ORIGINAL RESEARCH PAPER
Volume-9 | Issue-5 | May-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN IN NEONATAL SEPTICEMIA IN A TERTIARY CARE HOSPITAL, IN SOUTH INDIA

Microbiology

Dr.N.Praveen Deen Kumar Assistant Professor, Department of Paediatrics, Government Medical College, Ananthapuramu, Andhra Pradesh, India.

Dr.B.Praveena* Tutor, Department of Microbiology, Government Medical College, Ananthapuramu, Andhra Pradesh, India. *Corresponding Author

Dr.G.Swarna Latha Professor & HOD, Department of Microbiology, Government Medical College, Ananthapuramu, Andhra Pradesh, India.

ABSTRACT

Introduction: Neonatal septicemia is one of the most important causes of mortality and morbidity especially in developing countries. The aim of this study was to know the bacteria responsible for blood stream neonatal infections in this region and its antibiotic sensitivity pattern.
Materials and Methods: At Microbiology department blood samples of neonatal septicemia suspected cases were processed according to standard guidelines.
Results: Out of 219 cases, majority of neonatal septicemia were caused by Gram negative organisms 144(66%) compared to Gram positive organisms 66(30%) and Candida in 9(4%) cases. Pseudomonas species is the predominant pathogen, it was 27.3% (60/219) followed by 26.02% (57) of Klebsiella species, 15.9% (35) were Staphylococcus aureus.
Conclusion: Antibiotic sensitivity pattern should be studied in NICU s from time to time so as to prepare the protocols for the preferred line of antibiotics which helps in reducing the neonatal morbidity and mortality.

KEYWORDS

Neonatal Septicemia, Bacteria, Sensitivity.

INTRODUCTION

Neonatal septicemia is one of the most common causes of mortality and morbidity especially in middle and lower income countries including India [1]. Worldwide, approximately 2.5-3 million cases of neonatal morbidity and mortality were recorded annually [2].

Neonatal septicemia is a clinical condition characterized by systemic signs and symptoms due to bacteremia in the first month of life. The spectrum of causative organisms for neonatal septicemia and their sensitivity pattern varies from one region to another region.

Based on the time of presentation after birth, neonatal sepsis is categorized as early onset sepsis (EOS) that occurs at or before 72 hours of newborn life and late onset sepsis (LOS) which occurs after 72 hours of newborn life [3]. EOS usually occurs due to the transmission of pathogens from the female genitourinary system to the newborn or fetus [4]. LOS is generally caused by the transmission of pathogens from the environment after delivery [5].

The bacterial agents associated with neonatal sepsis are Group B streptococci, Escherichia coli, Listeria monocytogenes, Coagulase Negative Staphylococci, Staphylococcus aureus, Enterococci, Klebisella species, Enterobacter species, Pseudomonas species, Salmonella species, Haemophilus influenzae, Neisseria meningitides and Streptococcus pneumoniae [4,6,7].

Infections and antimicrobial resistance are the major problems globally, especially in India. The prevelance of multidrug resistance (MDR) is increasing day by day. MDR is defined as Gram negative isolates resistant to any three of the five antibiotic classes (extended-spectrum cephalosporins, carbapenems, aminoglycosides, flouroquinolones and piperacillin-tazobactum). In Gram positive isolates the resistance is expressed in terms of MRSA (Methicillin Resistant Staphylococcus aureus) indicated by resistance to cefoxitin. The aim of this study was to know the bacteria responsible for neonatal infections in this region and its antibiotic sensitivity pattern.

2. MATERIALS AND METHODS

An observational prospective study was conducted in the Department of Pediatrics, Government General Hospital, Anantapuramu, Andhra Pradesh from January 2019 to August 2019.

2.1. Study Population.

Inclusion criteria:

All the babies admitted in NICU (< 28 days) with confirmed sepsis by blood culture positivity during the period from January 2019 to August 2019.

Exclusion criteria:

Neonates with multiple congenital malformations.

Neonates with complex congenital heart diseases.

Among total of 325 samples of suspected sepsis cases sent for blood culture, 219 samples were shown positive blood culture. So the blood culture positive 219 neonatal sepsis cases were considered as the study population.

Clinically neonatal sepsis was diagnosed by symptoms, signs, complete blood picture, CRP, micro ESR. These clinically suspected cases were advised to undergo blood culture and sensitivity.

2.2. Sampling Procedure and Processing.

The trained laboratory technician wore sterile gloves during the procedure and prepared a patch of skin approximately 5 cm in diameter over the proposed site of veni-puncture. This area was cleaned thoroughly with70% isopropyl alcohol. The skin was allowed to dry for at least 1 minute before veni-puncture. About 1-2 ml sample of blood was drawn from a fresh veni-puncture site and added to a bottle containing brain heart infusion broth supplemented with 0.054% SPS (MicroXpress, Goa) under aseptic precautions which gives broth containing blood in a ratio of 1:5. These blood culture bottles were sent to the department of Microbiology immediately after collection.

At Microbiology department samples were processed by incubating bottles at 37˚C for 7 days. Daily bottles were checked for growth and turbidity, hemolysis of red cells, gas bubbles and clot formation of discrete colonies. Presumptive diagnosis by doing gram stain of these positive broth bottles was informed immediately to the clinicians. Sub cultures from BHI broth were performed after 24 hours, 3rd day and 7th day on Nutrient agar, Mac Conkey agar, Blood agar, Chocolate agar of HiMedia laboratories. Blood culture bottles showing no growth on subculture after 7 days of incubation were reported as negative. The colonies were observed and processed for identification of pathogen and antibiotic susceptibility testing by following standard guidelines. In babies in whom CONS was isolated, a repeat blood culture was performed to confirm the infection, since CONS is part of skin microbial flora.

2.3. Statistical Analysis.

After receiving report of blood cultures, all the data along with the patient details were entered into structured predesigned proforma. Later, the results were transferred into spread excel sheet to evaluate and tabulate the results.

3. RESULTS

3.1. Prevalence of Neonatal Septicemia.

A total 219 blood culture positive cases of neonatal sepsis were obtained from 325 suspected neonatal septicemia cases. So the culture positivity was 67%.

3.2. Prevalence of Neonatal Sepsis among Different Variables.

Out of 219 confirmed neonatal sepsis, 122(56%) were males and 97(44%) were females with predominant male to female ratio. Of these 219 neonates, 138(63%) were normal weight, 57(26%) were low birth weight and 24(11%) were very low birth weight. Of these, 142(65%) were of term gestation and 77(35%) were of preterm gestation with predominant normal weight and term gestation babies. Out of 219 neonates, out born were predominant and were 131(60%) and inborn babies were 88(40%) (Table 1).

Table 1. Prevalence of neonatal sepsis among different variables

Variable		Percentage
Sex	Male	122 (56%)
	Female	97 (44%)
Birth weight	>2500g	138 (63%)
	1500-2499g	57 (26%)
	<1500g	24 (11%)
Gestation	Term	142 (65%)
	Preterm	77 (35%)
Inborn/Out born	Out born	131 (60%)
	In born	88 (40%)

3.3. Distribution of Isolates from Blood Culture.

Out of 219 cases, majority of neonatal septicemia were caused by Gram negative organisms 144(66%) compared to Gram positive organisms 66(30%) and Candida in 9(4%) cases (Fig 1).

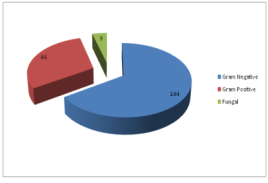

Fig 1. Distribution of Organisms from Blood Culture.

3.4. Causative Gram Negative Organisms in EOS and LOS

Of the 144 organisms, Pseudomonas species were isolated in 60 cases, which is followed by 57 of Klebsiella species, 20 of Escherichia coli, 2 of Citrobacter species, 4 of Acinetobacter species and 1 of other Gram negative bacilli (Table 2). Pseudomonas species and klebsiella species were the predominant gram negative organisms isolated both in EOS and LOS.

Table2. Causative Gram negative Organisms in EOS and LOS.

Organisms	No.ofCases	EOS	LOS
Pseudomonasspecies	60	35	25
Klebsiellaspecies	57	33	24
Escherichiacoli	20	11	9
Citrobacterspecies	2	2	0
Acinetobacterspecies	4	3	1
Non-Fermenting Gramnegativebacilli	1	1	0
Total no.of organisms	144	85	59

3.5. Causative Gram Positive organisms in EOS and LOS

Out of 66 Gram positive organisms, 35 were Staphylococcus aureus, 16 were Enterococcus species, 14 of Coagulase Negative Staphylococci (CONS) and 1 of Streptococcus agalactiae (Table 3). Staphylococcus aureus was the predominant pathogen isolated in both EOS and LOS cases.

Table 3: Distribution of gram positive organisms among neonatal sepsis.

Organism	No. of cases	EOS	LOS
S.aureus	35	24	12
Enterococcus sp	16	4	11
CONS	14	8	6
S.agalactiae	1	1	0
Total	66	37	29

3.6. Antibiotic profile of Gram negative isolates

Among 144 Gram negative isolates, 75.6% were sensitive to Meropenem, 70.1% to Tobramycin, 64.5% to Piperacillin+ tazobactum, 61.8% to Amikacin, 61.8% to Ciprofloxacin, 61.1% to Colistin, 60.4% to Cefaperazone+sulbactum, 56.9% to Ofloxacin, 55.5% to Netilimicin. The resistant rates to Ampicillin, Amoxiclav, Cefotaxime and Gentamycin is 97.9%, 95.8%,92.3% and 54.8% respectively .

Table 4. Anti biotic sensitivity pattern of Gram negative or ganisms

Antibiotics	Sensitive n (%)	Intermediate sensitive n(%)	Resistant n (%)
Meropenem	109 (75.6%)	4 (2.7%)	31(21.5%)
Tobramycin	101 (70.1%)	2 (1.3%)	41(28.4%)
Piperacillin+Tazobactum	93 (64.5%)	2 (1.3%)	49(34.02%)
Amikacin	89 (61.8%)	2 (1.3%)	53(36.8%)
Ciprofloxacin	89 (61.8%)	2 (1.3%)	53(36.8%)
Colistin	88 (61.1%)	1 (0.69%)	55(38.1%)
Cephalosporin+ sulbactum	87 (60.4%)	3 (2.08%)	54(37.5%)
Ofloxacin	82 (56.9%)	0	62(43.05%)
Netilmycin	80 (55.5%)	2 (1.3%)	62(43.05%)
Gentamycin	64 (44.4%)	1 (0.69%)	79 (54.8%)
Cefotaxime	09 (6.25%)	2 (1.3%)	133(92.3%)
Amoxiclav	04 (2.7%)	2 (1.3%)	138(95.8%)
Ampicillin	02 (1.38%)	1 (0.69%)	141(97.9%)

3.7. Antibiotic profile of Gram positive isolates

Among the 66 gram positive isolates, 100% were sensitive to linezolid, 90.9% to vancomycin, 78.7% to Amikacin, 72.2% to Ofloxacin and Meropenem, 68.1% to Ciprofloxacin and Piperacillin+tazobactum.

Table 5. Antibiotic sensitivity pattern of Gram positive organisms

Antibiotics	Sensitive n (%)	Intermediate sensitive n	Resistant n (%)
Linezolid	66 (100%)	0	0
Vancomycin	60 (90.9%)	2	4 (6.0%)
Amikacin	52 (78.7%)	4	10 (15.1%)
Ofloxacin	48 (72.7%)	2	16 (11.1%)
Meropenem	48 (72.7%)	6	12 (18.1%)
Ciprofloxacin	45 (68.1%)	4	17 (25.7%)
Piperacillin+Tazobactum	45 (68.1%)	5	16 (11.1%)
Cephalosporin+sul bactum	42 (63.6%)	6	18 (27.2%)
Amoxiclav	42 (63.6%)	6	18 (27.2%)
Cefoxitin	36 (54.5%)	6	24 (36.3%)
Ampicillin	17 (25.7%)	7	42 (63.6%)

3.8. MDR (MultiDrug Resistant) pattern

Incidence of MDR (Multidrug Resistant) organisms among Gram negative bacilli was 18.05% (26/144). 23% of Pseudomonas species, 20% of Escherichia coli, 12% of Klebsiella species and 50% of Citrobacter species were multi drug resistant. (Table 6).

Table 6. Distribution of MDR pathogens among gram negative organisms.

Organism(N)	FrequencyofMDRorganismsn(%)
Pseudomonassp.(60)	14(23%)
Escherichiacoli(20)	4(20%)
Klebsiellasp.(57)	7(12%)
Citrobactersp.(2)	1(50%)
TOTAL	26

DISCUSSION

Positivity of blood cultures varies from region to region; this may be due to a different epidemiology of bacteriological profile responsible for neonatal sepsis. The blood culture positivity rate in the present study was 67% (219/325). Lower incidence rate was reported by Pokhrel B et al in Nepal (20.5%) [8]. Higher incidence rate was reported by Jain NK et al (56%) [9] and 64% by Tallur et al [10].

As per this study, 66% (144/219) babies had Gram negative sepsis, 30% (66/219) babies had Gram positive sepsis and Fungal sepsis was diagnosed in nine 4% (9/219) neonates. Gram negative bacteria were the most commonly isolated organisms causing neonatal sepsis,

followed by Gram positive bacteria and Fungi respectively in our study. Jyothi P et al [11] Agnihotri et al [12] and in other studies [8,13] mentioned Gram negative pathogen predominance. In contrast to our study, Sharma P et al [14] from India, few other studies [15,16] documented a preponderance of Gram-positive organisms.

In this study, Pseudomonas species is the predominant pathogen, it was 27.3% (60/219) followed by 26.02% (57) of Klebsiella species, 15.9% (35) were Staphylococcus aureus, 9.13% (20) of Escherichia coli, 7.3% (16) were Enterococci species, 6.3% (14) of Coagulase Negative Staphylococci (CONS). We isolated Pseudomonas species, Klebsiella species, followed by staphylococcus aureus as the predominant isolates both in EOS and LOS. Similar to our study Bhat Y et al [17], Joshi et al [18] and Tallur et al [10] reported Pseudomonas as the predominant organism followed by Klebsiella species. Nikita Singh Yadav et al [19] reported Staphylococcus aureus as a predominant pathogen followed by Klebsiella . Jyothi P et al [11], Pokhrel B et al [8] reported Klebsiella as a predominant pathogen among Gram negative septicaemia and Coagulase Negative Staphylococci as a predominant pathogen among Gram positive septicaemia.

Out of 219 bacterial culture positive organisms, early onset septicemia (58.09%) was more common than late onset septicemia (41.9%). Jyothi P et al [11], Pokhrel B et al [8] also reported blood culture confirmed neonatal sepsis higher in EOS than LOS. The higher incidence of EOS in our study might be due to horizontal transmission of pathogens from delivery rooms and vertical transmission from maternal genital tract due to non adherence to infection control practices as majority of neonates were out-born.

In our study most of the gram negative isolates showed highest susceptibility to meropenem, tobramycin, pipericillin-tazobactum, ciprofloxacin, amikacin, colistin, cephalosporin-sulbactum, ofloxacin and netilmycin. Gram positive isolates showed 100% sensitivity to linezolid. Most of the gram positive isolates were sensitive to vancomycin, amikacin, ofloxacin, meropenem, pipericillin-tazobactum. Both gram negative organisms and gram positive organisms showed common sensitivity to amikacin and carbapenems in our study.

Jyothi P et al [11] noted among gram negative isolates 93% were sensitive to imipenem, followed by amikacin and netilmycin. Among gram positive isolates 91% were sensitive to linezolid, followed by tetracycline, piperacillin+tazobactum. The main reason for variation in antibiotic sensitivity pattern is due to differences in the common antibiotics used in different hospitals and emergence of antibiotic resistance due to indiscriminate use of antibiotics.

The resistance rates against ampicillin(99%), cefotaxime (92%), the most commonly used empiric antibiotics at our NICU were extremely high followed by high resistance to amoxyclav and gentamicin.

Management of Neonatal sepsis is a difficult and challenging task to clinicians due to emergence of antibiotic resistance of pathogens. In this study, incidence of MDR (Multidrug Resistant) organisms among gram negative bacilli was 18.05% and MRSA isolates were 36.3%.Among gram negative isolates high rates of multi drug resistance was observed in pseudomonas (23%), Escherichia coli (20%), Klebsiella species (12%). Thakur S et al [20] noted 48% of Multidrug resistant bacilli and 41% MRSA isolates among neonatal sepsis.

The survival rate of culture positive neonatal sepsis in the present study was 83.1%. Twenty one babies were referred and mortality rate was 7.3% (16). In blood culture positive neonatal deaths, the most common organism isolated is Klebsiella species, followed by Pseudomonas species. Imad R Makhoul et al [21] observed a sepsis events caused by Pseudomonas, Klebsiella and Serratia species were associated with highest risk of early mortality. Increase in antibiotic resistance is due to variety of reasons such as over-the-counter availability of antibiotics and misuse of antibiotics.

CONCLUSION

Gram negative septicemia noted in higher percentage in neonatal sepsis cases. Pseudomonas species, Klebsiella species, Staphylococcus aureus were the predominant pathogens isolated and were predominant in both EOS and LOS groups. Gram negative pathogens have shown higher sensitivity towards meropenem, tobramycin, piperacillin-tazobactum and amikacin. Gram positive pathogens have shown higher sensitivity towards linezolid, vancomycin amikacin, meropenem. Incidence of MDR organisms and MRSA is on rise which is of major concern. Resistance to commonly used first line antibiotics like ampicillin, cefotaxime and gentamycin is noted high. Antimicrobial stewardship programme in all hospitals should be followed to limit the emergence of antimicrobial resistant pathogens. Antibiotic sensitivity pattern should be studied in NICU s from time to time so as to prepare the protocols for the preferred line of antibiotics which helps in reducing the neonatal morbidity and mortality.

REFERENCES

1. Seale AC, Blencowe H, Manu AA, Nair H, Bahl R, Qazi SA et al. Estimates of possible severe bacterial infection in neonates in Sub Sahara Africa, South Asia and Latin America for 2012 : a systematic review and meta-analysis. Lancet Infect Dis. 2014 Aug; 14(8); 731-741.
2. Darmstadt GL, Zaidi AKM, Stoll BJ. Infectious diseases of the fetus and newborn Infant. Philadelphia: Elsevier; 2011. Neonatal infections: a global perspective; pp. 24–51.
3. Wynn JL. Defining neonatal sepsis. Curr. Opin. Pediatr. 2016 Apr; 28(2): 135-40.
4. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatr Clin N Am. 2013;60:367–389.
4. Raymond SL, Stortz JA, Mira JC, Larson SD, Wynn JL, Moldawer LL. Immunological Defects in Neonatal Sepsis and Potential Therapeutic Approaches. Front Pediatr. 2017;5:14.
5. Arora U, Devi P. Bacterial profile of blood stream infections and antibiotic resistant pattern of isolates. JK Sci. 2007;9:186–190.
6. Zakariya BP, Bhat V, Harish BN, Arun Babu T, Joseph NM. Neonatal sepsis in a tertiary care hospital in South India: bacteriological profile and antibiotic sensitivity pattern. Indian J Pediatr. 2011;78:413–417.
8. Pokhrel B, Koirala T, Shah G, Joshi S, Baral P. Bacteriological profile and antibiotic susceptibility of neonatal sepsis in neonatal intensive care unit of a tertiary hospital in Nepal. BMC Pediatr. 2018 Jun 27; 18(1):208.
9. Jain NK, Jain VM, Maheshwari S. Clinical profile of neonatal sepsis. Kathmandu Univ Med J. 2003;1:117–20.
10. Tallur SS, Kasturi AV, Nadgir SD, Krishna BVS: Clinico-bacteriological study of neonatal septicemia in Hubli. Indian J Pediatr. 2000, 67: 169-174. 10.1007/BF02723654.
11. Jyothi P, Metri C, Basavaraj, Peerapur V. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. J Nat Sci Biol Med. 2013 Jul-Dec;4(2):306-309.
12. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. Jpn J Infect Dis. 2004;57:273–5.
13. Kaistha N, Mehta M, Singla N, Garg R, Chander J. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. J Infect Dev Ctries. 2009;4:55–7.
14. Sharma P, Kaur P, Aggarwal A. Staphylococcus aureus- the predominant pathogen in the neonatal ICU of a tertiary care hospital in Amritsar, India. J Clin Diagn Res. 2013;7(1): 66–69.
15. Kausik Kumar Sarangi, Dipti Patnaik, Surya Narayan Mishra, Manas Kumar Nayak, Jagadananda Jena. Bacteriological profile and antibiogram of blood culture isolates done by automated culture and sensitivity method in a neonatal intensive care unit in a tertiary care hospital in Odisha, India. Int J Adv Med. 2015 Nov;2(4):387-392.
16. Ozkan H, Cetinkaya M, Koskal N, Celebi S, Hacimustafaoglu M. Culture proven neonatal sepsis in preterm infants in a neonatal intensive care unit over a 7 year period, CoNS as a predominant pathogen. Paediatr Int. 2014;56:60-6.
17. Ramesh Bhat Y, Leslie Edward S Lewis and Vandana KE. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. Italian Journal of Pediatrics 2011;37:32
18. Joshi SG, Ghole VS, Niphadkar KB: Neonatal gram-negative bacteremia. Indian J Pediatr 2000, 67:27-32.
19. Nikita Singh Yadav, Saroj Sharma, Dhiraj Kumar Chaudhary, Prabat Panthi, Pankaj Pokhrel, Anil Shrestha, Pappu Kumar Mandal. Bacteriological profile of neonatal sepsis and antibiotic susceptibility pattern of isolates admitted at Kanti Children's Hospital, Kathmandu, Nepal. BMC Res Notes. 2018;11:301.
20. Thakur S, Thakur K, Sood A, Chaudhary S. Bacteriological profile and antibiotic sensitivity pattern of neonatal septicaemia in a rural tertiary care hospital in North India. 2016;34(1):67-71.
21. Imad R Makhoul, Polo Sujov, Tatiana Smolkin, Ayala Lusky and Brian Reichman. Pathogen specific early mortality in very low birth weight infants with late onset sepsis: A National Survey. Clinical Infectious Diseases. 2005;40:218-24.