistance Gene Determinants

Molecular characterisation of the isolated pathogens revealed multidrug resistance (MDR) to be a significant issue. The primary carbapenemase determinant, either by itself or in combination with blaOXA-48, was the most common gene discovered. Patients between the ages of 61 and 80 years have had notably high percentages of *Klebsiella pneumoniae* isolates with blaNDM alone, blaNDM+blaOXA-48. This distribution is typical of the epidemiology of carbapenem resistance in South Asia, where the predominant drivers of clinical resistance are blaNDM and blaOXA-48 variants, in contrast to other Western settings [19]. Thus regional specific multiplex PCR (mPCR) target panels is necessary due to these geographic variations [20].

Furthermore, uncommon co-producer genotypes like blaNDM+ blaVIM and blaNDM+ blaKPChighlight the development of a complex resistance pattern. Vancomycin resistance in *Enterococcus species* (vanA/B) and methicillin resistance in *Staphylococcus aureus* (mecA, 30.8% MRSA prevalence) were identified among Gram-positive isolates, with VRE primarily seen in individuals

over 40. These genotypic results were confirmed by phenotypic susceptibility testing. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* also showed widespread pan-drug resistance (PDR), with susceptibility to most antimicrobial classes ranging from 20% to 40%. This is consistent with national surveillance data from India that shows increased carbapenem resistance in tertiary care facilities [21–23].

CONVENTIONAL BLOOD CULTURE & MULTIPLEX PCR

The findings of the present study further elucidate the complementary nature of conventional blood culture and multiplex PCR in diagnosing sepsis patients. Specifically, whereas conventional blood culture remains an indispensable tool in sepsis diagnostics enabling the isolation of viable microorganisms, their identification at the species level, and the determination of their susceptibility profile, multiplex PCR assay allows for the rapid identification of resistance-conferring genes, thus playing a critical role in guiding the choice of antimicrobial agents used to manage the infection. Particularly in the context of sepsis, rapid diagnostics allowing for the timely introduction of targeted antimicrobial therapy are paramount to improving the patient outcome.

The overall antimicrobial susceptibility profile of the isolated microorganisms further informs the choice of antimicrobial agents used to manage BSI and sepsis patients on a case-by-case basis. Notably, the carriage of carbapenemase genes among *K. pneumoniae* isolates rendered them resistant to carbapenems, whereas their susceptibility to aztreonam-avibactam and tigecycline could be used as a basis for selecting the antimicrobial agent for managing carbapenem-resistant NDM/OXA-48-producing *K. pneumoniae* isolates. In turn, the susceptibility of *E. coli* and non-fermenters to fosfomycin, tigecycline, and aminoglycosides detected in some isolates can similarly be utilized for guiding the selection of antimicrobial agents for managing patients with infections caused by these bacteria.

The presence of linezolid, vancomycin, teicoplanin, and daptomycin susceptibility among the Gram-positive bloodstream isolates was also noted and could be used as a reference when deciding on the antimicrobial regimen for treating Gram-positive blood stream infection or sepsis patients. In particular, the study detected mecA in *S. aureus* and coagulase-negative *staphylococci*, which can be utilized to guide the management of MRSA bacteremia and to adjust the empirical antimicrobial regimen, if such is chosen based on the PCR findings. Similarly, the finding of vanA/vanB in enterococci isolates has immediate therapeutic implications since these bacteria are resistant to vancomycin, and thus, the management of VRE bloodstream infection requires the use of specific antibiotics with activity against *Enterococcus spp*., such as linezolid, and other narrow-spectrum antimicrobial agents.

CROSS-SITE CONCORDANCE

The findings of the present study further demonstrate the significance of paired-isolate analysis to elucidate the etiology of bacteremia and sepsis and to guide the management of these conditions. In particular, the observation that some of the isolates obtained from blood cultures were found to share resistance profiles with those obtained from urine, highlights on the potential for the presence of similar microorganisms in paired specimens. This finding has significant clinical implications, given that, in many cases, blood cultures remain positive only after a few attempts, and therefore, there is a clinical tendency to disregard the results of a negative blood culture, particularly if the urine sample obtained from the same patient is positive. The finding of resistance genes specific to certain bacteria, such as ESBL genes specific to E*nterobacteriaceae*, or vancomycin resistance-specific to *Enterococcus spp*., could also benefit from such paired-isolate analysis.

The detection of a number of Gram-negative bacilli, including *E. coli* and *K. pneumoniae*, in paired blood and urine cultures, as well as *Enterococcus faecium* and *Staphylococcus epidermidis* in paired urine and respiratory cultures further demonstrate the potential for multiple bloodstream infection etiologies. In particular, the findings of such bacteria in paired respiratory cultures suggest the possibility of polymicrobial infection. Additionally, the fact that a number of BAL isolates exhibited, higher minimum inhibitory concentration (MIC) values to certain third-generation cephalosporins than their paired blood isolates could potentially be explained by the inducible chromosomal resistance genes, which were more likely to be expressed due to the exposure of BAL fluid to these agents. Therefore, the findings of the study suggest that the presence of similar microorganisms in paired specimens is rather common, which should be taken into consideration when performing diagnostic testing and determining the antimicrobial regimen for managing sepsis patients.

The overall data showed that Gram-negative bacilli (E. coli and K. pneumoniae) were again predominant in paired sites; *Enterococcus faecium* and *Staphylococcus epidermidis* were also recovered at secondary urinary and respiratory sites, which demonstrated polymicrobial presentations in critical illness. High phenotypic antimicrobial susceptibility concordance was observed between paired blood and urine isolates, especially for fluoroquinolones and [24, 25].

LIMITATION OF THE STUDY

The findings may not be as applicable in different hospital settings because the study was only carried out at one facility. Although the sample size of

97 isolates offers insightful information, it might not fully capture the range of microbial diversity and resistance patterns in larger populations. For some organisms, only a single isolate was available, resulting in 100% sensitivity to certain antibiotics. However, these findings should be interpreted cautiously, as large number of isolates and greater organism variability are required for reliable determination of antibiotic sensitivity patterns. Only 10 patients were included in the exploratory examination of cross-site concordance; larger cohorts are needed for validation.

Key Findings

Klebsiella pneumoniae was the leading bloodstream pathogen, followed by *S. aureus, E. coli, Enterococcus spp., A. baumannii, and P. aeruginosa*. Most positive cultures were detected within 20 h, showing that conventional culture was relatively rapid for initial signal detection. NDM and NDM+OXA-48 were the dominant resistance mechanisms, together representing a major share of PCR-positive isolates. MecA-positive *staphylococci* and VanA/VanB-positive *enterococci* contributed significantly to the resistant burden. Carbapenems were largely ineffective against major Gram-negative isolates, while aztreonam-avibactam and tigecycline had better activity against selected MDR strains. Blood and source-site isolates were often concordant, but some showed clinically important discordance. Multiplex PCR provided rapid resistance profiling, but conventional blood culture remained essential for definitive diagnosis.

CONCLUSION

This study presents evidence that septicaemia and bacteraemia cases in this cohort are characterized by a prevalence of multidrug resistant pathogens, presenting resistance to carbapenems, with *Klebsiella pneumoniae* being the most common isolate. The domination of blaNDM and blaOXA-48 among resistance genes, alongside the presence of mecA and vanA/B among resistance genes, suggests the local resistance pattern in South Asia and allows the customization of multiplex PCR panels for local pathogens in this area. Concordance between genotype and phenotypic results for mPCR panel testing and targeted tests, as well as between paired blood and non-blood cultures, supports the assumption that targeted mPCR could be used as a powerful tool to rapidly identify pathogens and their resistance mechanisms in cases of sepsis [26]. Compared to the 48–72-hour delay usually associated with conventional culture-based antibiograms, the use of rapid molecular multiplex assays significantly shortened the diagnostic turnaround time, allowing pathogen and resistance-marker detection within 2 hours of positive blood culture notification. This accelerated diagnostic window decreased the requirement for prolonged empiric broad-spectrum coverage by allowing the earlier initiation of targeted antibiotic therapy. Consequently, the study highlights the importance of improving hospital infection control management and stepping up efforts to detect and monitor antibiotic resistance.

Conflict of Interest: The authors declare no conflict of interest.

Author's Contribution:

Dr. Bhaskar Narayan Chaudhuri, Dr. Partha Guchhait, and Dr. Satadal Das designed the study procedure, and corrected the manuscript. Ms. Ruparna Kayal prepared the manuscript. All Authors checked and approved the manuscript.

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