Comparison of Broad Range 16S rDNA PCR and BACTEC System for Detection of Neonatal Sepsis

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ABSTRACT

Background: Bacterial sepsis is a feared complication of the newborn. A large proportion of infants admitted to the Neonatal Intensive Care Unit (NICU) for suspected sepsis receive treatment with potent systemic antibiotics while a diagnostic workup is in progress. The gold standard for detecting bacterial sepsis is blood culture. However, the sensitivity of blood culture is suspected to be low. Molecular assays for the detection of bacterial DNA in the blood represent possible new diagnostic tools for early identification of a bacterial cause.

Aim: The aim of this study was to compare a broad range 16S rDNA PCR done on blood samples without prior enrichment to conventional BACTEC blood culture for detecting bacteria in blood samples from neonates with suspected sepsis.

Methods: Fifty five neonates with clinically diagnosed sepsis were included in this study. A broad range 16S rDNA polymerase chain reaction (PCR) without preincubation was compared to conventional diagnostic work up for clinical sepsis, including BACTEC PEDS PLUS/F blood culture, for early determination of bacterial sepsis in each infant.

Results: Only 20 infants had a positive blood culture. Analysis by 16Sr DNA PCR revealed 21 samples positive for the presence of bacterial DNA. PCR failed to be positive in one sample from blood culture positive infant, and was positive in 2 samples with blood culture negative infants. Compared to blood culture the diagnosis of bacterial proven sepsis by PCR revealed 95.0% sensitivity, 94.3% specificity, 90.5% positive predictive value and 97.1% negative predictive value. PCR combined with blood culture revealed bacteria in 40.0% of the patients diagnosed with sepsis.

Conclusion: There is a need for PCR as a method to quickly point out the infants with sepsis. However, uncertainty about a bacterial cause of sepsis was not reduced by the PCR result, reflecting that blood culture is irreplaceable at present, since pure isolates are essential for antimicrobial drug susceptibility testing.

Key Words: Neonatal sepsis, broad range 16S rDNA PCR, BACTEC system.

BACKGROUND

Neonatal sepsis is a major cause of morbidities and mortalities mostly remarkable in the third world nations. Neonatal sepsis has been divided into two main categories including early (72 hours of age) and late-onset (after 72 hours of age) sepsis. Unfortunately signs and symptoms of neonatal sepsis such as respiratory distress and irritability are vague and not confined to the diseases; other non-infectious processes also may present with the same manifestations.

Early diagnosis and subsequent therapy for the infected infants or those with a higher risk may play a vital role in lowering such mortality and morbidity rates. However, diagnosing neonatal sepsis is difficult since clinical signs are often vague, and laboratory parameters are unspecific. Initiation of broad-spectrum systemic antibiotic treatment is based only on the suspicion of sepsis since no early definitive diagnostic test is yet available. The clinician accepts some over-treatment because of the high risk of mortality if sepsis is left untreated.

Conventional blood culture is considered the gold standard in the etiological diagnosis of neonatal bacterial sepsis. However, obtaining sufficiently large amounts of blood for culture from neonates are often difficult, and it often takes 48-72 hours to obtain a preliminary positive result. Some bacterial species, however, are difficult to isolate, or
grow slowly in the laboratory due to stringent growth requirements, while others may not grow because of prior empirical treatment of patients with antimicrobial agents.\textsuperscript{(16-19)} Detection of bacterial DNA in blood samples of neonates is suggested to represent a rapid and sensitive supplement to blood culture in diagnosing bacterial sepsis in neonates.\textsuperscript{(11,20,21)} Molecular diagnostic techniques, such as PCR, are being developed to aid in the diagnosis of bacterial infection by detecting bacterial genetic material.\textsuperscript{(22)} Unlike culture, most molecular assays are designed specifically for one organism. Broad-range assays, based on ribosomal genes (rDNA), are designed to overcome this limitation. Bacterial rDNA consists of highly conserved nucleotide sequences that are shared by all bacterial species, interspersed with variable regions that are genus- or species-specific. The DNA sequences of the variable regions form the basis of phylogenetic classification of microbes.\textsuperscript{(23)} By using PCR primers that are targeted at conserved regions of rDNA, it is possible to design broad-range PCRs capable of detecting DNA from almost any bacterial species.\textsuperscript{(24,25)}

The aim of this study was to compare a broad range 16S rDNA PCR done on blood samples without prior enrichment to conventional BACTEC PEDS PLUS/F blood culture for detecting bacteria in blood samples from infants with suspected sepsis.

METHODS

Patients

A six-month prospective study was done on 55 neonates admitted to the NICU at Maternity and Pediatric Hospital in Al-Madinah Al-Munawarah. The patients were discharged from the NICU with diagnosis of sepsis: infants with suspicion of sepsis or development of clinical signs consistent of sepsis. All infants included in the study were treated with systemic antibiotics.

Microbial analyses

A minimum of 1 ml full blood for conventional BACTEC Peds PLUS/F blood culture (Becton Dickinson, USA), and 1–2 ml EDTA blood for 16S rDNA PCR were obtained by standard sterile procedures before starting general systemic antibiotic treatment. Only one blood culture bottle was routinely drawn from each patient. The bottles for culturing were immediately incubated. The EDTA-blood samples for PCR were blinded and stored in room temperature for until 72 hours, divided into plasma and cell fractions, and were then stored at -70°C before analysis.

PCR reactions

PCR reactions were set up to amplify bacterial DNA using the primer 5’TGAAGAGTGTGATCGGCCTCAG, 5’AAGGAGGTGATCCAAACC. The primer reacts with highly conserved regions of the bacterial 16S rDNA gene to provide PCR products of approximately 1500 basepairs.\textsuperscript{(26)} The primer is routinely used in some laboratory for 16S rDNA based identification of unknown isolates (Eurogentec, Belgium). Each PCR reaction (50 μl) consisted of 1 × Amplitaq Gold buffer (Applied Biosystems,USA) supplemented with 2.5 U Amplitaq Gold Low DNA enzyme (Applied Biosystems,USA), 2 mM MgCl\textsubscript{2}, 0.2 mM dNTP (Roche Diagnostics, Germany), 20 μl template and PCR grade water (Roche Diagnostics, Germany). Cycling conditions included a 5 minute denaturing step at 94°C followed by 30 to 40 cycles of 20 seconds at 94°C, 20 seconds at 58°C and 60 seconds at 72°C. PCR results were considered positive when visible PCR products of the correct size were found in DNA isolated from either the plasma or the cell fraction. PCR results were considered negative when no visible PCR products of correct size were found.\textsuperscript{(27)}

RESULTS

Comparison of 16S rDNA PCR and blood culture

As presented in the table; twenty of the infants had a positive blood culture result. Blood cultures from 20 of 55 patients, counting for 36.4% (20/55) of the patients diagnosed with sepsis in this study. Twenty one patients had a positive PCR, so 38.2 % (21/55) of patients were diagnosed with sepsis by PCR. One patient with a positive blood culture had a negative PCR result. Two patients had a positive PCR in spite of a negative blood culture. The comparison between the PCR results and those obtained using BACTEC reveals a high level of agreement between the two methodologies, with PCR sensitivity, specificity, and positive and negative predictive values of 95.0, 94.3, 90.5, and 97.1%, respectively.
DISCUSSION

Neonatal sepsis is a frequent cause of morbidity and mortality, defined as clinical syndrome characterized by systemic signs of infection and bacteremia in the first month of life. Neonatal infections currently cause about 1.6 million deaths annually in developing countries. In the present study the diagnosis of bacterial sepsis in the newborn by PCR revealed 95.0% sensitivity, 94.3% specificity, 90.5% positive and 97.1% negative predictive value. The high negative predictive value that was calculated for the PCR assay compared to that of culture is indicative of the assay’s usefulness in accurately ruling out the diagnosis of bacterial sepsis in the uninfected term neonate admitted to the NICU for such an evaluation.

While neonatal sepsis was clinically diagnosed in 55 patients in the study, a pathogenic bacterium was detected in the blood culture of only 36.4% of these patients. With the molecular method of broad range 16S rDNA PCR, the detection of bacteria improved to 38.2%. Based on the criteria used for the diagnosis of sepsis, these two methods combined had a sensitivity of 40.0%.

Two patients tested positive for broad range bacterial PCR but had negative cultures. The blood cultures may have been negative due to inadequate amount of blood drawn for optimal detection of bacteria. Some investigators found that low-level bacteraemia (<10 cfu/ml) is far more common (up to 68%) in paediatric patients than previously believed. They concluded that it is necessary to collect up to 4.5% of the patient's blood volume (approximately 4 ml/kg) in at least two blood cultures to detect low concentrations of pathogens in the blood. However, as neonates are very sensitive to even small losses of blood, collecting more than 1–2 ml of blood is not an option for this group of patients.

In one patient, blood culture was positive with a concordant negative PCR result. This result could reflect the presence of a low level of live bacteria. Even if improving the detection limit, low level bacteraemia (<10 cfu/ml) in neonates will be difficult to identify by any method based on detection of bacterial DNA or growth. Jordan et al. showed a higher level of agreement between the two methodologies when preincubation was performed before PCR testing. They used 200–500 microl EDTA-fullblood preincubated at 37°C for 5 hours before PCR-testing, and found 96% sensitivity, 99.4% specificity, and 88.9% positive and 99.8% negative predictive values for PCR compared with the culturing of 0.5–1.0 ml full blood with BACTEC 9240. However, a drawback with this procedure is that only live bacteria, able to grow in blood culture bottles will be detected.

Second, a dramatic loss in sensitivity was observed when we attempted to detect the bacterial 16S rDNA gene from whole blood specimens with volumes of appreciably less than 200 µl. More specifically, culture-positive blood specimens whose paired samples for PCR testing had volumes ranging from 25 to 75 µl lacked detectable levels of the 16S rDNA gene. This observation led us to establish a minimum blood volume requirement of 200 µl for 16S rDNA PCR testing.

This study reiterates the current problem; the vast majority of term infants admitted to the NICU for suspected sepsis are not infected but have symptoms consistent with those of other medical conditions that mimic sepsis, such as hypoglycemia, delayed transition, or transient tachypnea. Despite this fact, these term infants was treated with antibiotics for at least 48 hours while awaiting the results of the preliminary blood culture report.

Automated blood culturing systems are good given time, but if a laboratory test could be developed that would rule out bacterial septicemia in less time than blood culturing, those infants whose symptoms had resolved could be taken off of antibiotics and discharged from the NICU sooner. NICU admissions and intravenous antibiotic therapies result in expensive hospital stays for infants that separate newborns from their mothers and create potential difficulties in successful bonding and breast-feeding, while exposing infants to antibiotics which are increasingly expensive and
overused. At best these practices increase the financial burden on our health care system, and at worst they contribute to the increasingly serious problem of antibiotic resistance. Although blood culturing will not be completely replaced by a nucleic acid amplification technology anytime soon, as pure isolates remain essential for antimicrobial drug susceptibility testing, PCR does appear to be an excellent diagnostic test choice for a rapid means of ruling out bacterial sepsis in certain select patient populations.\textsuperscript{(10,37)}

**CONCLUSION**

16S rDNA PCR method used in this study increased the sensitivity in detecting bacterial DNA in newborns with signs of sepsis. PCR has potential as a method for earlier detection of bacteria but this technology needs to be further developed and improved. Blood culture is irreplaceable at present, since pure isolates remain essential for antimicrobial drug susceptibility testing.

**REFERENCES**


مقارنة بين التفاعل التسلسلي البوليميرزي باستخدام 16س ريوسومال دون أو واسع المجال ووجها الباكاتك لتشخيص تلوث الدم البكتيري في الأطفال حديثي الولادة

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الخلافي:

يعتبر تلوث الدم البكتيري من المضاعفات المخيفة التي تصيب الأطفال حديثي الولادة. ولذلك يعنى هؤلاء الأطفال مضاعفات حيوية قوية في التشخيص الإكلينيكي بجرة العناية المركزية للأطفال حديثي الولادة (الحضانة) وذلك لحين التأكد من التشخيص معملا. وتعتبر مزرعة الدم هي الطرق القائمة والذكية لتشخيص تلوث الدم البكتيري. ولكن تعتبر حساسية هذه الطرق قليلا. ولذلك تمثل اختبارات اليونانية الجزءية للكشف الخاص الزمني البكتيري وسيلة حديثة لتشخيص المبكر.

الهدف من البحث: مقارنة بين التفاعل التسلسلي البوليميرزي باستخدام 16س ريوسومال دون أو واسع المجال ووجها الباكاتك لتشخيص تلوث الدم البكتيري في الأطفال حديثي الولادة.

خطوات البحث: انتهت هذه الدراسة على 55 طفل حديثي الولادة مشخصون كلينيكي بمساء بث الدم البكتيري بالحضانة. وقد تم التشخيص المعملي لكل طفل منهم باستخدام كل من التفاعل التسلسلي البوليميرزي باستخدام 16س ريوسومال دون أو واسع المجال دون تحصين ومرضوع الدم باستخدام جهاز الباكاتك.

النتائج: تم التأكد من التشخيص معملا لعشرين طفلًا عن طريق مزرعة الدم باستخدام جهاز الباكاتك وواحد عشر من طفل باستخدام التفاعل التسلسلي البوليميرزي باستخدام 16س ريوسومال دون أو واسع المجال. وقد وجد أن التفاعل التسلسلي البوليميرزي كان سلبيا في واحد من الأطفال المشخصين إيجابيا بمزرعة الدم وكانت المزرعة سلبية في ثمان من الأطفال المشخصين إيجابيا بالتفاعل التسلسلي البوليميرزي. وقد وجد أن استخدام كلتا الطريقيتين معا بالعمل تميز من حساسية التشخيص المعملي إلى 40%.

الاستنتاج: يعتبر استخدام التفاعل التسلسلي البوليميرزي باستخدام 16س ريوسومال دون أو واسع المجال طريقة أسرع من الطرق التقليدية باستخدام مزرعة الدم لتشخيص المعملي ولكنه يمكن الاستغلال عن التشخيص المعملي بمزرعة الدم لأنها تحدد نوع البكتيريا ومنها تجري مزرعة الحساسية للميكوب لتحديد المضاعفات الحيوية المناسبة.