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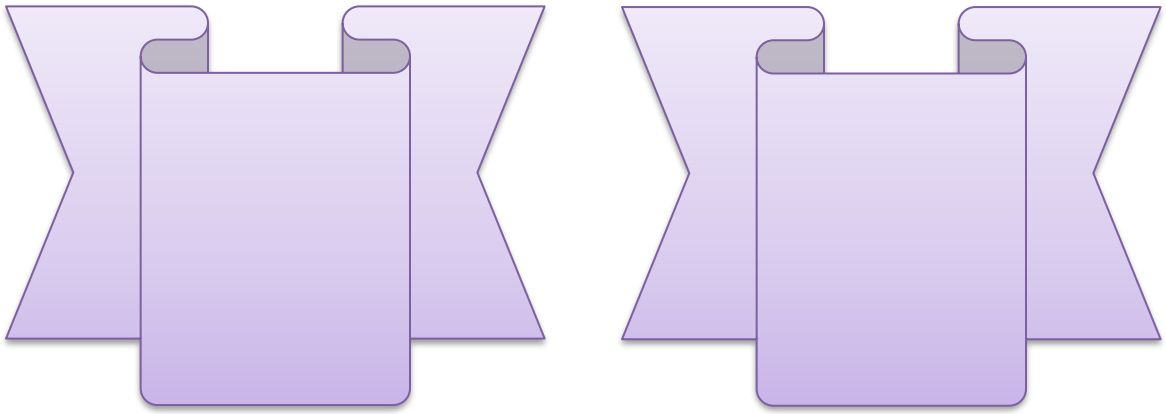

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Original Article

Microbiological Study for Comparison between Broad Range 16S rDNA PCR and Conventional Blood Culture for Diagnosis of Sepsis in The Newborn

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ABSTRACT

Background: Neonatal sepsis is major reason for mortality and morbidity in newborns. Blood culture is gold standard for identifying bacterial sepsis. Even so, it has low sensitivity and produces outcomes late. Bacterial DNA identification in blood samples could represent new diagnostic tool for identifying bacterial reason early on.

Aim of the work: To compare recognition of bacterial DNA in blood samples from neonates with assumed sepsis using broad range 16S rDNA PCR performed on all blood samples without prior enrichment and conventional blood culture.

Patients and Methods: This research included 90 neonates with clinically suspected neonatal sepsis in Al-Azhar Assiut University Hospital's Neonatal Intensive Care Units. Minimum of two-three ml blood was collected from each neonate using standard sterile processes, one-two ml for conventional blood culture and one ml EDTA blood for PCR.

Results: There seemed to be 52 [57.8%] neonates who had positive bacterial blood culture outcomes with isolation of important microorganisms and 69 [76.7%] neonates who had positive PCR outcomes [16S rDNA gene detected]. Even so, 38 [42.2%] of neonates had negative bacterial blood culture outcomes and 21 [23.3%] had negative PCR outcomes [16S rDNA gene not detected]. 4 neonates with positive bacterial blood culture had negative PCR outcome, whereas 21 neonates had positive PCR despite negative bacterial blood culture, with significant difference among PCR and blood culture outcomes [$p < 0.001$]. The sensitivity values of PCR and blood culture were 97.3%, and 89.8% respectively and the specificity values of PCR and Blood culture were 95.0% and 86.7% respectively.

Conclusion: When routine cultures are negative, PCR strategy seems to be simple, reliable and valued complementary way for diagnosing neonatal sepsis in samples collected throughout antimicrobial therapy.

Keywords: Sepsis; 16S rDNA PCR; Blood Culture.

INTRODUCTION

Neonatal sepsis, also known as septicemia, is scientific syndrome characterized by systemic marks of circulatory compromise due to bacteria invading bloodstream during first month of life. Even so, diagnosing neonatal sepsis is difficult because exposure to recognize sepsis risk factors is not required, medical marks are frequently vague and laboratory parameters are unspecific [1]. C-reactive protein elevation was used as useful marker of sepsis in several researches. Because no early definitive diagnostic for sepsis is accessible, broad-spectrum systemic antibiotic therapy is initiated solely on suspicion of sepsis [2].

Even so, obtaining large enough quantities of blood for culture from neonates is frequently difficult and it frequently takes forty-eight to 72 hours to achieve preliminary positive outcome [3]. Antigen detection methods enable detection and recognition of microorganisms without need for culture. Latex agglutination assay is most commonly used commercially available exam. These exams, even so, can only identify individual organisms like S. agalactiae and have high false positive and negative rate [4]. New pneumococcus urinary antigen exams are more promising, although they are related to false positives due to pneumococcal carriage [5].

Identification of bacterial DNA in neonatal blood samples is proposed as rapid and sensitive supplement to blood culture in diagnosis of bacterial sepsis in neonates [1]. Amplification of greatly conserved DNA sequences discovered in bacteria using polymerase chain reaction would allow for and sensitive recognition of bacteria in clinical specimens [6].

In this research, we compared broad range 16S rDNA PCR performed on all blood samples without prior enrichment to conventional blood culture for identifying bacterial DNA in blood samples from neonates in neonatal Intensive Care Units with suspected sepsis.

PATIENTS AND METHODS

This research was conducted from July 2020 to June 2021 at Medical Microbiology and Immunology Department, Faculty of Medicine, Al-Azhar Assiut University. This research included 90 neonates with clinically suspected neonatal sepsis in Al-Azhar Assiut University Hospital's Neonatal Intensive Care Units. Consent was obtained from authorized person in Neonatal Intensive Care Units from the neonates' parents, as accepted by Faculty of Medicine, Al-Azhar Assiut University's ethical committee.

All included neonates were revealed to NICU with clinical signs suggestive of neonatal sepsis, which included: core temperature higher than 38 °C or less than 36 °C and temperature instability, respiratory manifestation and gastrointestinal manifestation [7].

All information was recorded, such as years old, gender, birth weight, premature rupture of membranes and mode of delivery, antepartum hemorrhage and gestational years old. If sepsis manifests within first **72** hours of birth, case is considered early onset of sepsis, whereas late onset of sepsis manifests more than or equal to 72 hours after birth [7].

Specimens: Minimum of two-three ml was taken from each neonate using standard sterile procedures, one-two ml for conventional blood culture and one ml EDTA blood for PCR.

Blood culture: Blood culture bottles were incubated aerobically at 37 ºC for at least 24 to 48 hours. After overnight incubation on blood agar medium and MacConkey's agar medium, blood from bottles viewing positive growth was subcultured. They were deemed negative if there was no growth on blood culture bottles after 7 days. Isolated organisms were identified using colonial morphology, microscopic test of Gram stained films and biological activity [8].

Polymerase chain reaction: EDTA blood samples for PCR were wrapped and refrigerated for seventy-two hours before being separated into plasma and cell fractions and stored at seventy degrees Celsius until analysis. For purification of DNA from cells, Qiagen QIAampTM DNA blood mini kit [Qiagen Ltd, Crawley, UK] was used. To amplify bacterial DNA, PCR reactions were set with primers F: 5'-TCGTGGTGGACTTCTC-3' and R: 5'-ACAGTGGGGGAAAGCCC-3'[Bioline, United Kingdom]. 16 S rDNA gene was amplified on DNA extracts using PCR conditions: initial denaturation at 94 °C for seven minutes, 33 cycles of 94 °C for twenty minutes, 58 °C for sixty minutes, 72 °C for 60 minutes and final extension at 72 °C for seven minutes. predicted PCR product was 1100 base pairs, which were

detached by electrophoresis on 1.5 percent agarose gel with ethidium bromide and visualized by UV transillumination [Biorade, France] [UV star, Biometra, Germany].

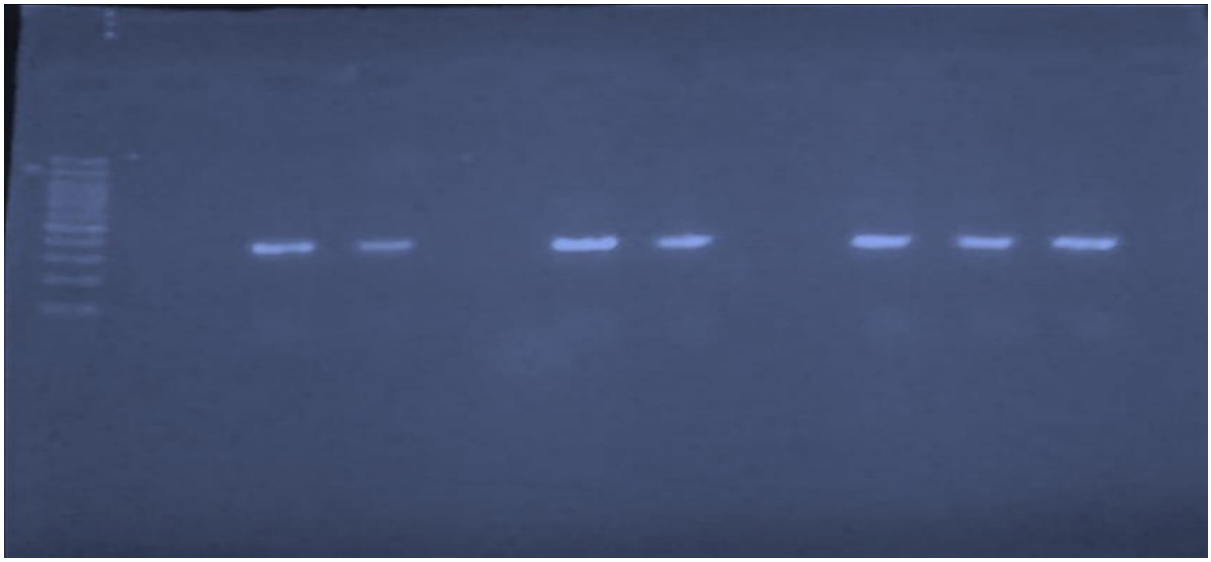

Figure [1]: Gel electrophoresis showing bands of PCR product of the 16S DNA gene. Lanes 2-3-5-6-8-9-10 show the amplified PCR product at 360 bp. Negative samples were detected in lanes 4 and 7. The causative microbes in positive sample were coagulase negative staphylococcus spp

Statistical analysis

Data was analyzed with Statistical Package for Social Science [IBM Corp., 2011]. IBM SPSS Statistics for Windows, Version 21. Numerical data was defined by mean and standard deviation. Non-numerical data was described by frequency and percentage. To compare various degrees of significance, Chi square test were used [for non-numerical data]. ROC curve was used to detect diagnostic statistics [sensitivity and specificity] for blood culture and PCR. Significance was set at P value < 0.05.

RESULTS

This study involved 90 neonates; their years old ranged from one to 17 days with median of six days. The majority of the patients were males 53/90 [58.9%]. About 32.2% of cases were full term, while 67.8% were preterm. There are 51 [56.7%] neonates were carried by normal vaginal delivery and 39 [43.3%] was delivered using caesarian section. There are 25 [27.8%] case were low birth weights and 65 [72.2%] case has normal birth weight. Also, 57 [63.3%] cases were presented with late onset of sepsis while there were 33 [36.7%] cases with early onset of sepsis [Table 1].

Regarding to clinical signs of sepsis among neonates; 68.9% of them had Fever > 38 °C, 52.2% had icterus, 50% had lung infection and 45.5% had feeding difficulties [Table 2].

According to association among PCR and blood culture outcomes; there were 52 [57.8%] neonates provided positive bacterial blood culture with isolation of important micro-organisms, whereas 69 [76.7%] neonates provided positive PCR outcomes [16S rDNA gene detected]. But, 38 [42.2%] neonates provided negative bacterial blood culture outcome and 21 [23.3%] neonates provided negative PCR outcomes [16S rDNA gene not detected]. Four neonates with positive bacterial blood culture had negative PCR outcome, 21 neonates had positive PCR in spite of negative bacterial blood culture with significant difference between results of PCR and blood culture [$p < 0.001$] [Table 3].

The Staphylococcus spp. was most common microorganism isolated from LOS cases 31/41 [75.6%], while only 3 cases [7.3%] were EOS [Table 4].

The least amount of antibiotic resistance among Gram-negative bacilli was observed in imipenem [14.3%]. In contrast, all Gram-positive bacteria were sensitive to vancomycin [Table 5].

Receiver operating curve [ROC] was used to determine the diagnostic performance of PCR and Blood culture in neonatal sepsis. Our ROC results revealed that PCR and Blood culture area under the ROC curve is equal to 0.973, and 0.892 respectively indicating that they are good diagnostic tool for neonatal sepsis. The sensitivity values of PCR and Blood culture were 97.3%, and 89.8% respectively and the specificity values of PCR and Blood culture were 95.0% and 86.7% respectively [Figures 2 and 3].

Table [1]: Demographic data of studied patients

		Cases [n=90]	
		No.	**%**
Age [Day]	Median Range	6 1-17	
Sex	Males Females	53 37	58.9% 41.1%
Gestational age [days]	Full term Preterm	29 61	32.2% 67.8%
Delivery	Vaginal Cesarean	51 39	56.7% 43.3%
Birth weight	LBW NBW	25 65	27.8% 72.2%
Sepsis onset	Early Late	33 57	36.7% 63.3%

LBW; low birth weight, NBW; normal birth weight

Table [2]: Distribution of clinical signs of sepsis among neonates

	Cases [n=90]	
	No.	**%**
Fever > 38 °C	62	68.9%
Icterus	47	52.2%
Irritability	35	38.9%
Feeding difficulties	41	45.5%
Omphalitis	18	20.0%
Lung infection[pneumonia]	45	50.0%

Table [3]: Relationship among blood culture outcomes and PCR

PCR	Blood culture		Total	P value
	Positive	**Negative**		
Positive [16S rDNA gene detected]	48 [53.3%]	21 [23.4%]	69 [76.7%]	**<0.001***
Negative [16S rDNA gene not detected]	4 [4.4%]	17 [18.9%]	21 [23.3%]	
Total	52 [57.8%]	38 [42.2%]	90	

Table [4]: Microbiological profile showed in blood culture from neonates according to onset of sepsis

	Blood culture		PCR [16S rDNA gene detected]	
	EOS	**LOS**	**EOS**	**LOS**
	No. [%]	No. [%]	No. [%]	No. [%]
Coagulase-negative Staphylococcus sp.	2 [2.2%]	29 [32.2%]	1[1.1%]	28 [31.1%]
Staphylococcus aureus	1 [1.1%]	2 [2.2%]	1[1.1%]	1[1.1%]
Enterococcus sp.	2 [2.2%]	2 [2.2%]	2 [2.2%]	2 [2.2%]
Group B Streptococcus	1 [1.1%]	1 [1.1%]	1 [1.1%]	1 [1.1%]
Streptococcus pneumoniae	2 [2.2%]	3 [3.3%]	2 [2.2%]	3 [3.3%]
Escherichia coli	1 [1.1%]	2 [2.2%]	1[1.1%]	2 [2.2%]
Klebsiella pneumoniae	0[0.0%]	1 [1.1%]	0[0.0%]	1 [1.1%]
Pseudomonas sp.	2 [2.2%]	1 [1.1%]	1 [1.1%]	1[1.1%]
Total	11 [12.2%]	41 [45.6%]	9 [10%]	39 [43.3%]

EOS: Early onset sepsis, LOS: Late onset sepsis.

Table [5]: Antimicrobial-resistant patterns of bacterial strains recognized by blood culture

Antibiotics	Gram-positive cocci resistance, n=forty five [86.5%]	Gram-negative bacilli resistance, n=7 [13.5%]
Ampicillin [10 µg]	45/45	7/7
Oxacillin [1 µg]	39/45	NT
Amoxicillin-clavulanic acid [30 µg]	34/45	7/7
Cefoxitin [30 µg]	44/45	6/7
Cefotaxime [30 µg]	44/45	5/7
Ceftazidime [30 µg]	43/45	5/7
Imipenem [10 µg]	31/45	1/7
Vancomycin [30 µg]	0/45	NT

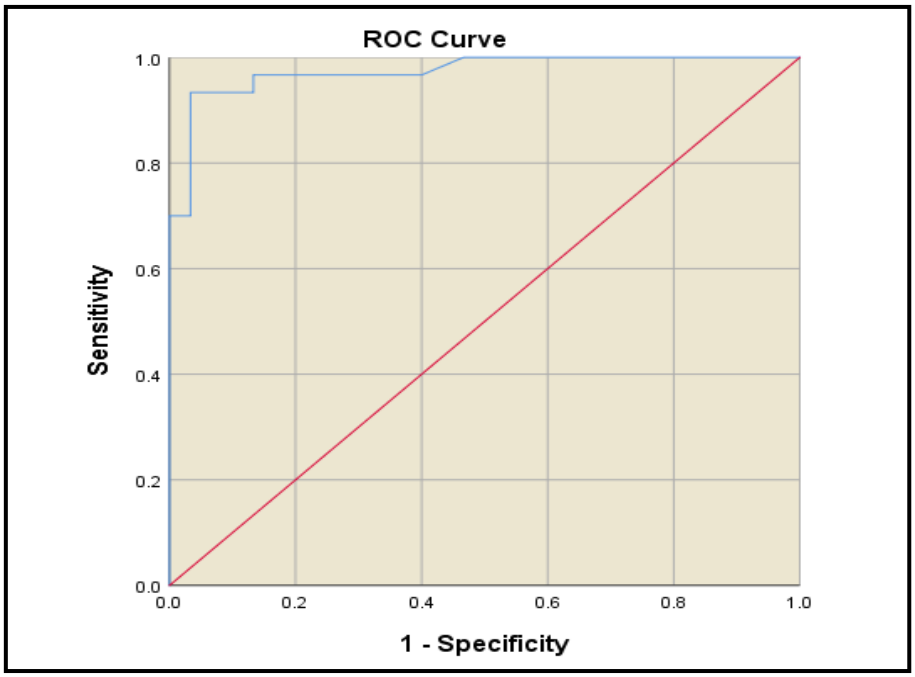

Figure [2]: ROC curve of PCR

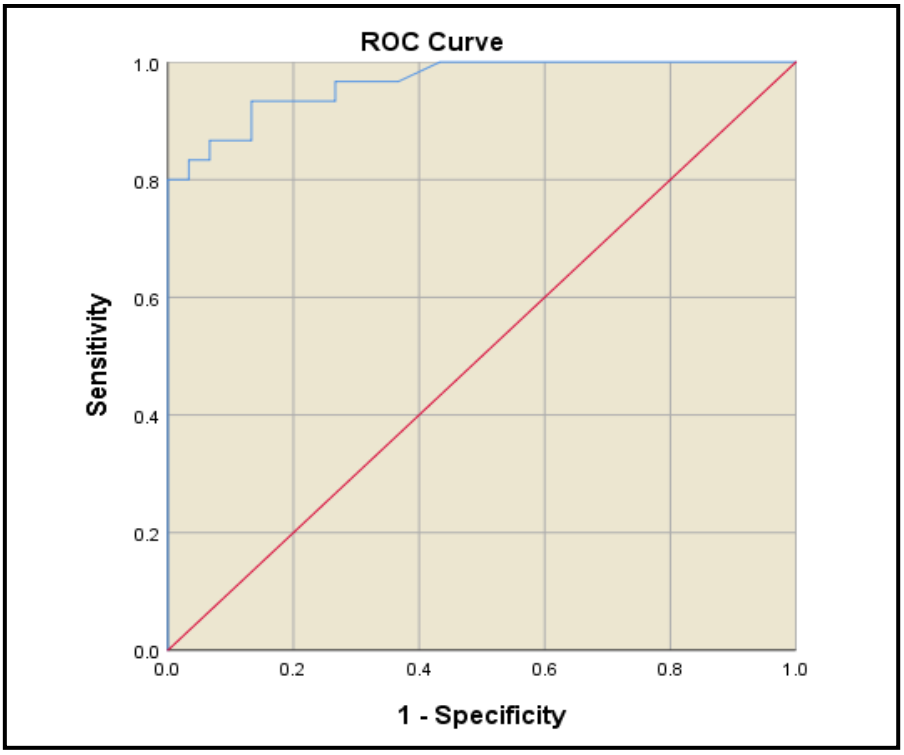

Figure [3]: ROC curve of Blood culture

DISCUSSION

Molecular methods like PCR have been used effectively to recognize large variety of pathogens from various samples. In clinical microbiology settings, PCR and other sequence-based recognition methods are used to identify infectious diseases. Amplification of 16S rDNA variable region by PCR has been used to diagnose neonatal sepsis and has proven useful in detecting pathogenic bacteria after blood culture outcomes are negative [9].

In the present study, the commonest organism in neonates with EOS was Enterococcus followed by Coagulase-negative Staphylococcus sp. In LOS, the commonest organism was Coagulase-negative Staphylo-coccus followed by Streptococcus pneumoniae. In the study of **Hassan *et al.*** [10], gram-negative organisms [84.13%] were a predominant causative agent for sepsis. Klebsiella [54%] followed by Pseudomonas aeruginosa [15.9%] and Escherichia coli [11.1%] were the most common isolates. A higher proportion of mortality was associated with a Gram-negative organism like P. aeruginosa [50%], followed by Klebsiella [32.4%] and E. coli [28.6%].

In our study, we discovered connection among PCR and blood culture outcomes. Fifty-two neonates had positive bacterial blood cultures with clinically important micro-organism isolation, while 69 had positive PCR outcomes [16S rDNA gene detected]. Even so, 38 neonates tested negative for bacterial blood cultures and 21 tested negatives for PCR [16S rDNA gene not detected]. Four neonates with positive bacterial blood culture contained negative PCR outcome, whereas 21 neonates contained positive PCR despite negative bacterial blood culture, with significant variation among PCR and blood culture outcomes [$p < 0.001$]. Our results were supported by research of **Reier-Nilsen *et al.*** [11], as they reported that one studied case with positive blood culture had negative PCR outcome, while another received inconclusive PCR outcome. Six cases had positive PCR despite negative blood culture, with significant difference in PCR and blood culture outcomes [$p < 0.001$].

In **Draz *et al.*** [12], despite negative bacterial blood culture, 15 neonates had positive PCR. Blood cultures could have been negative when non-enough blood is drawn to identify bacteria [13]. Low-level bacteremia [< ten CFU/ml] was discovered to be far more common [up to sixty eight percent] in pediatric studied cases than previously thought. To identify low dilutions of pathogens in blood, they deduced that up to 4.5 percent of studied case's blood volume should be collected in at least 2 blood cultures. Although, because neonates are extremely sensitive to minor blood losses, collecting more than one- two ml of blood is not choice for studied case category.

Our ROC curve results revealed that PCR and blood culture area under ROC curve is equal to 0.973 and 0.892 respectively indicating that they are good diagnostic tool for neonatal sepsis. The sensitivity values of PCR and Blood culture were 97.3%, and 89.8% respectively and the specificity values of PCR and Blood culture were 95.0% and 86.7% respectively.

While, in the study of **Reier-Nilsen *et al.*** [11], when compared to blood culture, PCR indicated 66.7 percent sensitivity, 87.5 percent specificity, 95.4 percent positive and seventy five percent negative predictive value for bacterial sepsis in newborns. Overall, PCR and blood culture identified bacteria in 35.5 percent of studied cases in sepsis. In their research, PCR had twenty nine percent sensitivity, 94.1 percent specificity, ninety percent positive and 44.4 percent negative predictive values when compared to sepsis diagnosis.

Draz *et al.* [12] demonstrated that, in comparison to blood culture, gold standard for diagnosing bacterial sepsis in newborns, study's sensitivity, specificity, PPV and NPV of PCR were 20/28 [71.42%], 7/22 [31.81%], 20/35 [57.14%] and 7/15 [46.66%], respectively. **Ohlin *et al.*** [14] reported that PCR assay demonstrated 79% percent sensitivity, 90% specificity, 59% positive predictive value and 96 % negative predictive value.

Conclusion: When routine cultures are negative, PCR strategy seems to be simple, reliable and valuable complementary way for diagnosing neonatal sepsis in samples collected throughout antimicrobial cure. Staphylococci spp. have been implicated in development of neonatal sepsis. To decrease neonatal sepsis, simple infection control measures with proven efficacy like hand washing, barrier nursing and promotion of clean deliveries must consider.

Conflict of Interest and Financial Disclosure: None.

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