Detection of Neonatal Bacteremia Using 16S rRNA Gene Amplification by Polymerase Chain Reaction

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ABSTRACT

The purpose of this study was to evaluate the potential of a broad diagnostic approach based on 16S rRNA gene amplification and sequencing for rapid detection of neonatal bacteremia.

Subjects and Methods: Blood samples were collected from fifty neonates admitted to the neonatal intensive care units with suspected sepsis, for paired analysis of bacterial growth using the BACTEC 9050 instrument and for bacterial 16S rRNA gene using a PCR assay with subsequent DNA sequencing for bacterial species identification.

Results: The specimens were positive for bacteria in 43 cases (86%) by blood culture and 44 cases (88%) by PCR out of a total of 50 specimens analysed. There was very good agreement between the results of PCR and blood culture for detection of neonatal bacteremia (kappa = 0.8). Taking blood culture as a reference method, the sensitivity, specificity, positive and negative predictive values for PCR were 100%, 75%, 95.5% and 100% respectively after exclusion of candida isolate which was detected by blood culture only and not by PCR (the primer being used was 16S rRNA not 18S rRNA needed to identify fungal sepsis). Concerning the time consumed to detect sepsis, blood culture method took more time (up to 5 days) while PCR took less time <4 hour. Our results revealed the ability of DNA sequencing to recognize two pathogens which were negative by culture, one was Klebsiella pneumoniae and the other was Staphylococcus epidermidis. In addition DNA sequencing identified 2 species (one Acinetobacter Iwoffii and one Acinetobacter baumannii) that couldn’t be identified by routine conventional biochemical reactions but only by Microbact test. Conclusion: This PCR-based approach is quite useful in detection and identification of neonatal pathogens and has the potential for excellent sensitivity and a shorter turn around time than those of culture based protocols.

Key words: Neonatal sepsis; 16S r RNA; DNA sequencing.

INTRODUCTION

Infections are still one of the most common causes of hospitalization and mortality in children and neonates, sepsis represents the most important cause of neonatal morbidity and mortality after congenital malformations (1). The incidence ranges from 1 to 8 cases for every 1000 infants, but much higher values are found in preterms, low birthweight newborn infants and generally, in newborn infants admitted to neonatal intensive care units. Mortality from septicemia has dropped from 40-50 % to 10-20 %, but in cases of early - onset fulminant sepsis, it is still around 70 % (2). Indeed, a majority of survivors have significant neurological sequelae as a consequence of central nervous system involvement, septic shock or hypoxaemia secondary to severe parenchymal lung disease or persistent pulmonary hypertension. Clinical diagnosis of sepsis is not easy, because symptoms and signs are not specific and moreover, a dramatic deterioration of clinical conditions can supervene very rapidly, even in asymptomatic newborn infants. Because the preliminary report of blood culture is not available before 48-72 h, it is customary to start treatment at birth in all neonates with risk factors for early sepsis and/or with alterations of some laboratory tests (i.e white cell count ,total neutrophil count , Immature / total ( I/T) ratio, CRP ). However, the sensitivity and specificity of each laboratory test are far from 100% (3,4), so that a large proportion of neonates not really infected are treated with broad-spectrum antibiotics. The possibility of having a 100% sensitive and specific method for the identification of bacteria in blood , with results available in a short period of time , could allow the onset of treatment only in neonates with infection , thus reducing the use of broad-spectrum antibiotics, the need of close observation of suspected cases and of course, medical costs . Molecular biology techniques, such as polymerase chain reactions (PCR) have been used as a specific and sensitive method for diagnosis of different bacterial, viral and protozoal infections, and the number of the diseases for which this diagnostic approach can be used is steadily increasing (5). DNA sequences present in all bacteria, such as portions of DNA encoding the 16 S ribosomal RNA (rDNA), have been used to define organisms as bacteria, those sequences have been amplified with PCR using an automated method allowing detection of even small amounts of bacteria and diagnosis of
sepsis. (6,7)

The aim of our study has been to determine the potential of a broad diagnostic approach based on 16S rRNA gene amplification and sequencing for rapid detection and identification of pathogens causing neonatal bacteremia.

SUBJECTS AND METHODS

This study was carried out in Pediatric and Clinical pathology departments, Benha University Hospital and the NICU department Benha Specialized Children Hospital during the period from January 2007 to June 2008.

History taking and inclusion criteria: Infants admitted to the NICU for sepsis evaluation were included in the study if the following criteria were fulfilled:

- The presence of one major or two minor risk factors (major risk factors were i- premature rupture of membranes PROM >24h, ii premature onset of labour before 37 weeks, iii chorioamnionitis, iv- intrapartum maternal fever >38°C – Minor risk factors were i PROM > 12< 24 h ii- low birthweight <1500g, iii- intrapartum maternal fever >37.5 < 38°C , iv- twin gestation, v- maternal apgar score at 1 min < 5.
- Sings of sepsis including, lethargy, irritability, apnea, cyanosis respiratory distress and poor capillary refill.
- Positive CRP (> 6 mg/L).
- Immature / total (I / T) ratio ≥ 0.2 (1).

Fifty neonates were included in our study and were subjected to the following:

Blood sampling: Paired blood samples were obtained from two different peripheral veins for paired blood cultures, and for PCR. Blood sample for PCR (0.5 ml) was placed in a sterile vacutainer containing EDTA and stored at -70°C till processed.

Methods:

Blood culture processing: Using an automated continuous monitoring blood culture system, BACTEC 9050 (Becton Dickinson, Sparks, MD) which use a fluorescent sensor for detecting microorganisms and relies primarily on the detection of CO2 produced by actively metabolizing microorganisms. (8) The pediatric - sample sized, blood culture bottles (Peds Plus, Becton Dickinson ) were sent from the NICU prefilled with blood from infants at their bedides. Between 0.5 and 1.0 ml of whole blood was added per blood culture bottle. The bottles were incubated immediately upon receipt in the microbiology laboratory in accordance with the manufacturer’s recommendations.

Positive samples were processed for identification of microorganisms as follow:

Subculture on blood, chocolate and MacConkey agars.

Identification of the growing colonies by (9, 10, 11):

- Colony morphology
- Gram staining
  - For Gram positive bacteria Catalase test, culture on mannitol salt agar, Coagulase test (slide and tube tests), Deoxyribonuclease (DNase) test, Novobiocin susceptibility test and CAMP test were done.
  - For Gram negative bacteria both conventional biochemical reactions (Triple sugar,llysine iron agar, citrate utilization, urease production, sulphide, indole, motility and oxidase tests (OXOID)) and Microbact test were used.

Microbact (12A) test (OXOID): The Microbact (12 A) gram negative system is a standardized micro-substrate system designed to simulate conventional biochemical substrates used for identification of enterobacteriaceae and common miscellaneous gram negative bacilli (oxidase negative, nitrate positive, glucose fermenter comprising 15 genera). Organism identification is based on PH changes and substrate utilization.

Procedure: was done according to the manufacturer’s instructions.

Interpretation: An octal coding system was adapted for Microbact, each group of 3 reactions produce a single digit of the code using the results obtained. The sum of these indices in each group of 3 reactions form the code number. This code was entered into the Microbact computer aided identification package for the identification choices. The percentage figure shown against the organism name is the percentage share of the possibility for that organism as apart of the total probabilities for all choices.

Polymerase Chain Reaction: The nucleic acid was extracted from the whole EDTA blood and
each sample was subjected to consensus primer mediated PCR method. The primers supplied by (Chem genes, U.S.A), were complementary to 16S r RNA gene sequences at base-pair positions 459 and 1349 respectively.

U 515F  5’ TGCCAGCAGCCGCGGTAAT  3’
EU 202950R  5’ GGGCGGCGTGTACAAGGC  -3’

Nucleic acid extraction: Nucleic acid was extracted from whole blood using Purogene kits supplied by Gentra, Minneapolis. U.S.A.

Procedure: Fifty microlitre of EDTA blood was added to 250 μl cell lysis solution and incubated at 65º C for 15 minutes till complete lysis, one 100 μl protein precipitation solution was added to the lysate and the sample was placed into an ice bath for 5 minutes and pelleted at 13.000 Xg for 3 minutes for protein precipitation. The supernatant containing DNA was added to 300 μl isopropanol and incubated for 5 minutes prior to centrifugation at 13.000 Xg for 5 minutes, the supernatant was poured of and 300 μl of 70% ethanol was added and then the tube was inverted several times to wash the DNA pellet, then the tubes were centrifuged again for one minute. To the remaining cell pellet 10 μl of DNA hydration solution was added and the pellet was incubated for one hour at 65 º C after that the nucleic acid was stored at –20 º C for later analysis.

PCR amplification and product detection:

Ten microliters of each prepared specimen was added to 40 μl of the PCR master mix (GeNet Bio- South Korea) The prepared PCR tubes were mixed by vortex to bring all the fluid to the bottom, the tube then placed in the Thermal Cycler (Hybaid Express thermal cycler) after addition of a drop of molecular biology grade paraffin oil to each tube to avoid evaporation of the mix and heated to 95 °C for 5 minutes and subjected to 35 cycles of 15 seconds at 94 ° C and another 15 seconds at 55 ° C and 30 seconds at 72 ° C. Following amplification the samples were held at 72 ° C for 10 minutes and twenty μl of each amplified samples was analysed by gel electrophoresis and stained by ethidium bromide. The gel was examined under ultraviolet light as ethidium bromide intercalated between the bases of the DNA and fluoresce. The negative control was examined to exclude any source of contamination, the positive control was examined for the presence of sharp band at 890 bp and the samples were compared with the controls.

DNA sequencing: Using ALFexpress AutoRead sequencing kits and ALFexpress II DNA Automated DNA sequencer (Amersham- pharmaica biotech-Amersham, United Kingdom) which is a fully automated and highly accurate system for DNA analysis. Its sophisticated detection system relies on laser-induced fluorescence of the carbocyanine dye Cy5 with which DNA fragments are labeled.

Principle: The term DNA sequencing encompasses biochemical methods for determining the order of the nucleotide bases, adenine, guanine, cytosine, and thymine, in a DNA oligonucleotide. Determining the DNA sequence is therefore useful in identification of pathogens and differentiates between broadly diverse bacteria commonly associated with neonatal sepsis (12).

Procedure: In accordance with the manufacturer’s recommendations, the fragments migrate according to the size, and each was detected as it passed a laser beam at the bottom of the gel.

Each type of dideoxynucleotide emitted a colored light of a characteristic wavelength and was recorded as a colored band on a stimulated gel image. The computer program interrupted the raw data and output an electropherogram with colored peaks representing each letter in the sequence.

Interpretation: The resulting bases of sequence generated from sequencing were compared with the same stretch of sequence within the various bacterial 16S r RNA gene sequences found in Gen Bank, EMBL Nucleotide Sequence Database, and the DNA Data Bank of Japan using the BLAST tool at the National Centre for Biotechnology Information (NCBI).Sequences were aligned using sequencer soft ware.

RESULTS:

Out of the 50 neonates included in the study 43 (86%) were diagnosed as having neonatal sepsis by blood culture technique – The mean gestational age was 33.5 ± 4.4 weeks (range was 24 – 42 weeks), the mean birth weight was 1,962 ± 478 gram (range was 560 to 3,939 g) and the mean age at the onset of presumed sepsis was 3.78 ± 0.57 day.
By gram stain out of 43 positive samples tested, 23 (53.5%) were gram positive cocci, 19 (44.2%) were gram negative bacilli and one (2.3%) was Candida.

PCR was successful in detecting neonatal sepsis in all patients with positive blood culture and in 2 of blood culture negative samples after exclusion of Candida isolate which need 18 S rRNA not 16S rRNA for its detection. There is very good agreement between PCR and BACTEC 9050 for detection of sepsis (kappa = 0.8) and this was of high statistical significant value.

Conventional biochemical reactions were done for both Gram positive and Gram negative isolates and Microbact 12 A was done for Gram negative oxidase negative, nitrate positive, glucose fermenter isolates for identification of the causative pathogens. Klebsiella pneumoniae and Coagulase negative staph (Staphylococcal.epidermidis) were the commonest detected pathogens (12 out of 43 positive blood culture (27.9%) for each), followed by Staphylococcal aureus in 9 out of 43 positive blood culture (20.9%), E.coli and Streptococcal agalactia in 2 out of 43 positive blood culture (4.7%) for each one, Pseudomonas aeruginosa, Acinetobacter bumannii, Acinetobacter Iwoffii, Citrobacter freundii, Enterobacter gerjerviane in 1 out of 43 positive blood culture (2.3%) for each and one isolate was detected as candida.

DNA sequencing was done for the 44 positive samples detected by PCR, and revealed Klebsiella pneumoniae and Staphylococcal epidermidis in 13 out of 44 positive samples (29.5%) for each, Staphylococcal aureus in 9 out of 44 positive samples (20.5%), Streptococcal agalactia and E.coli in 2 out of 44 positive samples (4.5%) for each, Acinetobacter lowfii, Acinetobacter bumannii, Enterbacter, gerjerviane, Citrobacter freundii and Pseudomonas aeruginosa in one out of 44 positive samples (2.3%) each one.

Our results revealed the ability of DNA sequencing method for identification of two pathogens (A. Iwoffii and A. bumannii) which could not be detected by routine biochemical reactions and detected only by Microbact test and also detect the ability of DNA sequencing to recognize two pathogens which were negative by culture and positive by PCR, one was Klebsiella pneumoniae and the other was Staphylococcus epidermidis. There is good agreement between the results of conventional biochemical method and DNA sequencing method for identification of the causative pathogens (kappa = 0.6).

The turnaround time from DNA extraction to detection of PCR product by agarose gel electrophoresis was less than 4 h, compared to cultures which became positive after 24 to 72 h.

The diagnostic validity test done for PCR considering blood culture as a reference method “after exclusion of candida isolates which need 18S rRNA not 16S rRNA for its detection” revealed that the sensitivity of the PCR method was 100%, while the specificity was 75%, the positive predictive and the negative predictive values were 95.5% and 100% respectively using primer complementary to 16S rRNA and there is excellent agreement between the results of DNA sequencing and the reference sequence using stringent criteria for species identification; i.e., 99% sequence similarity or higher for species assignment and 95% sequence similarity or higher for genus assignment.

<table>
<thead>
<tr>
<th>PCR method</th>
<th>BACTEC 9050 method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>*Positive</td>
<td>42</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
</tr>
</tbody>
</table>

Significance (p) = 0.01, kappa = 0.8, Agreement = 93.3%

*42 positive samples after exclusion of Candida isolate
Table (2): Sensitivity, Specificity, PPV, NPV, False +ve and False -ve results of PCR for detecting sepsis considering blood culture as reference method after exclusion of candida isolate

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>False +ve</th>
<th>False -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>100%</td>
<td>75%</td>
<td>95.5%</td>
<td>100%</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Table (3): Molecular identification in comparison with reference sequence

<table>
<thead>
<tr>
<th>Results of DNA sequencing</th>
<th>NO</th>
<th>Agreement(%) from reference sequence</th>
<th>reference sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13</td>
<td>99%</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>Staphylococcal epidermidis</td>
<td>13</td>
<td>99%</td>
<td>Staphylococcal epidermidis</td>
</tr>
<tr>
<td>Staphylococcal aureus</td>
<td>9</td>
<td>99%</td>
<td>Staphylococcal aureus</td>
</tr>
<tr>
<td>Streptococcus agalactia</td>
<td>2</td>
<td>98%</td>
<td>Streptococcus agalactia</td>
</tr>
<tr>
<td>E coli</td>
<td>2</td>
<td>100%</td>
<td>E coli</td>
</tr>
<tr>
<td>Acinetobacter baumanii</td>
<td>1</td>
<td>99%</td>
<td>Acinetobacter baumanii</td>
</tr>
<tr>
<td>Acinetobacter lwofii</td>
<td>1</td>
<td>99%</td>
<td>Acinetobacter lwofii</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1</td>
<td>98%</td>
<td>Citrobacter freundii</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>98%</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Enterobacter gergivaneae</td>
<td>1</td>
<td>98%</td>
<td>Enterobacter gergivaneae</td>
</tr>
</tbody>
</table>

Figure (1): Microbact (12A) test shows positive results for Klebsiella pneumoniae (a), E.coli (b) and Acinetobacter lwofii (c)

Figure (2): Agarose gel electrophoresis with amplified product size at 890 bp
DISCUSSION:

Accurate clinical diagnosis of neonatal sepsis is often difficult as signs and symptoms in the neonate may be subtle or vague. Although the incidence of bacteremia is relatively low, the risk of mortality is quite high ranging from 10 to 50% (1). Blood culturing is considered to be the golden standard for diagnosis of neonatal bacterial sepsis (13), however blood culturing technique can have unacceptable low sensitivities. The reason for this include intermittent seeding of low numbers of bacteria within the blood stream, the extremely small blood volume obtained from infant for culturing and the increasingly common practice of providing intrapartum antibiotics to mothers of high risk delivers. It is well known that the smaller the volume of blood obtained for culturing, the lower the chance of recovering the organism (14).

Bacterial DNA consensus sequences for example 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 use in PCR- based assay to diagnose blood stream infection (15,16).

For the foreseeable future, culture will not be superseded by PCR- based testing due to the requirement for purified culture isolates in antimicrobial susceptibility testing. However, if an amplification assay could reliably rule out neonatal sepsis in less time than bacterial culture, it would allow for the exclusive treatment of neonates with true infections, thus reducing the use of broad- spectrum antibiotics and the potential for infant who are not septic of acquiring drug resistant bacteria. This approach would also permit shorter hospital stays within the neonatal intensive car unite (NICU) and reduce significantly the overall medical costs to the health care system as well as the emotional burdens of the families of these infants. (8)

The aim of our study has been to determine the potential of a broad diagnostic approach based on 16S r RNA gene amplification and sequencing for rapid detection and identification of pathogens causing neonatal bacteremia.

In this study it is found that the highest level of sepsis occurred in males (68.2 %) with low birth weight (70.5 %) these results are in agreement with that of Shaw et al., (17) and with that of Schrag et al., (18). They found that the highest incidence of sepsis occurs in males with low birth weight, preterm babies and those with maternal complications.

The gold standard for establishing the diagnosis of neonatal sepsis is the isolation of pathogens from one or more blood cultures (19). In the present study 43 out of 50 (86%) neonates suspected to have sepsis were positive by blood culture technique (continuous monitoring system BACTEC 9050).These results were in agreement with Buttery, (20) who found that blood culture were positive in 80% of suspected cases of neonatal sepsis. However a lower rate of detection (44.9%) was reported by Shaw et al., (17). The difference may be due to the fact that in our study we selected cases with strong suspicion of sepsis as those with lethargy, irritability, apnea, cyanosis, respiratory distress, poor capillary refill, with one major or two minor risk factors or more, positive CRP (> 6 mg/l), abnormal leucocyte counts, together with immature / total (I / T ) ratio ≥ 0.2.

The above difference may also be due to the fact that the vast majority of infants admitted to NICU for suspected sepsis are not infected but have symptoms consistent with those of other medical condition that mimic sepsis such as
hypoglycemia, delayed transition, or transient tachypnea \(^{(8)}\). Another explanation for negative blood cultures in neonates with suspected sepsis is that a very small amount of the organism is present in the blood, a quantity that could not be enough to determine a visible growth in usual culture conditions \(^{(21)}\). Brozanski et al., \(^{(22)}\) found that blood culture as the gold standard may not be optimal in the newborn because of antibiotic exposure in utero and the small blood volume obtained for culture.

Detection of neonatal sepsis quickly is critical as it is a life threatening condition. Using an approach that could be more rapid than standard culture and identification techniques for detecting neonatal sepsis would be highly desirable. A molecular-based approach for detecting a highly conserved target like the 16S rRNA gene which is present among all bacteria desirable. A molecular-based approach for detecting a highly conserved target like the 16S rRNA gene which is present among all bacteria would be useful \(^{(23)}\).

In the present study 44 out of 50 (88\%) whole EDTA blood samples were positive for sepsis by PCR. Among all cases with negative blood culture, 2 cases were positive for sepsis only by PCR. These results were in agreement with Laforgia et al., \(^{(21)}\) and Jordan and Durso, \(^{(8)}\) who found 2 positive cases by PCR which were negative by blood culture. This may be due to the fact that PCR amplification is capable of identifying bacterial DNA, even if a very small amount is present in the blood, a quantity that could not be enough to determine a visible growth in usual culture conditions. Besides, according to the previous data it has been demonstrated that when a small amount of blood is drawn and then subseeded in different culture media, it is possible that up to 27\% of sepsis are not diagnosed particularly during neonatal age \(^{(13)}\). It is also possible that these cases represent “false positive “results secondary to amplification of contaminating DNA \(^{(21)}\) but in our study strict precautions were taken which dramatically reduces this possibility.

There is very good agreement between the results of PCR and blood culture methods for diagnosis of neonatal sepsis (kappa =0.8 ). In accordance with our results Makhoul et al., \(^{(24)}\) and Brozanski et al., \(^{(22)}\) also detected a very good agreement between PCR and blood culture results (p<0.001).

In the current study taking blood culture as a reference method, the sensitivity, specificity, positive and negative predictive values for PCR were 100\%, 75\%, 95.5\% and 100\% respectively after exclusion of candida isolate which were detected by blood culture not by PCR as the primer being used was 16S r RNA not 18S r RNA needed to identify fungal sepsis\(^{(25)}\). 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 neonates admitted to the NICU for such evaluation and this result is in agreement with that of Jordan and Durso, \(^{(8)}\) who reported a negative predictive value for PCR in comparison to blood culture for diagnosis of neonatal bacteremia to be 99.8\%.

NICU admission 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.

Concerning the time consumed to detect sepsis, blood culture method took more time (up to 5 days) while PCR took less time (<4 hour). Laforgia et al., \(^{(21)}\) agrees with this result and concluded from their results that PCR allows identification of common and uncommon pathogens and shorten the course of antibiotic. Jordan and Durso, \(^{(8)}\) disagree with our result (the time required by PCR was roughly 9 hour) this is explained by incubation of CBC sample for 5 hours after addition of 4ml of tryptic soy broth (TSB) because they use the discarded whole blood after CBC analysis for their PCR assay.

Accurate identification of bacterial isolates is an essential task of the clinical microbiology laboratory. For many organisms, traditional phenotypic identification is difficult and time consuming and sole reliance on biochemical methods limit the clinical microbiology
The approach of performing rapid, short base-read sequencing on a PCR amplicon could provide the physician with valuable information about the etiology of the micro-organism sooner than culture, thus enabling them to make more informed decisions sooner about the type of antibiotics to give the infant, eliminating the need to give the neonate an ineffective or unnecessary broad-spectrum antibiotic which would help reduce the risk of destroying the infant’s normal intestinal flora and/or promoting antibiotic resistance.\(^{(23)}\)

Our results revealed the ability of DNA sequencing to recognize two pathogens which were negative by culture one was Klebsiella pneumoniae and the other was Staphylococcus epidermidis. In addition DNA sequencing could identify 2 species (one Acinetobacter lwoffii and one Acinetobacter baumannii) that couldn’t be identified by routine conventional biochemical reactions but only by Microbact test. This agrees with Tang et al.\(^{(28)}\) who found that DNA sequencing could identify four Acinetobacter species that couldn’t be identified by exhaustive phenotypic methods.

The criteria used for species identification in our study was 99% sequence similarity or higher for species assignment and 95% sequence similarity or higher for genus assignment, our study revealed identification of all isolates for species level by ≥98% sequence similarity. This agrees with Bosshard et al.\(^{(29)}\) who found that > 98.9% of isolates could be identified at the species level by using DNA sequencing.

In the present study Klebsiella pneumoniae and Staphylococcus epidermidis were the most commonly isolated pathogens (13 out of 44 (29.5%)) for each. These results are in accordance with the study of Abo-Alam et al.\(^{(30)}\) in Egypt who found the most frequently isolated organisms from septic neonates was Klebsiella pneumoniae in 32.5% and with the finding of El-Badawy et al.\(^{(31)}\) who found that the frequency of K. pneumoniae in neonatal sepsis was 26% of the isolates.

Coagulase negative staphylococcal infections have emerged since the 1980s as the leading cause of late onset or nosocomial sepsis in NICUs in industrialized countries. In recent years, they have been reported as causing over 50% of all late – onset infections and most infected babies are less than 30 weeks gestation at birth.\(^{(32)}\) The reasons for the emergence of CoNS as such important pathogens are probably multifactorial attributable in part to invasive procedures (e.g indwelling central vascular catheter, central nervous system shunt, thoracostomy tube), the thin fragile skin of premature babies and the use of lipid emulsion in parental nutrition which provides a growth medium for the organisms.\(^{(33)}\)

In accordance with our results, CoNS were the most common cause of both early and late onset sepsis in the study of Haquet et al.,\(^{(34)}\) in England, the most common cause of late onset sepsis (50%) in the study of Kilani and Basamad\(^{(35)}\) in Saudi Arabia and the most common cause of early onset sepsis (35%) in the Egyptian study of Badrawi et al.\(^{(36)}\) They represent the second most prevalent organism in the Egyptian studies of El-Maraghi et al.,\(^{(37)}\) and El-Badawy et al.\(^{(31)}\) representing 20% of the isolates in both studies.

Staphylococcus aureus remains a very important neonatal pathogen in developing countries, responsible for 8-22% of blood stream isolates in different regions.\(^{(38)}\) In our study it accounted for 9 out of 44 isolates (20.9%) which was in accordance with the results of Ladhani\(^{(39)}\) who found a Staphylococcus aureus frequency of 20% of the isolates.

The group B streptococcus is the most common gram positive organism that cause septicaemia and meningitis during the 1st month of life in infants older than 37 weeks gestation.\(^{(40)}\) Vertical transmission from mother to infant is one route of infection; also nosocomial acquisition has been implicated. In our study Streptococcus agalactiae occurred in 2 out of 44 isolates (4.5%). Al-Zwaini et al.\(^{(41)}\) found that the frequency was 1% of isolates. The explanation of this low percentage is that the majority of cases in both studies are premature with gestational age < 37 weeks.
E.coli is one of the most important pathogens of newborns and young infants causing sepsis, meningitis, and urinary tract infection. In our study 2 out of 44 isolates were identified as E.coli (4.5 %), the most recent study from the NICU in Kasr El-Aini Hospital, found E.coli to represent 1% of the total isolates from neonates with EOS.

Acinetobacter spp. are important nosocomial pathogens associated with a number of hospital acquired infections worldwide. In hot, humid areas, acinetobacter infection is endemic, with higher incidence of nosocomial infection including bacteremia and pneumonia, than those reported elsewhere. In our study Acinetobacter spp were detected in 2 out of 44 cases with neonatal sepsis (4.5 %), however Mahmoud et al., in Pakistan and Badrawi et al. in Egypt reported a higher rates for Acinetobacter spp in septic neonates which were 10.3 % and 10% respectively.

The role of P.aeruginosa as an important pathogen in children, especially in premature infants has been known since 1960. P.aeruginosa is a well-known cause of nosocomial infections among infants in NICUs. In our study P. aeruginosa accounted for 1 out of 43 isolates (2.3%).This result was in accordance with Baltimore, who found a rate of 2% of P.aeruginosa isolates among his patients with neonatal septicemia.

Citrobacter a gram negative enteric bacillus, is a rare cause of septicemia and meningitis, seldom reported beyond the neonatal period. In our study which detected Citrobacter freundii in 1 out of 43 patients with positive blood culture (2.3%).

Regarding the time required to identify bacteria by conventional method it usually require 24-48 hour after a discrete colony has been isolated, in contrast to DNA sequencing which takes about 24 hour, Tang et al., agrees with our results and concluded from their study that DNA sequencing can be completed within 24 hour in contrast to conventional methods that require more than 48 hour and two weeks are required for identification of many slowly growing and fastidious organism.

This technology has proven to be quite useful in diagnosis of neonatal pathogens and has the potential for excellent sensitivity and a shorter turn around time than those of culture based protocols.

In summary: The initial results presented here show promise as a means of rapidly detecting and differentiating among the bacteria commonly associated with neonatal sepsis. The benefit of PCR is its rapid availability of results with a high negative predictive value as a tool to rule out sepsis. This type of patient management could lead to shorter antibiotic courses and NICU stays for the otherwise healthy newborn infants. We speculate that this decrease in NICU stays could facilitate infant – parental attachments and successful establishment of breast – feeding as well as reduce the hospital costs associated with prolonged treatment.

However more PCR positive whole blood samples will need to be analysed before this can be proven and the use of 18S r RNA with 16S r RNA for detection of fungal infection which show increased incidence in the last years is recommended.

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اكتشف تجرُم الدم عند الأطفال حديثي الولادة عن طريق مضاعفة جين 16 اس أر إن

أيه الريبووزومي بواسطة تفاعل تسلسل البلمرة

د. هشام كامل

قسم البيولوجيا الأكلينيكية - كلية طب بنها و قسم طب الأطفال مستشفى الأطفال التخصصي

تهدف هذه الدراسة للإكتشاف مقدرة طريقة التشخيص المعتمدة على مضاعفة جين 16 اس أر إن أية الريبووزومي بواسطة تفاعل تسلسل البلمرة ومعرفة تسلسل الحمض النووي في سرعة إكتشاف تجرُم الدم عند الأطفال حديثي الولادة

وقد تم تجميع عينات من 50 من الرضياء الأطفال حديثي الولادة والمصحية في أصابتهم بجرم الدم لعمل مزارع

دم لهم والتعرف على الميكروبر السيب بالطرق البيئولوجية المعتمدة واختبار الميكروباكت وعند الكشف عن وجود

جين 16 اس أر إن أيه الريبووزومي الدال على وجود تجرُم الدم ومعرفة تسلسل الحمض النووي

وقد تم إكتشاف 43 حالة مصابة بجرم الدم بواسطة مزارع الدم (باكتيك) بينما استطاع تفاعل تسلسل البلمرة في

إكتشاف 44 حالة مصابة وكانت نسبة التوافق بين الطرقتين 93.9%.

وكد أنتقفت الطرقتين في تشخيص 44 حالة بينما استطاع اختبار تفاعل تسلسل البلمرة في تشخيص إصابتين لم تتمكن

مزارع الدم من تشخيصهما بينما استطاعت مزارعة الدم في تشخيص حالة مصابة بفتر الكائدليا لم تتمكن اختبار تفاعل تسلسل البلمرة من اكتشافها لاستخدام نوع من البريمير خاص فقط لتشخيص الميكروباكت الحساسية للجرم و ليس الفترات.

وقد استطاعت معرفة تسلسل الحمض النووي من التعرف على نوع من الميكروباكت لا تستطيع الطرق

البيئولوجية المعتمدة من التعرف عليها إلا عن طريق اختبار الميكروباكت

وقد خلص هذا البحث إلى أن طريقة التشخيص المعتمدة على مضاعفة جين 16 اس أر إن أيه الريبووزومي بواسطة

تفاعل تسلسل البلمرة ومعرفة تسلسل الحمض النووي تمتاز بدرجة عالية من الدقة والحساسية بالإضافة إلى استطاعة

الحصول على النتيجة بعد فترة أقل بكثير من تلك المعتمدة على المزارع.