ORIGINAL ARTICLE

CAUSATIVE BACTERIA AND ANTIBIOTIC RESISTANCE IN NEONATAL SEPSIS

Shahzad Najeeb, Saima Gillani, Rifayyat Ullah*, Anees ur Rehman
Department of Paediatrics, Ayub Medical College and Hospital Complex, Abbottabad, *Kabir Medical College, Peshawar, Pakistan

Background: Neonatal sepsis is characterised by bacteraemia and clinical symptoms caused by microorganisms and their toxic products. Gram negative bacteria are the commonest causes of neonatal Sepsis. The resistance to the commonly used antibiotics is alarmingly high. The major reason for emerging resistance against antibiotics is that doctors often do not take blood cultures before starting antibiotics. We have carried out this study to find out various bacteria causing neonatal sepsis and their susceptibility to antibiotics for better management of neonatal sepsis. **Methods:** A total of 130 neonates with sepsis who were found to be blood culture positive were taken in this study. Culture/sensitivity was done, isolated organisms identified and their sensitivity/resistance was noted against different antibiotics. Data were arranged in terms of frequencies and percentage. **Results:** Out of 130 culture proven cases of neonatal sepsis, gram negative bacteria were found in 71 (54.6%) cases and gram positive bacteria in 59 (45.4%) cases. Staphylococcus aureus was the most common bacteria found in 35 (26.9%) cases followed by Escherichia coli in 30 (23.1%) cases. Acinetobacter species, Staphylococcus epidermidis, Klebseila, Streptococci, Enterobacter cloacae and Morexella species were found in 17 (13.1%), 17 (13.1%), 13 (10%), 7 (5.4%), 6 (4.6%), and 5 (3.8%) cases respectively. In most of the cases causative organisms were found to be resistant to commonly used antibiotics like ampicillin, amoxicillin, cefotaxime, and ceftriaxone (77.7%, 81.5%, 63.1%, and 66.9% respectively). There was comparatively less (56.9%) resistance to ceftazidime. Gentamicin had resistance in 55.1% cases, while amikacin and tobramycin had relatively less resistance (17.4% and 34.8% cases respectively). Quinolones and imipenem had relatively less resistance. Vancomycin was found to be effective in 100% cases of Staphylococcus group. **Conclusion:** Staphylococcus aureus are the most common gram positive bacteria and Escherichia coli are the most common gram negative bacteria causing neonatal sepsis. Resistance to commonly used antibiotics is alarmingly increasing. Continued surveillance is mandatory to assess the resistance pattern at a certain level.

Keywords: Neonatal sepsis, bacterial isolates, Antibiotic susceptibility, Drug resistance, gram-positive, gram-negative

INTRODUCTION

Neonatal Sepsis is characterised by bacteraemia and clinical symptoms caused by microorganisms and their toxic products.[1] Sepsis and meningitis are responsible for most of these deaths. Resistance to commonly used antibiotics is emerging and constitutes an important problem worldwide.[2] A close attention to preventive strategies would be necessary to reduce morbidity and mortality from neonatal septicaemia.[3]

Bacterial infection is the biggest cause of neonatal admissions to hospitals, and probably the biggest cause of morbidity in the community. Key pathogens are Escherichia coli, Klebseila species, Staphylococcus aureus, and Streptococcus pyogenes. The incidence of neonatal infections with Group B streptococcus is highly variable, as is the spectrum of antimicrobial resistance.[4] Gram-negative bacteria, especially members of the Enterobacteracae are important causes of bacteraemia in Neonatal ICUs. Although most isolates remain sensitive to the new antibiotics, emergence of resistant strains cannot be excluded in the future. For that reason, new strategies and continuous surveillance are required to monitor the changing epidemiology of pathogens, antibiotic susceptibilities and antibiotic use needed to overcome the increasing incidence of resistance to conventional drugs.[5]

Despite advances in supportive care and use of antibiotics, sepsis preserves its importance due to its high mortality and morbidity for neonates. Identifying the causative agents and antibiotic resistance yearly in a neonatal intensive care unit (NICU) helps the physician to choose the most appropriate empirical therapy.[6]

Achievement of the Millennium Development Goal for child survival, requires a significant improvement in the management of infections in young infants, reported antibiotic susceptibility, and value of clinical signs in identifying severe bacterial illness. Common pathogens in young infant infections change over time and vary within and across settings. Improved country level data on pattern of microorganisms' resistance and antibiotic use are required to help reduce mortality through development of local, evidence-based clinical guidelines.[7] Limited information is available on aetiology of serious bacterial infections in community settings. Hospital-based studies suggest that most

infections in the first week of life are due to gram-negative pathogens, and many may be environmentally rather than maternally-acquired, owing to unhygienic delivery practices. These findings have implications for developing prevention and management strategies in communities and hospitals.[8] E. coli and Staph aureus are the most common organisms causing neonatal sepsis and there is high degree of resistance to commonly used first line antibiotics.[9]

MATERIAL AND METHODS

This descriptive study was conducted from 10th April 2009, to 10th January 2010 in Neonatology Unit, Department of Paediatrics, Ayub Teaching Hospital Abbottabad. A total of 130 neonates of either gender admitted in neonatal unit through paediatric OPD, labour room and emergency room who presented with sepsis or develop sepsis in hospital and their blood or CSF culture was found to be positive were included in the study.

Inform consent was obtained from the parents or attendants. Detailed history and examination were carried out in all patients presenting with sepsis. Patients who had already received antibiotics or having dysmorphic features or having chromosomal or congenital anomalies were excluded from the study.

Blood sample was taken under aseptic measures. A 3–5 ml blood sample was taken in 5 cc disposable sterile syringe. The blood samples were added into blood culture bottles. BACTEC technique was used for obtaining bacterial growth after incubation of 24–48 hours. The blood culture and sensitivity, and a complete record of causative organisms and their resistance to antibiotics were recorded. Ampicillin, amoxicillin, gentamicin, amikacin, tobramycin, cefotaxime, ceftazidime, ceftriaxone, imipenem, ofloxacin, ciprofloxacin, and vancomycin were tested for sensitivity/resistance. Relevant haematological, biochemical and radiological investigations were also performed. Second blood culture was also performed in a few cases which were not improving after initial treatment. The data were analysed using SPSS-10.

RESULTS

A total of 130 patients of neonatal sepsis were included in the study. These patients were positive for sepsis on blood culture. Male patients were 74 (56.9%), while female patients were 56 (43.1%). Age of the patients ranged from 2 to 28 days. Gram-negative bacteria were more frequent than gram-positive bacteria, i.e., gram-negative bacteria were found in 71 (54.6%) cases, while 59 (45.4%) cases were caused by gram-positive bacteria. Late onset sepsis (LOS) was found in 92 (70.85%) cases, while early onset sepsis (EOS) was found in 38 (29.15%) cases. Staph. aureus and Staph. epidermidis were sensitive to Vancomycin. Frequency of various causative bacteria and antibiotic resistance pattern is shown in Table 1–3.

Table-1: Frequency of various bacteria causing neonatal sepsis [n (%)]

Causative bacteria	Total	EOS	LOS
Staphylococcus aureus	35 (26.1)	9 (6.9)	26 (20)
E. coli	30 (23.1)	11 (8.4)	19 (14.6)
Staphylococcus epidermidis	17 (13.1)	4 (3.1)	13 (10)
Acinetobacter spp.	17 (13.1)	5 (5.8)	12 (9.3)
Klebseila	13 (10)	0	13 (10)
Streptococci spp.	7 (5.4)	7 (5.02)	0
Enterobacter cloacae	6 (4.6)	2 (1.56)	4 (3.12)
Moraxella	5 (3.8)	0	5 (5.8)

Table-2: Pattern of resistance to selected antibiotics in neonatal sepsis (%)

Antibiotics	Percentage
Ampicillin/cloxacillin	77.7
Amoxicillin	81.5
Gentamicin	55.1
Amikacin	17.4
Tobramycin	34.8
Cefotaxime	63.1
Ceftriaxone	66.9
Ceftazidime	56.9
Ciprofloxacin	40
Ofloxacin	38.5
Imipenem	42.3
Vancomycin	0

Table-3: Resistance of various causative bacteria to antibiotics (%)

	Penicillins		3rd generation cephalosporins			Aminoglycosides			Quinolones		
Causative Bacteria	Ampicillin	Amoxicillin	Cefotaxime	Ceftriaxone	Ceftazidime	Gentamicin	Amikacin	Tobramycin	Ciprofloxacin	Ofloxacin	Imipenem
Staph aureus	77.1	77.1	80	74.3	70	---	---	---	30.4	74.2	74.2
Acinetobacter spp.	98.1	98.1	82.36	98	70.5	66.6	46.6	46.6	70.5	52.9	52.9
Staph epidermidis	82.6	85	63.35	60	50.34	---	---	---	58.8	70.5	70.5
Klebseila	92.3	100	14.6	14.6	0	48.9	26.6	50.4	26.37	7.6	7.65
Streptococci	14.28	20.8	20	40	0	---	---	---	20.8	13.5	13.5
E. coli	73.33	76.6	30.76	46	46.16	60	3.3	30	40.33	20	20
Enterobacter cloacae	80.3	80.3	76.4	80.2	58.7	66.6	0	0	0	0	0
Moraxella	80	100	66.6	80.33	33.33	0	0	20	0	0	0

DISCUSSION

Infants are generally more susceptible to infections than adults.[10] This is due to a number of factors including an inadequately developed immune system making sepsis a risk to the newborn, especially under poor hygienic conditions. In pre antibiotic era, mortality from neonatal sepsis exceeded 90% but now with availability of antibiotics, the mortality rate has been reduced to between 10 and 50% as describe in Rubin *et al*[11], and Yalaz *et al*[6] Blood culture is the gold standard for the confirmation of sepsis. In advance centres, blood culture is positive in 80% of genuine sepsis.[12]

Shaw *et al*[13], Moreno *et al*[14], Lim *et al*[15] have described that late onset sepsis (LOS) is more common

as compare to early onset sepsis (EOS) which is consistent with our study in which we found that LOS occurred in 56.9% of cases compare to EOS which was present in 43.1% of cases.

Gram positive and gram negative bacteria were isolated in different cases. But gram negative bacteria (54.6%) were more common than gram positive bacteria (45.4%), which is consistent with other studies[16,17]. In a study, gram positive bacteria were the main contributors to neonatal sepsis[18], this may be due to geographical distribution of microorganisms.

Stphylococcus aureus was the most common pathogen found in our study. This is consistent with study of Shaw *et al*[13]. Staphylococcus aureus was found in both EOS and LOS, but mainly were found in LOS compare to EOS which is consistent with WHO reports[19].

The second most common bacteria were E. coli. This is consistent with other studies; in some studies E. coli was the most common bacteria isolated, while in most studies it is second most common bacteria causing neonatal sepsis.[2,20]

Staphylococcus epidermidis was found in 13.1% of cases in our study contributing to both early onset sepsis (EOS) in 3.1% of cases and late onset sepsis (LOS) in 10% of cases. Waseem *et al*[17] described Staph epidermidis in 10.51% contributing 7.8% cases to EOS, and 2.77% cases to LOS. These findings are nearly the same as our study, except that Staph epidermidis was more common in LOS in our study which may be due to higher number of cases of LOS in our study.

Acinetobacter was isolated in same percentage as that of Staphylococcus epidermidis, i.e., 13.1%. Waseem *et al*[17] have found Acinetobacter in 7.67% of cases, with EOS in 2.15% of cases and LOS in 5.55% of cases. In our study Acinetobacter was more commonly isolated in LOS (9.3%) as compare to EOS (5.8%), which is near to the findings of Waseem *et al.*[17]

Klebseila species were isolated in 10% of cases, all of which were found in late onset sepsis (LOS). Shaw *et al*[16] and Waseem *et al*[17] found Klebseila the second most commonly isolated bacteria, i.e., in 18.32% and 30% of cases respectively. This difference may be due to geographical situation and organism distribution.

Streptococcal species were found in 5.4% of cases, and all cases were isolated in EOS. Awoniyi *et al*[21] have described that streptococci species causes neonatal sepsis only in 5% of cases, and mainly found in EOS, which is highly consistent with our study. Shaw CK *et al*[71] have found streptococci species in 10.72% of cases, which mainly have isolated in EOS (7.69%). These similar findings are also described in a study at Multan Aftab *et al*[22].

The least common organisms isolated were Enterobacter cloacae (4.6%) and Moraxella species (3.8%). In other studies Enterobacter species have been isolated in less than 10% of cases.[13,21] In our study we have found Enterobacter species mostly in late onset sepsis (3.1% in LOS and 1.56% in EOS). Waseem *et al*[17] have found Enterobacter species in 5% of cases, mostly in LOS, which is consistent with our study.

Our study revealed that most of the commonly used antibiotics had high resistance of bacteria. Bacteria were highly resistant to ampicillin and amoxicillin. Overall resistance rates for ampicillin and amoxicillin were 77.7% and 81.5% respectively. Staph. aureus, Acinetobacter, Klebseila, and E. coli showed 77%, 98.1%, 92.3% and 73.3% resistance to these antibiotics respectively. Waseem *et al*[17] described high resistance of these bacteria against ampicillin. Awoniyi *et al*[21] also have found these bacteria highly resistant to ampicillin. Both ampicillin and amoxicillin have been found having comparatively less resistance to Streptococci, i.e., resistance in 14.28% and 20.8% of cases respectively. Waseem *et al*[17] found Streptococci 100% sensitive to ampicillin.

Both gram-positive and gram-negative bacteria have been found resistant against 3rd generation cephalosporins. Cefotaxime and ceftriaxone had high resistance and ceftazidime had relatively less resistance. Many other studies[13,17,21] have also described the emerging pattern of resistance against cefotaxime and ceftriaxone, and relatively low resistance to ceftazidime. Waseem *et al*[17] found that this may be due changing pattern of resistance due to indiscriminate use of antibiotics. They have found that Streptococci are 100% sensitive to cephalosporins, consistent with our findings in case of ceftazidime, while both cefotaxime and ceftriaxone had sensitivity in 83.4% of cases.

Aminoglycosides shows variable pattern of resistance against different bacteria. Waseem *et al*[17] described that gentamicin had resistance in 82.4%, 75%, and 80% of these bacteria respectively. E. coli, Acinetobacter, Klebseila, and Enterobacter were resistant to Amikacin. Resistance to amikacin has been reported in 38.34%, 20%, and 30% cases respectively.[17]

Quinolones are not frequently used for neonatal sepsis. Resistant is emerging against them because of indiscriminate use of antibiotics, but still resistance to quinolones is low compare to commonly used antibiotics. Our study showed that ciprofloxacin had resistance in 40% cases, and ofloxacin had resistance in 38.5% of cases. Staph epidermidis and acinetobacter were resistant to both ciprofloxacin and ofloxacin. Enterobacter were sensitive to both antibiotics. Shaw *et al*[13] have found Staph aureus, Streptococci, and enterobacter highly resistant against ciprofloxacin. This difference in resistance pattern may

be due to emerging resistant strains of bacteria due to indiscriminate use of antibiotics.

Imipenem is widely used nowadays and has high sensitivity against both gram-positive and gram-negative bacteria. Shaw *et al*[13] have found 100% sensitivity of imipenem against Staph aureus, Acinetobacter, Klebseila, E. coli, Streptococci, and Enterobacter species. The present study showed that imipenem sensitivity rate was 57.1%, of which sensitivity against individual bacteria discussed above was 25.8%, 47.1%, 92.35%, 80%, 86.5% and 100% respectively. Waseem *et al*[17] also have found imipenem 100% effective against the above mentioned bacteria. In our study high resistance rate may be due to resistant strains of bacteria due to improper use of antibiotics.

Vancomycin is having excellent coverage against staphylococci.[13,22] The present study also has found 100% sensitivity of vancomycin against Staph aureus and Staph epidermidis.

CONCLUSION

Staphylococcus aureus and E. coli are the most common organisms causing neonatal sepsis. Both gram-positive and gram-negative bacteria have developed resistance against commonly used antibiotics like ampicillin, amoxicillin, cefotaxime, ceftriaxone, and gentamicin. Less commonly used antibiotics like amikacin and ceftazidime are relatively more effective.

REFERENCES

1. Waheed M, Laeeq A, Maqbool S. The etiology of neonatal sepsis and patterns of antibiotic resistance. J Coll Physicians Surg Pak 2003;13:449–52.
2. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath PT. Neonatal sepsis: an international perspective. Arch Dis Child Fetal Neonatal Ed 2005;90(3):F220–4.
3. Adejuyigbe EA, Adeodu OO, Ako-Nai KA, Taiwo O, Owa JA. Septicaemia in high risk neonates at a teaching hospital in Ile-Ife, Nigeria. East Afr Med J 2001;78:540–3.
4. Osrin D, Vergnano S, Costello A. Serious bacterial infections in newborn infants in developing countries. Curr Opin Infect Dis 2004;17:217–24.
5. Ellabib MS, Ordonez A, Ramali A, Walli A, Benayad T, Shebrlo H. Changing pattern of neonatal bacteremia. Microbiology and antibiotic resistance. Saudi Med J 2004;25:1951–6.
6. Yalaz M, Cetin H, Akisu M, Aydemir S, Tunger A, Kültürsay N. Neonatal nosocomial sepsis in a level-III NICU: evaluation of the causative agents and antimicrobial susceptibilities. Turk J Pediatr 2006;48:13–8.
7. Newton O, English M. Young infant sepsis: aetiology, antibiotic susceptibility and clinical signs. Trans R Soc Trop Med Hyg 2007;101:959–66.
8. Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants in developing countries. Pediatr Infect Dis J 2009;28:10–8.
9. Aftab R, Iqbal I. Bacteriological agents of neonatal sepsis in NICU at Nishtar Hospital Multan. J Coll Physicians Surg Pak 2006;16:216–9.
10. Anah MU, Udo JJ, Ochigbo SO, Abia-Bassesy LN. Neonatal septicaemia in Calabar, Nigeria. Trop Doct 2008;38:126–8.
11. Rubin LG, Sanchez PJ, Siegel J, Levine G, Saiman L, Jarvis WR. Evaluation and treatment of neonates with suspected sepsis: a survey of neonatologists practices. J Pediatr 2002;110:e42.
12. Buttery JP. Blood cultures in newborn and children: optimizing an everyday test. Arch Dis Child Fetal Neonatal Ed 2002;87:F25–8.
13. Shaw CK, Shaw P, Thapalial A. Neonatal sepsis bacterial isolates antibiotics susceptibility patterns at a NICU in a tertiary care hospital in western Nepal: A retrospective analysis. Kathmandu Uni Med J 2007;5(2):153–60.
14. Moreno MT, Vargas S, Poveda R, Sáez-Llorens X. Neonatal sepsis and meningitis in a developing Latin American country. Pediatr Infect Dis J 1994;13:516–20.
15. Lim NL, Wong YH, Boo NY, Kasim MS, Chor CY. Bacteraemic infections in a neonatal intensive care unit: a nine months survey. Med J Malaysia 1995;50:59–63.
16. Bhutta ZA. Epidemiology of neonatal sepsis in Pakistan. An analysis of available evidence and implication for care. J Coll Physicians Surg Pak 1996;6(1):12.
17. Waseem R, Khan M, Izhar TS. Neonatal sepsis. Professional Med J 2005;12:451–6.
18. Karlowicz MG, Buescher ES, Surka AE. Fulminant late onset sepsis in a neonatal intensive care unit, 1988–1997 and impact of avoiding empirical vancomycin therapy. J Pediatr 2000;106:1387–90.
19. The WHO young infants study group. Bacterial etiology of serious infections in young infants in developing countries: Results of a multicenter study. Pediatr Infect Dis J 1999;18:S17–22.
20. Bindayna KM, Jamsheer A, Farid E, Botta GA. Neonatal Sepsis 1991–2001: Prevalent bacterial agents and antimicrobial susceptibilities in Bahrain. Med Prince Pract 2006;15:131–6.
21. Awoniyi DO, Udo SJ, Oguntibeju. An epidemiological survey of neonatal sepsis in a hospital in western Nigeria. Afr J Microbiol 2009;3:385–9.
22. Aftab R, Iqbal I. Bacteriological agents of neonatal sepsis in NICU at Nishtar Hospital, Multan. J Coll Physicians Surg Pak 2006;16:216–9.

Address for Correspondence:

Dr. Shahzad Najeeb, Department of Paediatrics, Ayub Medical College and Hospital Complex, Abbottabad, Pakistan.
Cell: +92-334-8981399