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Isolation, Identification and Antibiotic Susceptibility Testing of Bacterial Pathogens from Blood Cultures of Neonates With Clinical Signs of Sepsis in A Tertiary Care Centre

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Abstract
Neonatal mortality rate is one of the indications for measuring the health status of a nation. Neonates harbor an immature immune system, rendering them highly susceptible to infections that can cause life threatening consequences. In developed countries, Group B streptococci and coagulase negative staphylococci are the common etiological agents of neonatal sepsis. But in developing countries, the bacteriological profile varies, not only from place to place but also from time to time. Blood culture is the gold standard for the diagnosis of septicaemia and ideally should be done prior to starting antibiotics. Developed to recover microorganisms from blood, but each having its own limitations. Newer blood culture techniques using highly enriched media and a continuous monitoring system, as BacT/Alert system have improved the turnaround time for cultures to about 12 hours. They are highly sensitive, detecting organisms as low as1-2 colony forming units per millilitre. A positive blood culture and antibiotic susceptibility profile is the best guide to effective antibiotic therapy This is a cross sectional with study sample size 142.Blood samples of neonates with sepsis for, isolation, identification and antibiotic susceptibility testing of bacterial pathogens from blood culture of neonates with clinical sign of sepsis from department of paediatrics in our hospital are included. 142 neonates with clinical signs of sepsis were enrolled in the study. 84(59.2%) were males and 58(40.8%) were females. Majority of the neonates (90, 63.4%) had birth weight greater 2.5kg, 37(26.1%) neonates had weight less than 2.5kg and 13(9.2%) <1.5kg and 2 of them <1 kg.116(81.7%) neonates presented with EOS and 26(18.3%) with LOS. 59(41.5%) neonates were born preterm. 80(56.3%) babies were born by lower segment caesarian section (LSCS), 56(39.4%) were by normal vaginal delivery and 6 of them by assisted vaginal delivery. 77(54.2%) neonates with clinical signs of sepsis had hyperbilirubinemia (NNHB)50(35.2%) neonates presented with respiratory distress. Among the 11 Gram positive organisms, all were susceptible to linezolid and vancomycin, 72.5% to amikacin,63.5% to ofloxacin, 54.5% to gentamicin, 9.09% each to ampicillin, cephalexin cloxacillin. Among the 7-gram negative organisms, all were susceptible to meropenem and colistin,57.1% each to gentamicin, amikacin piperacillin-tazobactam, 42.8% to ciprofloxacin28.5% to ofloxacin. There were 8 definite pathogens, 10 possible pathogens2 skin contaminants. In a reduced usage of antibiotics in the ICUs can prevent emergence of multidrug resistant bacteria. Moreover, hand hygiene forms the cornerstone in preventing the transmission of these resistant bugs.

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Key Words
Isolation, antibiotic, susceptibility, neonates, clinical signs

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INTRODUCTION

Neonatal mortality rate is one of the indications for measuring the health status of a nation. Neonates harbor an immature immune system, rendering them highly susceptible to infections that can cause life threatening consequences[1]. Sepsis is one of the leading causes of neonatal morbidity and mortality, particularly in the developing world. Neonatal sepsis is a clinical syndrome of bacteraemia characterised by systemic signs and symptoms of infection in the first month of life[2]. Septicaemia in neonates is documented by positive blood culture in the first 4 weeks of life[3].

Neonatal sepsis can be classified into two subtypes depending upon the age of onset of infection, the early-onset sepsis (within 72 hours of life) and the late-onset sepsis (after 72 hours of life). Common risk factors predisposing to both the syndromes are the gestational age and birth weight[4]. Early-onset sepsis is caused by organisms present in the maternal genital tract.

Low birth weight, foul smelling or me conium-stained liquor amnii, prolonged rupture of membrane is some of the high-risk factors associated with an increased risk of early-onset sepsis. The late-onset sepsis is either hospital acquired or community acquired. Factors that predispose to an increased risk of nosocomial sepsis include low birth weight, pre-maturity, invasive procedures, parenteral fluid therapy, ventilation use of stock solutions[2]. In developed countries, Group B streptococci and coagulase negative staphylococci are the common etiological agents of neonatal sepsis. But in developing countries, the bacteriological profile varies, not only from place to place but also from time to time[5].

Blood culture is the gold standard for the diagnosis of septicaemia and ideally should be done prior to starting antibiotics[2]. Developed to recover microorganisms from blood, each having its own limitations. Newer blood culture techniques using highly enriched media and a continuous monitoring system, as BacT/Alert system have improved the turnaround time for cultures to about 12 hours. They are highly sensitive, detecting organisms as low as1-2 colony forming units per millilitre[4,5]. A positive blood culture and antibiotic susceptibility profile is the best guide to effective antibiotic therapy.

MATERIALS AND METHODS

This is a cross sectional with study sample size n= 142. Blood samples of neonates with sepsis for, isolation, identification and antibiotic susceptibility testing of bacterial pathogens from blood culture of neonates with clinical sign of sepsis from department of paediatrics in our hospital after getting permission to conduct the study from institutional Ethics committee for the duration of 6 months. All neonates with clinical signs of sepsis are included in the study.

Blood Collection: 2.5 ml of blood was collected from a peripheral venepuncture site of the neonates after following strict aseptic precautions. The blood was inoculated into BacT/Alert PF blood culture bottles. The bottles were then sent to the Microbiological laboratory along with a properly filled requisition form. At the Microbiology lab, the blood culture bottles were logged into bacT/Alert 3D automated machines by entering the samples accession number and patient identifier into the computer. The bottles were then placed into the assigned wells. When an increasing concentration of CO2 is detected in a bottle, the light adjacent to the well is illuminated. The bottle was then removed from the instrument for further processing[7]. The data was entered in a computer spread sheet and summarized using tables, charts and graphs. The proportions and confidence limits of the various pathogens isolated and their antibiotic susceptibility patterns were obtained using SPSS software.

RESULTS AND DISCUSSIONS

142 neonates with clinical signs of sepsis were enrolled in the study. 84(59.2%) were males and 58(40.8%) were females. Majority of the neonates (90, 63.4%) had birth weight greater 2.5kg, 37(26.1%) neonates had weight less than 2.5kg and 13(9.2%) <1.5kg and 2 of them <1 kg.116(81.7%) neonates presented with EOS and 26(18.3%) with LOS. 59(41.5%) neonates were born preterm. 80(56.3%) babies were

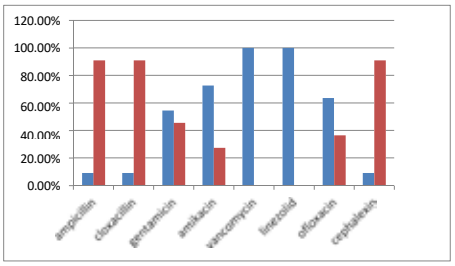

Fig. 1: Antibiotic susceptibility of Gram-positive organisms

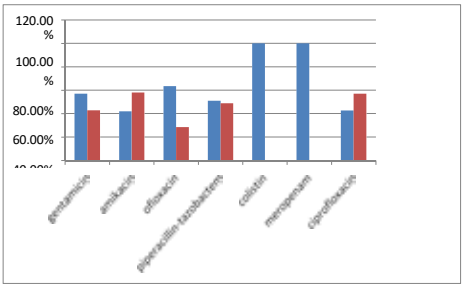

Fig. 2: Antibiotic susceptibility of Gram-negative organisms

Table 1: Maternal risk factors

Maternal factors	present	absent
GHTN	20(14.1%)	122(85.9%)
GDM	32(22.5%)	110(77.5%)
Pre-eclampsia	5(3.5%)	137(96.5%)
PROM	24(16.1%)	118(83.1%)
Hypothyroidism	18(12.7%)	124(87.3%)
MSAF	20(14.1%)	122(85.9%)
UTI	11(7.7%)	131(92.3%)
Polyhydramnios	3(2.1%)	139(97.9%)
Placenta praevia	5(3.5%)	137(96.5%)

Table 2: Symptoms and signs in neonates with clinical signs of sepsis

Symptoms/signs	present	absent
Respiratory distress	50(35.2%)	92(64.8%)
NNHB	77(54.2%)	65(45.8%)
Fever	14((9.9%)	128(90.1%)
Poor feeding/decreased activity	7(4.9%)	135(95.1%)
Abdominal distension	2(1.4%)	140(98.6%)
Vomiting	5(3.5%)	137(96.5%)
Jaundice	1(.7%)	140(99.3%)
Tachycardia	1(.7%)	140(99.3%)
Hypoglycemia	10(7%)	132(93%)
Convulsions	3(2.1%)	139(97.9%)
UTI	7(4.9%)	135(95.1)
Umbilical discharge	2(1.4%)	140(98.6%)
Pleural effusion	2(1.4%)	140(98.6%)
URTI	1(.7%)	141(99.3%)

born by lower segment caesarian section (LSCS), 56(39.4%) were by normal vaginal delivery and 6 of them by assisted vaginal delivery.

The maternal risk factors included pre-eclampsia, premature rupture of membrane (PROM), maternal fever, placental abruption, convulsions, antenatal steroids, gestational hypertension (GHTN), gestational diabetes mellitus (GDM), hypothyroidism, oligohydramnios, Me conium-stained aspiration fluid (MSAF), urinary tract infection (UTI), placenta previa, anaemia polyhydramnios. (Table 1)

77(54.2%) neonates with clinical signs of sepsis had hyperbilirubinemia (NNHB) 50(35.2%) neonates presented with respiratory distress. (Table 2)

Out of 142 samples, 20 were culture positive. Of these, 20 isolates, 16(80%) organisms were from neonates with EOS and 4(20%) organisms from those with LOS. Organisms isolated from EOS were Coagulase negative Staphylococci (CoNS)(8), Pantoea(2), Klebsiella pneumoniae(2), Enterobacter spp.(1), Acinetobacter spp.(1), Pseudomonas aeruginosa (1), aerobic spore bearing bacilli(1) micro cocci(1).The organisms isolated from LOS were CoNS(2), Pseudomonas aeruginosa(1), Staphylococcus aureus(1). There were 8 definite pathogens, 10 possible pathogens 2 skin contaminants. 100% definite pathogens isolated during 12-36 hrs. In case of possible pathogens 90% organisms isolated during 12-36 hrs and 10% were isolated during 36-48 hrs. Out of 18 possible and definite pathogens, 11 (61.1%) were gram positive and 7(38.8%) were Gram negative organisms. The Gram positive bacteria included CoNS(10) and S. aureus(1). The Gram negative bacteria obtained were K. pneumoniae(2), Pantoea spp.(2), Acinetobacter spp.(1), P.aeruginosa(1) and Enterobacter spp.(1).

Among the 11 Gram positive organisms, all were susceptible to linezolid and vancomycin, 72.5% to amikacin, 63.5% to ofloxacin, 54.5% to gentamicin, 9.09% each to ampicillin, cephalexin cloxacillin. (Fig. 1).

Among the 7-gram negative organisms, all were susceptible to meropenem and colistin, 57.1% each to gentamicin, amikacin piperacillin-tazobactam, 42.8% to ciprofloxacin 28.5% to ofloxacin. (Fig. 2).

In our study, there were 84 males (59.2%) and 58 females (40.8%). In a study done by S. Thakur, out of the total 450 neonates, 117 (62%) were males and 71(38%) were females inborn neonates were 111(59%), more common compared to out born neonates which were 77(41%)12. 116 neonates (81.7%) were clinically diagnosed with EOS 26 (18.3%) had signs of LOS. In another study by P. Jyothi. EOS was found to be 3 times higher than LOS. Out of 131 cases, 98(74.8%), had EOS and 33(25.2%) had LOS[8].

Maternal risk factors for neonatal septicaemia in our study included gestational diabetes mellitus 32(22.5%), gestational hypertension 20(14.1%), MSAF 20(14.1%), premature rupture of membrane 24(16.9%) maternal fever 6(4.2%). The common neonatal risk factors were respiratory distress 50(35.2%), poor feeding 7(4.9%), NNHB 77(54.2%), hypoglycemia 10(7%). In a study by Pragathi Abhimanyu Bulle, maternal risk factors for neonatal septicemia such as maternal fever 17.1% and prolonged rupture of membranes (22.17%) were found to be more common. Low birth rate, prematurity, neonatal resuscitation and me conium aspiration were common risk factors. Most of the neonates presented with lethargy (69.74%), no sucking (68.42%) and temperature changes (42.98%)[10,11,12].

Out of 18 possible and definite pathogens, 10 were possible pathogens and 8 were definite pathogens. 100% definite pathogens isolated during 12-36 hrs. In case of possible pathogens 90% organisms isolated during 12-36 hrs and 10% were isolated during 36-48 hrs. In a study by Y. Kumar, out of 2268 blood samples obtained 451 shows positive blood culture. Definite bacterial pathogens isolate during 0-12 hrs 11.6%, 12-24hrs 65.1%, 24-36 hrs 14%, 36-48hrs 2.3%, 48-60 hrs 4.3%, 60-72hrs 2.3%. Possible pathogens isolated during 0-12 hrs were 1.25%, 12-24hrs 47.4%, 24-36hrs 39.3%, 36-48hrs 8.3%, 48-60 hrs1.25%, 60-72hrs 1.25%, >72hrs 1.25%[8].

Out of the 20 isolates, 16(80%) organisms were isolate from neonates with EOS and 4(20%) organisms from LOS. According to the study by P. Jyothi, of 131 positive blood culture samples 98 (74.8%) were early onset septicaemia cases, while 33(25.2%) were late onset septicaemia.8 20 blood cultures were positive in our study. There were 8 definite pathogens, 10 possible pathogens 2 skin contaminants. Out of 18 possible and definite pathogens, 11 (61.1%) were gram

positive organisms and 7(38.8%) were gram negative organisms. In a study by S. Thakur, of 180 positive blood cultures from 450 blood samples, Gram positive bacteria and Gram-negative bacteria accounted for 60% and 40% respectively. This finding is in parallel with the results obtained in our study[9].

Among the 11-gram positive organisms, all were susceptible to linezolid and vancomycin, 72.5% to amikacin, 63.5% to ofloxacin, 54.5% to gentamicin, 9.09% each to cephalexin, ampicillin, cloxacillin. Among the 7 gram negative organisms, all were susceptible to meropenem and colistin, 57.1% to gentamicin, amikacin, piperacillin-tazobactem, 42.8% to ciprofloxacin, 28.5% to ofloxacin. In a study by P. Jyothi, best overall sensitivity among gram negative isolates was to imipenem (93%), followed by amikacin (52%) and netilmicin (52%). Gram positive isolates had 91% susceptibility to linezolid, 68% to tetracycline, 64% to piperacillin/tazobactam erythromycin 52% to ciprofloxacin.

CONCLUSION

142 blood samples from neonates with clinical signs of sepsis reached the microbiology lab for culture. of these, 20 samples yielded bacterial growth. 80% organisms were isolated during the period of EOS. There were 8 definite pathogens, 10 possible pathogens 2 skin contaminants. Among the pathogens, 11 (61.1%) were gram positive-and 7(38.8%) were gram negative organisms. The Gram positive bacteria included CoNS(10) and S. aureus(1}.The gram negative bacteria obtained were K. pneumoniae(2) Pantoea spp.(2), Acinectobacter spp.(1), P.aeruginosa(1) and Enterobacter spp.(1). CoNS was found to be predominant organism isolated from neonatal blood cultures in our institution. Among the 11-gram positive organisms, all were susceptible to linezolid and vancomycin, 72.5% to amikacin, 63.5% to ofloxacin, 54.5% to gentamicin, 9.09% each to cephalexin, ampicillin, cloxacillin. Among the 7 gram negative organisms, all were susceptible to meropenam and colistin, 57.1% to gentamicin, amikacin, piperacillin-tazobactem, 42.8% to ciprofloxacin, 28.5% to ofloxacin. In a reduced usage of antibiotics in the ICUs can prevent emergence of multidrug resistant bacteria. Moreover, hand hygiene forms the cornerstone in preventing the transmission of these resistant bugs.

Summary: Neonatal sepsis is a clinical syndrome of bacteraemia characterised by systemic signs and symptoms of infection in the first month of life. The main objective of the study is for isolation, identification and antibiotic susceptibility testing of bacterial pathogens from blood culture of neonates with clinical sign of sepsis and to compare the bacterial pathogens between early-onset sepsis and late-onset sepsis. All blood samples of neonates with clinical signs of sepsis reaching the microbiology department were considered for study. It is a cross-sectional study and the study. During the study, 142 samples were collected from neonates with clinical signs of sepsis. Out of 142 samples, 20 were found to be positive in BacT/Alert. The isolated the organisms by inoculating the blood samples (in tryptic soy broth) on blood agar, chocolate agar and MacConkey agar. A gram smear of the sample was also prepared and examined. The inoculated culture plates were incubated at 37 degree C for 18-24 hours. On the second day, the culture plates were examined. The bacterial growth obtained was identified by following standard microbiological procedures like study of colony morphology, Gram staining and a set of biochemical tests. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Muller-Hinton agar.

Of the 142 neonates enrolled in the study, 84(59.2%) were males and 58(40.8%) were females. 90(63.4%) neonates had birth weight greater 2.5kg, 37(26.1%) <2.5kg, 13(9.2%) with <1.5kg and 2 of them <1 kg. 116(81.7%) neonates were diagnosed with EOS and 26(18.3%) with LOS. 59(41.5%) neonates were born preterm. 80(56.3%) babies delivered by LSCS, 56(39.4%) by normal vaginal delivery 6 by vaginal assisted delivery. The maternal risk factors included GHTN 20 (14.1%), GDM 32(22.5%), pre-eclampsia 5(3.5%), PROM 24(16.1%), hypothyroidism 18(12.7%), MSAF 20(14.%), UTI 11(7.7%). 77(54.2%) neonates with clinical signs of sepsis had hyperbilirubinemia.

20 blood cultures were positive in our study. 80% organisms were isolated during the period of EOS. There were 8 definite pathogens, 10 possible pathogens 2 skin contaminants. Out of 18 possible and definite pathogens, 11 (61.1%) were gram positive and 7(38.8%) were gram negative. The Gram positive bacteria included CoNS(10) and S. aureus(1). The gram negative bacteria obtained were K. pneumoniae(2) Protoea spp.(2), Acinectobacter spp.(1), P.aeruginosa (1) and Enterobacter spp.(1). Of the 18 samples positive for pathogens, 17(94%) became positive between 12 and 36 hours and one turned positive during 36-48 hrs. Among the 11 gram positive organisms, all were susceptible to linezolid and vancomycin, 72.5% to amikacin, 63.5% to ofloxacin, 54.5% to gentamicin, 9.09% each for cephalexin, ampicillin, cloxacillin. Among the 7 gram negative organisms, all were susceptible meropenam and colistin, 57.1% to gentamicin, amikacin,

piperacillin-tazobactem , 42.8% to ciprofloxacin, 28.5% to ofloxacin.

Suggestions:

- Collection under proper aseptic percussions must be ensured prior to collection of blood from neonates, which help to minimize the use of antibiotics
- If a computer link between microbiology lab and the neonatal unit becomes available, the unnecessary usage of antibiotics can further reduce.
- Proper hand hygiene should be maintained to prevent the transmission of infections among the neonates.

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