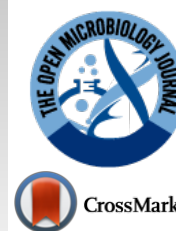




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RESEARCH ARTICLE

Rapid Detection of Pathogenic Bacteria in Whole Blood Samples Using 23S rRNA PCR Assays

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Abstract:

Purpose:

Bloodstream infections are a general cause of death among hospitalized patients. Rapid diagnosis and timely treatment can reduce mortality. The aim of this investigation was to evaluate the 23S rRNA PCR assays as a rapid detection method for diagnose of sepsis in patients with suspected bacteremia.

Methods:

A cross-sectional study was conducted at Shahid Beheshti University Hospital in Kashan from November 2017 to December 2018. The blood samples of 265 patients with suspected bacteremia were studied by blood culture and 23S rRNA PCR techniques. The results were analyzed using SPSS version 16 and Chi-square test.

Results:

Eighty (30.2%) blood samples of 265 suspected patients, were identified as positive by PCR assays, whereas 27 (10.2%) were identified as positive by the blood culture technique. The statistical analysis showed a significant association between the results of PCR assays and blood culture and factors such as prior antibiotic use and underlying diseases ($P < 0.05$). Also a significant correlation was observed between laboratory and clinical criteria and the results of both PCR assays and blood culture ($P < 0.05$).

Conclusion:

The 23S rRNA PCR method is a rapid and sensitive technique specially for diagnosing sepsis among patients in whom bacteremia is difficult to diagnose with culture method including neonates and patients who have taken antibiotics before microbial culture.

Keywords: Bloodstream infections, Blood culture, 23S rRNA, PCR, Microbial culture, Neonates.

Article History

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1. INTRODUCTION

Bloodstream Infections (BSIs) are the second cause of death among patients who are hospitalized in the Intensive Care Unit (ICU) [1]. The mortality rates due to blood stream infections vary in different parts of the world and range from

20-70 percent [2 - 4]. Despite the fact that sepsis was known 2000 years ago, but the definition of it is still challenging for clinicians and there is no 'gold standard' in this context. Therefore for evaluation of the intensity of sepsis, a reliable method is needed. The availability of standard guidance helps clinicians to better manage patients and increase patient survival [5]. The presence of an infection and two or more Systemic Inflammatory Response Syndrome (SIRS) criteria including (respiratory rate more than 20 breaths/minute, heart rate more than 90 beats/minute, leukopenia or leukocytosis and

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fever or hypothermia) were used to define sepsis previously [6]. Recently sepsis has been redefined using a new set of criteria included in the quick Sepsis-Related Organ Failure Assessment (qSOFA) criteria. According to the Surviving Sepsis Campaign (SSC), qSOFA criteria is a tool for the distinction of patients who are at higher risk of death or long-term hospitalization in ICU. The presence of two out of three qSOFA criteria including: (altered mental status, systolic arterial blood pressure less than or equal to 100 mmHg, and respiratory rate greater than or equal to 21 breaths/minute) are associated with high risk of death [6]. Bacteria are recognized as the main causes of BSIs [7]. Gram-positive bacteria have been recognized as the most common causes of BSIs and include 30-50% of all cases of bloodstream infections whereas 25-30% of these infections are due to gram-negative bacteria [8]. Although the gold standard method for the identification of bloodstream infections is blood culture method but this method has several limitations [9]. One of the main problems of this approach is that the time needed for identification is too long (2 days or more), also blood culture has low sensitivity [10]. It has been documented that only about 34% of cases of nosocomial bacteremia are detected with blood culture [10].

A series of rapid and sensitive molecular methods have been introduced for rapid detection of a wide range of pathogenic bacteria directly in blood specimens [11]. The molecular methods have the ability to rapidly identify infective bacteria even if the patient has taken antibiotics [12]. These useful methods have the potential to reduce mortality rates, duration of hospitalization and ICU stays due to the shortened time of detection, and identify the pathogenic bacteria which are missed by blood culture [10]. The aim of the current survey was to speed the diagnostic trend and detection of the bacterial causes of BSIs directly in the whole blood samples using 23S rRNA PCR assays and compare the results obtained with the results of standard blood culture.

2. METHODS

2.1. Patients

This cross-sectional study was performed in the ward of the Infectious Diseases at Shahid Beheshti University Hospital in Kashan from November 2017 to December 2018 and a total of 265 patients were studied. Criteria for selection of patients for the study included hospitalized patients with fever ($> 38^{\circ}\text{C}$), respiratory rate (> 20 breaths/minute), and white blood count (WBC) more than $12,000/\text{mm}^3$ or less than $4000/\text{mm}^3$. Demographic information of the patients including age, gender, prior antibiotic use, and underlying diseases was obtained by filling questionnaires and informed consent was obtained from all patients or their parents.

2.2. Sample Collection

For blood culture, blood samples were obtained from the catheter blood of patients at the onset of fever prior to the initiation of antimicrobial therapy. For PCR assay, 4 ml blood in EDTA (VACUETTE[®] K3EDTA TUBE, Greiner Bio-One, USA) was used which was obtained from the same catheter blood and was sent within 1-2 hours to Research Laboratory at the Kashan University of Medical Sciences and stored at 4°C

until use. A Bactec 9050 (Becton Dickinson, Sparks, MD) automated blood culture system was used. Blood samples were inoculated into aerobic and anaerobic Bactec bottles and were incubated at 35°C for 7 days. Bottles were studied with gram staining and then sub-cultured. Bacterial isolates from positive bottles were identified by standard bacteriological tests [13].

2.3. PCR Assays

DNA extraction from the EDTA whole blood tube was done using DNA Blood Mini Kit (QIAamp DNA Blood Mini Kit Qiagen, Germany) according to the protocol of the manufacturer. The Optical Density (OD) of the extracted blood DNA was measured by spectrophotometry method using the NanoDrop (BioTek Epoch Microplate). The confirmed extracted DNA was stored at -20°C until PCR assay. For the DNA amplification and PCR assay, the thermal cycler (Eppendorf master cycler[®], MA) was applied and the reaction mixture had a total volume of 50 μL . Thermal program of the PCR assays contained initial denaturation at 95°C for 5 min; 35 cycles including 30 sec at 95°C , 1 min at 58°C , 1 min at 72°C ; and finally 10 min at 72°C for final extension. The conserved 23S rRNA gene of the bacteria and specific primers including: F (5'-TCGCTCAACGGATAAAAAG-3') and R (5'-GATGAN-CCGACATCGAGGTGC-3') were used [14]. Positive and negative controls were included for each PCR reaction.

2.4. Statistical Analysis

All statistical analyses were carried out using SPSS for windows version 16.0. Statistical evaluation was performed by Chi-square test and $P < 0.05$ was considered significant

3. RESULTS

Of 265 hospitalized patients suspected to have BSIs, only 27 (10.2%) showed positive blood culture. Of these patients, 117 (44.2%) were females and 148 (55.8%) were males and their ages ranged from 2 months to 96 years with the mean age of 68.1 years. One hundred and twenty eight (48.3%) patients had an underlying disease including 47 (17.7%) having diabetes mellitus, 22 (8.3%) having kidney failure, 13 (4.9%) having respiratory failure, 30 (11.3%) having infectious disease, 14 (5.3%) having malignancies, and 2 (0.8%) hospitalized infants in NICU (Neonatal Intensive Care Unit) (Table 1). Of 27 patients with positive blood culture, 22 (81.5%) were of age 60 years or more. Of all patients, 110 (41.5%) received empirical antibiotic treatment. The PCR assays using 23S rRNA genes identified 80 (30.2%) of all patients with BSIs. Of 27 blood culture positive samples, 24 (88.9%) were found positive by PCR assays including (6 coagulase-negative staphylococci, 6 *Staphylococcus aureus*, 5 *Escherichia coli*, 3 *Enterococcus* spp. 3 *Acinetobacter baumannii*, and 1 *Klebsiella pneumoniae*) whereas 3 blood culture positive samples were PCR negative which were identified as coagulase-negative staphylococci. Forty-nine patients among 80 PCR positive patients (61.3%) have had prior antibiotic use. The results of the statistical analysis revealed a significant association between the results of PCR assays and blood culture and factors such as prior antibiotic use and the underlying disease ($P < 0.05$) (Table 1). Also, there was a significant correlation between laboratory and clinical criteria of patients such as

WBC, C-reactive protein (CRP), ESR (Erythrocyte sedimentation rate) and fever and the results of both PCR assays and blood culture ($P < 0.05$) (Table 2).

4. DISCUSSION

BSIs is one of the most important causes of mortality among hospitalized patients [15]. Rapid diagnosis and proper treatment of this infection could be effective in reducing the mortality rate associated with bloodstream infections [15]. In the present study, we used 23S rRNA PCR assays for rapid diagnosis of bacteremia in patients with suspected bloodstream infections. The results obtained were compared to the results of blood culture. Previous studies showed that the amplification of conserved genes such as 23S rRNA and 16S rRNA genes is an accepted method for the detection of bacterial pathogens in blood samples of patients with bacteremia [7]. The results of PCR assays using 23S rRNA gene revealed that of all patients

with suspected BSIs 30.2% had bacteremia. In a report in Germany on 342 blood samples of patients with suspected sepsis, 25.7% had positive PCR results using 16S rRNA gene [7]. In other studies in China and Egypt, from 172 and 62 hospitalized neonates with suspected septicemia, 9.8% and 25.8% were identified as positive by the PCR method using 16S rRNA genes, respectively [16]. In these studies in accordance with our report, a higher rate of identification of bacteremia in comparison to blood culture has been reported [16, 17]. These results show that the molecular methods have higher sensitivity and can detect lower levels of bacteria in the blood samples in comparison to blood culture method. All techniques have advantages and disadvantages. However using a culture method, multiple bacterial species can be detected simultaneously, but the most important disadvantages of this method are: 1) it is time-consuming, 2) the bacterial species that are not cultivable are eliminated, and 3) it is incapable to identify bac-

Table 1. Association between the results of PCR assays and blood culture regarding patients factors.

P-Value	Blood Culture		P-Value	PCR		Factors
	Negative, N (%)	Positive, N (%)		Negative, N (%)	Positive, N (%)	
						Age (years)
0.82	3 (1.1)	0 (0.0)	0.43	2 (0.8)	1 (0.3)	0-20
	12 (4.5)	1 (0.3)		7 (2.7)	6 (2.3)	21-40
	58 (21.9)	7 (2.7)		47 (17.7)	17 (6.4)	41-60
	114 (43.0)	13 (4.9)		91 (34.3)	37 (14.0)	61-80
	51 (19.3)	6 (2.3)		38 (14.3)	19 (7.2)	80-100
						Gender
-	98 (36.9)	21 (7.9)	-	99 (37.4)	46 (17.4)	Male
	140 (52.9)	6 (2.3)		86 (32.4)	34 (12.8)	Female
						Underlying disease
0.004*	37 (14.0)	10 (3.7)	0.1	29 (10.9)	17 (6.4)	Diabetes mellitus
	19 (7.2)	3 (1.1)		11 (4.1)	10 (3.8)	Kidney failure
	11 (4.1)	2 (0.8)		8 (3.1)	5 (1.9)	Respiratory failure
	24 (9.0)	6 (2.3)		18 (6.8)	12 (4.5)	Infectious disease
	12 (4.5)	2 (0.8)		11 (4.1)	3 (1.1)	Malignancies
	2 (0.8)	0 (0.0)		2 (0.8)	0 (0.0)	Hospitalized infants in NICU
	133 (50.2)	4 (1.5)		106 (40.0)	33 (12.5)	Without underlying diseases
						Prior antibiotic use
0.39	94 (35.4)	16 (6.1)	< 0.001*	61 (23.0)	49 (18.5)	With prior antibiotic use
	144 (54.3)	11 (4.1)		124 (46.8)	31 (11.7)	Without prior antibiotic use

* $P \leq 0.05$ is significant.

Table 2. Association between the results of PCR assays and blood culture regarding laboratory data and clinical criteria of the patients.

P-Value	Blood Culture		P-Value	PCR		Factor
	Negative (Median Factors)	Positive (Median Factors)		Negative (Median Factors)	Positive (Median Factors)	
0.015*	10.5	13.0	0.018*	10.3	12.8	WBC
0.001*	23.0	53.0	0.003*	35.6	55.7	ESR
0.002*	38.3	38.6	0.05*	38.4	38.9	Fever
0.009*	45.1	77.3	0.001*<	39.8	68.2	CRP

* $P \leq 0.05$ is significant.

teria in patients who have taken antibiotics. Although the molecular methods do not have the mentioned limitations, these techniques specifically identify only a group of desired bacterial species. Also, these methods generally need either the use of multiple qPCRs or an additional sequencing step, which leads to increased costs. Therefore, the molecular methods are useful and complementary to the blood culture. The automated molecular techniques have high value, although as a novel approach, the interpretation of their results requires more experience and standardization. Among all patients with positive blood culture, 3 of them had negative PCR results. As there were no laboratory findings and clinical symptoms in two of these three cases, and also the isolated bacteria were diagnosed as coagulase-negative staphylococci, the positive blood culture could be due to contamination during sample collection. Our results showed that the previous long-term antibiotic use was a risk factor associated with bacteremia. This finding is similar to other researcher findings and can help clinicians to consider a higher probability of bacteremia in hospitalized patients who have received longer courses of antibiotic therapy [18, 19]. A number of studies have shown that clinical and laboratory diagnostic criteria such as fever, WBC count, CRP and ESR are approved screening tests with high sensitivity for the detection of sepsis [20]. A combination of these tests and the molecular methods enhance the accuracy of diagnosis of bacteremia [20]. Similar to other studies in this context, we used these clinical and laboratory criteria as predictive factors for the diagnosis of bacteremia. The findings showed that fever, WBC count, CRP and ESR were significantly higher in patients with bacteremia. Isaacman *et al.*, (1998) have used the PCR and blood culture methods to evaluate blood samples of patients with suspected bacteremia and showed that patients with positive blood culture had a higher degree of fever and WBC count in comparison to blood culture negative patients regardless of PCR results [21]. Therefore clinical and laboratory criteria, especially in cases where the results of cultures and molecular methods are not fully consistent, can be helpful in interpreting the results.

CONCLUSION

Finally, the findings of the present survey revealed that the 23S rRNA PCR method is a rapid and sensitive technique specially for the diagnosis of sepsis among patients in whom bacteremia is difficult to diagnose with culture method including neonates and patients who have taken antibiotics before microbial culture.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethical Clearance Committees of the Kashan University of Medical Sciences (No: IR.Kaums. Rec.1395.13).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Participants provided written informed consent.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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