**Bacteriological Profile and antibiotics resistance pattern of Isolates in Neonatal Septicemia in tertiary care center in North India**

**RC, Guleria. VK, Bhardwaj\*, M.Singh, S A Ganju, L R Chandel, R Singh, A, Sharma, K, Parmar, A. Singh , N. Saini**

**Department Of Microbiology, Department of Pediatrics**

**Shri Lal Bahadur Shastri Govt. Medical College**

**Mandi at Nerchowk, H.P, India**

**ABSTRACT:**

**Background:** Septicemia in neonates refers to generalized bacterial infection documented by the presence of bacteria, toxins in bloodstream that confirm by positive blood culture. Emergence of drug resistant organisms is a major problem in the management of septicemia. The aim of present study was undertaken to determine bacteriological profile and antibiotics resistance pattern of isolates in Neonatal Septicemia in tertiary care center in North India.

**Methods:** The presentstudy was carried out in the department of Microbiology, and Pediatrics, SLBSGMCH Nerchowk Mandi HP**.** A total 226 blood samples of septicemic neonate were studied bacteriological. Antibiotics susceptibility testing was done by Kirby Bauer disc diffusion method in accordance to Clinical laboratory Standards Institutes (CLSI) 2022 guidelines.

**Results:** A 21.68% (49 out of 226) case of septic neonate’s blood culture was positive. Staphylococcus aureus was the most common isolates (73.4%) encountered followed by E.coli (16%), Acinetobacter spp (4%), Enterococcus spp(4%) and Klebsiella spp(2%) Gram-positive isolates shows excellent sensitivity to Linazolid and Vancomycin.Gram-Negative organisms E coli is 100% resistant to Ceftazidime ,87.3%, to Ampicillin-Sulbactum and Klebsiella spp are resistant to all drugs.

**Conclusion**: The studies conclude that the isolates organism exhibited higher resistance towards commonly drug used antimicrobials. Health care personal and common population should be aware of the antibiotics resistance to frequently used antibiotics.

**Key words:** Antibiotics resistance, CONS, Septicemia, blood culture

**INTRODUCTION**

Neonatal sepsis is a clinical syndrome in infants’ up to 28 days of life manifested by systemic sign of infection and isolation of a bacterial pathogen from the blood stream. A consensus on definition for neonatal sepsis is lacking.1 Neonatal sepsis is a catastrophic worldwide health disease that causes substantial neonatal morbidity and mortality, accounting for 18.6% of all neonatal fatalities.2 Early onset and late onset sepsis are defined on the basis of presentation within 72 hours or after 72 hours of life respectively [2,3].

The incidence of neonatal sepsis was 30 per 1000 live births, according to the National Neonatal Prenatal Database 2002-03.4 In an another systematic review and meta analysis of population- based studies, the incidence of neonatal sepsis was 22 per 1000 live births and mortality was 11 to 19 percent5,6

In underdeveloped nations like India, it is one of the primary causes of newborn mortality.7The isolation of microorganisms from a patient's blood culture is critical for diagnosis and prognosis.8

Septicemia is caused by both gram positive and gram negative organisms, and the predominance of one type over the other changes from place to place and even over time in the same place. The gold standard method for diagnosing septicemia is the isolation of the pathogen from blood culture. Resistant strains have emerged as a result of the uncontrolled use of numerous strong and broad spectrum antibiotics.9

Early empirical therapy with wide spectrum antibiotics in patients presenting with clinical symptoms suggestive of septicemia or bacteremia is usual practice. Such empirical therapy may be justifiable given the severity of septicemia, but targeted therapy based on the antibiogram of the isolate will undoubtedly improve the therapeutic outcome.10

The goal of this study was to examine the bacteriological characteristics and antibiotic susceptibility patterns of pathogens isolated from the blood causing neonatal sepsis in the Neonatal Intensive Care Unit (NICU).

**MATERIAL & METHOD:**

This study was a retrospective cohort study. Data was collected from the hospital record of the patients. All neonates who were admitted with clinical syndrome of septicemia in NICU of Shri Lal Bahadur Shastri Government Medical College, Nerchowk,Mandi between January 2022 to June 2022 were enrolled retrospectively and analysed. During this time, 226 blood samples of neonates with clinical suspicion of septicemia were obtained from deptt of Pediatrics were studied bacteriologicaly. 1-2 ml of venous blood samples were collected under aseptic conditions and inoculated in 10-20 ml of Brain-Heart Infusion Broth (BHI) (HiMedia Laboratories Pvt. Ltd). The culture bottles were aerobically incubated at 37°C. Blind Subculture was performed on 5 percent sheep blood agar and Mac-Conkey's agar after an overnight incubation period.

If no growth observed on plates by the next day, subcultures were again repeated from the broth on day 3, day 4 and finally on day 7. Antibiotic susceptibility testing was carried out using conventional procedures as per CLSI 2022 guidelines.11

The Colony Characteristics, Gram's staining, motility testing, and biochemical testing were used to identify bacterial isolates. Catalase and coagulase tests (slide and tube test) were used to identify Gram positive isolates; for Gram negative organisms, Simons citrate test, motility, indole test, Christensen urease test, sugar fermentation (glucose, lactose, sucrose, and mannitol), and triple sugar iron test (TSI) were used.

The antimicrobial susceptibility testing was performed using the Kirby- Bauer disc diffusion method on Muller Hinton agar media and antibiotic discs (Hi Media Lab Pvt limited).

**RESULTS:**

Out of 226 blood culture received from neonates of clinically suspected sepsis, 49(21.68%) were culture Positive. Susequently 38(77.5%) were found to be Gram-positive, 11(22.4%) were Gram-Negative. There was no fungal growth obtained. Staphylococcus aureus was commonest organism isolated seen in 36 (73.4%) of positive cultures. It was followed by Escherichia coli 8(16. %), Enterococcus spp 2(4%) Acinetobacter spp 2(4%) and Klebsiella spp in 1(2%) of isolates (Table 1).

**Table 1- Isolation of organisms from blood culture:**

|  |  |  |
| --- | --- | --- |
| **S.No.** | **Organisms** | **Number/Percentage (%)** |
| 01. | Staphylococcus aureus | 36(73.4%) |
| 02. | Escherichia Coli | 8(16%) |
| 03 | Acinetobacter spp | 2(4%) |
| 04 | Enterococcus spp | 2(4%) |
| 05 | Klebsiella spp | 1(2%) |
|  | **TOTAL** | **49** |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  | | --- | |  | |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |

**Fig: 1- Type of Organisms isolated in blood culture of Newborn**

**Table-2: Antibiotics Resistance Pattern Of Gram Positive Organisms:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S.No. | Antibiotics | | | Organisms ( n-38) | |
|  | |  | Staphylococcus aureus (36) | | Enterococcus spp (2) |
| 01 | | Penicillin | 100%(36) | | 100%(2) |
| 02 | | Ampicillin | 58.3%(21) | | 100%(2) |
| 03 | | Erythromycin | 50%(18) | | 100%(2) |
| 04 | | Clindamycin | 50%(18) | | 100%(2) |
| 05 | | Trimethoprim-sulfamethoxazole | 33.3%(12) | | 100%(2) |
| 06 | | cefoxitin | 58%(21) | | 100%(2) |
| 07 | | Gentamycin | 11%(4) | | 100%(2) |
| 08 | | Linazolid | 0%(36) | | 100%(2) |
| 09 | | Vancomycin | ------ | | 0(2) |

**Fig:-2. Antibiotics Resistance pattern of Staphylococcus aureus and Enterococcus spp**

**ANTIBIOTICS**

Isolated staphylococcus aureus stains were 100% resistant to penicillin followed by 58% to Ampicillin and cefoxitin (MRSA), 50% to Clindamycin, and Erythromycin, 33.3% Trimethorim- Sulfa-methoxazole , 11% to Gentamycin. Staphylococcus aureus was 100% sensitive to Linazolid. Enterococcus spp were resistant to all of above antibiotics and sensitive to Vancomycin only (Table 2).

**Table 3: Antibiotics resistance pattern of Gram Negative Organisms:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.No.** | | **Antibiotics** | **Organisms** | | |
|  |  | | E.coli (8) | Acenitobacter spp (2) | Klebsiellla spp (1) |
| 01 | Ampicillin-sulbactum | | 87.5%(7) | 0(2) | 100%(1) |
| 02 | Ciprofloxacin | | 37.%(3) | 100%(2) | 100%(1) |
| 03 | Gentamycin | | 87%(7) | 100% (2) | 100%(1) |
| 04 | Ceftaz-Clavulanic | | 50%(4) | 100%(2) | 100%(1) |
| 05 | Ceftazidime | | 100%(8) | 100%(2) | 100%(1) |
| 06 | Imipenam | | 25%(2) | 100%(2) | 100%(1) |
| 07 | Netilmycin | | ------ | -------- | 100%(1) |
| 08 | Tetracyclin | | ---- | ----- | 100%(1) |
| 09 | Ticarcillin | | -------- | --------- | 100%(1) |

Among Gram-negative organisms E.coli were 100% resistant to ceftazidime followed by 87.5% to Ampicillin-Sulbactum and 85% to Gentamycin. It was 50% resistant to ceftazidime- clavulanic acid and 25% Imipenam. Acenitobactor spp were sensitive to Amicillin-sulbactum only and resistant to all other antibiotics. Klebsilaa spp was multidrug resistant (Table 3).

**DISCUSSION:**

Blood culture is still a gold standard method for diagnosis of Neonatal septicemia. Information provided by culture, type of organisms and their antibiotics sensitivity report can guide the Pediatrician to empirical therapy.

In this study blood culture positivity was found 21.68%. Pevious studies reported blood culture positivity rate from 20% to 45%. Mahapatra 12 khanal 13and Murthy et al 14 have reported culture positivity of 40% to 45% and 24% respectively.

In India the variation of culture positivity is due to the fact that most of patients are referred from peripheral health facilities and already have recieved broad range of empirical antibiotics.

In present study, Gram-positive organisms are being predominantly responsible for 77.5% of neonatal septicemia (table 1). Staphylococcus aureus was cultured in 36 (73.4%) and was major organisms causing neonatal septicemia. Karthikeyan et al.15also reported Staphylococcus aureus as most common organisms in neonatal septicemia seen in 61.5% of cultures. In our study Enterococcus spp was 2(4%), followed by Gram-negative organisms E.coli 8(16%), Acenitobacter spp 2(4%) and klebsiella spp 1(2%). Whereas many studies reported Klebsiella spp and Staphylococcus aureus to be the commonest organism. 16,17,18

These findings has implications for treatment and infection control measures. The Staphylococcus aureus survive in the hospital environment and Health care facility linens up to 3 to 6 months19. The Staphylococcus aureus is normally part of human flora. About 20-40% of healthy population is carrier of S, auerus, colonizing the organism persistently or transiently?

The most common site of colonization are anterior nares, oropharynx; followed by skin, vagina, axilla, and perineum. These colonization sites serve as reservoir for infections. Therefore, there is need of effective infection control measure at the time of birth of baby. In health care facility, the health care providers and attendents are potential carriers of S. aureus. The hospital stains are often multidrug resistant strains spread to patients either from hospital staff or hospital environment.20 E.coli, Enterococcus spps, Acinetobacter spps, and Klebsiella spps are also responsible for a substantial percentage of Nosocomial infections in a modern era and represent vast majority of multidrug resistance. They have potential for being transmitted from environment to the neonates during birth that breach infections control measure.21

Antibiotics resistance, a major global problem of multidrug resistant bacteria causing neonatal sepisis in the developing countries is increasing. In this study, there is high resistance against Penicillin, Ampicillin and Clindamycin in most of the isolates. 58% isolates of Staphylococcus aureus are Methicillin Resistant as comparable to study by Ahirwar SK et al22, 62%, and Michael et al. 85%. Our isolate shows maximum sensitive to Trimethoprim-sulphoxamizole and Gentamycin. Staphylococcus aureus is 100% sensitive to Linazolid, and Enterococcus spps resistant to all antibiotics only except to Vancomycin .

Among gram-negative organisms, Klebsiella spp are multidrug resistant and resistant to all antibiotics while Acinetobacter spp are only sensitive to Amicillin- sulbactum. E.coli is 100% resistant to ceftazidime, 87.3% to ampicillin-Sulbactum, and 87% Gentamycin. E.coli was 37% resistant to Ciprofloxacin and 25% to Imipenam.

Less sample size is major Limitation of our study . There is no data about the receiving of pre-referral antibiotics by the patient, which has probable reduced possibility of more culture positivity in the current study.

This study also does not have details about the early onset or late onset sepsis. As it is retrospective cohort study and data available was limited.

**REFERENCES:**

1. Wynn JL, Wong HR, et al.: Time for Neonatal for Neonatal Specific Concess definition for sepsis. Pediatr Criti Care Med.2014 Jul;15(6):523-8
2. Lesser CF, Miller SI. Septicemia. In: Braunwald E, Hauser SL, Fauci AS, Longo DL, Kasper DL, Jameson JL, eds. Harrison's Principles of Internal Medicine. 15th ed. McGraw-Hill Company, 2001; vol.1: 970-974
3. VrishaliAvinashMuley, DnyaneshwariPurushottamGhadage, ArvindVamanraoBhore Bacteriological Profile of Neonatal Septicemia in a Tertiary Care Hospital from Western India Journal of Global Infectious Diseases / Apr-Jun 2015 / Vol-7 / Issue-2.
4. Report of the National Neonatal Perinatal Database. Report 2002-2003. NNPD Network. Available from: <http://www.newbornwhocc.org/pdf/> nnpd\_report\_2002-03.PDF. [Last accessed on 2010 July 17].
5. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N.The global burden of paediatric and neonatal sepsis: a systematic review. Lancet Respir Med. 2018;6(3):223.
6. Kimberlin DW, Brady MT, Jackson MA, Long SS (Eds), American Academy of Pediatrics. Group B streptococcal infections. In: Red Book: 2018 Report of the Committee on Infectious Diseases, 31st ed, American Academy of Pediatrics, Itasca, IL 2018. p.762
7. Kumaravel K S, Rameshbabu B. A Study of the Bacteriological Profile and Antibiotic Sensitivity in Neonatal Septicemia. International Journal of Contemporary Medical Research. Volume 3 | Issue 6 | June 2016 | ICV: 50.43

1. Meenakshi Kante\*, Muni Lakshmi P, Sreenivasulu Reddy P Bacterial profile of blood stream infections and their antibiograms. *Int J Res Med Sci. 2015Mar;3(3):698-704*.
2. Kenneth C. Iregbu, Olufumilayo Y. Elegba, Iretiola B. Babaniyi Bacteriological profile of neonatal septicaemia in a tertiary hospital in Nigeria *African Health Sciences* 2006; 6(3): 151- 154.
3. Murty D S, Gyaneshwari M. Blood cultures in paediatric patients: A study of clinical impact. Indian J Med Microbiol 2007;25:220-4.
4. Clinical and Laboratory Standards Institute[CLSI]. Performance for Antimicrobial disk Susceptibility Testing-Approved Standard. 27th information supplement.Wayne, PA. M100-S27; 2017
5. Mahapatra, A., Ghosh, S.K., Mishra, S. etal. Enterobacter cloacae predominant pathogen in neonatal septicaemia. Indian J. Med. Microbiology 2002,20: 110-12:
6. Khanal B, Harish BN, Sethuraman KK Shrinivasan S. infective endocarditis. Report of prospective study in an Indian Hospital. Trop Doct. 2002, 32. 83-5.
7. Murthy, DS, Gynaneshwari M. Blood culture in paediatrics patient: A study of clinical impact. Indian J Med Microbiol 2007;25:220-4.
8. Karthikeyan G, Premkumar K. Neonatal sepsis: Staphylococcus aureus is predominant pathogen. Indian J Pediatr 2001, 68:715-7
9. Meenakshi Kante, Muni Lakshmi P, Sreenivasulu Reddy P. Bacterial profile of blood stram infections and their antibiograms. Int J Res Med Sci. 2015 Mar;3(3):698-704.
10. Shrestha S, Shrestha NC, Singh Shrestha RPB, Keyestha S, Shrestha M, al. Bacterial Isolates and its antibiotics susceptibility pattern in NICCU.. Kathmandu Univ Med J. 2013: 41;66-70.
11. Patel D, Nimbalkar S, Sethi A, Kungwan A, Nimbalkar S. Blood culture isolates neonatal sepsis and their sensitivity IN Anand District of India. Indian Journel Pediatr 2014;81:785-90.
12. Modi P. Micrococcaceae. Note book of Microbiology, 3rd edit. Page 260; 2020.
13. Sastry AS, Bhat S. Staphylococcus infections. Essential of Medical Microbiology, 3rd edit P 513. 2021
14. Iregbu KC, Elegbra OY, Babaniyi IB. Bacteriological profile of neonatal septicemia in a tertiary hospital in Nigeria. Afr Health Sci 2006;24:81.
15. Ahirwar SK, Purohit M, Mutha A. Bacteriological profile and antibiotics resistance pattern of isolates from neonatal septicemia patients in tertiary care hospital central India. J Med Sci and Cli research. Vol 06;10: 458-63, 2018.