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# AEROBIC BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF BLOOD CULTURE ISOLATES IN CLINICALLY DIAGNOSED SEPTICEMIA PATIENTS IN NEONATE INTENSIVE CARE UNIT AT TERTIARY CARE FACILITY RAJASTHAN

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ABSTRACT

**Background:** Neonatal sepsis is caused by Gram-positive and Gram-negative bacteria and Candida. This study was done to determine the Aerobic Bacteriological Profile and Antibiotic Sensitivity patterns of the organisms isolated from the clinically suspected cases of neonatal sepsis.

**Materials and Methods:** This was an observational cross-sectional study for the duration of one year from April 2021 to March 2022. Total 100 neonates were enrolled in the study. This study was conducted at tertiary care facility, Rajasthan. Demographic characteristics and antibiotic sensitivity were done in all the neonates. Data was analysed using Microsoft Excel 2019.

**Results:** Mean age of neonates were 6.37±7.66 days. Around one third (38%) were culture positive. Early and late onset of sepsis was seen in 55.3% and 44.3% neonates respectively. Association of bacterial culture was only significant with gender (p value<0.05). Among gram positive cocci, Staph. aureus was most common and among gram negative, Klebsiella species were most common.

**Conclusion:** The blood stream infection was more associated with gram negative bacilli than gram positive cocci. Gram negative bacilli showed high resistance to Ampicillin, Amikacin and Ciprofloxacin. Gram positive cocci showed high resistance to Ampicillin, Cotrimoxazole. This study highlights the changing trends in the susceptibility Pattern of the isolates to routinely used antibiotics.

**Keywords:** Aerobic Bacteriological Profile, Antibiogram, Neonatal sepsis, Septicemia.

## INTRODUCTION

Neonatal mortality rate is one of the main indicators to assess the health status of a Nation. Globally one of the leading causes of neonatal mortality and morbidity is septicemia.[1] The spectrum of pathogens causing paediatric Blood Stream Infection differs broadly as per age, presenting symptoms and immune status of affected.[2] Nowadays because of widespread coverage of vaccination program, H. influenza and S. pneumonia are infrequent bloodstream pathogens. In a study done in 2012 on infants of <3 months of age, showed the leading causes of bacteraemia was Escherichia coli, group B Streptococcus (Streptococcus agalactiae), and Staphylococcus aureus.[3]

In United states, incidence of neonatal sepsis was 1-2 per 1000 live births which is much lesser than India. Culture proven sepsis may occur in about 20% of neonatal intensive care unit (NICU) admissions.[4] Previously, Gram negative organisms like Klebsiella was the main isolated pathogens in neonates with bacteraemia, incidence is about 47.5%-64%.[5-7] According to the data from National Neonatal Perinatal Database (NNPD, 2002-03), the incidence

of neonatal sepsis is 30 per 1000 live births and sepsis was leading to 19% of all neonatal deaths in India.[8-10]

Neonatal sepsis is defined as a clinical syndrome in an infant of up to 28 days of age, manifested by systemic signs of infection and isolation of a bacterial pathogen from the bloodstream.[11] The clinical spectrum of sepsis begins when a systemic infection (e.g. bacteremia, fungemia, viremia) or localized infection (e.g.: meningitis, pneumonia, pyelonephritis) progresses from sepsis to severe sepsis (the presence of sepsis combined with organ dysfunction), septic shock (severe sepsis plus the persistence of hypo perfusion or hypotension for >1 hour despite adequate fluid resuscitation or a requirement for inotropic agents or vasopressors), multiple organ dysfunction syndrome (MODS), and ultimately death.[12] Neonatal sepsis is generally classified into two major categories on the basis of onset of symptoms. Early onset sepsis (EOS) presents within the first 72 hours of life and Late onset sepsis (LOS) usually presents after 72 hours of birth.[13]

Neonatal sepsis is caused by Gram-positive, Gram-negative bacteria and Candida.[14] In neonatal sepsis, the diversity of affecting organisms varies depends primarily on place and time.[15,16] This diversity in pathogen spectrum is mainly attributed to the change in lifestyle and pattern of antibiotic use. Incidence of neonatal sepsis also differs from NICU to NICU, depending on conditions of facilities and services patients are availing at facility, stay at the facility, in combination causing infants to infection.[17-19] There are no specific features of neonatal sepsis and clinical presentation varies widely.[20]

To diagnose sepsis in neonates there is no laboratory test with 100% sensitivity and specificity. The gold standard test to diagnose neonatal sepsis is blood culture, which is confirmatory but the results of this test are available only after 48-72 hours. Due to no pathognomic symptoms and signs of neonatal sepsis, it is hard to make clinical diagnosis of Sepsis.[21] Positive blood culture may not always establish diagnosis of neonatal septicaemia. There may be other reasons of blood culture being positive like sample contamination or transient bacteraemia.[13] Thus there is requirement of the initiation of empirical antibiotic therapy until the suspected neonatal sepsis is ruled out. At this time, increased multidrug resistant among organisms make only lesser number of treatment options available and this results in delay in getting effective treatment.[22] Empirical antibiotic therapy should be unit-specific and usually shortlisted by the prevalent spectrum of etiological agents and their antibiotic sensitivity pattern. After getting sensitivity reports antibiotics once started should be modified.[8]

Although many assumed markers are reported in various clinical research settings, most of them are not available to the routine diagnostic laboratory.[13] With this background, the current study was undertaken to determine the Aerobic Bacteriological Profile and Antibiotic Sensitivity patterns of the organisms isolated from the Clinically suspected cases of neonatal sepsis admitted in NICU of Government Medical College and Associated Hospitals, Kota.

## MATERIALS AND METHODS

This Analytical cross-sectional type of observational study was conducted in the Department of Microbiology and Immunology, Government Medical College and Associated Hospitals, Kota. Data collection for the study was started in April 2021 after clearance from the Institutional Ethical Committee (No.F.3()Acad/Ethical Clearance/2021/44 dated 15/03/2021) of the institution, and data collection was completed in March 2022.

**Methodology:** All neonates with clinical suspicion of bacteraemia were included in our study. Total 100 neonates were included in the study. Blood samples were collected from neonates admitted with clinical diagnosis of neonatal septicaemia. Detailed history and clinical findings of suspected neonates were recorded in the proforma designed for this study. Age, sex, premature rupture of membranes (PROM), early onset septicaemia (EOS), late onset septicaemia (LOS), fever, hypothermia, diarrohea, respiratory distress, lethargy, jaundice and any evidence of sepsis on skin/ umbilical cord were recorded. The sensitivity testing for antibiotics was done by Kirby Bauer method based on the approach and zone size interpretive criteria established for the commonly tested anti-microbial agents published in the CLSI, M-100 (2021) 31st edition “Performance Standards for Antimicrobial Susceptibility Tests”.

**Statistical analysis:** Data was entered in excel spreadsheet. Descriptive was represented in form of frequency and percentage. Discrete data was presented in form of percentage and analysed using chi-square test or Fischer exact test. Statistical significance was kept at 95% confidence.

## RESULTS

In present study, mean age and mean birth weight of neonates was 6.37±7.66 days and 2.25±0.66 kgs respectively. Mean gestational age of neonates was 36.64±3.37 weeks. Around two thirds (64%, 64/100) were male and rest were female. Out of 100 suspected neonates, 38(38%) were culture positive. Early onset sepsis was seen in 55.3% (21/38) neonates and late onset sepsis in 44.3% (17/38) neonates.

[Table 1] depicts the association of Bacterial culture with different variables, out of 64 male neonates almost half (30/64, 46.9%) were found positive and among female 22.2% found positive, this difference was statistically significant (p value<0.05). While association of culture positivity with Place of Delivery, Term, Mode of Delivery, PROM, Birth weight and Neonatal period was found to be statistically insignificant (p value>0.05)

[Figure 1] depict the result of blood culture, almost half (47.4%, 18/38) were having gram positive cocci and rest (52.6%, 20/38) had gram negative bacilli). Among gram positive cocci, Staph. aureus was most common and among gram negative, Klebsiella species were most common.

[Table 2] depicts Antibiotic sensitivity and resistance pattern in Gram negative bacilli (n=20). Klebsiella spp. showed 75% sensitivity to Tigecyclines followed by Meropenem (62.5%) and Ciprofloxacin (37.5%) Piperacillin (25%) and Amikacin (25%). E.coli had 100% sensitivity to Tigecyclines, 83.33% to Meropenem, and 33.33% for Amikacin. Pseudomonas spp. Showed 100% sensitivity to Aztreonam and meropenem.

[Table 3] depicts that coagulase negative Staphylococcus spp. showed 80% sensitivity to Vancomycin, Linezolid and Teicoplanin and for Gentamycin (40%). Staphylococcus aureus. showed 88.8% sensitivity to Vancomycin and Linezolid, and for Teicoplanin (77.7%) and Gentamycin (44.4%). Enterococcus spp. showed 100% sensitivity to Linezolid followed by Vancomycin (66.6%), and Gentamycin (66.6%). Streptococcus spp. Showed 100% sensitivity to Linezolid and Vancomycin.

**Table 1: Association of Bacterial culture with different variables**

| Variable | | Culture Negative (n=62) | Culture Positive (n=38) | Total | Test of significance |
|---|---|---|---|---|---|
| Sex | Female | 28(77.8) | 8(22.2) | 36(100) | X2 = 4.943, Df=1; p value = 0.026 |
| | Male | 34(53.1) | 30(46.9) | 64(100) | |
| Place of Delivery | Hospital | 60(64.5) | 33(35.5) | 93(100) | *p value = 0.101 |
| | Home | 2(28.6) | 5(71.4) | 7(100) | |
| Term | Full Term | 42(61.8) | 26(38.2) | 68(100) | X2 = 0.023, Df=1; p value = 0.881 |
| | Pre term | 20(62.5) | 12(37.5) | 32(100) | |
| Mode of Delivery | LSCS | 23(62.2) | 14(37.8) | 37(100) | X2 = 0.258, Df=2; p value = 0.879 |
| | NVD | 37(62.7) | 22(37.3) | 59(100) | |
| | Other | 2(50) | 2(50) | 4(100) | |
| PROM | No | 43(67.2) | 21(32.8) | 64(100) | X2 = 1.465, Df=1; p value = 0.226 |
| | Yes | 19(52.8) | 17(47.2) | 36(100) | |
| Birth weight | VLBW <1.5 kg | 6(50) | 6(50) | 12(100) | X2 = 2.740, Df=2; p value = 0.254 |
| | LBW 1.5-2.5 kg | 30(57.7) | 22(42.3) | 52(100) | |
| | NBW >2.5 kg | 26(72.2) | 10(27.8) | 36(100) | |
| Neonatal period | Early Neonates (0-7 days) | 42(59.2) | 29(40.8) | 71(100) | X2 = 0.476, Df=1; p value = 0.490 |
| | Late Neonates (8-28 days) | 20(69) | 9(31) | 29(100) | |
| *Fisher exact test was used for analysis. | | | | | |

**Table 2: Antibiotic sensitivity and resistance pattern in Gram negative bacilli (n=20)**

| Organism | | AK | A/S | AT | CPM | CAZ | CAC | CIP | COT | IPM | MRP | P | PI | TGC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Klebsiella Spp. (8) | S | 2 | 0 | 1 | 1 | 2 | 1 | 3 | 3 | | 5 | | 2 | 6 |
| | % | 25 | 0 | 12.5 | 12.5 | 25 | 12.5 | 37.5 | 37.5 | | 62.5 | | 25 | 75 |
| | R | 6 | 8 | 7 | 7 | 6 | 7 | 5 | 5 | | 3 | | 6 | 2 |
| | % | 75 | 100 | 87.5 | 87.5 | 75 | 87.5 | 62.5 | 62.5 | | 37.5 | | 75 | 25 |
| E. Coli (6) | S | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 | | 5 | | 3 | 8 |
| | % | 33.33 | 33.33 | 16.66 | 16.66 | 16.66 | 33.33 | 16.66 | 33.33 | | 83.33 | | 50 | 100 |
| | R | 4 | 4 | 5 | 5 | 5 | 4 | 5 | 4 | | 1 | | 3 | 0 |
| | % | 66.66 | 66.66 | 83.33 | 83.33 | 83.33 | 66.66 | 83.33 | 66.66 | | 16.66 | | 50 | 0.0 |
| Acinetobacter (3) | S | 2 | 1 | 0 | | 1 | 2 | 0 | 1 | | | 0 | 1 | 2 |
| | % | 66.66 | 33.33 | 0 | | 33.33 | 66.66 | 0.0 | 33.33 | | | 0.0 | 33.33 | 66.66 |
| | R | 1 | 2 | 3 | | 2 | 1 | 3 | 2 | | | 3 | 2 | 1 |
| | % | 33.33 | 66.66 | 100 | | 66.66 | 33.33 | 100 | 66.66 | | | 100 | 66.66 | 33.33 |
| Enterobacter Spp. (1) | S | 1 | 0 | | 1 | | | 1 | | | 1 | | 1 | |
| | % | 100 | 0.0 | | 100 | | | 100 | | | 100 | | 100 | |
| | R | 0 | 1 | | 0 | | | 0 | | | 0 | | 0 | |
| | % | 0 | 100 | | 0 | | | 0 | | | 0 | | 0.0 | |
| Pseudomonas Spp. (1) | S | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | | | 0 |
| | % | 100 | 0.0 | 100 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 100 | | | 0 |
| | R | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | | | 1 |
| | % | 0.0 | 100 | 0.0 | 0 | 100 | 100 | 0.0 | 100 | 100 | 0.0 | | | 100 |
| Citrobacter (1) | S | | 0 | | 0 | | | | | 1 | 1 | | | 1 |
| | % | | 0.0 | | 0.0 | | | | | 100 | 100 | | | 100 |
| | R | | 1 | | 1 | | | | | 0 | 0 | | | 0 |
| | % | | 100 | | 100 | | | | | 0.0 | 0.0 | | | 0.0 |

**Table 3: Antibiotic sensitivity and resistance pattern in Gram positive cocci (n=18)**

| Organism | | A/S | CIP | COT | CX | CD | E | GEN | LZ | P | TEI | VAN | CXM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Staphylococcus aureus (9) | S | 2 | 1 | 1 | 2 | 4 | 2 | 4 | 8 | 0 | 7 | 8 | 4 |
| | % | 22.2 | 11.1 | 11.1 | 22.2 | 44.4 | 22.2 | 44.4 | 88.8 | 0.0 | 77.7 | 88.8 | 44.4 |
| | R | 7 | 8 | 8 | 7 | 5 | 7 | 5 | 1 | 9 | 2 | 1 | 5 |
| | % | 77.7 | 88.8 | 88.8 | 77.7 | 55.5 | 77.7 | 55.5 | 11.1 | 100 | 22.2 | 11.1 | 55.5 |
| CONS (5) | S | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 4 | 0 | 4 | 4 | 2 |
| | % | 40 | 20 | 40 | 20 | 40 | 20 | 40 | 80 | 0.0 | 80 | 80 | 40 |
| | R | 3 | 4 | 3 | 4 | 3 | 4 | 3 | 1 | 9 | 1 | 1 | 3 |
| | % | 60 | 80 | 60 | 80 | 60 | 80 | 60 | 20 | 100 | 20 | 20 | 60 |
| Enterococcus Spp. (3) | S | 1 | 0 | | 1 | 0 | 1 | 3 | 3 | 1 | 2 | 3 | |
| | % | 33.3 | 0.0 | | 33.3 | 0.0 | 33.3 | 100 | 100 | 33.3 | 66.6 | 100 | |
| | R | 2 | 3 | | 2 | 3 | 2 | 0 | 0 | 2 | 1 | 0 | |
| | % | 66.6 | 100 | | 66.6 | 100 | 66.6 | 0 | 0.0 | 66.6 | 33.3 | 0.0 | |
| Streptococcus Spp. | S | | 0 | 1 | | 0 | 1 | 1 | 1 | | | 1 | |
| | % | | 0 | 100 | | 0 | 100 | 100 | 100 | | | 100 | |
| | R | | 1 | 0 | | 1 | 0 | 0 | 0 | | | 0 | |
| | % | | 100 | 0 | | 100 | 0 | 0 | 0 | | | 0 | |

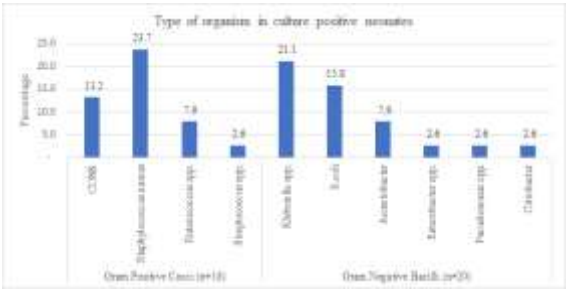


**Figure 1: Type of organism in culture positive neonates**

## DISCUSSION

In Present study, blood culture positivity was 38%, which was higher compared to studies reported by Pokhrel et al. 2018, Thapa et al. 2019, Almohammady et al. 2020, Sneha V et al. 2019,[23-26] which was 20.5%, 10.8%, 31.7% and 36.22% respectively while it was lesser than Thakur et al. 2016,[27] they reported 42% culture positivity. One possible explanation for the difference in blood culture results could be because of the routine utilization of antibiotics during obstetric care which might affect the blood culture yield of the neonates as there is significant transplacental transfer of these antibiotics to the foetus.[28] In our study proportion of early onset sepsis was higher than late onset (55.3% vs 44.3%) similar finding was also reported by Thapa et al. 2019 in their study.[24]

M:F ratio was found 1.77:1. Male neonates suspected for sepsis were admitted more than females. In Niza Monga et al. study sex ratio was 1.63:1 which was like current study. In the current study culture positivity was observed 46.9% in male and 22.2% in female neonates. In Sorsa et al.'s study culture confirmed neonatal sepsis, was 59.1% in male while 40.9% in females.[29] In both study culture positivity was higher in males compared to females. It may be due to more male being admitted in NICU or less attention of parents on female new-borns in our society. Secondly the male new-borns are more prone to septicemia as compared to females. In current study it was observed that to be preterm is risk factor for neonatal sepsis. The reason for more preterm neonatal sepsis may be due to extensive manipulation and resuscitation predisposing them for possible invasive colonization with Gram-positive bacteria.[28] In the current study, very low birth weight neonates (<1.5 kg) had more incidence of blood culture positivity (50%) compared to low birth weight neonates (42.3%) and normal birth weight neonates (27.8%). In study of Shehab et al. 2015 blood culture positivity was like our study, the highest incidence was found in VLBW group.[30] In Pokhrel et al.'s study predominantly isolated bacteria were Klebsiella (33.3%),E. Coli(20.5%) and Staphylococcus aureus (18%),[23] which were concordant to our study. Gram-negative bacteria were the most isolated organisms which is also in congruent with study reports from Egypt, Uganda and other developing countries.

In Sorsa et al.'s study resistance rate of E. coli against Gentamycin were 55.6% while Klebsiella spp. were resistant 82%.[29] In our study higher resistance was also found to Aminoglycosides 63.6% in E.coli and 83.3% Klebsiella spp. In a study done by Pokhrel et al. 2018, CONS showed good susceptibility (100%) to Vancomycin and Linezolid, almost similar to the current study.[23] In Thakur et al.'s study, gram-positive microorganisms were found to have resistance to Penicillin and Gram-negative organisms showed high resistance to Cephalosporins and aminoglycosides.[27] Enterococcus showed more resistance to CD and streptococcus to CX and CIP. In Pokhrel et al.'s study, Klebsiella showed high resistance to Cefotaxime, Gentamicin, Ciprofloxacin, Ofloxacin and Chloramphenicol.[23] It showed good sensitivity to Carbapenems, Colistin and Tigecycline. In the current study almost, similar results were observed.

## CONCLUSION

Our study concluded that the blood stream infection was more associated with gram negative bacilli than gram positive cocci. Gram negative bacilli showed high resistance to Ampicillin, Amikacin and Ciprofloxacin. It showed sensitivity to Tigecycline, Meropenem and Piperacillin. Gram positive cocci showed high resistance to Ampicillin,

Cotrimoxazole. It showed sensitivity to Vancomycin, Teicoplanin and Linezolid. Hence the antimicrobial susceptibility pattern showed that the isolates were increasingly becoming resistant to both first line as well as second line antibiotics with only few options left to treat the patients. This study highlights the changing trends in the susceptibility Pattern of the isolates to routinely used antibiotics. A regular epidemiological study of neonatal sepsis cases with respect to the pathogens and their antibiotic susceptibility patterns are thus necessary to guide the clinicians to choose appropriate empirical therapy as well switch over to the best regime based on antibiotic susceptibility pattern to improve the overall outcome of the patient's health and to avoid emergence of resistant strain.

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