THE STUDY OF BACTERIOLOGICAL PROFILE AND ANTIBIogram OF NEONATAL SEPTICEMIA

Varun Dwivedi¹, R. Murthy²

HOW TO CITE THIS ARTICLE:
Varun Dwivedi, R. Murthy. “The Study of Bacteriological Profile and Antibiogram of Neonatal Septicemia”. Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 75, September 17; Page: 13057-13062, DOI: 10.14260/jemds/2015/1880

ABSTRACT: Septicemia accounts for a significant proportion of morbidity and mortality in the newborn and is therefore a major problem in pediatric practice worldwide. The objective of this study was to know the bacteriological profile and antibiogram of neonatal septicemia of NICU (Neonatal Intensive Care Unit) of CIMS. Under aseptic precautions, blood was drawn from 500 neonates with suspected septicemia and inoculated in biphasic media. Isolates obtained were identified as per standard protocol and antibiotic susceptibility was done by Kirby Bauer disc diffusion method as per CLSI (Clinical and laboratory standards institute) guidelines. A total number of 78(15.6%) patients had positive blood cultures. The most common pathogens isolated were Staphylococcus aureus (n=22, 28.20%),  followed by Escherichia coli (n=19, 24.3%), Enterobacter spp (n=10, 12.82%), Coagulase negative Staphylococcus (n=9, 11.53%), Proteus spp. (n=8, 10.25%), Pseudomonas spp. (n=5, 6.41%), Acinetobacter spp (n=4, 5.12%), Klebsiella spp (n=1, 1.28%). The Gram negative organisms were more resistance to commonly used antibiotics like Cefotaxime, Ampicillin but highly sensitive to Imipenam. The Gram positive bacteria showed high resistance to Chloramphenicol, Penicillin but they were highly susceptible to Vancomycin and Gentamycin. As the Gram negative organisms were the most common isolates in neonatal septicemia, As there is increasing incidence of resistant strain in common isolates of neonatal septicemia.

KEYWORDS: Neonatal sepsis, Blood culture, Antibiogram, Bacteriological profile.

INTRODUCTION: Neonatal sepsicaemia is one of the commonest causes of neonatal mortality and morbidity throughout the world. The world health organization (WHO) estimates that 85% of newborn deaths are due to infections including sepsis, pneumonia and tetanus. It is also estimated that 20% of all neonates develop sepsis and is responsible for 30-50% of total neonatal death in developing countries.¹⁻³

Incidence of neonatal septicaemia varies from 2.2/1000 live births in developed countries to 10- 50/1000 live births in developing countries, though underreporting is common on both.⁴

Gram negative neonatal septicemia are more common and are mainly caused by Klebsiella, Escherichia coli, Enterobacter, Proteus spp., Pseudomonas. The gram positive organisms, Staphylococcus aureus, Coagulase negative staphylococci (CONS), Streptococcus pneumonia and Streptococcus pyogenes are most commonly isolated.²⁻⁵

Neonates are considered immunocompromised in view of their relatively immature immune defense mechanisms. Specifically they have quantitative as well as qualitative deficiency in their humoral immunity. The preterm neonate is at further risk, as trans placental transfer of antibodies starts after 32 weeks of gestation and endogenous synthesis does not being until about 24 weeks after birth.⁴

A number of studies of the microbial flora and sensitivity patterns in neonates from other parts of the world but weaimed to determine the Gram-positive and Gram-negative bacteriological

DOI: 10.14260/jemds/2015/1880

ORIGINAL ARTICLE

J of Evolution of Med and Dent Sci/ eISSN- 2278-4802, pISSN- 2278-4748/ Vol. 4/ Issue 75/ Sept 17, 2015  Page 13057
profile of bacteremia and antibiotic susceptibilities in Neonatal Intensive Care Unit (NICU) of Chhattisgarh institute of medical sciences Hospital CIMS (Bilaspur)

MATERIALS AND METHODS: The study was carried out between March 2013 to July 2015 in the Department of Microbiology, Chhattisgarh institute of medical sciences (CIMS) Hospital Bilaspur. Blood for culture was collected aseptically from 500 clinically suspected septicemia. The predominant clinical presentations report disclosed following symptoms like respiratory distress, refusal to feed, lethargy, restlessness and irritability, hypothermia or fever and seizures.6-7 Cases admitted in NICU. One ml neonates blood was collected and inoculated into biphasic media (Pediatric use) Himedia, Mumbai.

The bottle was shaken gently and incubated at 37°C aerobically for a maximum period of 7 days. The bottles were observed daily and as soon as signs of growth like turbidity, air bubbles or colonies over the solid slant portion of the biphasic medium were detected in either medium, subculture was done on blood agar and MacConky agar on 3rd and 5th day.

The identification tests for gram positive bacteria were gram stain, coagulase test, catalase test and for gram negative bacteria were gram stain, IMViC (Indole, methyl red, voges-proskauer, citrate), motility, urease, TSI, oxidase were done from an isolated colony. I used amino acid test for Pseudomonas and Acinetobacter spp.

Antibiotic sensitivity tests of the isolates were performed by the Kirby bauer disc diffusion method on Mueller Hinton agar for antibiotics according to CLSI (Clinical and laboratory standards institute) guideline.8

RESULT: Out of the 500 blood culture from neonates, 78(15.6%) showed bacterial growth and 422 samples were negative. Out of 78 bacterial isolate, 31(39.74%) were gram positive cocci and 47(60.25%) were gram negative bacilli.

The most common gram-positive bacteria causing septicemia infection was Staphylococcus aureus 22(28.20%) followed by coagulase-negative Staphylococcus (CONS) 9(11.53%). In gram-negative bacteria Escherichia coli 19(24.35%) was the most common pathogen following Enterobacter 10(12.82%), Proteus spp. 8(10.25%), Pseudomonas 5(6.41%), Klebsiella 1(1.28%) and Acinetobacter 4(5.12%) (Table 1).

| Microorganisms                  | Number of Organisms | Percentage of Total Organisms |
|---------------------------------|---------------------|-------------------------------|
| Gram-positive organisms         |                     |                               |
| Staphylococcus aureus           | 22                  | 28.20%                        |
| Coagulase-negative Staphylococcus (CONS) | 9 | 11.53%                              |
| Gram-negative organisms         |                     |                               |
| Escherichia coli                | 19                  | 24.35%                        |
| Enterobacter                    | 10                  | 12.82%                        |
| Proteus spp.                    | 8                   | 10.25%                        |
| Pseudomonas spp.                | 5                   | 6.41%                         |
| Klebsiella spp.                 | 1                   | 1.28%                         |
| Acinetobacter spp.              | 4                   | 5.12%                         |
| Total                           | 78                  | 100%                          |

Table 1: Number and percentage of organisms isolated from blood culture
The tables I have provided represents the antibiotic sensitivity. In gram positive bacteria the antibiotic Sensitivity of Staphylococcus aureus and CONS (coagulase negative staphylococci) were Vancomycin (100%), Gentamycin (90.32%) (Table 2).

Sensitivity in gram negative bacteria were Imipenam (85.10%), Piperacillin/Tazobactum (65.95%). Klebsiella spp were 100% resistant to Ampicillin, Cefotaxime, Gentamycin (Table 3).

| MICROORGANISMS | Antibiotics | Staphylococcus Aureus N=22 | Coagulase negative Staphylococcusus N=9 | Total N=31 |
|----------------|-------------|-----------------------------|----------------------------------------|------------|
|                | Vancomycin  | 22(100%)                    | 9(100%)                                | 31(100%)  |
|                | Gentamycin  | 21(95.45%)                  | 7(77.77%)                              | 28(90.32%)|
|                | Erythromycin| 13(59.09%)                  | 6(66.66%)                              | 19(61.29%)|
|                | Cefoxitin    | 12(54.54%)                  | 2(22.22%)                              | 14(45.16%)|
|                | Clindamycin  | 11(50%)                     | 5(55.55%)                              | 16(51.61%)|
|                | Penicillin   | 10(45.45%)                  | 3(33.33%)                              | 13(41.93%)|
|                | Cefazolin    | 9(40.90%)                   | 5(55.55%)                              | 14(45.16%)|
|                | Ciprofloxacin| 9(40.90%)                   | 6(66.66%)                              | 15(48.38%)|
|                | Chloramphenicol| 8(36.36%)              | 4(44.44%)                              | 12(38.70%)|

Table 2: Antibiotic susceptibilities of gram positive organisms

| MICROORGANISMS | Antibiotics | Escherichia Coli N=19 | Enterobacter Spp N=10 | Proteus Spp N=8 | Pseudomonas N=5 | Klebsiella N=1 | Acinetobacter N=4 | Total N=47 |
|----------------|-------------|-----------------------|-----------------------|-----------------|----------------|----------------|------------------|-----------|
|                | Amoxicillin/ Sulbactum | -                     | -                     | -                | -              | -              | 1(25)            | 1(2.12)  |
|                | Ciprofloxacin       | 2(10.52)              | 3(30)                 | 6(75)           | 2(40)          | 1(100)         | 2(50)            | 16(34.04) |
|                | Piperacillin/ Tazobactum | 13(68.42)            | 4(40)                 | 7(87.5)         | 3(60)          | 1(100)         | 3(75)            | 31(65.95) |
|                | Amikacin            | 12(63.15)             | 2(20)                 | 8(100)          | 3(60)          | 1(100)         | 4(100)           | 30(63.82) |
|                | Gentamycin           | 9(47.36)              | 2(20)                 | 8(100)          | 2(40)          | 0              | 1(25)            | 22(46.80) |
|                | Imipenem            | 18(94.73)             | 7(70)                 | 6(75)           | 4(80)          | 1(100)         | 4(100)           | 40(85.10) |
|                | Ceftriaxone          | -                     | -                     | -                | 2(40)          | -              | 4(100)           | 6(12.76)  |
|                | Ampicillin           | 2(10.52)              | 1(10)                 | 2(25)           | -              | 0              | -                | 5(10.63)  |
|                | Piperacillin         | 9(47.36)              | 5(50)                 | 3(37.5)         | 2(40)          | 1(100)         | 2(50)            | 22(46.80) |
|                | Ticarcillin          | -                     | -                     | -                | 4(80)          | -              | -                | 4(8.51)   |

Table 3: Antibiotic susceptibilities of gram negative organisms

**DISCUSSION:** Severe sepsis remains one of the leading causes of death in Neonates. 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.
We processed 500 blood samples from clinically diagnosed septicemia cases. The rate of bacterial isolation in blood culture in this study was 15.6% (78/500). This was in concordance with the other studies by Roy I et al.,9 and Kayange N et al.10 The incidence of culture-proven neonatal septicemia was 14.4 per 1000 live births. This was comparable to the study done by Karthi-keyan et al.11 The weaker immune system in neonates and children explains this higher rate of isolation.12

In a report from Karachi, the incidence of gram positive and gram negative were almost equal.13 In our study Gram negative bacterial isolates (60.25%) were more than Gram positive isolates (39.74%). This is in contrast to developed countries, where Gram positive bacteria were more commonly reported. This was in concordance with National Neonatal Perinatal Database (NNPD) (2003),14 Aletayeb SMH et al.,15 and Sundaram V et al. Kamath et al.,17 reported that 71.8% of BSIs in India were caused by Gram negative bacteria, with Klebsiella species accounting for 16.4%, Pseudomonas spp. 13.6%, Escherichia coli 11.8%, Enterobacter spp. 11.4% and Acinetobacter spp. In our study we have found that the presence of Klebsiella spp was 1.28%, Pseudomonas spp. 6.41%, Escherichia coli 24.35%, Enterobacter spp. 12.82%, Acinetobacter spp. 5.12%. Couto,18 et al reported that 51.6% of blood stream infections in Brazil were caused by Gram-negative bacteria, with Klebsiella spp accounting for 26.6%, Escherichia coli 9.7% and Pseudomonas spp. 6.4%. Bizzarro et al.,19 reported that 32.8% of BSIs in the USA were caused by Gram-negative bacteria, with Escherichia coli accounting for 37%, K. pneumonia 17% and Pseudomonas aeruginosa 12%.10. Among the Gram negative organism Escherichia coli was the most common organism followed by Enterobacter and Proteus spp.20, 21, 22, 23 In our country, the most frequent Gram-negative Microorganisms grown in blood cultures are Klebsiella spp. and Escherichia coli.24 In our study we have found that the predominant isolates was Escherichia coli followed by Enterobacter spp, Proteus spp.

In this study, the predominant isolates was Staphylococcus aureus which is in agreement with other reports.11, 25 Mustafa M,26 reported that Gram positive bacteria were having better susceptibility to Amikacin (68%), Cephalosporins and Ciprofloxacin (63%); but were more resistant to Ampicillin (13.6%) and Gentamicin (45%) in their present study (2014). Sudarshan raj27 reported that Gram negative bacteria were resistant to most commonly used antibiotics like Ampicillin (70.04%), Ofloxacin (56.09%) and Co-trimoxazole (80.48%). Resistance was least with Imipenem (0%), Netilmicin (29.26%) and Ceftazidime (25.60%). In our study, We have found that the gram negative bacteria were resistant to most commonly used antibiotics like Ampicillin (10.63%), Ciprofloxacin (34.04%), Cefotaxime (36.17%). Kamble R,28 reported that Among the Klebsiella pneumonia (56.25%) were sensitive to Ciprofloxacin (64.28%) and Amikacin (75%), and 62.5% of the Klebsiella isolates were complete resistance to Ampicillin, Amoxyclav, Cefazolin, Cephalothin, Cefuroxime and Cefoperazone. 80% of Acinetobacter and Pseudomonas spp. were sensitive to Piperacillin+Tazobactum (95.23%) while 90% of Acinetobacter and 86.67% of Pseudomonas spp. were sensitive to Imipenem (95.65%). In our study all Gram negative bacteria were having considerable sensitivity to Imipenam (85.10%), Amikacin (83.82%) and Piperacillin/Tazobactum (65.95%). All Gram positive isolates were highly sensitive to Vancomycin (100%), Gentamycin (90.32%), Erythromycin (61.29%).

**CONCLUSION:** In the present study, Gram negative organisms were the predominant cause of neonatal sepsis. Escherichia coli was the most common species, followed by Enterobacter and Proteus species. The organisms were resistant to most commonly used antibiotics. Organisms responsible for neonatal sepsis are different from developed and developing countries.
antibiotic susceptibility studies will help pediatricians to choose an appropriate antimicrobial for empirical treatment of neonatal septicemia.

REFERENCES:
1. Sankar MJ, Agarwal R, Deorari AK and Paul V K. Sepsis in the newborn. Indian Journal of Pediatrics.2008; 75(3): 261-266.
2. Vergnano S, Sharland M, Kazembe P, M wansambo C, Heath PT. Neonatal sepsis: An international perspective. Arch dis child fetal neonatal ed.2005; 90(3): 220-224.
3. Kumhar GD, Ramachandran VG, Gupta P. Bacteriological analysis of blood culture isolates from neonates in a tertiary care hospital in India: Journal health population & nutrition.2002; 20(4): 343-347.
4. Ahmed SS, Chowdhury M, Hoque MM, Begum D, Ahmed A. Role of intravenous immunoglobulin (IVIG) as an adjuvant in the treatment of neonatal sepsis in preterm babies. Journal of Bangladesh College of physicians and surgeons.2006; 24(3): 97-104.
5. Jaswal RS, Kaushal RK, Goel A and Pathania K. Role of C-reactive protein in deciding duration of antibiotic therapy in neonatal septicemia. Indian pediatrics.2003; 40(9): 880-883.
6. Cloherty JP, Eichenwald EC, Stark AR. Manual of neonatal care: Sixth edition: Lippincott Williams & wilkins.2007; 274-300.
7. Jain NK, Jain VM, Maheshwari S. Clinical profile of neonatal sepsis. Kathmandu university medical journal.2003; 1(2): 117-120.
8. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Tech Bull Regist Med Technol.1966Mar; 36(3): 49-52.
9. Roy I, Jain A, Kumar M, Agarwal SK. Bacteriology of neonatal septicaemia in a tertiary care hospital of northern India. Indian J Med Microbiol.2002; 20: 156–9.
10. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC Pediatrics 2010; 10: 39.
11. Karthikeyan G and Premkumar K. Neonatal sepsis: Staphylococcus aureus as the predominant pathogen. Indian J Pediatr 2001; 68(8): 715-717.
12. Meremkwer MM, N wachukwu CE, Asuquo AE, Okebe J, Utsalo SJ. Bacterial isolates from blood cultures of children with suspected septicemia in Calabar, Nigeria. BMC Infect Dis.2005; 5: 110–5.
13. Anwar SK, Mustafa S, Pariyani S, Ashraf S, Taufiq KM. Neonatal sepsis: an etiological study. J Pak Med Assoc.2000; 50: 91-94.
14. National Neonatal Perinatal Database 2002-2003 report http://www.nnfi.org/images/NNPD_2002-03.pdf. (Last ac-cessed on October 22, 2013).
15. Aletayeb SMH, Khosravi AD, Dehdashtian M, Kompani F, Mortazavi SM, Aramesh MR. Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis. African Journal of Microbiology Research 2011; 5(5): 528-531.
16. Sundaram V, Kumar P, Dutta S, Mukhopadhy K, Ray P, Gautam V, Narang A. Blood culture confirmed bacterial sepsis in neonates in a North Indian tertiary care center: Changes over the last decade. Jpn.J.Infect.Dis.2009; 62: 46-50.
17. Kamath S, Mallaya S, Shenoy S. Nosocomial infections in neonatal intensive care units: profile, risk factor assessment and antibiogram. Indian J Pediatr 2010; 77: 37-9.
18. Couto RC, Carvalho EA, Pedrosa TM, Pedroso ER, Neto MC, Biscione FMA. A 10-year prospective surveillance of nosocomial infections in neonatal intensive care units. Am J Infect Control. 2007; 35: 183-9.

19. Bizzarro MJ, Raskind C, Baltimore RS, Gallagher PG. Seventy five years of neonatal sepsis: 2003. Pediatrics. 2005; 116: 595-602.

20. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. Jpn J Infect. Dis. 2004; 57: 273-275.

21. Jyothi P, Basavaraj MC, Basavaraj PV. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. J Nat Sc Biol Med. 2013; 4: 306-9.

22. Shah AJ, Mulla SA, Revdiwala SB. Neonatal sepsis: High antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit of a tertiary Care hospital. J Clin Neonatol. 2012; 1: 72-5.

23. 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.

24. Indian Neonatal Society; Nosocomial Infections Study Group. Nosocomial infections in Neonatal units in Delhi: epidemiology, problems, unit policies and opinions of health care workers. 2010; 52: 50-7.

25. Thomas M, Padmini B, Srimathi G, Sundararajan V, Rajni BA. Microbial Profile Of Neonatal Infection in Coimbatore. Indian J Pediatr. 1999; 66: 11-4.

26. Mustafa M. Ahmed SL. Bacteriological profile and antibiotic susceptibility patterns in neonatal septicemia in view of emerging drug resistance. JMAS. 2014; 4(1): 2-8.

27. Dr. Sudarshan raj C, Dr. Pradeep reddy M, Dr. Neelima A. Bacteriological profile of neonatal septicemia in a tertiary care hospital. WJPPS. 2013; 2(6): 5709-5717.

28. Kamble R, Ovhal R. Bacteriological profile of neonatal septicemia. IJCM. 2015; 4(2): 172-182.

AUTHORS:
1. Varun Dwivedi
2. R. Murthy

PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Department of Microbiology, Dr. C. V. Raman University, Bilaspur, (C. G.).
2. Professor, Department of Microbiology, Chhattisgarh Institute of Medical Sciences, Bilaspur (C. G.).

FINANCIAL OR OTHER COMPETING INTERESTS: None

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Varun Dwivedi,
Department of Microbiology,
Chhattisgarh Institute of Medical Sciences,
Bilaspur-495001,
Chhattisgarh.
E-mail: varun.setwin@gmail.com

Date of Submission: 01/09/2015.
Date of Peer Review: 02/09/2015.
Date of Acceptance: 14/09/2015.
Date of Publishing: 15/09/2015.