Bacteriology of neonatal septicaemia

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ABSTRACT

Background: Septicaemia is one of the important causes of mortality and morbidity in neonates. Blood culture is the gold standard for its diagnosis. Emergence of multidrug resistant bacterial strains is a major problem in the management of neonatal sepsis. Present study was undertaken to identify the common bacterial pathogens associated with neonatal sepsis and to determine their antibiotic susceptibility pattern.

Materials and Methods: Blood cultures from 125 suspected cases of neonatal sepsis were examined. The growths from the sub-cultures were identified by conventional biochemical tests. Antibiotic susceptibility testing was performed by Modified Kirby-Bauer disc diffusion method and drug resistant strains in primary screening were further processed for ESBL and MRSA status by combination disc method (ESBL) and Oxacillin disc diffusion method (MRSA).

Results: Out of the 125 cultures obtained from suspected cases, 57(45.6%) were culture positive. 35.08% of the culture isolates were Gram negative bacilli. 63.15% of the isolates were Gram positive cocci. The prevalence of MRSA in 26 strains of Staphylococcus aureus was found to be 23.07% (6 strains). The overall prevalence of ESBL producers among Gram negative bacterial isolates was 35% (7 strains).

Conclusion: This study stresses the need for the continuous screening and surveillance for antibiotic resistance in NICU.

Key words: neonatal sepsis, MRSA, ESBL, blood culture

INTRODUCTION

Neonatal septicaemia is a clinical syndrome characterised by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life. Incidence of Neonatal septicaemia in India is 30/1000 live births.[1]

Neonatal septicaemia may be categorised as early onset and late onset. Early onset neonatal septicaemia is associated with acquisition of the microorganisms from the mother. The organisms implicated are Group B Streptococci, E. Coli, coagulase negative staphylococci, Haemophilus influenza and Listeria monocytogenes. Late onset neonatal septicaemia occurs within 4-90 days of life and is acquired from the care giving environment organisms Coagulase negative Staphylococci, Staphylococcus aureus, Escherichia coli, Klebsiella species, Pseudomonas species, Candida species, Acinetobacter and anaerobes. Pneumonia is common in early onset septicaemia, whereas meningitis and bacteraemia are common in late onset septicaemia.[2] The risk factors for early onset neonatal septicaemia include premature rupture of membrane, prolonged labour, prematurity, low birth weight, birth asphyxia, congenital anomalies, urinary tract infection of the mother and poor maternal nutrition. Prematurity and central venous catheterization poses a risk for the late onset neonatal septicaemia.[3]

The signs of neonatal septicaemia include diminished spontaneous activity, less vigorous sucking, apnoea, bradycardia, temperature instability, respiratory distress, nausea, vomiting, diarrhoea and jaundice. Microbial invasion of the bloodstream can have serious consequences such as shock, multi-organ failure, disseminated intravascular coagulation (DIC) and death.[4]

Early diagnosis is important and requires awareness of the risk factors, particularly in low birth weight neonates. The gold standard for the diagnosis of neonatal septicaemia is the isolation of the bacterial agent from blood culture. For

effective management of neonatal septicaemia, study of their antibiotic sensitivity plays a significant role.[5] Emergence of multidrug resistant bacterial strains is a major problem in the management of neonatal septicaemia. Present study was undertaken to identify the common bacterial pathogens associated with neonatal septicaemia and to determine their antibiotic susceptibility pattern.

MATERIALS AND METHOD

We conducted the study between March 2009 and February 2010, wherein we collected blood samples from 125 clinically diagnosed neonatal septicaemia cases through strict aseptic precautions. One ml blood was collected and inoculated into 10 ml of brain heart infusion broth (1:10 dilution). The culture bottles were incubated at 37^{0}C aerobically and periodic subcultures were done onto MacConkey's agar, blood agar and chocolate agar after overnight incubation 2, 3, 5 and finally on day 7. The growth obtained was identified by using standard protocol.

Antibiotic susceptibility test: The standard disc diffusion tests for susceptibility to routine antibiotics were done by Modified Kirby Bauer method. Antibiotic panel was based on the hospital antibiotic policy. Zone sizes were measured and interpreted according to CLSI standards.[6] Drug resistant strains in primary screening were further processed for the detection of extended spectrum beta lactamases (ESBL) in gram negative bacterial isolates and Methicillin resistance in Staphylococcus aureus strains.

Detection of MRSA was done by oxacillin disc diffusion method by placing 1 µg oxacillin disc on the bacterial lawn culture of Staphylococcus aureus. After incubation at 35^0 C for 24 hours, the zone of inhibition was measured. An inhibition zone of diameter less than or equal to 10 mm indicates MRSA. Staphylococcus aureus ATCC 25923 was used as quality control for oxacillin susceptibility.[7]

Gram negative bacilli that exhibited intermediate resistance or resistance to third generation cephalosporins were screened for ESBL production. ESBL producers were confirmed by combination disc method using cefotaxime (30 µg) and cefotaxime/ clavulanate (30/10 µg) (Himedia). An increase of 5 mm in the zone of inhibition in a disc containing clavulanate compared to the drug alone is considered as positive for ESBL producers.[8]

RESULTS

Of the 125 neonates screened for sepsis, there were 57 (45.6%) positive blood cultures. 20(35.08%) of the culture isolates were Gram negative bacilli. Thirty six (63.15%) of the isolates were Gram positive cocci. The frequency of isolation of the bacterial isolates is shown in Table 1 and 2. The antibiogram of the Gram positive and Gram negative isolates are shown in Table 3 and 4 respectively.

Table 1: Incidence and distribution of Gram positive cocci isolated from blood culture

Bacterial isolates	Number and percentage
Staphylococcus aureus	26(45.61%)
Coagulase negative staphylococci	10(17.54%)
Total	36

Table 2: Incidence and distribution of Gram negative bacilli isolated from blood culture

Bacterial isolates	Number and percentage
Escherichia coli	1(1.75%)
Klebsiella pneumoniae	10(17.54%)
Enterobacter species	1(1.75%)
Citrobacter species	1(1.75%)
Salmonella typhi	1(1.75%)
Proteus mirabilis	1(1.75%)
Pseudomonas species	3(5.26%)
Acinetobacter species	2(3.51%)
Total	20

Table 3: Antibacterial resistance pattern of the Gram positive blood stream isolates from NICU.

Bacterial isolates	Number and percentage
Escherichia coil	1(1.75%)
Klebsiella pneumoniae	(10(17.54%)
Enterobacter species	1(1.75%)
Citrobacter species	1(1.75%)
Salmonella typhi	1(1.75%)
Proteus mirabilis	1(1.75%)
Pseudomonas species	3(5.26%)
Acinetobacter species	2(3.51%)

P/A=Penicillin/Ampicillin, Cf = Ciprofloxacin, Va = Vancomycin , G =Gentamycin, Ce = Cefotaxime, Ak= Amikacin, Co =Cotrimoxazole

Table 4: Antibacterial resistance pattern of the Gram negative blood stream isolates from NICU.

	P/A	Cf	Va	G	Ce	Ak	Co
S.aureus(26)	17 63.3%	7 23.92%	0 0%	2 7.69%	3 11.3%	1 3.84%	5 19.23%
Coagulase negative staphylococci (10)	4 40%	2 20%	2 20%	5 50%	3 30%	5 50%	6 60%

A=Ampicillin, Ak= Amikacin, G= Gentamycin, Cf= Ciprofloxacin, Ce=Cefotaxime, Cu=Cefuroxime, I= Imipenem, Pt= Piperacillintazobactam, NT= not tested

DISCUSSION

Sepsis is the commonest cause of neonatal mortality, responsible for about 30-50% of the total neonatal deaths in developing countries.[9] Physical signs and symptoms, though useful in identifying possible cases have limited specificity. Definitive diagnosis is by bacteriologic culture of blood samples to identify organisms and establish antibiotic susceptibility.[3]

We have processed 125 blood samples from clinically diagnosed neonatal septicaemia cases. The rate of bacterial isolation in blood culture in this study was 45.6%, which is comparable to the previous studies.[3,10] The weaker immune system in neonates explains this higher rate of isolation.

The common isolates in blood culture in our study are Staphylococcus aureus 26 (45.61%), Coagulase negative staphylococci 10 (17.54%) and Klebsiella pneumoniae 10 (17.54%). The findings are consistent with the previous studies.[1,11] This suggests that infections by these agents constitute a significant threat to child survival in this local and other developing country settings.

The in vitro susceptibility tests of Klebsiella pneumoniae showed high levels of resistance to commonly used antibiotics such as Ampicillin(40%), Gentamicin(50%) and third generation cephalosporins (30%). For multiple resistant strains of Klebsiella spp. and other gram negative bacilli, a combination of a third generation cephalosporin (cefotaxime or ceftazidime) with amikacin may be appropriate. However, recent reports suggest that at least 60-70% of the gram negative organisms are resistant to the above antibiotics and routine use of these antibiotics might increase the risk of infections with ESBL organisms.[12]

n our study, out of the 20 strains of Gram negative isolates tested for ESBL production, 7(35%) strains were ESBL producers. Thus there is a need for continuous screening and surveillance for ESBL producers in NICU.

Imipenem and piperacillin- tazobactam proved to be the most effective antibiotics for all the Gram negative bacterial isolates including non fermenters in units with higher incidence of resistant strains.

63.08% of the Staphylococcus aureus strains showed resistance to penicillin. Penicillin resistant Staphylococcus aureus should be treated with cloxacillin, nafcillin or methicillin, but the worrisome fact is the emergence of MRSA. The incidence of MRSA in the 26 strains was 23.07% (6 strains). The occurrence of MRSA is more in NICU because of indiscriminate use of higher antibiotics as an emergency empirical therapy. Vancomycin remains the drug of choice for the Gram positive bacterial isolates in our setup. MRSA should be treated with a combination of ciprofloxacin or vancomycin with amikacin.

This study has shown that S. aureus and gram-negative rods including Klebsiella pneumoniae are the leading causes of septicemia in neonates, a pattern similar to that of other low income countries. Observed decline in susceptibility of these common pathogens to common antibiotics calls for increased efforts to ensure more rational use of these drugs.

The main forces driving the increase in antimicrobial resistant bacteria appear to be poor infection control practices and inappropriate use of antibiotics. Specific antibiotic utilization strategies like antibiotic restriction, combination therapy and antibiotic cycling may help decrease or prevent the emergence of resistance. Of these, one that is currently attracting considerable interest particularly in the intensive care unit setting is antibiotic cycling or rotation. It is the scheduled rotation of one class of antibiotics with one or more different classes exhibiting comparable spectra of activity; in order to fulfill the definition, the cycle must be repeated.

AUTHOR NOTE

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