ORIGINAL ARTICLE

BACTERIOLOGICAL ANALYSIS AND RESISTANCE PATTERN AMONG VARIOUS CULTURE ISOLATES FROM NEONATAL SEPTICEMIA AT TERTIARY CARE HOSPITAL OF AHMEDABAD

Sanjay D Rathod[1], Palak V Bhatia[2], Parimal H Patel[3], Jayshri D Pethani[1], Lata R Patel[3], Bimal Chauhan[4]

[1]Associate Professor; [2]Resident; [3]Tutor; [4]Assistant Professor, Department of Microbiology, NHL Municipal Medical College, Ahmedabad

Correspondence: Dr.Sanjay D.Rathod, Email:dr_sanjay1976@yahoo.co.in

ABSTRACT

Introduction: Septicemia remains a significant cause of morbidity and mortality in the newborn. Shortly after the introduction of potent and broad-spectrum antibiotics, the emergence of resistant strains became a major problem in various Intensive care units. So, determination of bacterial etiology and antibiotic susceptibility patterns of isolates from septicemia in Neonatal intensive care units (NICU) is now crucial to abate neonatal mortality. This study was under taken to know the bacterial etiology of septicemia in neonate and antibiotic susceptibility pattern of the isolates.

Materials and Methods: During Jan-2011 to June -2011, 626 Blood samples were collected aseptically from newborns admitted with sepsis in NICU, Sheth Vadilal Sarabhai General Hospital, Ahmadabad and processed by standard conventional method. Antibiotic susceptibility pattern of isolates was studied by Kirby Bauer Disc diffusion technique.

Results: Total 626 samples were received during the study period of which 107 (17.09%) samples were found to be positive. Out of 107 isolated organisms 59 (55.14%) were *Klebsiella* sp., 11 (10.28%) *Staphylococcus aureus*, 10 (9.34%) *Escherichia coli*, *9 (8.41%) Coagulase negative staphylococci,* 7 (6.54%) *Pseudomonas aeruginosa*, 5 (4.67%) *Enterobacter sp.*5, 4(0.63%) *Enterococcus* sp., 1 (0.93%) *Acinetobacter baumanii* and *1* (0.93%) *Proteus-mirabilis*. Majority of organisms isolated were resistant to commonly used antibiotics. Imipenem showed 100% sensitivity for gram negative organisms. Methicillin resistance was found in 9.01% *Staphylococcus aureus.* Isolated all *Enterococcus sp.* were sensitive to vancomycin and high level gentamycin.

Conclusion: Multi-drug resistance organisms were isolated from septicemia in neonates. This study would guide the clinicians to formulate appropriate treatment strategy as well as to take various preventive measures which ultimately would help to decrease neonatal mortality.

Keywords: Septicemia, Antibiotic susceptibility, Drug resistence, neonates

INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the 1st month of life. It is an important cause of morbidity and mortality among neonates in India, with an estimated incidence of approximately 4% in intramural live births. An early and accurate etiological diagnosis is not always easy, especially since the disease may start with minimal or non-specific symptoms. Delayed treatment until clinical recognition of signs and symptoms of sepsis

entails risk of preventable mortality, notwithstanding the fact that presumptive antibiotic therapy may result in over-treatment. Of necessity, many more babies are evaluated and treated for sepsis than the number who actually have the condition. Etiological causes also do not remain the same.[1] The varying microbiological pattern of septicemia in children warrants the need for an ongoing review of the causative organisms and their antimicrobial susceptibility pattern. The incidence of bacteremia in children varies widely.[2] Group B streptococci is a common cause of neonatal sepsis in west but infrequent in India and other tropical countries. *Staphylococcal aureus, Klebsiella sp., E.coli* along with *Coagulase negative staphylococcus* and *Pseudomonas sp.* are the main organisms responsible for neonatal septicemia in India.[3] Uncontrolled use of various potent and broad-spectrum antibiotics has lead to emergence of resistant strains which has become a

major problem in various Intensive care units. Early diagnosis and to treat neonatal infections by empirical use of antimicrobial drugs as soon as possible is must to reduce the mortality. Various diagnostic tests (hematological, biochemical and radiological) can be performed easily and results may be available in an hour or so. However, blood culture remains the gold standard for the diagnosis of neonatal septicemia.[4] The uncertainty surrounding the clinical approach to treatment of neonatal septicemia can be minimized by periodic epidemiological surveys of etiological agents and their antibiotic susceptibility patterns leading to recognition of the most frequently encountered pathogens in a particular neonatal setting. The rational and correct use of antibiotics requires understanding of common pathogens and their

drug sensitivity pattern in the regions. Due to constantly evolving antimicrobial resistant patterns there is the need for constant antimicrobial sensitivity surveillance. This will help clinicians provide safe and effective empirical therapies, develop rational prescription programs and make policy decisions and finally assess the effectiveness of all.[2] As antibiotic sensitivity pattern to common pathogen has been changing day by day, so it has been necessary to study about bacteriological analysis and antibiotic sensitivity pattern. Determination of antibiotic sensitivity patterns in periodic intervals is mandatory in each region for choosing appropriate antibiotic therapy. The present study was undertaken to study the bacteriological profile of neonatal septicemia cases and their antibiotic susceptibility pattern for planning strategy for the management of these cases.

MATERIALS AND METHOD

Total 626 blood culture samples from suspected patients of neonatal septicemia were obtained from NICU of Sheth Vadilal Sarabhai General Hospital, Ahmedabad. The samples were collected with proper aseptic precautions. The samples were inoculated in the sterile pediatric blood culture bottle which contain glucose broth with 0.5% sodium polyenatholsulfonate.[5] Bottles were incubated at 37ºC for 7 days. Serial subcultures were made from the bottles at regular intervals on Nutrient agar, Mac Conkey agar and Blood agar[4]. The isolates were identified by standard methods, including colony morphology, Gram stain, bacteriologic and biochemical methods.[6]After the isolation of bacteria, antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion method on Muller Hinton (MH) agar as per CLSI recommendations.[7]

RESULTS

Of total 626 samples received, 107(17.09%) samples showed bacterial growth 22.42% (24/107) isolates were Gram positive and 77.57%(83/107) isolates were Gram negative.

Klebsiellae sp. was the most common organism accounting for 55.14% (59) followed by *Staphylococcus aureus* 10.28% (11), *E.coli* 9.34% (10), *Coagulase negative staphylococcus* 8.41% (9), *Pseudomonas- aeruginosa* 6.54%(7) *Enterobacter sp.* 4.67% (5), *Enterococcus sp.*3.73% (4), *A.baumanii* 0.93% (1) and *Proteus mirabilis* 0.93% (1). Frequency of isolates from neonate with clinically suspected septicemia shown in Table 1.

Table 1: Distribution of bacterial isolates from neonate with clinically suspected septicemia

Bacteria	Isolates (n=626)
Klebsiella sp.	59(9.24%)
S. aureus	11(1.75%)
E.coli	10(1.59%)
Coagulase negative staphylococi	9(1.43%)
P. aeruginosa	7(1.11%)
Enterobacter sp.	5(0.79%)
Enterococcus sp.	4(0.63%)
Acinetobacter baumanii	1(0.15%)
Proteus mirabilis	1(0.15%)

Table 2 and 3 show the antibiotic susceptibility patterns of organisms isolated.

Table 2: Resistance patterns of Gram-negative isolates

Antibiotics	Resistant isolates (%)					
	Klebsiella sp.	E.coli	P.aeruginosa	Enterobatcer sp.	Proteus mirabilis	A.baumanii
Ampi/sulbactam	40 (67.7)	7 (70.0)	-	3 (60.0)	00	00
Cefotaxime	56 (94.9)	9 (90.0)	-	4 (80.0)	00	1 (100)
Ceftazidime	56 (94.9)	9 (90.0)	2 (28.6)	4 (80.0)	00	1 (100))
Imipenem	00	00	00	00	00	00
Amikacin	40 (67.7)	6 (60.0)	00	2 (40.0)	00	1 (100)
Co-trimoxazole	46 (77.9)	1 (10.0)	-	4 (80.0)	1 (100)	1 (100)
Doxycycline	44 (76.2)	8 (80.0)	-	3 (60.0)	00	1 (100)
Pip.Tazobactam	-	-	1 (14.3)	-	-	-
Aztreonam	-	-	1 (14.3)	-	-	-

- = Antibiotic not tested; Doxycycline was tested in 58 stains of *klebsiella sp.*.

Most of the Gram-negative organisms were resistant to commonly used antibiotics. Isolated enterobacteriaceae

showed maximum resistant for 3rd generation cephalosporins followed by cotrimoxazole, doxycycline and aminoglycosides. Isolated *A.baumanii* was only sensitive to imipenem and Ampicillin salbactam. Imipenem showed 100% sensitivity for Gram negative organisms.

Combinations of antibiotics ampicillin/sulbactum were sensitive in about 34% of cases.

Table 3: Antibiotic sensitivity patterns of Gram positive isolates

Antibiotics	Resistant isolates (%)		
	S.aureus	Coagulase negative *staphylococi*	*Enterococcus sp.*
Amoxicillin	11(100)	6 (66.7)	4 (100)
Erythromycin	11(100)	6 (66.7)	-
Cefprozil	4 (37.5)	2 (22.2)	4 (100)
Cefoxitin	1 (9.1)	3 (33.3)	-
Amikacin	1 (9.1)	00	-
Vancomycin	00	00	00
Gentamycin(High level)	-	-	00
Cotrimoxazole	7 (63.6)	3 (33.3)	4 (100)
Doxycycline	6 (54.5)	3 (33.3)	3 (75.0)

- = Antibiotic not tested.

Isolated *P.aeruginosa* showed less resistance than other isolated Gram negative bacilli. Of them 28.6% were resistant to ceftazidime and 14.3% to aztreonam and 100% sensitive to imipenem and amikacin.

Gram positive organism showed maximum resistance to penicillin, erythromycin, aminoglycoside as well as tested cephalosporin. Methicillin resistance was found in 9.01% *Staphylococcus aureus*. Of the amino glycosides studied, amikacin showed good sensitivity for *S.aureus*.

Isolated all *Enterococci* were sensitive to high level gentamycin and vancomycin while only one strain was sensitive to doxycycline.

DISCUSSION

For the effective management of neonatal septicemia cases, study of the bacteriological profile with their antibiotic pattern plays a significant role. In this study, blood culture positivity rate in neonatal septicemia cases was 17.09%, similar results found by Kenneth C Iregbu *et al.*[8] Among the samples received from NICU, *Klebsiellae sp.* was the most common isolate (59.14%) followed by, *S.aureus*(10.28%), *E.coli*(9.34%), *Coagulase negative staphylococci*(1.43%) *Pseudomonas aeruginosa* (6.54%), *Enterobacter sp.*(4.67%), *Enterococcus sp.*(0.63%), *A.baumanii* (0.93%)and *proteus mirabilis*(0.93%). In the present study Gram-negative organisms constituted the major group of isolates from neonatal septicemia cases, among this group *Kleibsella sp.* and in Gram positive organisms *S.aureus* has been found to be the prominent pathogen, which correlates with the findings of Dr.Kairavi *et al.*[9] In our study *Enterococci* were isolated while in other study *Enterococci* were not isolated.[9, 10] The results of antibiotic sensitivity pattern revealed that majority of Gram-negative organisms were resistant to commonly used antibiotic. Though not tested by a standard method, sensitivity pattern of Gram negative organisms (i.e resistance to 3rd generation cephalosporins in more than 50% cases) was suggestive ESBL production which would pose a major problem for treatment of neonates. Imipenem showed 100% sensitivity for Gram negative organisms which was similar to study by Kenneth C Iregbu *et al.*[8] Gram positive organisms were highly sensitive to Vancomycin similar to study done by A.K.Mane *at el.*[10] Vancomycin resistant *Enterococci* and methicillin resistant in *Staphylococci* was not a common finding. We did not distinguish between community- and hospital-acquired infections for analyzing the results. Being a retrospective study of microbiological records, correlation with neonatal morbidity and mortality and other markers of sepsis was also not possible. Inclusion of these data would have definitely enhanced the utility of this study. Clinical recognition of neonatal sepsis is not always straight-forward. Appropriate intervention requires an early etiological diagnosis. Microbial etiology of neonatal septicaemia is diverse. Several studies on neonatal sepsis have documented the diversity of bacteria and their temporal variability. The present study reiterates the earlier findings and emphasizes the importance of periodic surveys of microbial flora encountered in particular neonatal settings to recognize the trend.

CONCLUSION

This study brings us to conclusion that, being a microbiologist, our duty is not only to identify the organism and report about its sensitivity but, also to guide the clinicians about the proper preventive measures to be taken by them. Neonatal septicemia is a life threatening emergency, and rapid treatment with antibiotics is essential for a favorable outcome. Classical empirical treatment of neonatal sepsis consists of amoxicillin & an aminoglycoside. In present study, *S.aureus* & Gram-negative isolates were frequently found to be resistant to amoxicillin & an aminoglycoside also, thus indicating that the use of these drugs might be ineffective. Therefore great caution is required in selection of antibiotic therapy. This highlights the variable nature of antibiotic susceptibility patterns both in time and location therefore; it is advisable to continuously evaluate the sensitivity-resistance pattern of isolates so as to make a rational use of antibiotics. These data support the hypothesis that determination of antibiotic sensitivity patterns in periodic intervals is mandatory in each region for choosing appropriate antibiotic therapy.

In the view of above, the strategy of antibiotic usage in the hospital must be reviewed. The determination of various enzymes production by various bacterial isolates like beta-lactamase, ESBL, Amp-c beta

lactamase, etc. in laboratory would prove a great help for the clinicians. Preventive measures necessary for reduction of neonatal mortality are hand washing compliance, proper nursing care, use of invasive procedures to the minimum, proper cleaning of NICU, never handle a baby without sterile gloves, proper NICU design and prudent use of antibiotics.

REFERENCES

1. Ghanshyam D. Kumhar, V.G. Ramachandran, and Piyush Gupta.Bacteriological Analysis of Blood Culture Isolates from Neonates in a Tertiary Care Hospital in India J Health Popul Nutr Dec 2002.; 343-347.
2. Karki S1, Rai GK2, Manandhar R3. Bacteriological Analysis and Antibiotic Sensitivity Pattern of Blood Culture Isolates in Kanti Children Hospital. J. Nepal Paediatr. Soc. 2010/Vol 30 ;94-97
3. Rao PS, Baliga M, Shivananda PG. Bacteriology of neonatal septicaemia in a rural referral hospital in south India. J Trop Pediatr. 1993; 39: 230-233
4. Sharma A, Kutty CV, Sabharwal U, Rathee S, Mohan H. Evaluation of sepsis screen for diagnosis of neonatal septicemia. Indian J Pediatr. 1993; 60: 559-563
5. Washington Winn Jr. Stephen Allen, William Janda, Elmer Koneman, Gary Procop, Paul Schreckenberger, Gail Woods. Koneman's Color Atlas and Textbook of diagnostic Microbiology. Lippincott Williams and Wilkins.6th edition page no.102.
6. Finegold SM, Markin WJ, Scott EJ. Bailey & Scott's Diagnostic Microbiology 5th edition. St. Louis: The CV Mosby Company.1978
7. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement. CLSI document M100-S18. Clinical and Laboratory Standards Institute 2010.
8. Kenneth C Iregbu, Olumilayo Y Elegba and Iretiola B Babaniyi.Bacteriological profile of neonatal septicaemia in a tertiary hospital in Nigeria. Afr Health Sci 2006;6(3): 151-154.
9. Kairavi J. Desai, Saklainhaider. S. Malek. Neonatal Septicemia: Bacterial Isolates & Their Antibiotics Susceptibility Patterns. NJIRM 2010; Vol. 1(3).12-15
10. A.K.Mane, N.V. Nagdeo, V.R. Thombare. Study of neonatal septicemia in a tertiary care hospital in rural Nagpur. Journal of recent advances in applied sciences 2010;25:19-24.