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Original Article

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Multiplex PCR for Rapid and Accurate Diagnosis of Bloodstream Pathogens in Patients with Suspected Sepsis

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Abstract

Background: Timely diagnosis of Bloodstream Infections (BSIs) is crucial for effective sepsis management. Conventional culture methods, though considered the gold standard, exhibit limitations. This study aimed to evaluate the efficacy of multiplex Polymerase Chain Reaction (PCR) for rapid and accurate detection of bloodstream pathogens in suspected sepsis patients.

Methods: The study was conducted between March 2021 and March 2022 at Valiasr Hospital, Zanjan, Iran. One hundred patients with suspected sepsis were recruited, and blood samples were collected for both methods. Demographic and clinical data were collected, and genomic DNA was extracted for PCR. Data were analyzed using SPSS software.

Results: Most patients were elderly (>60 years), and Multiplex-PCR demonstrated higher detection rates than culture. Age, antibiotic history, and infection site were associated with bacterial frequency. A significant relationship existed between bacterial frequency and patient outcome. Pulmonary infections were most common, with specific imaging patterns observed.

Conclusion: Multiplex PCR is a rapid and sensitive tool for diagnosing sepsis, offering superior sensitivity compared to culture. Further research is needed to validate its broader clinical application.

Keywords: Bacteria, Demography, DNA, Hospitals, Iran, Multiplex polymerase chain reaction, Sepsis, Genomics

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Introduction

Sepsis, a life-threatening condition characterized by a Systemic Inflammatory Response Syndrome (SIRS) following infection, remains a significant public health challenge worldwide (1,2). Early and accurate diagnosis of Bloodstream Infections (BSIs) is crucial for promptly initiating appropriate antimicrobial therapy, the cornerstone of sepsis management (3). While considered the gold standard for BSI diagnosis, conventional culture-based methods have limitations, including extended Turnaround Times (TATs), susceptibility to contamination, and suboptimal sensitivity when samples are obtained from patients on antibiotics. These limitations emphasize the need for rapid and accurate diagnostic tools to guide timely treatment decisions and improve patient outcomes (4,5).

In recent years, multiplex Polymerase Chain Reaction (PCR) has emerged as a promising alternative for rapidly and sensitively detecting pathogens in blood specimens (6). Multiplex PCR assays amplify multiple target sequences simultaneously, enabling the detection of a broader range of pathogens in a single reaction (7). Compared to conventional culture methods, multiplex PCR offers several advantages, including faster TATs, reduced risk of contamination, and the ability to detect fastidious or antibiotic-resistant pathogens (8). Several studies have evaluated the performance of multiplex PCR for diagnosing BSIs in patients with suspected sepsis. These studies have demonstrated that multiplex PCR can achieve high sensitivity and specificity comparable to or even better than conventional culture methods (9-11). Moreover, multiplex PCR has been shown to identify pathogens that culture may miss, particularly in patients receiving antibiotics (12).

The introduction of multiplex PCR assays can potentially transform the diagnosis and management of BSIs (13). By providing rapid and accurate pathogen identification, multiplex PCR can enable the timely initiation of appropriate antimicrobial therapy, potentially improving patient outcomes and reducing healthcare costs (14). Therefore, this study aims to rigorously evaluate and compare the diagnostic efficacy of traditional bacterial culture and modern multiplex PCR in identifying sepsis-causing micro-organisms. Also, this study provides a comprehensive understanding of the demographic and clinical factors associated with sepsis and seeks to unravel critical associations influencing sepsis outcomes. The findings of this study may pave the way for the broader implementation of multiplex PCR in routine clinical practice, potentially transforming the landscape of sepsis diagnosis and management.

Materials and Methods

Patient recruitment and sample collection

This cross-sectional study included 100 patients with suspected sepsis admitted to the Infectious Diseases Department of Valiasr Hospital, Zanjan, Iran, between March 2021 and March 2022. Convenience sampling was used to recruit patients aged 17 to 96 years. Due to the limited study duration and access to eligible patients during hospitalization, this type of sampling was used. Inclusion criteria were suspected sepsis based on clinical signs, laboratory findings, and absence of antibiotic treatment within 48 *hr* before sample collection. Two blood samples were drawn per patient: 8-10 *ml* of venous blood for conventional blood cultures and 2 *ml* of EDTA-treated whole blood for molecular analysis. Samples were collected under aseptic conditions, each set taken from the same venipuncture to minimize patient discomfort and prevent contamination.

Infection control protocols

Standard infection control practices were followed to ensure consistency in clinical management and reduce contamination risks, including daily patient bathing if needed, regular intravenous line changes every 72 *hr*, and strict adherence to debridement protocols for burn wounds. Carbapenems were the primary empiric choice in cases of suspected sepsis. These protocols were implemented to limit biases related to institutional practices. The infection control protocols followed were based on the standard guidelines of the Infection Control Committee of Valiasr Hospital, adapted from the Centers for Disease Control and Prevention (CDC) guidelines for infection prevention (15).

Microbiological culture methods

Blood culture samples were inoculated into aerobic and anaerobic bottles with tryptic soy broth containing

SPS anticoagulant. Cultures were incubated using the automated Bactec blood culture system (Becton Dickinson, USA) at 35°*C* for up to seven days. Samples exhibiting microbial growth were subjected to Gram staining, subculturing, and identification according to standard microbiological procedures. No selective media were used in this process to avoid excluding potentially relevant pathogens.

Multiplex PCR for pathogen detection

Genomic DNA was extracted from EDTA whole blood samples using the GeneAll Blood DNA extraction kit following the manufacturer's protocol, which involved cell lysis, protein digestion, and elution of purified DNA. The quality and purity of extracted DNA were verified by NanoDrop spectrophotometry (Thermo Scientific, USA), with an optimal 260/280 ratio of approximately 1.8. Additionally, DNA integrity was assessed by gel electrophoresis (0.8% agarose gel) (16). PCR amplification targeted five major bloodstream pathogens frequently associated with sepsis: *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), and *Klebsiella pneumonia* (*K. pneumoniae*). Specific primers were designed for the following genes: femA (*S. aureus*), sesC (*S. epidermidis*), oprL (*P. aeruginosa*), K1 (*K. pneumoniae*), and MetH (*E. coli*). Primers were synthesized by Metabion (Germany) and are detailed in table 1. PCR was conducted on a SimpliAmp thermal cycler (Applied Biosystems, USA) with the following parameters: initial denaturation at 95°*C* for 5 *min*, followed by 30 cycles of denaturation at 95°*C* for 25 *s*, primer annealing at optimal temperatures for 40 *s*, and extension at 72°*C* for 50 *s*. A final extension was performed at 72°C for 6 *min*. Amplification products were visualized on a 1.5% agarose gel stained with ethidium bromide (17).

Ethical approval

The Ethics Committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1399.451) approved the study protocol. All eligible patients gave oral informed consent before participating in the study.

Data collection and analysis

Demographic and clinical data were collected using a structured questionnaire, including age, gender, risk factors (*e.g.*, immunodeficiency, chronic diseases, prosthetic implants), clinical symptoms, and imaging results. Statistical analyses were conducted using SPSS 22 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro-Wilk test and histogram plots. Categorical variables were analyzed using Chi-square or Fisher's exact tests. A p-value of <0.05 was

Table 1. Primers sequences used for amplification of specific genes

Primers		Sequences 5′ → 3′	Size (*bp*)	References
femA	Forward	AAAAAAGCACATAACAAGCG	132	(39)
femA	Reverse	GATAAAGAAGAAACCAGCAG		
sesC	F	GTTGATAACCGTCAACAAGG	388	(40)
SesC	R	CATGTTGATCTTTTGAATCCC		
oprL	F	AACAGCGGTGCCGTTGAC	131	(41)
oprL	R	GTCGGAGCTGTCGTACTCGAA		
K1	F	GGTGCTCTTTACATCATTGC	1283	(42)
K1	R	GCAATGGCCATTTGCGTTAG		
MetH	F	CGTGGTGGTCGCTTTTACCACAGAT	106	(43)
MetH	R	TCCACTTTGCTGCTCACACTTGCTC		

considered statistically significant.

Results

Demographic characteristics of patients

The study included 100 patients aged between 17 and 96 years. The majority of patients, 74 cases (74%), were over 60 years old. Sixteen patients (16%) were aged 41–60, while 10 patients (10%) were under the age of 40. In terms of residency, 71 patients resided in urban areas and 29 in rural areas.

Comparison of PCR and culture for pathogen detection

Multiplex PCR results were obtained within 4–6 *hr* after blood sample collection, enabling faster clinical decision-making. The bacterial pathogens detected included *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*. PCR showed higher sensitivity compared to culture, detecting more pathogens overall. Table 2 provides a detailed comparison of detection frequencies between PCR and culture methods.

Bacterial distribution according to gender

Analysis showed a statistically significant relationship between gender and the frequency of *S. epidermidis* (p=0.001). However, no significant gender-based differences were observed for the other pathogens (*S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*). Details are presented in table 3.

Antibiotic history and pathogen distribution

Of the 100 patients, seven had received antibiotics prior to hospitalization, while 93 had not. A significant association was found between previous antibiotic use and the detection of *S. epidermidis*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (p=0.001). This finding suggests prior antibiotic exposure may influence pathogen distribution.

Clinical outcomes and pathogen frequency

Clinical outcomes included 56 deaths, 42 recoveries, and 2 ICU admissions. A statistically significant relationship was identified between patient outcome and the frequency of all five bacterial species ($p<0.05$), as summarized in table 5. These findings highlight the prognostic value of pathogen detection in septic patients.

Infection site and pathogen prevalence

Pulmonary infections were the most frequent (31 cases), followed by cardiac/pulmonary (14 cases), pulmonary/hepatic (8 cases), and pulmonary/renal (8 cases). A significant association was observed between the infection site and the frequency of *E. coli* ($p<0.001$), *K. pneumoniae* ($p<0.001$), and *P.*

Table 2. Summary of bacterial detection by Multiplex PCR and culture, stratified by demographics, antibiotic history, and outcome

Variable	*S. aureus*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Detection method	Culture: 1 PCR: 6	Culture: 7 PCR: 8	Culture: 0 PCR: 4	Culture: 2 PCR: 6	Culture: 3 PCR: 8
Gender (positive cases)	Male: 1 Female: 5 p=0.092	Male: 3 Female: 5 p=0.001	Male: 2 Female: 2 p=0.920	Male: 3 Female: 3 p=0.940	Male: 4 Female: 4 p=0.912
Antibiotic history (positive cases out of patients with vs. without prior antibiotic use)	0 out of 7 *vs.* 6 out of 93 p=0.914	1 out of 7 *vs.* 7 out of 93 p=0.001	0 out of 7 *vs.* 4 out of 93 p=0.001	0 out of 7 *vs.* 6 out of 93 p=0.001	1 out of 7 *vs.* 7 out of 93 p=0.001
Outcome (positive cases)	Improved: 4 ICU: 0 Deceased: 2 p=0.001	Improved: 2 ICU: 0 Deceased: 6 p=0.001	Improved: 0 ICU: 0 Deceased: 4 p=0.001	Improved: 2 ICU: 0 Deceased: 4 p=0.001	Improved: 1 ICU: 1 Deceased: 6 p=0.028

* p-value level based on Fisher's Exact Test; *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), and *Klebsiella pneumonia* (*K. pneumoniae*).

Table 3. Frequency of sources of infection by type of bacteria

Bacterial infection / Sources of infection	*S. aureus*		*S. epidermidis*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
	P	N	P	N	P	N	P	N	P	N
Pulmonary	2	29	3	28	1	30	1	30	2	29
Hepatic	1	0	0	1	0	1	0	1	0	1
Pulmonary/hepatic	2	6	2	6	0	8	0	8	0	8
Cardiac/pulmonary	0	14	0	14	1	13	2	12	1	13
Hepatic/renal	0	2	0	2	0	2	1	1	0	2
Pulmonary/kidney	0	8	1	7	2	6	0	8	0	8
Pulmonary/articular	1	1	0	2	0	2	0	2	0	2
Pulmonary/nervous	0	5	0	5	0	5	1	4	3	2
Pulmonary/cutaneous	0	2	0	2	0	2	1	1	0	2
Cardiac/pulmonary/renal	0	1	1	0	0	1	0	1	0	1
Pulmonary/kidney/skin	0	1	0	1	0	1	0	1	1	0
other	0	25	1	24	0	25	6	27	1	24
p-value	0.068		0.075		<0.001		<0.001		0.038	

Staphylococcus aureus (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), and *Klebsiella pneumonia* (*K. pneumoniae*).

Table 4. Frequency of bacterial strains based on history of antibiotic use

Taking antibiotics	Bacterial infection	Have number (%)	Does not have number (%)	p-value*
S. aureus	Positive	0(0)	6(6)	0.914
	Negative	7(7)	87(87)	
S. epidermidis	Positive	1(1)	7(7)	0.001
	Negative	6(6)	86(86)	
E. coli	Positive	0(0)	4(4)	0.001
	Negative	7(7)	89(89)	
K. pneumoniae	Positive	0(0)	6(6)	0.001
	Negative	7(7)	87(87)	
P. aeruginosa	Positive	1(1)	7(7)	0.001
	Negative	6(6)	86(86)	

* p-value level based on Fisher's Exact Test.

aeruginosa (p=0.038). Table 6 presents the infection site distribution in detail.

Imaging findings

Chest radiographs and CT scans revealed pulmonary abnormalities in 86 patients. The most common radiological findings were pleural effusion, followed by reticular opacities and Ground-Glass Opacities (GGO). These results indicate that pulmonary involvement is a predominant feature in patients with suspected sepsis.

Table 5. Relationship between the outcome and the abundance of bacteria (Frequency of bacterial strains according to outcome)

Age group / Bacterial infection		Improved number (%)	ICU- required number (%)	Deceased number (%)	p-value*
S. aureus	Positive	4(4)	0(0)	2(2)	0.001
	Negative	38(38)	2(2)	54(54)	
S. epidermidis	Positive	2(2)	0(0)	6(6)	0.001
	Negative	40(40)	2(2)	50(50)	
E. coli	Positive	0(0)	0(0)	4(4)	0.001
	Negative	42(42)	2(2)	52(52)	
K. pneumoniae	Positive	2(2)	0(0)	4(4)	0.001
	Negative	40(40)	2(2)	52(52)	
P. aeruginosa	Positive	1(1)	1(1)	6(6)	0.028
	Negative	41(41)	1(1)	50(50)	

* p-value based on Chi-square Test.

Table 6. Frequency of sources of infection by type of bacteria

Bacterial infection / Sources of infection	*S. aureus*		*S. epidermidis*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
	P	N	P	N	P	N	P	N	P	N
pulmonary	2	29	3	28	1	30	1	30	2	29
Hepatic	1	0	0	1	0	1	0	1	0	1
Pulmonary/Hepatic	2	6	2	6	0	8	0	8	0	8
Cardiac/Pulmonary	0	14	0	14	1	13	2	12	1	13
Hepatic/renal	0	2	0	2	0	2	1	1	0	2
Pulmonary/kidney	0	8	1	7	2	6	0	8	0	8
Pulmonary/articular	1	1	0	2	0	2	0	2	0	2
Pulmonary/nervous	0	5	0	5	0	5	1	4	3	2
Pulmonary/cutaneous	0	2	0	2	0	2	1	1	0	2
Cardiac/Pulmonary/Renal	0	1	1	0	0	1	0	1	0	1
Pulmonary/kidney/skin	0	1	0	1	0	1	0	1	1	0
Other	0	25	1	24	0	25	6	27	1	24
p-value	0.068		0.075		<0.001		<0.001		0.038	

Discussion

In this study, the aim was to evaluate the utility of multiplex PCR for the rapid and accurate detection of bloodstream pathogens in patients with suspected sepsis. The results revealed that most patients included in the study were elderly, a pattern that has also been observed in previous studies (18-20). Interestingly, consistent with other studies, the PCR results demonstrated a higher detection rate of bacterial pathogens than traditional culture methods, suggesting the superior sensitivity of molecular testing in identifying bloodstream pathogens (21-23).

Although various studies around the world have identified various bacteria as the most common cause of sepsis (24,25), the present study is consistent with the findings of Gheybi *et al*, Tabatabaei *et al*, and Ghadiri *et al*. coagulase-negative *Staphylococcus* and *P. aeruginosa* identified as the most common cause of sepsis (26-28).

The findings of the present study also highlighted significant associations between patient demographics and the frequency of bacterial strains. Specifically, a notable relationship was observed between age and the prevalence of bacterial pathogens, indicating a potential age-related susceptibility to bloodstream infections. These results align with the findings of several studies conducted in America, Taiwan, and Saudi Arabia (29-31). Additionally, the history of antibiotic use was significantly correlated with the frequency of specific bacterial strains, underscoring the influence of prior antibiotic exposure on microbial composition in septic patients (32-34). Furthermore, the study identified a significant correlation between the outcome of sepsis patients and the frequency of bacterial pathogens. Consistent with previous literature, our results suggest that the presence of specific bacterial strains may contribute to the severity and mortality of sepsis cases (35).

Moreover, the infection site was associated with the prevalence of particular bacterial species, emphasizing the importance of understanding infection localization for targeted treatment strategies (36,37). Notably, pulmonary infections were the most common type of infection observed in the present study cohort, with imaging findings indicative of pleural effusion and distinct patterns such as reticular and GGO patterns. These findings align with previous reports highlighting the prevalence of pulmonary involvement in septic patients, emphasizing the need for prompt diagnosis and management of respiratory complications in sepsis (37,38).

In summary, the present study underscores the potential of multiplex PCR as a valuable tool for the rapid and accurate detection of bloodstream pathogens in patients with suspected sepsis. The findings contribute to the growing body of evidence supporting the use of molecular diagnostics in improving the timely identification and management of septic patients, ultimately facilitating better clinical outcomes.

Limitations

The small sample size may limit the generalizability of the findings, and larger cohorts are necessary to validate the broader application of the multiplex PCR method. Also, relying on data from a single center may introduce institutional biases, and a multicenter approach may increase the external validity of the results. Although demographic associations were explored, deeper correlations between molecular findings and specific clinical outcomes were not extensively studied, leaving room for further exploration. Furthermore, the use of convenience sampling may have introduced selection bias, potentially limiting the generalizability of the findings. Additionally, due to the lack of per-patient matched data between PCR and culture results, sensitivity and specificity could not be calculated. Future studies should include individual-level diagnostic comparisons to enable such analysis. These limitations emphasize the need for future research efforts to address these limitations and strengthen the evidence base supporting the implementation of multiplex PCR for diagnosing sepsis.

Conclusion

The present study demonstrates the utility of multiplex PCR as a rapid and sensitive diagnostic tool for identifying bloodstream pathogens in patients with suspected sepsis. The results highlight the superior sensitivity of multiplex PCR over traditional culture methods, with a higher detection rate of bacterial pathogens. Significant associations between patient demographics, antibiotic history, and outcomes emphasize the multifactorial nature of sepsis. The study underscores the potential of multiplex PCR to transform sepsis diagnosis and management by providing timely and accurate pathogen identification, contributing to improved patient outcomes. Further research and implementation studies are warranted to establish the broader integration of multiplex PCR into routine clinical practice, thereby enhancing the sepsis diagnosis and treatment landscape.

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Ethical approval

The Ethics Committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1399.451) approved the study protocol. All eligible patients gave oral informed consent before participating in the study.

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Conflict of Interest

The authors declare no conflict of interest.

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