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ORIGINAL ARTICLE

Automated Blood Culture versus Amplification of 16S rRNA Gene Method for Detection of Neonatal Septicemia

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ABSTRACT

Background*: Accurate and rapid diagnosis of neonatal septicemia is highly warranted because of high associated morbidity and mortality. Blood culture serves as a routine method for diagnosis of bacterial sepsis. However, it sometimes takes ≥ 72 hours for the results, with low sensitivity. Full automated blood culture method is superior to conventional methods in terms of speed and sensitivity. The polymerase chain reaction–based detection of 16S rRNA has reduced the laboratory turnaround time and has good sensitivity.* ***Objectives:*** *The aim of this study was to compare automated blood culture and amplification of 16srRNA gene by PCR in detection of aerobic bacterial infection in blood samples of hospitalized neonates with suspected neonatal sepsis and to determine the most common types of bacteria causing neonatal sepsis.* ***Methodology:*** *Blood samples collected from 40 neonates clinically suspected as neonatal sepsis were subjected to bacterial identification through automated blood culture by BacT/ALERT PF Plus Culture Bottles, and bacterial detection of 16S rRNA gene by PCR.* ***Results:*** *Out of 40 neonatal blood samples with suspected sepsis, 37(92.5%) neonates showed concordance between automated blood culture and PCR; 17(42.5%) showed positive results while 20 (50%) gave negative results by both methods. The remaining 3 cases (7.5%) were positive only by PCR. PCR sensitivity, specificity, positive and negative predictive values were 100%, 86.9%, 85% and 100% respectively. So, accuracy of PCR in relation to automated blood culture is 92.5%. The most common pathogens in cases of early onset neonatal sepsis were Klebsiella pneumoniae (7/15, 46.7%) followed by Streptococcus agalactiae (1/15, 6.6%) while the most common pathogens found in cases of late onset neonatal sepsis were Klebsiella pneumoniae and coagulase negative staphylococci (4/25,16% for each) then Staphylococcus aureus (1/25, 4%).* ***Conclusion:*** *16S rRNA PCR showed accurate rapid diagnosis with higher sensitivity in diagnosis of neonatal septicemia. Klebsiella peumoniae was the main causative bacteria in early onset neonatal sepsis and late onset neonatal sepsis.*

INTRODUCTION

Neonatal sepsis is one of the most common reasons for admission to neonatal intensive care units (NICUs) in developing countries. It is also a major cause of mortality in both developed and developing countries. It is responsible for about 30-50% of the total neonatal deaths in developing countries [1].

Neonatal sepsis is a type of neonatal infection involving the blood stream of infants less than 28 days old. It may be categorized as early onset neonatal sepsis (EONS) or late onset neonatal sepsis (LONS) [2].

EONS is defined as sepsis in neonates at or before 72 hours of life, while LONS refers to sepsis occurring after 72 hours of life[3]. So LONS describes cases diagnosed on 4th-30th days of life [4].

In order to improve the outcome associated with neonatal sepsis, it is necessary for a diagnostic test to be rapid and sensitive to decrease delay in treatment and to avoid unnecessary exposure to antibiotics and invasive procedures. Also, this minimizes the evolution of multidrug resistant bacteria in neonatal units [5].

Blood cultures are excellent method for diagnosis of sepsis [6]. However, the rate of positivity is low and is affected by prenatal use of antibiotics, bacteremia level, inoculated blood volume and laboratory abilities [7]. Also, the inability to isolate causative microorganisms of neonatal sepsis by blood culture does not exclude sepsis[8].

Several automated systems, which can efficiently detect bacterial growth earlier than the conventional culture methods, have been developed in the recent years. One such system is the BacT/Alert, an automated

system for blood cultures. BacT/Alert system allows higher yields and decreased detection time for the various organisms, as compared to the conventional method [9].

Identifying conserved regions of the 16S ribosomal RNA gene (rRNA gene) common to all bacteria, has been an area of interest for researchers. Amplification of the variable region of 16S rDNA by PCR has been used for neonatal sepsis diagnosis, and it has proven to be useful to detect pathogenic bacteria after blood culture gives negative results [10].

The aim of the present study was to evaluate the diagnosis of neonatal sepsis by amplification of the 16S rRNA conserved gene in bacteria by PCR in comparison with automated blood culture results and to detect the most common bacteria causing neonatal sepsis.

METHODOLOGY

This prospective study was conducted at the Medical Microbiology and Immunology Department, Faculty of Medicine, Benha university in the period from July 2019 to March 2020. The neonates were enrolled in the study after written informed consents were obtained from their parents. The study was approved by the Ethics Committee, Faculty of Medicine, Benha University.

Subjects:

Forty neonates (23 males and 17 females) clinically suspected as neonatal sepsis were included in this study. EONS cases were (15/40) while (25/40) were LONS cases.

Inclusion criteria included neonates admitted to NICU at Benha University Hospital and Benha Children Hospital with clinical manifestations suggestive of neonatal septicemia (at least two clinical signs) which included: core temperature greater than 38.5°C or less than 36°C and/or temperature instability, cardiac manifestation as (bradycardia, tachycardia, poor perfusion, or hypotension), respiratory manifestation as (tachypnea, apnea, cyanosis, or respiratory distress), gastrointestinal manifestation as (feeding difficulty or abdominal distension), non-specific manifestation as (lethargy, hypotonia, or irritability). Exclusion criteria included, neonates with chromosomal anomalies, severe birth asphyxia and exposure to surgical procedures.

Full history was taken, including age, sex, birth weight, gestational age, premature rupture of membranes (PROM), maternal fever and mode of delivery. If sepsis manifestations appear at the first 72 hours after birth, the case is considered as EONS, while LONS manifestations appear after 72 hours of birth. Laboratory and clinical data were also included (blood pressure, temperature and CRP).

Two ml of blood were collected from each neonate with clinically suspected neonatal sepsis under complete aseptic precautions. One ml of blood was inoculated into BacT/ALERT PF plus Bottles (Automated culture bottles) at the bedside of the patient. The other one ml blood was inoculated in sterile EDTA containing tube for further PCR assay. One ml of the liquid of inoculated BacT/ALERT PF plus positive bottles was withdrawn by syringe and transferred into a sterile plugged glass tube, under complete aseptic precautions for complete bacteriological identification.

Amplification of the bacterial 16S rRNA gene by PCR: bacterial DNA was extracted from blood samples of the studied neonates following the manufacturer instructions **Quick-DNA™ Miniprep (Zymo Research, USA).** The 16S rRNA primer sequences included RW01 fwd, 5'- AAC TGG AGG AAG GTG GGG AT-3' and RW01 rev, 5'- AGG AGG TGA TCC AAC CGC A-3' **(Biosearch technologies, USA) [17].**

In a PCR tube, a PCR amplification reaction of a total volume 50µl containing 5µl of the extracted DNA template, 25µl of **2×EasyTaq® PCR SuperMix (TransGen Biotech Co, China)**, 1µl of the forward primer, 1µl of the reverse primer and 18µl of nuclease free water. The amplification reaction in the thermal cycler followed these steps: initial denaturation at 95°C for 3 mins, followed by 35 cycles of (94°C for 1 min, 53°C for 2 mins, 72°C for 2 mins), and a final extension at 72°C for 10 mins. The expected PCR product was 380 base pairs and was separated by electrophoresis on a 1.5% agarose gel using ethidium bromide and visualized by UV transillumination.

RESULTS

Forty neonates with clinically suspected neonatal sepsis were enrolled in our study. Out of them 15/40 (37.5%) neonates and 25/40 (62.5%) neonates were diagnosed according to their clinical data as EONS and LONS respectively. A total of 23/40 (57.5%) were males and 17/40 (42.5%) were females, with a mean birth weight of 2.02±0.65 kg and mean gestational age of 34.7±3.1 weeks. Most of the cases were preterm 26/40 (65%) and low birth weight (LBW) 27/40 (67.5%). 37/40 (92.5%) of neonates were delivered by cesarean section (C.S). Out of 40 mothers of neonatal sepsis cases 8 cases (20%) were suffering from urinary tract infection (UTI), 4 cases (10%) of them had PROM while only 2 cases (5%) of them had fever (table 1).

Table 2 showed that out of 40 neonatal blood samples 17 (42.5%) show bacterial infection by automated blood culture method while 20 cases (50%) were positive for bacterial infection by detection of 16S rRNA by PCR (figure 1). No cases were positive by blood culture and negative by PCR. Three (7.5%) cases show bacterial infection by PCR only. So, no false-positive results for blood culture were detected, but false-negative results were 3/40 (7.5%). The comparison revealed that the sensitivity, specificity, positive

predictive value and negative predictive values of 100%, 86.9%, 85% and 100% respectively for PCR. So, the accuracy of PCR test in relation to automated blood culture was 92.5%.

Table 3 showed that the most common causative organisms isolated from cases of EONS was K*lebsiella pneumoniae* with a percentage of 46.7% while the most common causative organisms isolated from cases of LONS was *Klebsiella pneumoniae* and Coagulase negative staphylococci (16% for each). *Streptococcus agalactiae* was isolated from 1/15 (6.6%) of EONS cases while *Staph aureus* was isolated from 1/25 (4%) LONS cases.

Table 1: Perinatal history of early and late onset neonatal sepsis cases.

Variables	EONS (=15) No., (%)	LONS (=25) No., (%)	Chi^2 test	P value	OR (95% CI)
Antenatal history					
-Maternal fever					
+ve	2(13.3)	0(0)	3.5	>0.05	-
-ve	13(86.7)	25(100)			
-PROM*					
+ve	3(20)	1(4)	2.66	>0.05	-
-ve	12(80)	24(96)			
-UTI*					
+ve	6(40)	2(8)	6.0	<0.05* (0.02)	7.6 (1.29-45.2)
-ve	9(60)	23(92)			
Natal history:					
-Sex					
Male	7(46.7)	16(64)	1.15	>0.05	-
Female	8(53.3)	9(36)			
Mode of delivery					
Normal	2(13.3)	1(4)	1.17	>0.05	-
C.S*	13(86.7)	24(96)			
-Gestational age					
Full term	6(40)	8(32)	0.26	>0.05	-
Preterm	9(60)	17(68)			
-Birth weight					
Normal weight	3(20)	10(40)	1.7	>0.05	
LBW	12(80)	15(60)			

* PROM: Premature rupture of membrane, UTI: Urinary tract infection, C.S: Ceserian section, OR….Odds ratio, CI…..Confidence interval, *Significant P-value≤0.05.

Table 2: Results of automated blood culture versus PCR in neonatal sepsis studied cases.

PCR	Automated blood culture		Total
	Positive	Negative	
Positive	17(42.5%)	3(7.5%)	20(50%)
Negative	0	20(50%)	20(50 %)
Total	17(42.5%)	23(57.5%)	40(100%)

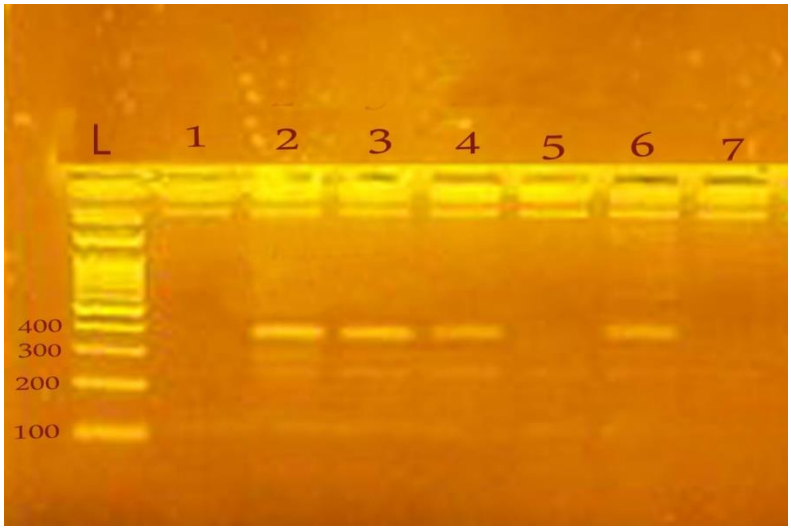

Fig. 1: Gel electrophoresis of 16srRNA gene

Gel electrophoresis showing bands of the PCR product of the 16S rRNA gene. (A) Lanes 2,3,4 and 6 show the amplified PCR product at 380 bp. (B) Negative samples were detected in lanes 1,5 and 7. L is 100 bp DNA ladder (Fermentas, Thermo Fisher Scientific, USA).

Table 3: Results of positive automated blood culture in early onset and late onset neonatal sepsis cases

Organisms	EONS (=15), (%)	LONS (=25), (%)	Total nnmber of microorganisms (=40), (%)
Klebsiella pneumonia	7(46.7%)	4(16%)	11(27.5%)
Streptococcus agalactiae	1(6.6%)	-	1(2.5%)
Coagulase negative staphylococci	-	4(16%)	4(10%)
Staphylococcus aureus	-	1(4%)	1(2.5%)

DISCUSSION

In the present study 25(62.5%) of neonates had LONS while 15 (37.5%) had EONS. The results agreed with *Rahman et al.* [11] who showed that the prevalence of LONS was 42/76 (55.26%) neonates in comparison to EONS 34/76 (44.74%) neonates.

As regards the prenatal history of the studied neonates, the present study showed that 2 (5%) of neonatal mothers had fever. Perera et al. [12] also found that six (3.7%) of their neonatal mothers had fever.

Herbst and Källén[13] concluded that the risk of neonatal sepsis increases with the duration of membrane rupture. Zakariya et al. [14] found that PROM ≥18 hours prior to delivery is a risk factor for EONS. In this study 10% of mothers of neonatal sepsis cases were suffering from PROM. Perera et al. [12] found that mothers of 15% of sick, term neonates had PROM lasting 18 hours or more. Also, EL-Amir et al. [15] reported that about one-third of their patients were exposed to PROM. Stoll. [16] explained that the cause of neonatal septicemia may be due to immature immunological responses of the neonates in the first week of life that make them more prone to infections in this period, also may be due to the ascending infection following membrane rupture or through the infected birth canal or acquired at the time of the newborn resuscitation in the labour room.

In our study 8/40 (20%) of mothers of neonatal sepsis cases were suffering from UTI. 6/15 (40%) of mothers of EONS cases were associated with UTI with a significant *P*-value ($P<0.05$; OR=7.6). This result agreed with Leal et al.[17] who stated that UTI of any cause raises the risk of sepsis in the neonate due to raising the risk of chorioamnionitis.

In our study, neonatal sepsis was predominant in males (57.5 %) which is comparable to that observed in other studies carried by Kung et al. [18]; Hammoud et al. [19] and EL-Amir et al. [15] who found that males represent 52.8, 54.9% and 53.3% respectively. None of the previous studies found a significant difference between males and females in neonatal sepsis, which is similar to the present results. The possible cause of the preponderance of neonatal septicemia in males may be attributed to the X-linked immunoregulatory gene factor

sharing in the susceptibility of host to infections in males [20].

In this study, out of 40 neonates sepsis was diagnosed in 37(92.5%) cases delivered by C.S. *Anwar et al.*[21] reported that neonatal sepsis was diagnosed in 53.8% of their neonates delivered also by C.S. Nehal et al. [22] and EL-Amir et al. [15] showed that neonatal sepsis occurs more frequently in neonates delivered by normal vaginal delivery with percentage of 70% and 65.3% respectively.

In the current study neonatal sepsis was predominant in preterm neonates (26/40, 65%). Also, neonatal sepsis in the present study was predominant in (27/40, 67.5%) neonates with birth weight ranges between 0.98 to 3 kg with mean ± Sd (2.02± 0.65) which agreed with a study conducted by *Salama et al.* [23], who reported that sepsis was more common among LBW neonates. Our findings coincide with *EL-Amir et al.* [15] who found in their study that 68% of the cases were LBW and 64% were preterm.

This study also agreed with other study carried by Ghosh et al. [24] who found that LBW and prematurity were the prevalent risk factors. Chacko[25] found that LBW was associated with high risk of sepsis due to prematurity which is a great risk factor in developing sepsis earlier.

These findings disagreed with Nehal et al. [22] who reported that out of 30 neonates with positive blood culture, three neonates were of low birth weight and 27 were of normal birth weight.

This study showed that the automated blood culture test gave positive results in 42.5% (17/40) of clinically diagnosed sepsis cases. This result agreed with *Gupta et al.* [26] who found that the result of the automated blood culture test was 39% of clinically diagnosed sepsis cases.

In this study 16S rRNA PCR test showed a positivity of 50% (20/40). Gaikwad and Mujawa[27] reported that in the neonates with suspected sepsis 16S rRNA test showed a positivity of 72%.

In the current study three neonatal sepsis cases (7.5%) were positive by PCR test while negative by automated blood culture (false negative). No cases were positive by blood culture and negative by PCR. No false-positive results for blood culture were detected (specificity 100%).

In a study by Gupta et al. [26] six episodes in that study showed only PCR positivity highlight its higher sensitivity. False negative results of blood culture may be explained by presence of fastidious or dead organisms. The patients also may be on prior antibiotics, which could act as inhibitors during culture but did not affect the results of PCR as explained by Handschur et al. [28].

In this study, the sensitivity and specificity of 16srRNA is 100% and 86.9% respectively. Positive predictive value, negative predictive value and accuracy were 85%, 100% and 92.5% respectively. Molloy et al.[29] in their study reported that the sensitivity and specificity of PCR was 100% and 97.85%, respectively. Gaikwad and Mujawar[27] in their study on neonates with suspected sepsis, the sensitivity of 16S rRNA was 100%, and specificity was 41.18%. Also, *Gupta et al.* [26] showed that PCR assay sensitivity, specificity, positive and negative predictive values were calculated as 100%, 91.8%, 87.5% and 100%, respectively. The diagnostic accuracy of PCR was 94.8%.

This study also showed that the most common organisms in cases of neonatal sepsis (both EONS and LONS) was *Klebsiella pneumoniae*. This result agreed with Sands et al.[30] who found that *Klebsiella pneumoniae* was the main causative agent of neonatal sepsis, and it also agreed with Ahmed et al.[31] who reported that both community acquired and hospital acquired infections caused by Enterobacteriaceae are among the most common human infections. They conducted their study in our hospital and detected *Klebsiella pneumoniae* in 34.4% from positive blood culture bottles. However, our results disagreed with a study conducted by Shaikh et al. [32] who found that *Staphylococcus aureus and* Pseudomonas were the most causative organisms of neonatal sepsis.

This study showed that the most causative organism of EONS cases was *Klebsiella pneumoniae* (46.7%) followed by *Streptococcus agalactiae* (6.6%). These results agreed with Seliem and Sultan. [33] and Salama et al. [23] who reported that the most causative agent of EONS was *Klebsiella pneumoniae*. EL-Amir et al. [15] demonstrated a prevalence of Gram-negative bacteria among patients with EONS but they found that the most common Gram-negative bacteria causing EONS was *E.coli*. Also, Silva-Junior et al. [34] showed that *E. coli*, *Staphylococcus aureus*, and *Streptococcus agalactiae* were the main causative organisms of EONS.

It is suggested that after the introduction of antepartum prophylactic antibiotics, *Streptococcus agalactiae* has shown a significant decline so Gram-negative bacteria, that commonly colonize maternal enteric canal, have become more common, especially among the preterm population [35,36]

This study demonstrated that the most causative organisms found in LONS cases were *Klebsiella pneumoniae* and coagulase negative staphylococci (16% for each) then *Staphylococcus aureus* (4%). Other studies conducted by Salama et al.[23] found that coagulase-negative staphylococci was the most causative organism (39.2%) of LONS. Hammoud et al.[19] found that coagulase-negative staphylococci and Klebsiella species were the most common organisms, causing 272 (34.65%) and 179 (22.80%) of LONS cases, while other studies conducted by Kung et al. [18], EL-Amir et al.[15] found higher percentage of Staphylococcus species: 45.2%,42.5% respectively.

CONCLUSION

Automated blood culture bottle is an excellent method in term of speed and sensitivity in diagnosis of neonatal sepsis. Amplification of 16srRNA gene method by PCR is faster, more specific and sensitive than conventional methods in diagnosis of neonatal sepsis. The 16S rRNA PCR showed high sensitivity in diagnosis of neonatal sepsis. So, it can be used as a good screening test. *Klebsiella pneumoniae* was the most common organism causing EONS and LONS in the present study.

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