Archives of Razi Institute, Vol. 78, No. 1 (2023) 221-226

Copyright © 2023 by
Razi Vaccine & Serum Research Institute
DOI: 10.22092/ARI.2022.358608.2259

Original Article

Comparison between Polymerase Chain Reaction and Blood Culture for Diagnosis of Neonatal Sepsis

Hayder Hamad, M[1], Eidan Hadi, M[1], Ajam, I. K[2]

1. Al Mustaqbal University College, Babylon, Iraq
2. Islamic University, Babylon, Iraq

Received 7 May 2022; Accepted 8 June 2022
Corresponding Author: mohammedhaider@mustaqbal-college.edu.iq

Abstract
Neonatal sepsis can be defined as any systemic bacterial infection confirmed by a positive blood culture in the first month of life. This study evaluated the polymerase chain reaction as the diagnostic approach to identify neonatal sepsis instead of blood culture. In this study, 85 blood specimens were collected from 85 patients with suspected septicemia; ages ranged between 1 to 28 days from both sexes (53 males and 32 females) from November 2014 to March 2015. From each neonate, a minimum of 1–3 ml of blood was collected by standard sterile procedures, 2 ml for blood culture, while 1 ml was used for DNA extraction. A minimum of 2 ml of blood is taken through venipuncture and injected into two or more "blood bottles" with specific media for aerobic and anaerobic organisms. The blood is collected using an aseptic technique. The recorded data showed that the bacterial culture was positive in 7.06% of patients versus 92.9%, revealing a negative bacterial culture. The most common types of bacteria isolated were three isolates of *Klebsiella* spp. (50.0%), followed by one isolate of *Staphylococcus aureus* (16.67%), one *E. coli* (16.67%) isolate, and one *Enterobacter* spp. (16.67%) isolate. Finally, molecular detection for bacterial sepsis was done using specific primers (*16 sRNA*, *rpoB* and *its*). It was found that *16 sRNA* genes were present in 20% of samples, and *rpoB* gene was present in (18.8%). While *its* gene used for the detection of fungi revealed negative results in all samples.
Keywords: Neonatal sepsis, 16 sRNA, PCR, Septicemia

1. Introduction

Neonatal sepsis can be defined as any systemic bacterial infection confirmed by a positive blood culture in the first month of life (1). Neonatal septicemia remains one of the main causes of mortality and morbidity despite the progress in hygiene, the introduction of new and potent antimicrobial agents for treatment and advanced measures for diagnosis. Up to 10% of infants have infections in the first month of life, resulting in 30-50% of neonatal deaths in developing countries (2). Early onset neonatal sepsis (EOS) occurring in the first 72 hours of life remains a significant cause of illness and death among very low birth weight (VLBW) preterm infants (3). Late-onset sepsis (LOS) is present during 7-28 days of age. The source of infection in LOS is either nosocomial (hospital-acquired) or community-acquired, and neonates usually present with septicemia, pneumonia or meningitis. The spectrum of infecting organisms has evolved over the last two decades from a predominance of Gram-negative organisms to Gram-positive organisms. Most community-acquired infections are due to *Streptococcus* spp. and *Escherichia coli*, other *Enterobacteriaceae*and non-ferment Gram-negative bacteria like *Pseudomonas* and *Acinetobacter* spp. (4).

Group B streptococcus (GBS, *Strep. agalactiae*) remains

the leading cause of neonatal sepsis and meningitis in the United States. Although less common, *Listeria monocytogenes* is associated with invasive disease in newborns. *Enterobacter* spp, *Klebsiellapneumoniae*, *P. aeruginosa* and *Staphylococcus aureus* is also microorganisms commonly isolated from infants with EOS (5). The bacteria responsible for late-onset septicemia include those acquired from the maternal genital canal and organisms acquired after birth from human contacts or contaminated equipment or materials (6).

The clinical diagnosis of sepsis in neonates is difficult because many signs of sepsis are nonspecific and are observed with other noninfectious conditions. Although a routine physical examination is evidence that sepsis is not present, bacteremia can occur without clinical signs (7). Microbial cultures of blood or other sterile body fluids are the gold standard in diagnosing neonatal sepsis. Blood cultures are generally assumed to have low sensitivity in neonates.

Molecular assays have the advantage of direct pathogen detection in a more rapid turnover time than blood cultures, with results available within a few hours. Molecular assays might eventually replace blood cultures but will continue as a supplement to blood cultures until they are adequately evaluated (8).

2. Materials and Methods

2.1. Patients

This study was conducted on 85 neonates (1-28 days) with clinical or laboratory findings suggestive of sepsis admitted at NICUs and premature infant unit of the Babylon Hospital for Pediatric and Gynecology from November 2014 to March 2015. Complete history taking, thorough clinical examination and routine laboratory investigations were made on all neonates with suspected sepsis.

2.2. Specimens

From each neonate, a minimum of 1–3 ml of blood was collected by standard sterile procedures, 2 ml for blood culture, while 1 ml was used for DNA extraction.

2.3. Blood Culture

A minimum of 2 ml of blood is taken through venipuncture and injected into two or more "blood bottles" with specific media for aerobic and anaerobic organisms. The blood is collected using an aseptic technique. This requires that both the tops of the culture bottles and the venipuncture site of the patient are cleaned prior to collection by swabbing with 70% isopropyl alcohol (povidone and left to dry before venipuncture).

2.4. Polymerase Chain Reaction

DNA (extracted from bacterial cells) was used as a template in specific PCR to detect *16s RNA*, *rpoB* and *ITS* genes. A pair of specific primers were used to amplify a fragment gene. While the conditions of PCR methods are listed in table 1. A single reaction mixture contained 2.5 µl of upstream primers, 2.5 µl of downstream primer, 5 µl of DNA extraction, 12.5 µl of master mix and 2.5µl of nuclease-free water to obtain a total volume of 25 µl. The resulting PCR products were run in 2% agarose gel.

Table 1. The primer sequences and PCR condition

Gene's name	Primer sequence (5'- 3')	Size BP	Condition
16s RNA	Sense –AGATTTGATCCTGGTCAG Antisense –GGACTACCAGGGTATTAAT	861	94°c 3min 1x 95°c 1min 54°c 1min 30x 72°c 1min 72°c 7min 1x
rpoB	Sense –CAGGTCGTCACGGTAACAAG Antisense –GTGGTTCAGTTTCAGCATGTAC	512	94°c 5min 1x 94°c 30sec 60°c 30sec 35x 72°c 1min 72°c 10min 1x
its	Sense–GCATCGATGAAGGCAGC Antisense–TCCTCCGCTTATTGATATGC	400	95c 1min 1x 95°c 1min 60°c 2min 28x 72°c 1min 72°c 10min 1x

3. Results and Discussion

3.1. Etiologic Agents of Septicemia

Out of the 85 samples were collected from the neonatal unit. Only 6 (7.06 %) samples yielded bacterial growth, of which *Staphylococcus aureus* recorded 1 isolate (16.67%), *Klebsiella* sp. 3 (50.0%), *Enterobacter* species1 (16.67%), *E. coli* 1 (16.67%)and 79 showed no bacterial growth (Table 2).

Table 2. Bacterial etiologic agents isolated from neonates with suspected sepsis

Etiologic agents	EOS No. (%)	LOS No. (%)	Total No. (%)
Klebsiella spp.	2 (33.33)	1 (16.67)	3 (50.0)
Escherichia coli	1 (16.67)	-	1 (16.67)
Staphylococcus aureus	-	1 (16.67)	1 (16.67)
Enterobacter spp.	1 (16.67)	-	1 (16.67)
Total	4 (66.67)	2 (33.33)	6 (100.0)

The low frequency of blood culture is attributed to the differences in blood volume withdrawn (9), blood culture techniques and exposure to antimicrobials of the mother or the possibility of infection with viruses, fungi or anaerobes (10). Another factor that may highly influence the sensitivity of the blood culture is starting antibiotic treatments at birth in all neonates with risk factors for early sepsis prior to diagnosis (11).

The isolation rate of bacteria in this study is comparable to rates reported in previous studies done by Fanaroff, Stoll (1) (8.7%), and Ahmed, Chowdhury (12) (4.2%). However, in contrast with many studies done by Nathoo, Mason (13) (31.75%), Aftab and Iqbal (14) (54.0%), and Kellogg, Ferrentino (15) (52.6%), where isolation rates are high, because of large samples that are used in their studies. The frequency of isolation of Gram-positive and harmful bacteria from blood culture in this study was 16.67% and 83.34%, respectively.

This finding is similar to other studies, which showed that Gram-negative bacteria were responsible for most cases of neonatal sepsis (16). This was in contrast to other studies where Gram-positive bacteria were the most typical cause of neonatal sepsis (17, 18), while another study showed that the frequency of isolation of both Gram-positive and negative was equal (19).

Klebsiella spp were the most common isolates (50.0%) causing neonatal sepsis in this study. Previous studies have reported similar findings (11, 20, 21). *Klebsiella* species have often been isolated in a hospital setting and are often implicated in nursery outbreaks (7).

3.2. Molecular Detection of Neonatal Sepsis

3.2.1. Detection Using *16s RNA* Gene

The results of gel electrophoresis for DNA bands based on genes specific for bacterial *16S RNA* revealed that 17:85 patients (20%) were positive for target specific for a bacterial gene (*16S rRNA*) in their blood samples with length (861) base pairs (Figure 1).

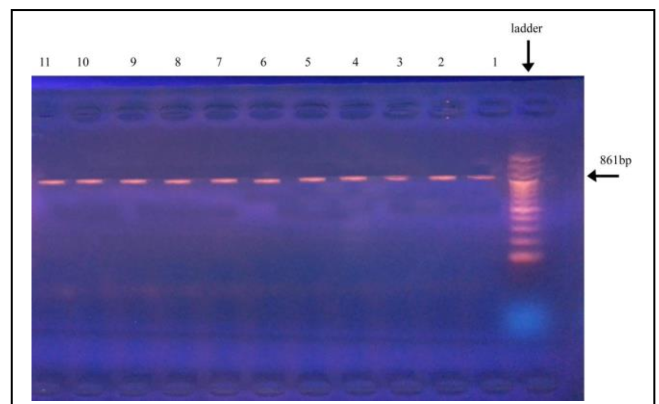

Figure 1. Gel electrophoresis of PCR products of *16s rRNA* gene in neonatal sepsis

The result in this study shows that six neonates gave positive bacterial blood cultures with the isolation of clinically significant microorganisms, while 17 neonates gave positive PCR results. However, 79 neonates gave a negative bacterial blood culture result, and 68 neonates gave negative PCR results. Six neonates had positive bacterial blood culture / PCR results, while 68 had negative bacterial blood culture / PCR results. Eleven neonates with a positive PCR had a negative bacterial blood culture result, as shown in table 3.

Table 3. PCR and bacterial blood culture results

		Blood culture		
		Negative	Positive	Total
PCR	Positive	11	6	17
	Negative	68	0	68
	Total	79	6	85

This difference includes intermittent seeding of low numbers of bacteria within the bloodstream, the extremely small blood volumes obtained from infants for culture, and the increasingly common practice of providing intrapartum antibiotics to mothers of high-risk deliveries (13). It is well known that the smaller volume of blood obtained for culturing lower the chances of recovering the organisms (5, 9). Although some infants may have a somewhat greater magnitude of bacteremia than adults (10 to 300 and 1 to 30 CFU/ml, respectively), the amount of blood sampled from newborn infants is significantly less than that taken from adults (0.5 to 1.0 and 10 to 30 ml, respectively) (8).

Haque, Jahan (11) found that bacterial DNA consensus sequences, e.g., the *16S rRNA* gene, have been identified to define an organism as a bacterium. With such sequence information available, numerous DNA primers and probes have been described for PCR-based assays to diagnose bacterial sepsis. These assays may prove helpful for detecting or ruling out septicemia in term infants born to mothers who received intrapartum antibiotics. However, data describing the use of molecularly-based testing for diagnosing viral, bacterial, or candidal infections in the adult and pediatric populations exist.

The result of this study agreed with Shang, Chen (22), who found that PCR amplification of 861 bp was positive in (13%) and 4% with negative cultures.

Schuchat, Whitney (23) found that the number of positive specimens identified by PCR by using *16 sRNA* (9.88%) was significantly higher than the number of positives identified by blood culture (4.65%).

3.2.2. Detection by Using the *rpob* Gene

Primers of the *rpoB* gene were used to detect *Enterobacteriaceae* in neonate blood samples. It has been found that 16:85 patients (18.8%) were positive for target-specific bacterial genes (*rpoB*) with a length of 512 base pairs. The amplicon was detected in gel electrophoresis and compared with an allelic ladder, as shown in figure 2.

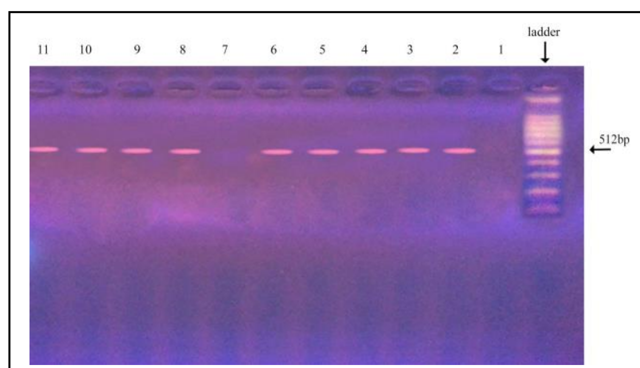

Figure 2. Gel electrophoresis product of *rpoB* gene

The *rpoB* gene coding for the beta subunit of the RNA polymerase has emerged as a core gene candidate for phylogenetic analyses and identification of bacteria, especially when studying closely related isolates. *rpoB* has been proposed as an alternative biomarker for microbial community studies. This gene is described as possessing the same key attributes as 16s rRNA in that it is common in all bacteria and is a mosaic of conserved as well as variable sequence domains (24).

Bindayna, Jamsheer (25) demonstrate that *rpoB* displays other important characteristics as an ecological marker, including (i) its universal presence in all prokaryotes, (ii) the presence of slowly and quickly evolving regions for the design of probes and primers of differing specificities, (iii) having a housekeeping function, making it less susceptible to some forms of lateral gene transfer, and (iv) a large enough size to contain phylogenetic information, even after removal of regions that are difficult to align.

Elamreen (18) showed that the *rpoB* gene is the most promising target for detecting *Enterobacteriaceae* by

PCR amplification for the detection of neonatal sepsis.

3.2.3. Detection of Fungi Using *its* Gene

After specific DNA extraction from blood samples, DNA was amplified with primer ITS F and ITSR in 25µl reaction mixtures. Then, the PCR products were purified by agarose gel electrophoresis, and it was found that no samples were producing fungal DNA with primer in pairs (400) bp fragments.

Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities of DNA (due to the high copy number of rRNA genes) and has a high degree of variation even between closely related species. This can be explained by the relatively low evolutionary pressure acting on such non-functional sequences (26).

Detection of fungemia by means of fungal blood culture is notoriously difficult, and invasive fungal infections are diagnosed better by molecular assay as PCR can detect naked DNA due to the presence of dead and degrading fungi within circulating phagocytes (27).

Unfavourable results can be from (i) the need for an appropriate gene sequence or (ii) faulty reagents and/or thermal cycler (28).

Authors' Contribution

Study concept and design: M. E. H.
Acquisition of data: M. H. H.
Analysis and interpretation of data: M. E. H.
Drafting of the manuscript: M. H. H.
Critical revision of the manuscript for important intellectual content: I. K. A.
Statistical analysis: M. H. H.
Administrative, technical, and material support: M. H. H.

Ethics

The study was conducted in accordance with the ethics committee of the Al Mustaqbal University College, Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol. 2007;196(2):147.
2. Ottolini MC, Lundgren K, Mirkinson LJ, Cason S, Ottolini MG. Utility of complete blood count and blood culture screening to diagnose neonatal sepsis in the asymptomatic at risk newborn. Pediatr Infect Dis J. 2003;22(5):430-4.
3. Shah AJ, Mulla SA, Revdiwala SB. Neonatal sepsis: high antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit of a tertiary care hospital. J Clin Neonatol. 2012;1(2):72.
4. Mutlu M, Aslan Y, Saygin B, Yilmaz G, Bayramoğlu G, Köksal I. Neonatal Sepsis Caused by Gram-negative Bacteria in a Neonatal Intensive Care Unit: A Six Years Analysis 新生兒深切治療部中由革蘭氏陰性細菌所導致的醫院內感染性膿毒癥: 一個持續六年的分析. HK J Paediatr (new series). 2011;16(4):253-7.
5. Baltimore RS, Huie SM, Meek JI, Schuchat A, O'Brien KL. Early-onset neonatal sepsis in the era of group B streptococcal prevention. Pediatrics. 2001;108(5):1094-8.
6. Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. 2010.
7. Murty D, Gyaneshwari M. Blood cultures in paediatric patients: A study of clinical impact. Indian J Med Microbiol. 2007;25(3):220-4.
8. Ishibashi M, Takemura Y, Ishida H, Watanabe K, Kawai T. C-reactive protein kinetics in newborns: application of a high-sensitivity analytic method in its determination. Clin Chem. 2002;48(7):1103-6.
9. Aurangzeb B, Hameed A. Neonatal sepsis in hospital-born babies: bacterial isolates and antibiotic susceptibility patterns. J Coll Physicians Surg Pak. 2003;13:629-32.
10. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. Jpn J Infect Dis. 2004;57(6):273-5.
11. Haque S, Jahan N, Mannan M, Hasan M, Begum

M, Rob S, et al. Identification of bacterial isolates in neonatal sepsis and their antimicrobial susceptibility. Mymensingh Med J. 2014;23(4):709-14.
12. Ahmed AS, Chowdhury MA, Hoque M, Darmstadt GL. Clinical and bacteriological profile of neonatal septicemia in tertiary level pediatric hospital in Bangladesh. Indian Pediatr. 2002;39(11):1034-9.
13. Nathoo K, Mason P, Gwanzura L, Kowo H, Mubaiwa L. Severe Klebsiella infection as a cause of mortality in neonates in Harare, Zimbabwe: evidence from postmortem blood cultures. Pediatr Infect Dis J. 1993;12(10):840-4.
14. Aftab R, Iqbal I. Bacteriological agents of neonatal sepsis in NICU at Nishtar Hospital Multan. J Coll Physicians Surg Pak. 2006;16(3):216-9.
15. Kellogg JA, Ferrentino FL, Goodstein MH, Liss J, Shapiro SL, Bankert DA. Frequency of low level bacteremia in infants from birth to two months of age. Pediatr Infect Dis J. 1997;16(4):381-5.
16. Ahmed Z, Ghafoor T, Waqar T, Ali S, Aziz S, Mahmud S. Diagnostic value of C-reactive protein and haematological parameters in neonatal sepsis. J Coll Physicians Surg Pak. 2005;15(3):152-6.
17. Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M. The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin Microbiol Rev. 2010;23(1):235-51.
18. Elamreen FA. Neonatal sepsis due to multidrug-resistant Klebsiella terrigena in the neonatal intensive care unit in Gaza, Palestine. Crit Care. 2007;11(4):1-.
19. Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem. 2004;50(2):279-87.
20. Kapoor L, Randhawa V, Deb M. Microbiological profile of neonatal septicemia in a pediatric care hospital in Delhi. J Commun Dis. 2005;37(3):227-32.
21. Afroza S. Neonatal sepsis--a global problem: an overview. Mymensingh Med J. 2006;15(1):108-14.
22. Shang S, Chen G, Wu Y, Du L, Zhao Z. Rapid diagnosis of bacterial sepsis with PCR amplification and microarray hybridization in 16S rRNA gene. Pediatr Res. 2005;58(1):143-8.
23. Schuchat A, Whitney CG, Zangwill K. Prevention of perinatal group B streptococcal disease: a public health perspective. 1996.
24. Chen Y-C, Eisner JD, Kattar MM, Rassoulian-Barrett SL, Lafe K, Bui U, et al. Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. J Clin Microbiol. 2001;39(11):4042-51.
25. Bindayna KM, Jamsheer A, Farid E, Botta GA. Neonatal Sepsis 1991–2001: Prevalent bacterial agents and antimicrobial susceptibilities in Bahrain. Med Princ Pract. 2006;15(2):131-6.
26. Brown DR, Kutler D, Rai B, Chan T, Cohen M. Bacterial concentration and blood volume required for a positive blood culture. J Perinatol. 1995;15(2):157-9.
27. Ahmad S, Khan Z, Mustafa AS, Khan ZU. Seminested PCR for diagnosis of candidemia: comparison with culture, antigen detection, and biochemical methods for species identification. J Clin Microbiol. 2002;40(7):2483-9.
28. Fazzeli H, Arabestani MR, Esfahani BN, Khorvash F, Pourshafie MR, Moghim S, et al. Development of PCR-based method for detection of Enterobacteriaceae in septicemia. J Res Med Sci. 2012;17(7):671.