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A Study On Clinico-Bacteriological Profile, Antibiogram And Outcome Of Bacterial Sepsis In Neonates In A Tertiary Care Hospital, West Bengal

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Abstract
Introduction: *Sepsis is an important cause of neonatal mortality. Developing countries bear a higher burden of neonatal mortality due to neonatal sepsis. Early diagnosis by identifying the clinical signs and symptoms and starting evidence-based empirical antibiotic therapy is key to a better outcome of neonatal sepsis. Thus, identifying the bacteriological profile and their antibiotic sensitivity pattern is important to formulate the antimicrobial policy.*
Aim: *The present study was undertaken to identify the clinic-bacteriological profile of neonatal sepsis cases admitted in the NICU and also to find out the antibiotic sensitivity pattern of isolated organisms.*
Methods: *Two hundred and sixty-two neonates were included in the study who were admitted with possible neonatal sepsis in the NICU in a tertiary care hospital, in West Bengal from January 2023 to January 2025. Neonatal sepsis was confirmed by blood culture and an antibiogram was performed to get the antibiotic sensitivity pattern as per CLSI guidelines. Data were analysed using Microsoft Excel version 2019 and SPSS 25.*
Result: *The prevalence of culture-positive sepsis was 20.73%. Gram-negative organisms have been isolated in 45.3% of cases and the remaining 54.7% were gram-positive sepsis. Staphylococcus aureus was the predominant organism. Burkholderia cepacia, CONS, and Klebsiella spp. are other important causative organisms. A higher prevalence of antibiotic resistance has been observed in the present study.*
Conclusion: *Regular follow-up on bacteriological profiles and antibiotic sensitivity tests are important to identify the predominant local bacteriological trends and this can help in formulating the antibiotic policy.*
Keywords: *neonatal sepsis; antibiotic resistance; EOS; LOS; gram-negative sepsis; gram-positive sepsis*

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I. Introduction:

Background of the Study:

Neonatal sepsis is a bloodstream infection that occurs in infants younger than 28 days. It remains a major cause of morbidity and mortality, particularly in middle- and low-income countries.[1] Neonatal sepsis ranks third among the causes of neonatal death following prematurity and intrapartum-related complications.[2] Neonatal sepsis can be potentially fatal and associated with increased health care burden. Neonatal sepsis can be caused by bacteria, viruses, or fungi and may present with varied manifestations, such as hemodynamic abnormalities, signs or symptoms, and associated with an elevated risk of mortality and morbidity. Neonates can present with subclinical infection or sometimes with severe focal or systemic manifestations. Its incidence ranges from 1 to 5 per 1000 live births, depending on the population being investigated and the case definition.[3,4] A recent systematic review and meta-analysis again reported a global incidence of 3930 neonatal sepsis cases per 100,000 live births (95% CI 1937–7812).[5] Approximately 24% of neonatal mortality has been attributed to neonatal sepsis globally.[6]

Classification of neonatal sepsis:

Based on the timing of symptom onset after birth, neonatal sepsis is classified as either early-onset sepsis (EOS) or late-onset sepsis (LOS), with experts using either 72 hours or 7 days as the cutoff.[7] Although the infectious agent may originate from maternal or intrauterine flora, it can also originate in a hospital or the community. Considering the mechanism of neonatal sepsis, it can be vertical transmission, community-acquired, or nosocomial infection. While the latter two are most prevalently seen in late-onset type, vertical transmission sepsis is usually observed in the first 72 hours of life.

Early-onset sepsis (EOS) primarily results from the transmission of pathogens from the maternal genitourinary system to the newborn or fetus. These pathogens can ascend through the vagina, cervix, and uterus, potentially infecting the amniotic fluid. Neonates may acquire the infection in utero or during delivery while passing through the vaginal canal. Common bacterial pathogens associated with EOS include Group B Streptococcus (GBS), Escherichia coli, coagulase-negative Staphylococcus, Haemophilus influenzae, and Listeria monocytogenes. Several maternal factors elevate the risk of neonatal sepsis, including chorioamnionitis, GBS colonization, preterm delivery (before 37 weeks), and prolonged rupture of membranes exceeding 18 hours.[6]

Late-onset sepsis (LOS), on the other hand, typically occurs due to pathogen transmission from the postnatal environment, such as contact with healthcare workers or caregivers. In some cases, LOS may arise as a delayed manifestation of an infection transmitted vertically. Infants who undergo invasive procedures, such as intravascular catheter insertion or other interventions that compromise mucosal integrity, face an increased risk of LOS. Additionally, preterm neonates are more vulnerable to sepsis and infections compared to full- term neonates. Preterm neonates are at higher risk of developing LOS due to several reasons, such as a weak immune system because of decreased IgG antibodies and incompetent opsonization and complement activation. Other important causes are compromised innate immune system due to immature epithelial barrier. Apart from these, preterm neonates require a lot of invasive procedures, like vascular access, endotracheal tubes, feeding tubes, urinary catheters, etc.[6] The data shows a trend of decreased early-onset sepsis over time with increased use of intrapartum antibiotic therapy, whereas increased survival of low birth weight and very low birth weight babies led to increased incidence of late-onset sepsis.[7,8]

Risk factors of neonatal sepsis[9]:

- EOS presents where the maternal genital tract is the source of ascending infection. Maternal risk factors like premature rupture of membranes (PROM), chorioamnionitis, peripartum fever, urinary tract infection within 2 weeks before delivery and prolonged rupture of membranes > 18 hours, multiple gestations, and caesarean sections are associated with increased risk of EOS.
- LOS occurs due to postnatal nosocomial infections or community-acquired infections. The risk factors associated with LOS are prematurity, prolonged invasive interventions like mechanical ventilation and intravascular catheterization, failure of early enteral feeding with breast milk, long duration of parenteral nutrition, hospitalization, surgery, and underlying respiratory and cardiovascular diseases.

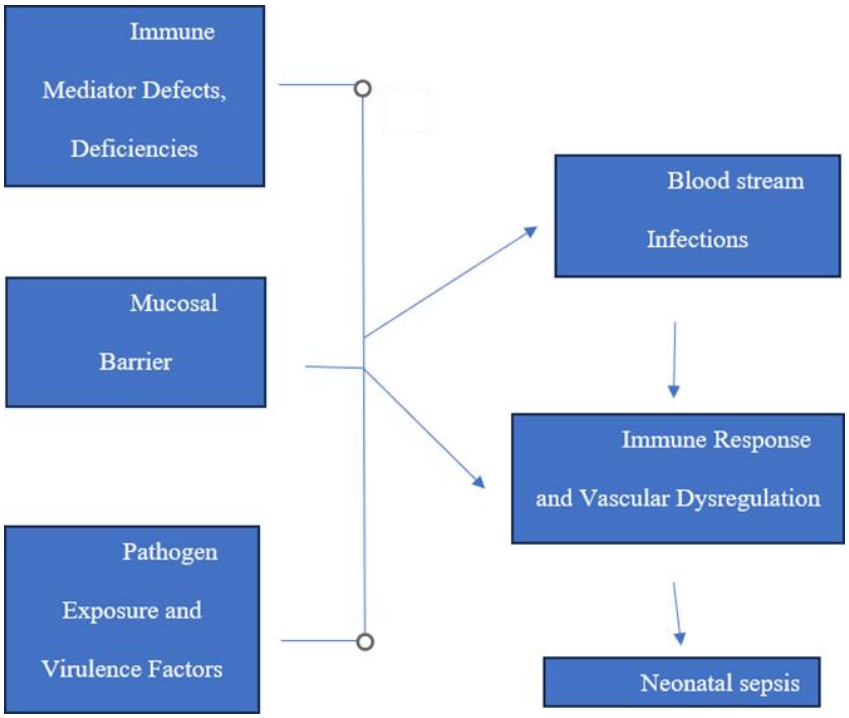
Pathophysiology of neonatal sepsis:

The unique characteristics and weakly developed budding immune system of neonates can easily be breached by the pathogenic organisms. For the same reason, the preterm are more vulnerable to the insult of pathogenic organisms and more profound & severe manifestations in comparison to the term neonates.

- The stratum corneum in the epidermis of the skin acts as the first barrier or defence of innate immunity. But it takes nearly 10 days after birth to gain its full functionality and considering the aspect of early delivery leading to prematurity, it further extended several weeks in the case of preterm neonates. The vernix which usually develops in the third trimester in utero, provides antimicrobial peptides (AMPs) like lactoferrin and lysozymes and acts as a mechanical barrier to invading pathogens. Thus, extremely low preterm neonates are susceptible to EOS owing to a lack of vernix.
- Secondly, the maternal IgG antibody usually gets transferred to foetus during the third trimester and preterm delivery automatically reduces the concentration of maternally derived protective antibodies in preterm neonates.
- The increased number of goblet cells in the respiratory mucosa of preterm neonates enhances the viscosity of respiratory secretions. The thick secretion decreases mucociliary clearance and premature neonates also lack the protective surfactant proteins to maintain the respiratory defence mechanisms.
- Chorioamnionitis or intra-amniotic infection is another important maternal factor causing neonatal infections. The foetus usually swallows and inhales the pathogenic bacteria present in the amniotic fluid leading to vertical transmission of infection. Besides, maternal infection can also contribute to EOS by hypermethylation owing to decreased expression of genes involved in foetal immune development.
- In the already weak neonatal immune system, interventions like central venous catheters, intravenous lines, and other invasive procedures further provide an optimum environment for the development of sepsis, especially LOS. Antibiotic usage and hypoxia are cited as other important culprits, which further contribute to LOS by intestinal mucosal injury and change in the protective gut microbial environment in neonates. Interleukin-17, a product of neonatal intestinal cells plays an important role as a preventive cytokine.
- The inability to contain infection locally leads to a response that leads to dysregulated systemic inflammatory response syndrome (SIRS). A series of events take place once the physical barrier is breached. The bacterial Lipopolysaccharide (LPS- a Pathogen Associated Molecular Pattern (PAMP) molecule) starts interacting with

Toll-like receptor 4 (a Pattern Recognition Receptor on myeloid cells). This interaction is important to instigate a pro-inflammatory cascade that causes upregulation of the production of various cytokines, namely tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). A specific level of these cytokines is protective and important for the removal of pathogens, while excess production of these endotoxins can impair the balance and lead to out-of-proportion vasoactive changes, leading to increased vascular permeability and end-organ damage.

- A specific and unique characteristic reported in neonates is lower L-selectin adhesion molecule expression and impaired formation of neutrophil extracellular traps (NETs) that prevent neutrophil killing of pathogens at the local level.



Key risk factors and events in the pathophysiology of neonatal sepsis

Clinical signs and symptoms:

Early identification and appropriate treatment are the keys to the favourable outcome of neonatal sepsis. The important challenge is to identify the clinical manifestations early, as very commonly the signs & symptoms are non-specific.

Clinical manifestations of neonatal sepsis are varied and often nonspecific. Neonatal sepsis symptoms might include hemodynamic collapse as well as vague or nonspecific symptoms. Lethargy, poor eating, and irritability are examples of early symptoms. Others may rapidly experience shock, poor perfusion, fever, hypotension, or respiratory distress. In many cases, the diagnosis may only be suspected based on test results that show hyperbilirubinemia, acidosis, hyperglycaemia, or hypoglycaemia. Thus, prompt diagnosis requires a high index of suspicion. Therefore, any circumstances that could raise an infant's risk of sepsis should be known to doctors.[6]

System-wise distribution of clinical manifestations of neonatal sepsis[9]:

- **Gastrointestinal system:** feeding intolerance, abdominal distension, vomiting, loose motion, hepatomegaly, jaundice
- **Respiratory system:** dyspnoea, apnea, tachypnea, intercostal muscle retractions, nasal flaring, grunting, stridor, wheezing, cyanosis
- **Renal system:** oliguria, anuria, haematuria, acidotic breathing, pedal edema, facial puffiness
- **Cardiovascular system:** dyspnoea, tachypnea, pedal edema, cyanosis, tachycardia, bradycardia, hypotension, cold clammy periphery
- **Central nervous system:** fever, irritability, lethargy, convulsion, hypotonia, hypertonia, tremors, twitching, bulging fontanelle, neck rigidity
- **Haematological system:** jaundice, splenomegaly, hepatomegaly, fever, purpura, pallor, abnormal bleeding

Bacterial Causes Of Systemic Neonatal Infections
Gram Positive organisms
Staphylococcus aureus
Coagulase negative staphylococcus Enterococci species
Group B streptococcus Streptococcus pneumoniae Viridans streptococcus Listeria monocytogenes

Gram Negative organisms
Klebsiella pneumoniae Escherichia coli
Proteus mirabilis Pseudomonas aeruginosa Citrobacter freundii Enterobacter cloacae Salmonella typhi
Serratia marcescens
Haemophilus influenza

Pathogen profile of EOS vs LOS in High-Income Countries vs Low-Income Countries:[10]

Studies published between 1976 and 2019 reported Group B Streptococcus (36.4%), *Escherichia coli* (24.8%), and *Coagulase negative staphylococcus* (15.4%) as the top three dominant causative pathogens of EOS in high-income countries (HIC), whereas *Klebsiella spp.* (32.4%), *CoNS* (14.7%), *E.coli* (10.3%), *Staphylococcus aureus* (9.7%), and *Pseudomonas* (6.7%) were identified as the most prevalent pathogen causing EOS in low-income countries (LIC). As EOS commonly occurs due to vertical transmission as the baby passes through the vaginal canal during delivery or in-utero pathogens get retrogradely moved from the vagina or cervix to the uterus and into the amniotic fluid.[11]

GBS being the common commensal of female genitourinary and gastrointestinal tracts, many studies have identified GBS as the most common causative organism of EOS. However, many literatures also cited *Escherichia Coli* as an important cause, even more than GBS. Although, relatively less common, *Staphylococcus aureus*, *coagulase-negative Staphylococcus*, *Haemophilus influenzae*, and *Listeria monocytogenes* are worth to be mentioned as other causes of EOS.

In the case of LOS episodes, gram-positive organisms like CoNS (45%), especially *Staphylococcus epidermidis, S. aureus* (13.8%), and *Enterococcus spp.* (7.0%) were identified most frequently in HICs. This denotes that gram-positive organisms predominate over gram- negative organisms causing LOS in HIC. However, for both EOS as well as LOS, the proportion of gram-negative organisms was relatively higher, especially *Klebsiella spp.* in both middle-income and low-income countries. This trend of the bacteriological profile of neonatal sepsis puts more emphasis on gram-negative organisms, especially *Klebsiella spp.*, and a relatively lesser relevance on GBS and CoNS

Research problem:

Sepsis is an important cause of neonatal mortality globally. Of the 6.9 million neonatal sepsis burden, 3.5 million cases are only reported in South Asia annually. India with its approximately 140 crore population, claims a huge burden of neonatal sepsis. No systematic surveillance and registry are in place to document and monitor the data on estimating the actual burden of neonatal sepsis. Many a time, the causes of neonatal mortality are not documented. Advancement in medical fields leads to decreased neonatal mortality comparatively. Conversely, it has increased the incidence of preterm births and low birth weight babies who require prolonged hospitalization, especially neonatal intensive care. This in turn leads to an increased incidence of neonatal sepsis. Due to inadvertent and unregulated antibiotic therapy, the trend of emerging pathogens and their resistance pattern has changed drastically over time. Even the prevalence of various bacteria and their resistance pattern varies from place to place. Antibiotic resistance is a pressing issue and it is a public health problem. The current study has been conducted to target this pressing issue realising its importance and contributing to the evidence pool.

Research Hypothesis:

Identification of the clinical profile of neonates suffering from sepsis and confirmation of that with blood culture followed by their antibiogram, leading to the identification of bacteriological profile and the resistance pattern of bacterial isolates help in better understanding of neonatal sepsis. Finding out the association of clinic-bacteriological profile with outcome in neonatal sepsis will further guide in the formulation of the correct approach and antibiotic policy for the management of neonatal sepsis for a better outcome.

Significance Of The Study:

Neonatal sepsis is a significant cause of morbidity and mortality in newborn, particularly in low-resource settings. Prompt diagnosis and appropriate antibiotic treatment are crucial for improving outcomes in affected neonates. However, the prevalence, aetiology, and antibiotic susceptibility patterns of neonatal sepsis can vary across different geographical regions, emphasizing the need for localized studies to guide effective

clinical management. According to the Indian Academy of Paediatrics, empirical antibiotic therapy should be based on local culture and sensitivity data and the profile of organisms for the last 6-12 months. Therefore, conducting a study in this region is essential to understand the prevailing bacterial pathogens, their antibiotic susceptibility patterns, and the clinical outcomes associated with neonatal sepsis. The findings will provide valuable insights for choosing antibiotics, implementing infection control strategies, and ultimately reducing the morbidity and mortality associated with neonatal sepsis in this hospital setting.

Objectives of the research:

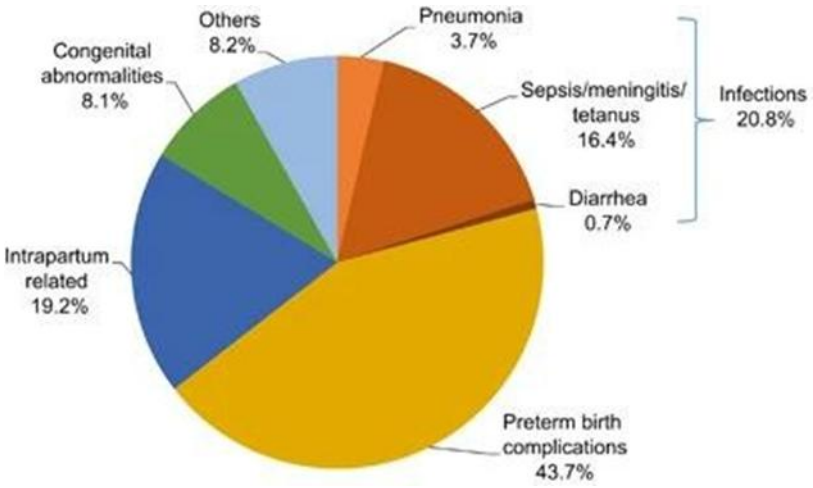
- To determine the prevalence of blood-culture-positive neonatal sepsis.
- To identify the bacteria causing neonatal sepsis.
- To study the antibiotic susceptibility pattern of the bacterial isolates and detect emerging patterns of resistance. (Methicillin-resistant Staphylococcus aureus, Extended-spectrum beta-lactamase)
- To describe the clinical features and outcome of the neonates with bacterial sepsis.

II. Review Of Literature

Historical Background:

Indian scenario:

The neonatal period i.e., the first 28 days of life has the highest daily risk of mortality compared to any other stage of childhood. During the first four weeks, the risk of demise is approximately 30 times higher than in the post-neonatal period i.e., 1 to 59 months of age. This important aspect has been neglected until the last decade resulting slow decline in neonatal mortality (NMR) in many countries, including India. India contributes to approximately one- fifth of the global burden of neonatal mortality. Data showed around 70% of total infant deaths and more than half of under-five deaths occur in the neonatal period in India.



Causes of neonatal deaths in India

A pooled analysis of data from three studies on the timing of neonatal deaths reveals that approximately three-fourths of all neonatal deaths occur within the first week of life. Notably, the first 24 hours alone account for more than one-third (36.9%) of all neonatal deaths.

The data from the Million Death Study from India revealed an important fact that infections of various types contributed to around 20.8% of neonatal mortality, whereas another 43.7% of neonatal deaths occurred due to preterm births and related complications as shown in the above diagram.[13]

Baqui et al. further potentiate the evidence that about three-fourths of neonatal mortality happens in the first week of life and even 30% of them occur in the first 24 hours of birth. About half of this mortality was associated with sepsis in the first week of life.[14] Amongst the rest 30% occur in the second week and around one-fifth in the third to fourth week of life.[14] While exploring the burden of neonatal sepsis in India, it was observed through hospital-based studies that the incidence of neonatal sepsis was about 30 per 1000 live births.[15] The community-based studies revealed that the incidence of neonatal sepsis varies from 2.7% to 17% of all live births.[15,16] The Delhi Neonatal Infection Study Group stated the fact that nearly 20% of neonates suffering from sepsis usually die and even this number rises to 50% in case of culture-proven sepsis cases.[17] This prospective cohort study was conducted in three hospitals, namely Vardhaman Mahavir Medical College, Maulana Azad Medical College, and All India Institute of Medical Sciences and 13530 neonates were recruited for this study. The incidence of total sepsis was 14.3% (95% CI: 13.8-14.9) and the incidence of culture-

positive sepsis was 6.2% (5.8-6.6). Among them two-thirds of sepsis cases were EOS and the rest were LOS. Two-thirds of total isolates were gram-negative pathogens and the most common organism found in this study was Acinetobacter spp. (22%), Klebsiella spp. (17%), Escherichia coli (14%), Pseudomonas spp. (7%), and Enterobacter spp. (4%). The predominant gram- positive bacteria were Coagulase-negative staphylococci (15%), Staphylococcus aureus (12%), Enterococcus spp. (6%), and Group B streptococci (1%). The pathogen pattern was so different in EOS and LOS cases. The case fatality rate was 26% for all sepsis cases and 48% for culture-positive sepsis cases. Of Acinetobacter spp. isolates 38% were resistant to ES cephalosporins, 78% of carbapenems, and 82% were multidrug-resistant (MDR) cases. Of all Klebsiella spp. isolates, 62% were resistant to ES cephalosporins, 35% were resistant to carbapenems, and 54% were MDR cases. Among 137 isolated Escherichia coli cases, 47% were resistant to ES cephalosporins, 15% were resistant to carbapenems, and 38% were MDR. Among the Pseudomonas spp. cases, 47% were resistant to ES cephalosporins, 31% were resistant to carbapenems, and 19% were MDR. Among 44 Enterobacter spp. isolates, 45% were ES cephalosporins, 20% were found resistant to carbapenem, and 50% were MDR. While among 150 isolated CONS, 61% were MRSA and none of them were vancomycin resistant. Among other Staphylococcus aureus cases, 38% were resistant to Methicillin, and again no vancomycin-resistant case was found among them. Among the Enterococcus spp, (n=56), 79% were Methicillin-resistant and 27% were vancomycin-resistant.[17]

Review of previous research:

In 2018, **Kurma VR et al.**[18] conducted a study at Government General Hospital, Guntur Medical College, involving 1,314 neonates admitted to the NICU during the study period. Of these, 519 neonates were suspected of having clinical septicaemia. Respiratory distress (31.2%) was the major presenting symptom, followed by the refusal of feeds (23.7%), prematurity (16%) and neonatal seizures (14.3%). Early onset sepsis (EOS) was diagnosed in 285 neonates (54.9%), while late-onset sepsis (LOS) occurred in 234 neonates (45.1%). Blood cultures from the 519 suspected cases revealed a positive result in 183 neonates (35.2%).

Gram-negative bacteria were identified as the leading cause of septicaemia, accounting for 68.3% of cases, while Gram-positive bacteria were responsible for 31.7%. The most frequently isolated microorganism was *Klebsiella pneumoniae* (34.7%), followed by *Acinetobacter* species (9.83%), *Pseudomonas aeruginosa* (9.23%), and *Escherichia coli* (8.10%). Among Gram-positive organisms, *Staphylococcus aureus* was the most common (21.8%), followed by coagulase-negative staphylococci (9.83%). In terms of antibiotic sensitivity, Gram-negative organisms showed high susceptibility to imipenem (84%), followed by piperacillin-tazobactam (45.3%), amikacin (36.8%), and ciprofloxacin (36.4%). For Gram-positive organisms, the most effective antibiotics were vancomycin (84.4%), teicoplanin (62.7%), and imipenem (41.6%). Both Gram- positive and Gram-negative bacteria showed significant resistance to ampicillin and ceftriaxone.

Additionally, the study found that preterm infants and those with low birth weight were more likely to develop septicaemia compared to their full-term counterparts.

A retrospective study by **Ibrahim et al.**[19] analysed data from the Ministry of Health's electronic database in the West Bank between 2019 and 2021. The study focused on neonates admitted to the Neonatal Intensive Care Unit (NICU) with suspected sepsis. Out of 6,090 suspected neonatal sepsis (NS) episodes, blood cultures identified pathogenic organisms in 884 samples. After excluding contaminants, 659 confirmed cases of neonatal sepsis were recorded. Among these, 545 were classified as primary infections. The prevalence of culture-proven neonatal sepsis was 12 per 1,000 live births. Of the primary infection cases, 26.6% were preterm neonates, and 122 (22.4%) neonates died due to NS. Late-onset sepsis (LOS) accounted for the majority of cases (70.6%), while 29.4% were early-onset sepsis (EOS). Among the culture-positive cases, 2.4% showed growth of yeast, while most (63.5%) were positive for gram-positive bacteria. Coagulase-negative staphylococci (CoNS) were the most prevalent gram- positive organisms, accounting for 49.2% of cultures, and 69.1% of these CoNS strains were multidrug-resistant organisms (MDRO).

For the gram-negative bacteria, *Klebsiella* spp. was the most commonly isolated pathogen (18.1%), followed by *Escherichia coli* (6.4%) and *Acinetobacter* spp. (4.4%). In total, the most common pathogens isolated from blood cultures were CoNS (49.2%), *Klebsiella* spp. (18.1%), and *Streptococcus* spp. (7.9%). Notably, 29% of the positive samples were identified as MDROs.

Gram-positive bacteria were significantly more likely to cause LOS (61.7%) and EOS (72.9%). In contrast, gram-negative bacteria had a higher likelihood of being MDROs compared to gram-positive bacteria (odds ratio [OR] = 38.17, $P < 0.001$). EOS cases had a 66.1% lower chance of being associated with MDROs in blood cultures compared to LOS (OR = 0.339, P = 0.048). Furthermore, gram-negative bacteria were found to be 1,097 times more likely to be MDROs than gram-positive bacteria (OR = 1,097.7, P

< 0.001). Regarding antibiotic susceptibility, gram-negative bacteria exhibited high sensitivity to amikacin (63%), meropenem (70%), piperacillin-tazobactam (65.6%), and colistin (100%). However, they were less sensitive to ampicillin (7.1%), cefotaxime (21.8%), and ceftazidime (29%). In contrast, gram-positive

bacteria showed high sensitivity to vancomycin (99.8%), and 81.4% of gram-positive organisms, excluding *Staphylococcus* spp., were susceptible to ampicillin.

Deress et al.[20] conducted a cross-sectional study at a tertiary care hospital in Ethiopia, recruiting 1,236 neonates admitted with suspected neonatal sepsis (NS) between January 2019 and December 2021. Blood cultures were positive in 314 neonates (25.4%). Of the isolates, 23% were bacterial pathogens, with gram-negative bacteria being more prevalent (75.3%, 214 cases) compared to gram-positive bacteria (24.7%, 70 cases). The most frequently isolated pathogens included *Klebsiella pneumoniae* (38.7%), followed by *Staphylococcus aureus* (13%), *Acinetobacter* spp. (8.1%), *Escherichia coli* (6.3%), and non-fermenting gram-negative rods (6%). The study also revealed significant antimicrobial resistance, particularly among *Coagulase-negative staphylococci* (CoNS), *S. aureus*, and *Enterococcus* spp. CoNS showed complete resistance (100%) to penicillin, oxacillin, and gentamicin. *S. aureus* demonstrated high resistance, with 94.6% of isolates resistant to penicillin, 64.9% to oxacillin, and 40.5% to gentamicin. *Enterococcus* spp. exhibited 85.7% resistance to penicillin, 57% to vancomycin, and 71.4% to chloramphenicol. Among the gram-negative bacteria, *K. pneumoniae* showed a concerning resistance profile, with high rates of resistance to meropenem (88.1%), ceftazidime (83.6%), ceftriaxone (83.6%), and amoxicillin- clavulanate (69%). *Acinetobacter* spp. exhibited moderate resistance to ceftriaxone (52.2%), ceftazidime (47.8%), and amoxicillin-clavulanate (47.8%), but remained largely susceptible to meropenem (13%) and ciprofloxacin (21.7%). Multidrug resistance (MDR) was observed in 61.6% of the isolated organisms. *K. pneumoniae* had the highest MDR rate (90.9%), while *Klebsiella ozaenae*, *Klebsiella oxytoca*, and *Proteus mirabilis* exhibited complete MDR (100%). *S. aureus* (40.5%), *Acinetobacter* spp. (43.5%), *E. coli* (61.1%), and non-fermenting gram-negative rods (41.2%) also showed high MDR rates. However, *Enterococcus* spp., *Streptococcus viridans*, *Streptococcus agalactiae*, CoNS, *Proteus stuartii*, and *Streptococcus pyogenes* did not display any multidrug resistance.

In 2016, **Jatsho et al.**[21] conducted a prospective study at a tertiary care hospital in Bhutan, involving 321 neonates with suspected clinical sepsis. Of the 314 neonates suspected of sepsis, 44 had positive blood cultures, resulting in a blood culture positivity rate of 14%. Among these culture-positive cases, 24 (54.5%) were early-onset sepsis (EOS), while 20 (44.5%) were late-onset sepsis (LOS). Clinical features significantly associated with culture-positive sepsis (CPS) compared to culture- negative cases included seizures, respiratory distress, bulging fontanels, hypothermia, and neonatal jaundice. Risk factors for culture-positive EOS were identified as prematurity, low birth weight, low APGAR scores at 1 and 5 minutes, and maternal intrapartum antibiotic use ($p < 0.05$). For LOS, preterm birth, low birth weight, and the use of total parenteral nutrition (TPN) were statistically significant risk factors. Gram- negative organisms (64.6%) were more commonly isolated than gram-positive organisms. The most frequent gram-negative pathogen was *Klebsiella pneumoniae*, followed by *Acinetobacter* spp. and *Escherichia coli*. Over 90% of *K. pneumoniae* isolates were resistant to third-generation cephalosporins but remained sensitive to carbapenems. Around 89% of *Acinetobacter* isolates were carbapenem-resistant (CRAB), although they were 100% sensitive to polymyxin-B.

Among all culture-positive neonates, 20.5% died, and gram-negative bacteria, especially *Klebsiella pneumoniae spp.* was identified as the primary cause.

In 2015, **Yadav et al.**[22] conducted a study at Kanti Children's Hospital in Kathmandu, Nepal, involving 350 neonates with clinically suspected neonatal sepsis. The overall incidence of neonatal sepsis was 16.9% among the 350 blood samples included in the study. Among the culture-positive cases, the bacteriological profile revealed that 27 (46%) were gram-positive cocci and 32 (54%) were gram-negative bacilli. The most commonly isolated bacterial strains were *Staphylococcus aureus* (35.6%), followed by *Klebsiella pneumoniae* (15.3%), *Acinetobacter* spp. (11.9%), *Enterobacter* spp. (10.2%), coagulase-negative staphylococci (CoNS) (10.2%), *Pseudomonas aeruginosa* (6.8%), *Escherichia coli* (6.8%), *Citrobacter* spp. (1.7%), and *Salmonella typhi* (1.7%).

Significant risk factors associated with neonatal sepsis included neonates under 3 days of age (71.2%), low birth weight (62.7%), preterm birth (31.4%), and caesarean delivery (63.3%) ($p < 0.05$). Antibiotic susceptibility testing showed that *S. aureus* was most sensitive to gentamicin (90%) and ofloxacin (90%), while it exhibited the highest resistance to ampicillin (76%). Among *K. pneumoniae* isolates, amikacin, gentamicin, meropenem, and imipenem demonstrated 100% sensitivity, whereas ampicillin, cefotaxime, and ceftazidime showed 100% resistance. Piperacillin was the most effective antibiotic against all *Acinetobacter* and *P. aeruginosa* isolates. However, ampicillin was 100% resistant against *Acinetobacter* strains. All CoNS strains were sensitive to amikacin and gentamicin but showed the highest resistance to ampicillin and ciprofloxacin (83% each).

Gamit et al.[23] conducted a study at Dhiraj Hospital, Gujarat, from June to December 2019, involving 228 neonates suspected of having neonatal septicaemia and admitted to the NICU. The study aimed to identify the bacteriological profile and antibiotic susceptibility patterns in these neonates. Out of the 228 clinically suspected cases of neonatal sepsis, 75 (32.89%) had positive blood cultures. Early-onset sepsis (EOS) was more prevalent (48 cases, 64%) compared to late-onset sepsis (LOS) (27 cases, 36%). Among the isolated pathogens, gram-positive bacteria (n=46) were more frequently identified than gram-negative bacteria (n=29). Coagulase-negative staphylococci (CoNS) were found in 46.67% of the positive blood cultures (35 out of 75), followed by gram-negative *Klebsiella* spp. in 17.33% (13 out of 75), *Acinetobacter* spp. in 16% (12 out of 75), and *Staphylococcus aureus* in 14.67% (11 out of 75).

An important finding of the study was the antibiotic susceptibility pattern. Gram- positive bacteria were most sensitive to vancomycin and linezolid, followed by ciprofloxacin and gentamicin. Nearly all gram-positive isolates showed resistance to penicillin. As for gram-negative bacteria, they demonstrated sensitivity to tigecycline, imipenem, and piperacillin/tazobactam, with ciprofloxacin and amikacin also showing effectiveness. However, all gram-negative bacteria were resistant to ampicillin and co- trimoxazole.

In their study conducted at a rural hospital in North India, **Thakur et al.**[24] reported a 42% culture positivity rate among 450 neonates admitted to the NICU with clinically suspected neonatal septicaemia. Among the culture-positive cases, 92 (49%) were diagnosed with early-onset sepsis (EOS) and 96 (51%) with late-onset sepsis (LOS). The study found a predominance of gram-positive organisms (60%) over gram-negative isolates (40%). The most common pathogens responsible for neonatal sepsis were *Staphylococcus aureus* (40%), coagulase-negative staphylococci (16%), non- fermenting group organisms (NFGOs) (15%), and *Klebsiella pneumoniae* (10%). Major risk factors associated with neonatal sepsis included the use of nasal cannulas (54%), birth asphyxia (48%), and prematurity (38%).

The study also highlighted significant antimicrobial resistance patterns. Gram-positive organisms showed high resistance to penicillin (87%), while gram-negative isolates exhibited notable resistance to third-generation cephalosporins (53–89%) and aminoglycosides (50–67%). Among the *S. aureus* isolates, 41% were methicillin- resistant, and 48% of gram-negative isolates produced extended-spectrum beta- lactamases (ESBL).

Bhat M et al.[25]**,** in their study among 2520 neonates admitted to the NICU in SGMS hospital, North India from 2014 through 2015, over one year identified 89 clinically suspected septicaemia, resulting in an incidence rate of 35.3 per 1,000 neonatal admissions. Among the 89 clinically suspected cases with positive screening tests for neonatal sepsis, 48.31% were confirmed as culture-positive cases. *Klebsiella* was identified as the most prevalent pathogen, accounting for 34.88% of the culture-positive cases, followed by *Staphylococcus aureus* (32.5%) and *Escherichia coli* (9.30%). Klebsiella was the commonest organism isolated in both early onsets (32%) and late- onset sepsis (38.9%) followed by Staphylococcus aureus (32% EOS and 33.3% LOS respectively). The majority of the isolated organisms exhibited resistance to commonly used antibiotics, including ampicillin, cloxacillin, and ceftriaxone. Aminoglycosides were found to be more effective against gram-negative organisms, while vancomycin proved to be the most effective treatment for *Staphylococcus aureus*.

Another study by **Zakariya BP et al.**[26] aimed to identify the bacteriological profile of neonatal sepsis and their antibiotic sensitivity patterns, and showed that among 120 clinically suspected cases of neonatal sepsis, 50 (41.6%) were confirmed by positive blood cultures. Besides, among 120 clinically suspected cases of neonatal sepsis, 69 (57.5%) were early-onset and 51 (42.5%) were late-onset sepsis. Gram-negative bacteria were more prevalent isolates, accounting for 82% of all CPS. *Klebsiella pneumoniae* (66%) had been identified as the most common among all gram-negative isolates and significantly associated with more EOS than LOS. Among the gram- positive isolates, Coagulase-negative staphylococci (12%) were the most common gram-positive agent. The 33 *Klebsiella pneumoniae* isolates were fully susceptible to meropenem (100%) and mostly to amikacin (82%). However, they showed varying levels of susceptibility to chloramphenicol (24%), ciprofloxacin (18%), ceftriaxone (3%), and ceftazidime (3%). None of the isolates were susceptible to gentamicin. Thirty-two percent of *Klebsiella pneumoniae* were ESBL producers.

A prospective study was conducted by **Jaybhay D et al.**[27] from 2021 to 2022 in the NICU, Mahatma Gandhi Mission Medical College and Hospital Aurangabad Maharashtra. Of total admissions, 45% were diagnosed with proven sepsis or probable sepsis. Out of the 162 proven sepsis cases, 91 cases (56.9%) were early onset and 71 cases (43%) were late onset. One neonate died from sepsis. Klebsiella spp. was the commonest isolated bacteria from blood cultures (36%), followed by Enterococci (23.6%), Staphylococcus aureus (19.8%), and Acinetobacter spp. (6.8%). Eos Cases were treated with an average of 4.27 antibiotics, with 93.1% receiving piperacillin and tazobactam 87.3% receiving a meropenem, and 52.4% receiving cefotaxime, while the LOS received an average of 3.15% antibiotics, with 80.1% receiving piperacillin and tazobactam,

79% receiving, meropenem, 54% cefotaxime and 12% colistin and 54% amikacin.

Oo NAT et al.[28] conducted a study in two tertiary care hospitals in Myanmar over two years (2017 to 2019) among 1705 neonates admitted to NICU with suspected neonatal sepsis. A majority of them (n=1235, 72.4%) belonged to the age group of <3 days age on admission and were males (1008, 59.1%). Among 1705 neonates, blood cultures were performed for 1615 neonates. Bacteriologically confirmed sepsis was detected in 672 neonates accounting for 41.6% of cases. Gram-negative organisms (62.6%) in blood cultures are more commonly isolated than gram-positive organisms (37.4%). EOS was observed in 43.7% of culture culture-positive sepsis cases, while LOS was in 56.3% of cases. Overall, the most commonly isolated pathogen from blood cultures was CoNS (both Staphylococcus epidermidis and CoNS others-22.2 %) and Klebsiella pneumoniae (13.4%), followed by Staphylococcus aureus (10.9 %). The predominant Gram-positive pathogens included S. aureus, S. epidermidis, and CoNS (others). The most common Gram-negative pathogens were K. pneumoniae, Acinetobacter spp., Serratia marcescens, and Enterobacter spp. Among isolated pathogens, Klebsiella spp. was found in 16.1% of cases. Gram-negative organisms were more prevalent in LOS than in EOS (58% vs 42%), while in EOS both gram-positive & gram-negative organisms were equally distributed (53.4% vs 46.6%).

Among neonates with confirmed sepsis, 496/672 (73.8%) were resistant to at least one first-line antibiotic (ampicillin, amikacin, gentamicin, or cefotaxime). Neonates with MDR organisms were 395 in number i.e., accounting for 58.8%. No difference was observed in neonatal mortality between antibiotic-sensitive and resistance cases (16.5% vs 16.8%). While analysing the risk factors associated with neonatal septicemia with positive blood cultures, it was identified that neonates who were born by emergency caesarean section had higher odds of having sepsis than those born by normal delivery [aPR: 1.2 (95%CI:1.1-1.4)]. Late-onset sepsis was more likely to have positive blood cultures than early-onset sepsis [aPR: 1.2 (95% CI: 1.1-1.4; p=0.008]. However, no significant difference was observed between home and institutional delivery. Resistance to first-line antibiotics was significantly higher for gram-negative organisms than gram-positive (79.6% vs 64.1%, p<0.001). Among Gram-positive isolates, 49% (123/251) were MDR, whereas 65% (272/421) of Gram-negative isolates were MDR. The case fatality rate of culture-positive sepsis caused by MDR isolates was higher than that of culture-positive sepsis caused by non-MDR isolates (65% versus 35%).

Mohakud et al.[29] conducted an observational study in special newborn care units (SNCUs) of Capital Hospital in Bhubaneswar, Odisha from May 2017 to October 2019. All neonates admitted to SNCU with blood culture-positive sepsis without any congenital malformations were included in this study. Among 445 neonates who had signs of clinical sepsis, organisms were isolated from blood cultures of 115 cases accounting for 25%. Among the culture-positive sepsis cases, the majority (72.2%) were LOS, while 27.8% were EOS. The most common presenting symptom among culture-positive neonatal sepsis was feeding intolerance (33.9%). Preterm and low birth weight neonates showed more culture-positive sepsis than their counterparts. Gram-positive organisms (60%) were prevalent in this study. Among isolated organisms, 42 (35.6%) cases were S. aureus followed by Coagulase negative Staphylococcus (CONS) (20.8%), E. coli (19.1%), K. pneumoniae (10.4%), Acinetobacter baumannii (2.7%), Enterobacter spp. (4.3%), Enterococcus spp. (4.3%), and Pseudomonas aeruginosa (2.7%). S. aureus was the most common organism isolated in both EOS and LOS. Gram-negative organisms showed resistance to ampicillin, while 68% of cases exhibited resistance to cephalosporins. Among carbapenem-resistant Gram-negative bacilli, 80% were sensitive to colistin. Extended-spectrum beta-lactamase (ESBL)- producing bacteria were identified in 40% of neonates, whereas multidrug-resistant (MDR) strains were detected in 16 cases (35.2%). All Gram-positive organisms are sensitive to vancomycin whereas resistance to penicillin was seen in 92.3% of cases. The MRSA-producing strains were isolated in 13 (18%) neonates.

A retrospective study conducted by **Dalal P et al.**[30] from July 2010 to September 2013 at a tertiary care teaching hospital in North India aimed to determine the bacteriological profile of neonatal sepsis and the patterns of antibiotic sensitivity. Out of 28,927 neonates born during the study period, blood cultures tested positive for 336 neonates, indicating an incidence of neonatal sepsis of 11.62 per 1,000 live births. Gram-negative organisms were identified in 81.8% of the cases, while gram-positive organisms were found in 18.82%. Pseudomonas aeruginosa emerged as the most common organism in both early-onset sepsis (EOS) (43.82%) and late-onset sepsis (LOS) cases (51.35%). The incidence of gram-positive sepsis was higher in late-onset sepsis (21.89%) compared to early-onset sepsis (15.7%). Staphylococcus aureus was the most prevalent gram-positive pathogen in both early-onset sepsis (7.3%) and late-onset sepsis (17.41%). The study found that carbapenems (92%) and piperacillin-tazobactam (90%) were the most effective antimicrobials against gram-negative organisms. Among aminoglycosides, gentamicin and amikacin showed moderate sensitivity, with amikacin being more effective against E. coli (70%) and Klebsiella (73%) compared to Pseudomonas (66%) and Acinetobacter (67%). There

was widespread resistance to cephalosporins, ciprofloxacin, and aztreonam, though ciprofloxacin showed better sensitivity to E. coli (75%) and Klebsiella (73%). Most gram-positive organisms showed high sensitivity to linezolid (90%) and vancomycin (78%). However, 50% of the enterococci (4 out of 8) were resistant to vancomycin. Resistance to amoxyclav (70%) and penicillin (85%) was also frequently observed.

Gupta et al.[31] conducted a retrospective study at a tertiary care hospital in India from July 2017 to July 2018. The study analysed blood culture reports from 452 neonates admitted with suspected neonatal sepsis. Among these 452 neonates, 138 (30.54%) showed growth of pathogenic organisms in their blood cultures. Gram-positive infections were observed in 28.26% of the cases, while gram-negative organisms were identified in 71.74% of blood culture-positive samples. The most prevalent gram- positive organism was Staphylococcus aureus (64.10%), followed by Coagulase- negative Staphylococcus (30.76%) and Enterococcus faecalis (5.12%). Among the gram-negative organisms, the most commonly isolated pathogen was Klebsiella pneumoniae (55.55%), followed by Citrobacter freundii (23.23%), Escherichia coli (14.14%), Pseudomonas aeruginosa (6.06%), and Acinetobacter baumannii (1.01%). The isolated gram-positive bacteria were mostly sensitive to vancomycin and linezolid, but showed significant resistance to ampicillin (80-100%). Of the Staphylococcus aureus isolates, 32% were methicillin-resistant (MRSA). Regarding gram-negative bacteria, susceptibility to imipenem was 60-100%, to levofloxacin was 70-100%, and to third-generation cephalosporins was 80-100%. The study also identified key risk factors for neonatal mortality, including prematurity (32%), sepsis (19%), and birth asphyxia (16%).

Minarey et al.[32] conducted a retrospective study at a tertiary care hospital in Central India, focusing on neonatal sepsis cases from February 2018 to February 2019. Among the 223 newborns included in the study, 91 were inborn, while the remaining 132 were born at other healthcare facilities, such as primary health centers, community health centers, district hospitals, or even en route to the hospital. A total of 120 blood cultures tested positive, yielding a confirmed neonatal sepsis prevalence of 53.8%. The most commonly isolated organism was Staphylococcus aureus (39.3%), followed by Klebsiella pneumoniae (34%) and Escherichia coli (15%). Other pathogens identified in smaller numbers included pathogenic Streptococci (3.4%), coagulase-negative staphylococci (3.1%), Pseudomonas spp. (1.2%), Acinetobacter spp. (1%), and Enterobacter spp. (0.5%). Candida albicans was also isolated in 1% of cases. The two most significant contributors to mortality in this study were sepsis (32.23%) and prematurity (18.83%).

Rajyaguru et al.[33] conducted a cross-sectional study from December 2020 to September 2021 to identify the bacteriological profile and antibiotic susceptibility patterns among 103 blood culture-proven neonatal sepsis cases at a tertiary care hospital in Vadodara. Among these cases, 53.4% were male, 73.4% were preterm, 61.2% had low birth weight, and 51.5% were delivered via normal vaginal delivery. Coagulase-negative Staphylococcus (CONS), specifically methicillin-resistant strains, was identified as the most prevalent cause of neonatal sepsis (28.15%). The isolated gram- positive organisms showed the highest sensitivity to vancomycin, followed by linezolid, while gram-negative organisms demonstrated maximum sensitivity to piperacillin/tazobactam, followed by meropenem.

Reddy et al.[34] conducted a study at the NICU of Malla Reddy Medical College for Women in Hyderabad over one year, from January 2013 to December 2014. Using consecutive sampling, they recruited 100 neonates admitted with suspected neonatal sepsis. Among the 100 cases, 35 (35%) were confirmed by blood culture. Of these 35 confirmed neonatal sepsis (NS) cases, 19 (54%) were classified as early-onset sepsis, and the remaining 16 (46%) were late-onset cases. The majority of isolated organisms were gram-positive bacteria (20, 57.14%), while 15 (42.85%) were gram-negative bacteria. Among the gram-positive organisms, 9 (45%) were *Coagulase-negative Staphylococcus* (CONS), 9 (45%) were *Staphylococcus aureus*, and 2 (10%) were *Streptococci* spp. Among the 15 isolated gram-negative organisms, *Klebsiella pneumoniae* was the most common (8, 52%), followed by *Escherichia coli* (3, 20%), *Pseudomonas aeruginosa* (3, 20%), and *Acinetobacter baumannii* (1, 8%).

The antibiotic susceptibility of the isolates was as follows:

- *Coagulase-negative Staphylococcus* was sensitive to 78% ampicillin, 75% amoxiclav, 90% ciprofloxacin, 87% ceftriaxone, 76% cefotaxime, 80% piperacillin, and 100% vancomycin.
- *Staphylococcus aureus* was sensitive to 59% ampicillin, 85% amoxicillin, 60% ciprofloxacin, 75% ceftriaxone, 76% cefotaxime, 80% piperacillin, and 100% vancomycin.
- *Streptococci* spp. was sensitive to 54% ampicillin, 66% amoxiclav, 87% ciprofloxacin, 67% ceftriaxone, 76% cefotaxime, 85% piperacillin, and 100% vancomycin.
- *Klebsiella* spp. was sensitive to 67% amoxiclav, 64% ciprofloxacin, 31% ceftriaxone, 33% cefotaxime, 75% cefoperazone, 33% piperacillin, 100% imipenem, and 14% gentamicin.

- *Escherichia coli* was sensitive to 78% amoxiclav, 66% ciprofloxacin, 44% ceftriaxone, 44% cefotaxime, 65% cefoperazone, 35% gentamicin, 56% piperacillin, and 100% imipenem.
- *Pseudomonas* spp. was sensitive to 80% amoxiclav, 86% ciprofloxacin, 72% ceftriaxone, 75% cefotaxime, 80% cefoperazone, 20% gentamicin, 80% piperacillin, and 100% imipenem.
- *Acinetobacter* spp. was sensitive to ceftriaxone, cefotaxime, cefoperazone, piperacillin, and imipenem.
- **Gap in existing status:** there are few existing literatures on this topic in West Bengal but there is a lack of such studies in this hospital setting. The lack of local data on the prevailing bacterial pathogens causing neonatal sepsis, their antibiotic resistance patterns, and the associated clinical outcomes hinders physicians in choosing appropriate antibiotic therapies. These gaps in knowledge necessitate conducting a study to address these unresolved issues and provide valuable insights into the management of neonatal sepsis in this hospital setting.

III. Materials And Methods:

Type of study: Institution-based observational study

Study design: cross-sectional study.

Study setting: The study has been done in the College of Medicine and JNM Hospital, WBUHS, Kalyani, Nadia District.

After approval of the synopsis, the study has been started. Data were collected in the first 12 months. The next 12 months of data analysis were done and after the presentation of the result, the thesis was written.

Place of study: The present study was conducted in the Dept of Microbiology, College of Medicine & J.N.M Hospital WBUHS, Kalyani.

Period of study: 2 years approximately.

Sample size/design: 261.87 ≈ 262

(By using the formula $N = \frac{Z^2PQ}{d^2}$)

Where P= 21.79% prevalence of blood culture-positive neonatal sepsis taken from another study (Paulami Dutta et al.)[35]

d= margin of error taken as 5%, Q= 100-P,

Z= 1.96 using 95 % Confidence interval

Sampling procedure: There were a total of twenty-five beds in SNCU, at College of Medicine and JNM Hospital, Kalyani. Every day nearly 6-10 neonates get admitted to the SNCU. Excluding the Sundays and holidays, in one year of the data collection period, we get nearly 250 days. Thus, on average, one to two patients were needed to be recruited after the application of predetermined inclusion and exclusion criteria. Every day simple random sampling technique using the lottery method was applied in this study to recruit study participants. Every day in the one year of data collection, among all the new admissions in SNCU, the cases of possible sepsis were identified. Then among them, one patient was selected by lottery technique. On the next day, among the cases of possible sepsis excluding the already recruited neonate, a lottery method was applied to recruit and this process was followed till the required sample size, i.e., 262 neonates were selected.

Data duplication was prevented as a unique identification number was given to every patient included in the study.

Inclusion criteria:

i) All newborns from age 0 to 28 days.

ii) All the clinically suspected neonatal sepsis cases.

Exclusion criteria: Neonates with the following were excluded

1. Patients whose antibiotic therapy has been started.
2. Respiratory-distress syndrome caused by pneumothorax, hyaline membrane disease, and atelectasis.
3. Primary gastrointestinal disease such as an anatomical obstruction.
4. Hematologic diseases e.g., Iso-immune haemolytic disease, red cell enzyme defects, and congenital leukaemia.
5. Recognised major congenital anomalies.

6. Central nervous system injury.

Study variables:

a. Age
b. Sex
c. Duration of hospital stay
d. Birth history (gestational age, birth weight, mode of delivery, place of delivery, birth asphyxia, history of invasive procedure, mechanical ventilation)
e. Signs and Symptoms of sepsis (lethargy, poor feeding, respiratory distress, temperature instability, convulsions, diarrhoea or vomiting)
f. Bacteria found
g. Extended spectrum beta lactamase (ESBL)/ Methicillin resistant Staphylococcus (MRS)
h. AST
i. Condition at the time of discharge (death/recovered)

Data collection procedure:

The neonates who met the inclusion criteria were selected for the study. Data were collected in a pre-designed proforma after obtaining due consent from parents.

Standard operating procedure for blood sample collection:

- Trained doctors in the SNCU collected blood from the selected neonate for blood culture under strict aseptic conditions before administering antibiotics.
- The local site from which the blood was drawn was cleaned with povidone-iodine 1% and washed with 70% alcohol.
- 1 ml of blood was collected and inoculated into a blood culture bottle for the Paediatrics age group (BacT/ALERT PF Plus bottle). **FIG-1**

Sample processing:

- The inoculated bottles were immediately brought to the microbiology department for further processing.
- The blood culture bottles were then put in BacT/ALERT (bioMérieux) machine.

FIG2

- All the blood culture bottles flagged positive were sub-cultured on Blood Agar & McConkey Agar media (HiMedia Laboratories Pvt. Limited).
- Isolates were identified by conventional methods (colony morphology, gram staining, and standard biochemical tests like catalase, coagulase, oxidase, urease, indole formation, triple sugar iron test, and citrate utilization test)- in case of any problem, the help of VITEK-2 was taken.
- Subsequently, Antibiotic sensitivity testing was done by the Kirby-Bauer disc diffusion method following antibiotic disks (Hi Media Laboratories Pvt. Ltd. Mumbai, India) as recommended in the CLSI guidelines M100-Ed34. MRSA and ESBL were detected phenotypically as per CLSI guidelines. Also, VITEK-2 was used.
- The blood culture was considered negative if no growth occurred after 7days of incubation.

Laboratory investigations, parameters and procedures:

I. Blood culture by BacT/ALERT machine. (If there is growth then the machine will alert about it)
II. Blood subculture in MacConkey Agar, Blood Agar. (Different organisms will show different culture characteristics)
III. Gram staining. (Will show if gram-positive [violet colour] or gram- negative [pink colour] bacteria, along with morphology i.e., cocci, bacilli, coccobacilli)
IV. Biochemical tests like Indole, Citrate, Urease, TSI, Oxidase, Catalase and Coagulase. (Different bacteria will give respective biochemical test results)
V. Antibiotic Susceptibility Testing by Kirby-Bauer Disk Diffusion method according to CLSI guidelines. (Zone size will be measured and recorded according to CLSI guidelines)
VI. If needed identification and AST by VITEK 2 system. **FIG-3 METHOD OF GRAM STAINING**

- Slides are made grease-free by passing it 6-12 times through a Bunsen burner.
- Slides were flooded with crystal violet and allowed to act for 30 sec.
- Tip of the stain and the tilled slide were flooded with iodine solution and allowed to act for 30 sec.
- Tilt the slide and wash the iodine with 95% alcohol and allow it to act for 2sec before washing it with

water from the tap.
- Next the slide was flooded with counter stain Safranin allowed to act for a minute.
- And then it was washed with water and dried.
- Observed under the microscope.

Biochemical Tests:
Catalase Test: Excluding the Streptococci, Enterococci, and a few other genera, most aerobic and facultative bacteria possess catalase activity Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism. Catalase converts hydrogen peroxide into oxygen and water. The catalase test is most commonly used to differentiate members of the Micrococococcaceae, which are positive, from members of the Streptococcaceae.

Reagent: -
- Hydrogen peroxide 3% stored in a brown bottle under refrigeration.
- An 18-24 hours culture of the organism to be tested.

Quality Control:
The hydrogen peroxide reagent must be tested with positive and negative control organisms each day or immediately before unknown bacteria are tested.
- Positive control: *Staphylococcus aureus*
- Negative control: *Streptococcus species.*

Procedure:
With an inoculating needle or a wooden applicator stick growth from the center of a colony is transferred to the surface of a glass slide.
One drop of 3% hydrogen peroxide is added and observed for bubble formation.

Interpretation: The rapid and sustained appearance of bubbles or effervescence constitutes a positive test.
Tube Coagulase Test: detects free coagulase of S. aureus. A small amount of the colony growth of the organism was emulsified in a tube containing 0.5ml of coagulase plasma. The tube was incubated at 37^0c for 4 hours and observed for clot formation by gently tilting the tube. If no clot is observed at that time, the tube was re-incubated at room temperature and read again after 18 hours. The tube coagulase test is considered positive if any degree of clotting is noted. The tube should be gently tilted and not agitated, because this may disrupt partially formed clotted material.

Quality Control:
- Positive: *Staphylococcus aureus*
- Negative: *Staphylococcus epidermidis*

Slide Coagulase: A staphylococcal colony was emulsified in a drop of saline to form a smooth milky suspension. Similar suspensions were made with positive and negative control strains. To the suspension, a loopful of plasma was added. Coarse clumping of cocci visible to the naked eye within 10 seconds was considered positive and absence of clumping was negative.

Quality Control:
The coagulation of properties of the plasma may be tested, for quality control purposes, by adding one drop of 5%-calcium chloride to 0.5 ml of the reconstituted plasma. A clot should form within 10-15 seconds. A known Staphylococcus aureus strain and a Staphylococcus epidermidis strain serve as positive and negative control, respectively. Each reconstituted vial of plasma with EDTA should be tested with 18- 24 hours cultures of the control strain.

Indole Test: This is tested in a peptone water culture after 48 or 96 hours of incubation at 37^0C. This test demonstrates the production of indole from tryptophane. 0.5ml of Kovac's reagent was added to it and was shaken gently. A red colour indicates a positive reaction. **FIG 9**

Quality Control:
- **Positive:** *Escherichia coli*
- **Negative:** *Klebsiella pneumoniae*

Citrate Utilization Test:

Solid (Simmon's) or liquid (Koser's) media can be used. A bacterial colony is picked up by a straight wire & inoculated into solid media. Incubate for overnight at 37^0C. The media turning blue colour indicates a positive reaction. **FIG 9**

Urease Test:

This test is done in Christensen's urease medium. The slope was inoculated heavily and was incubated at 37^0C. The result was examined after four hours and after overnight incubation. Urease-positive culture produces a purple-pink colour. Urease-producing bacteria reduce urea to ammonia. Ammonia makes the medium alkaline and thus phenol red indicator turns pink/ red in colour. **FIG 9**

Triple Sugar Iron Medium:

A popular composite medium is the triple sugar iron (TSI) medium which indicates whether a bacterium ferments glucose only, or lactose and sucrose also, with or without gas formation, besides indicating H2S production as well. The medium is distributed in tubes, with a butt and slant. After inoculation, if the slant remains red and the butt become yellow, all the sugar-glucose, lactose, and sucrose are fermented. Bubbles in the butt indicate gas production and the blackening of the medium shows the formation of H2S. The TSI medium facilitates the preliminary identification of Gram-negative bacilli. **FIG 9**

K/A= (red/yellow) = only glucose fermented.

A/A= (yellow/yellow) = glucose, lactose, sucrose also fermented. K/K= (red/red) = no sugar fermented.

K=alkaline, A=acidic.

Identification of *Staphylococcus aureus:*

- **On nutrient agar:** after incubation for 24 hours, the colonies are large (2-4 μ in diameter), circular, convex, smooth, and shiny, and most strains produce golden-yellow pigment. **FIG 4**
- **On blood agar:** Colonies were surrounded by a narrow zone of beta haemolysis.
- **On MacConkey agar:** they produce smaller colonies that appear pink due to lactose fermentation.
- **Catalase:** Positive **FIG 5**
- **Coagulase:** Positive **FIG 6**

In cases where Coagulase Negative Staphylococci (CoNS) were isolated from a blood culture, the initial isolate was interpreted as a possible skin contaminant. A repeat blood culture sample was requested for confirmation. If the second sample also yielded CoNS the organism was reported as a bloodstream infection isolate, considering its clinical significance.

Identification of *Klebsiella pneumoniae*

- **Nutrient agar:** Large dome-shaped, mucoid colonies
- **Blood agar:** large, greyish, white, mucoid colonies

- **MacConkey agar:** Lactose fermenting mucoid colonies **FIG 7**

Species identification was confirmed by biochemical reactions as mentioned below.

Biochemical reactions:

Indole	Negative
Citrate	Utilized
TSI	Acid / Acid with gas no H_2S
Urease	Positive

Identification of *Escherichia coli*:

- **Nutrient agar:** Colonies were 1-3 mm in diameter, circular, low convex, smooth with no pigmentation and colour
- **Blood agar:** Greyish-white colonies with haemolysis
- **MacConkey agar:** Flat and lactose fermenting colonies **FIG 8**

Biochemical reactions:

Indole	Positive
Citrate	Not utilized
TSI	Acid / Acid with gas no H_2S
Urease	Negative

Identification of *Pseudomonas aeruginosa*:

- **Nutrient agar:** Colonies were large, low convex with serrated margins, with bluish green pigmentation and earthy odour.
- **Blood agar:** Diffuse haemolysis was observed
- **MacConkey agar:** Non-lactose fermenting colonies with pigmentation

Biochemical reactions:

Indole	Negative
Citrate	Utilized
TSI	Alkali / no change in the butt
Oxidase test	Positive (FIG 10)
Urease	Positive

Identification of *Acinetobacter baumannii*:
Blood agar: Smooth, opaque, raised, creamy colonies
MacConkey agar: Non-lactose fermenting colonies. **FIG 11**

Species were further confirmed by VITEK 2.

Identification of *Burkholderia cepacia*:
Blood agar: colonies having a round shape with a white, rounded shape, smooth edges, and convex elevation.
MacConkey agar: colonies were opaque with rounded shape, smooth edges, and convex elevation.
Gram stain: Gram negative bacilli (rod) shapes with pink color

VITEK-2 was used for further identification as well as antibiotic susceptibility assay.

Identification of *Serratia marcescens*:
Nutrient agar: smooth, circular, and raised non-lactose fermenting colonies of around 2 to 4 mm. when grown at 20-30 deg C, red pigmentation was observed after overnight (24 hours) incubation.
MacConkey agar: produces smooth, raised, circular (2 to 4 mm), and non-lactose fermenting pink to red colonies.
Blood agar: produces beta-hemolytic with narrow zone of hemolysis, medium-sized (2 to 4 mm) circular greyish colonies.

Identification of Group B streptococcus.
Blood agar: Small to medium sized grayish white colonies produces narrow beta hemolysis.
Catalyst test: negative.
Bacitracin sensitivity: resistant.

Antibiotic susceptibility testing:

After isolating and identifying the organism their antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion technique. The test was performed on Muller Hinton agar using commercially available antibiotic discs. At least 3-5 colonies of the same morphological type are transferred into a tube containing 4-5 ml of nutrient broth and incubated for 2-6 hours at 37° C. Turbidity of the actively growing broth culture was adjusted so that it is optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing 1.5 X 10^8 CFU/ml. To perform this step properly, adequate light was needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines. A sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The dried surface of a Muller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The antimicrobial discs were dispensed onto the surface of the inoculated agar plate. The discs were evenly distributed so that they were not closer than 24mm from centre to centre. Ordinarily, not more than six discs were placed on a 100mm plate. The plates were incubated aerobically at 37 deg C for 16 to 18 hours.

After 16 to 18 hours of incubation, each plate was examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition would be uniformly circular and there would be a confluent lawn of growth. The diameter of the zones of complete inhibition was measured, including the diameter of the disc. The sizes of the zones of inhibition were interpreted by referring to the CLSI 2024 Ed34 Guidelines and reported as either susceptible, intermediate, or resistant to the agents that were tested (**FIG 12).** For

automated identification and AST, we used VITEK 2 -compact, Card no N280.

For Gram-negative organisms

Antibiotic Name	Disk Concentration (µg)
Amikacin (AK)	30 µg
Ampicillin (AMP)	10 µg
Ampicillin-Sulbactam (A/S)	20/10 µg
Cefepime (CPM)	30 µg
Cefoperazone-Sulbactam (CFS)	75/30 µg
Cefotaxime (CTX)	30 µg
Ceftriaxone (CTR)	30 µg
Ceftazidime (CAZ)	30 µg
Ciprofloxacin (CIP)	5 µg
Colistin (CL)	10 µg
Gentamicin (GEN)	10 µg
Imipenem (IPM)	10 µg
Levofloxacin (LE)	5 µg
Meropenem (MRP)	10 µg
Minocycline (MIN)	30 µg
Piperacillin-Tazobactam (PTZ)	100/10 µg
Tigecycline (TGC)	15 µg
Tobramycin (TOB)	10 µg
Trimethoprim-Sulfamethoxazole (COT)	1.25/23.75 µg

For Gram-positive organisms

Antibiotic Name	Disk Concentration (µg)
Ampicillin (AMP)	10 µg
Cefoxitin (CX)	30 µg
Cefepime (CPM)	30 µg
Cefotaxime (CTX)	30 µg
Ceftriaxone (CTR)	30 µg
Ciprofloxacin (CIP)	5 µg
Clindamycin (CD)	2 µg
Gentamicin (GEN)	10 µg
Levofloxacin (LE)	5 µg
Linezolid (LZ)	30 µg
Trimethoprim-Sulfamethoxazole (COT)	1.25/23.75 µg
Vancomycin (VA)	30 µg

Criteria for performing ESBL test:
Isolates resistant to 3rd generation cephalosporins:

Phenotyping confirmatory disc diffusion test:

Dried MHA plates were inoculated with 0.5 McFarland matched test microbial inoculums. On inoculated plate this was done by placing a disk of ceftazidime (30 µg) or cefotaxime (30 ug) alone and ceftazidime + clavulanic acid (30/10 µg) or on cefotaxime + clavulanic acid (30/10 µg) a Mueller-Hinton Agar plate at least 20 mm apart from each other. After overnight incubation plates were examined. A ≥ 5mm increase in a zone diameter for either antimicrobial tested in combination with clavulanate vs the zone diameter of the agent when tested alone which indicates the ESBL producers. (For Example- ceftazidime zone = 16; Ceftazidime- clavulanate zone = 21).

Methicillin-resistant Staphylococcus (MRSA):

MRS is defined by Cefoxitin or oxacillin testing, as appropriate to the species are considered resistant to other β-lactam agents, i.e., penicillins, β-lactam combination agents, cephems and carbapenems. The cefoxitin Disc Diffusion test are recommended all Staphylococci spp. Except for *S. pseudintermedius, S. schleiferi*, which requires Oxacillin disk diffusion test. The susceptibility testing for Cefoxitin are same as antibiotic susceptibility testing but for oxacillin susceptibility it requires 2% NaCl in Muller Hinton agar medium. The sensitive and resistant zones size for *S. aureus* and *S. lugdunesis* is ≥ 22 and ≤ 21 mm resp. While for other species ≥ 25 and ≤ 24 mm resp. and For *S. pseudintermedius, S. schleiferi* oxacillin zone ≥ 18 and ≤ 17 mm respectively.

Outcome definition and parameters:

- **Clinical sepsis:** is defined as neonates who have signs and symptoms of neonatal sepsis with or without risk factors. The clinical features considered were fever (>38.0 deg C), hypothermia (≤36.5 deg C), convulsions,

lethargy, poor feeding, respiratory distress, vomiting, bulging fontanels, jaundice, and umbilical pus infections.

- **Culture-positive/ proven sepsis:** means neonates who have clinical sepsis with positive blood culture growths.
- **Early onset sepsis (EOS):** Occurrence of sepsis on or before 72 hours of life.
- **Late-onset sepsis:** Occurrence of sepsis after 72 hours of life.

Statistical analysis plan:

After data collection, data were entered in Microsoft Excel version 19. Data were analysed using Statistical Package for the Social Sciences (version 25; IBM Corp., Armonk, NY, USA). Normality was checked by histogram and Shapiro-Wilk test.

The continuous variable, like age and duration of stay in hospital were expressed in median and interquartile range as they were not normally distributed. Age and duration of stay in hospital were also expressed in proportion and percentage. Other categorical data were also expressed in proportion and percentages. Differences in proportions were analysed by Chi-square or Fischer Exact test. The result was considered statistically significant if $p<0.05$.

Ethical clearance: Written informed consent was obtained from the parents or legal guardians of every neonate in our study.
Ethical clearance was obtained from the Institute Ethics Committee. The copy is attached.

Work plan:

a. Neonates with clinical suspicion of sepsis will be selected on the basis of inclusion and exclusion criteria.
b. Consent Taking (guardian/parents will be provided with information sheets in their preferred language, and will be asked to fill out consent forms if they wish to proceed with the study)
c. History Taking of Patient (patient medical history will be collected and recorded)
d. 1ml blood will be collected from the patient in a blood culture bottle (BacT/Alert PF Plus) maintaining proper aseptic condition.
e. The blood culture bottles will be brought to the microbiology department and put into BacT/ALERT machine.
f. The samples flagged positive by the machine will be taken out for subculture in Blood Agar and MacConkey Agar media.
g. Colony characteristics will be studied, gram staining will be performed and biochemical tests will be done to identify the causative bacteria.
h. After bacterial identification Antibiotic Susceptibility will be performed by the Kirby Bauer Disk Diffusion method according to CLSI guidelines.
i. The results will be reported and analysed. Statistical Analysis of Study Results (data will be analysed using the latest software version)

IV. Result

Table 1. Distribution of neonates with probable sepsis according to their gender (n=262)

Gender	Frequency	Percentage
Male	139	53.1%
Female	123	46.9%
Total	262	100%

Comments: The above table shows the distribution of neonates with probable sepsis according to their gender. It showed that among 262 neonates, 139 (53.1%) were male and 123 (46.9%) were females.

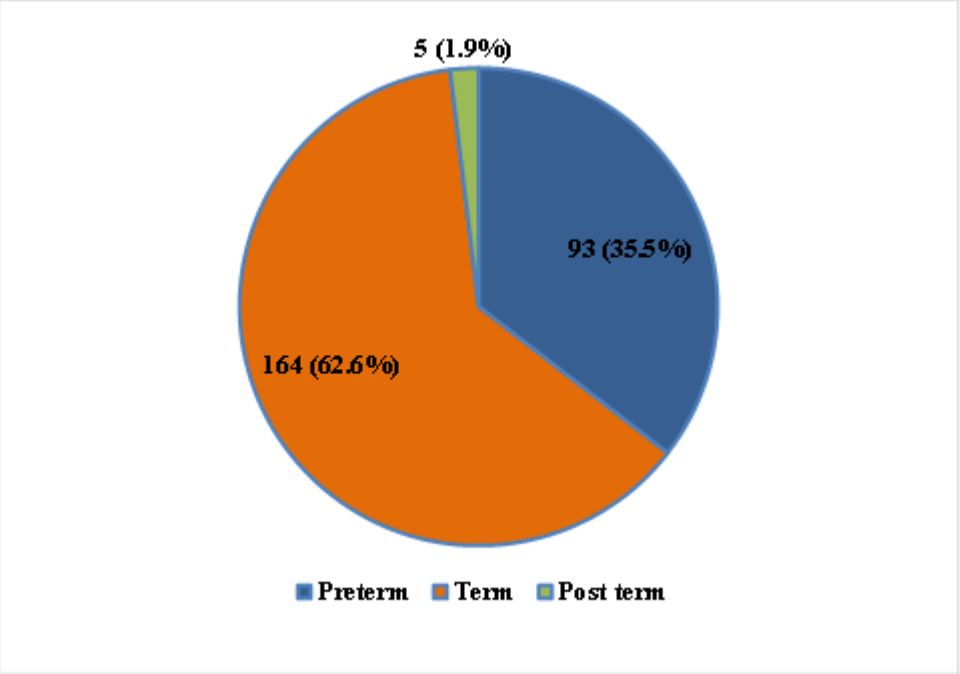


Figure1. Distribution of neonates according to their gestational age (n=262)

Comment: The above pie diagram shows that among 262 neonates, 164 (62.6%) were delivered at term, 93 (35.5%) were post term, and only 5 of them (1.9%) were preterm neonates.

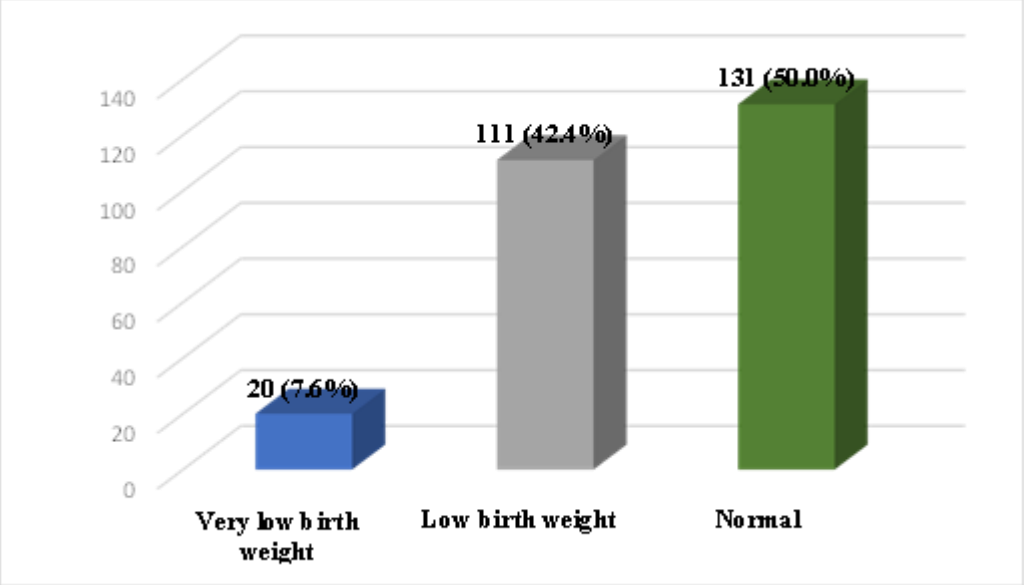


Figure 2. Distribution of neonates according to their birth weight (n=262)

Comment: The simple bar diagram shows distribution of neonates according to their birth weight. Among a total of 262 neonates, half of them (131, 50%) had normal birth weight i.e. ≥2500gm. About 42.4% had low birth weight i.e., between <2500 gm to 1500 gm. Twenty of them (7.6%) had birth weight <1500gm and they were considered very low birth weight.

Table 2. Distribution of neonates according to their place of delivery (n=262)

Place of delivery	Frequency	Percentage
Hospital	257	98.1%
Home	5	1.9%
Total	262	100%

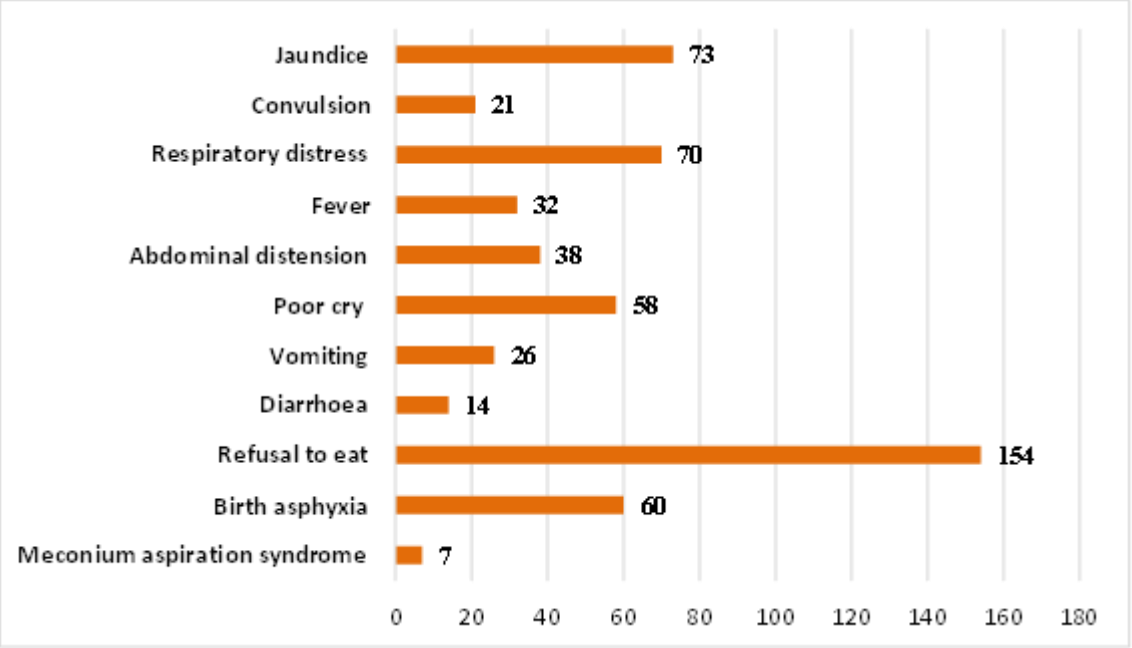
Comment: The above table shows the distribution of neonates according to their place of delivery. Among a total of 262 neonates, majority (257, 98.1%) were delivered in hospital, whereas 5 of the neonates were delivered at home.

Table 3. Distribution of neonates according to mode of delivery (n=262)

Mode of delivery	Frequency	Percentage
Normal vaginal delivery	131	50.0%
LUCS	131	50.0%
Total	262	100%

Comment: The above table shows the distribution of neonates according to mode of delivery. Among 262 neonates, half of them were delivered by normal vaginal delivery, while the rest half by lower uterine segment caesarean section.

Figure 3. Distribution of neonates according to their clinical signs or symptoms (n=262)



Comment: The above horizontal bar diagram shows the distribution of neonates according to their clinical signs or symptoms. Among a total of 262 neonates, majority i.e., 154 had history of refusal to feed, 73 were suffering from jaundice, 70 had respiratory distress, 60 of them had history of birth asphyxia. Another 58 of them had history of poor crying. 38 of them gave history of abdominal distension, while 32 of them suffered from fever. 21 neonate experienced convulsion, 26 had diarrhoea, 14 of them had vomiting. Meconium aspiration syndrome was noted in 7 neonates.

Table 4. Distribution of neonates according to their blood culture test result (n=262)

Blood culture test	Frequency	Percentage (%)
Growth of microorganism	53	20.73%
No growth	209	79.77%
Total	262	100%

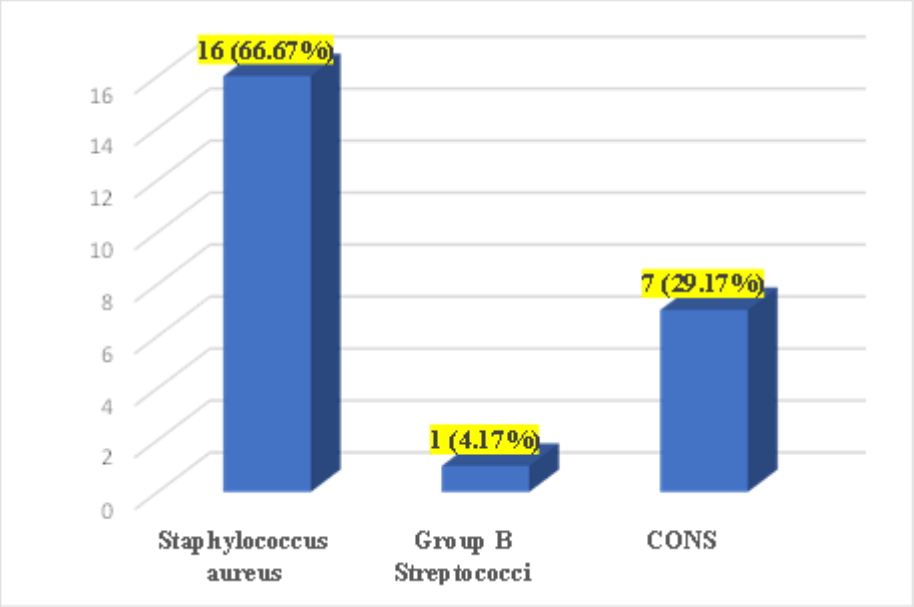
Comment: The above table shows the distribution of neonates according to their blood culture test results. Among the total 262 neonates having suspected or possible sepsis, 20.73% were confirmed as having sepsis as they showed bacterial growth in blood cultures. Whereas 209 (79.77%) did not show any bacterial growth in blood cultures. THUS, PREVALANCE OF BLOOD CULTURE POSITIVE BACTERIAL SEPSIS IS 20.73%

Table 5. Neonates with blood culture positive results showing type of microorganism grown (n=53)

Blood culture positive	Frequency	Percentage
Gram positive organism	24	45.28%
Gram negative organism	29	54.72%
Total	53	100%

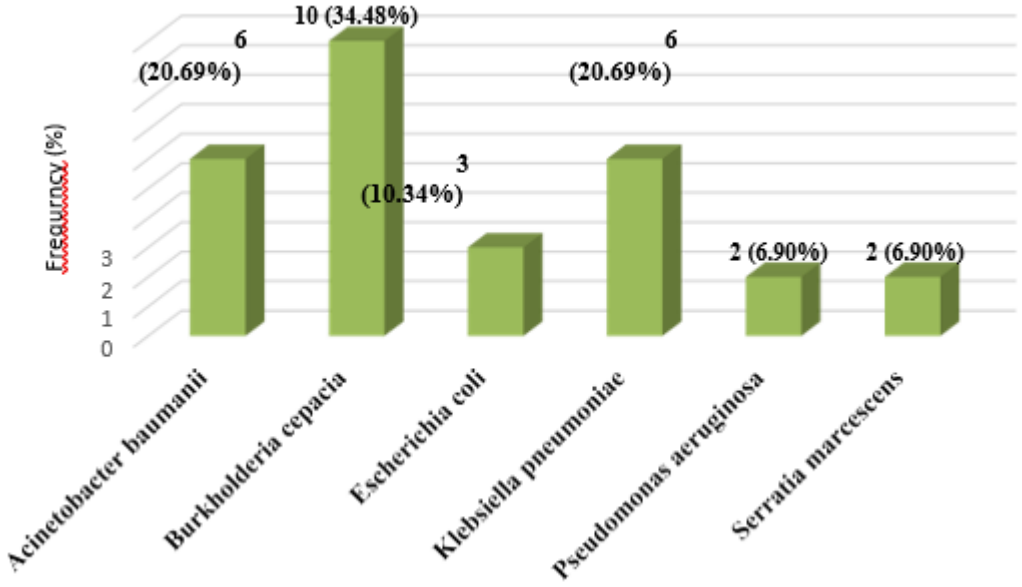
Comment: The above table shows the type of organism that grew in their blood cultures among the blood culture-positive neonatal sepsis cases. Among a total of 53 culture-positive sepsis cases, 24 (45.28%) showed growth of gram-positive bacteria, while 29 (54.72%) showed growth of gram-negative bacteria.

Figure 4. Percentage of growth of different type of gram-positive bacteria among the neonates (n=24)



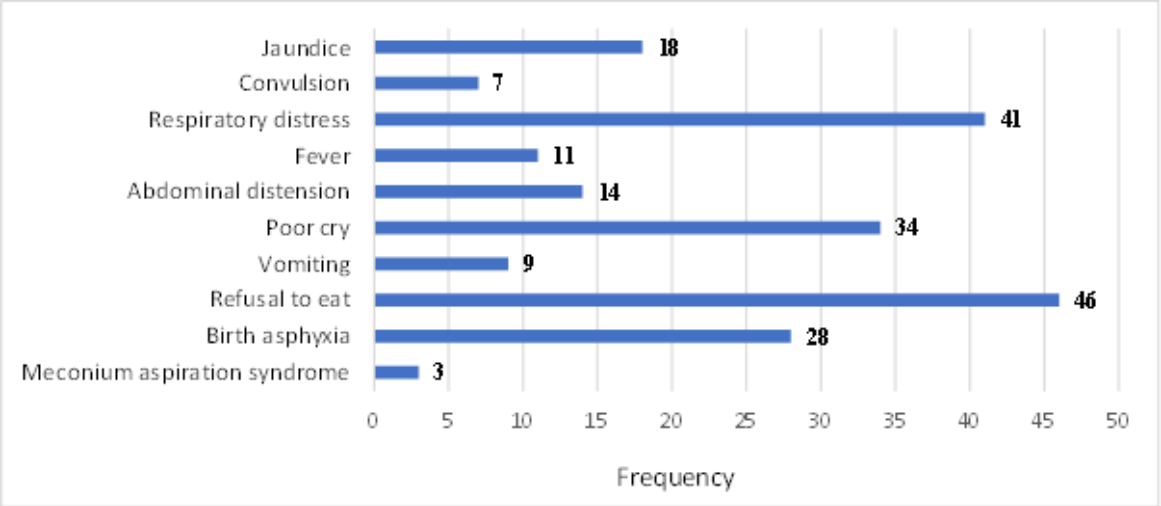
Comment: The above simple bar diagram shows the growth of different type of gram-positive bacteria among the neonates. Among all gram-positive sepsis cases (n=24), majority (16, 66.67%) showed growth of *Staphylococcus aureus*. 7 of 24 cases, showed growth of Coagulase-Negative Staphylococci. Only 1 case of Group B Streptococci positive case was identified among them.

Figure 5. Distribution of different gram-negative organism in positive blood culture neonates (n=29)



Comment: The above simple bar diagram shows the distribution of different gram-negative bacteria in blood culture-positive neonatal sepsis cases (n=29). Among these 29 cases, 10 (34.38%) showed growth of *Burkholderia cepacia*. 6 of them (20.69%) had *Acinetobacter baumannii* and another 6 (20.69%) had *Klebsiella pneumoniae* in their blood cultures. 3 of them showed growth of *Escherichia coli*, while 2 cases of *Pseudomonas aeruginosa* and another 2 cases of *Serratia marcescens* were isolated.

Figure 6. Distribution of culture positive neonatal sepsis according to their presenting clinical signs and symptoms (n=53)



Comment: The above horizontal bar diagram shows various clinical signs and symptoms among the culture-positive sepsis cases. Among a total of 53 culture-positive cases, 46 presented with symptoms of refusal to feed, while 41 cases had respiratory distress. 34 of them had poor crying as a manifestation. 28 neonates had a history of birth asphyxia and another 18 neonates had jaundice. 14 of them had abdominal distension and 11 had a history of fever. 9 neonates had vomiting and 7 of them had convulsions. The remaining 3 neonates had meconium aspiration syndrome.

Table 6. Association of gender with blood culture positive neonatal sepsis (n=262)

Gender	Blood culture		Inferential statistics
	Positive	Negative	
Female (n=123)	24 (19.5%)	99 (80.5%)	χ^2= 0.074; df=1 P= 0.878
Male (n=139)	29 (20.9%)	110 (79.1%)	
Total (n=262)	53 (20.2%)	209 (79.8%)	

Comment: The above table shows the association of gender with blood culture positivity status. Among 123 girl babies, 24 (19.5%) showed growth of pathogenic bacteria, while among 139 boy babies, 79.1% exhibited growth of organisms in blood culture. However, no association was observed between gender and blood culture positivity (p= 0.074) by a chi- square test.

Table 7. Association of gestational age with blood culture-positive neonatal sepsis (n=262)

Gestational age	Blood culture		Inferential statistics
	Positive	Negative	
Preterm (n=93)	35 (37.6%)	58 (62.4%)	**Fischer exact= 34.843, df=2; p<0.001**
rm (n=164)	15 (9.1%)	149 (90.9%)	
Post-term (n=5)	3 (60.0%)	2 (40.0%)	
Total (n=262)	53 (20.2%)	209 (79.8%)	

Comment: The above table shows the association of gestational age with blood culture- positive neonatal sepsis with a chi-square test. Among 93 preterm neonates, 37.6% showed growth of bacteria in their blood cultures and 37.6% did not. Among 164 term neonates, 9.1% had blood culture-positive results. Among 5 post-term neonates, 60% showed blood culture- positive results. The above finding suggests that preterm and post-term neonates have a higher risk of having neonatal sepsis than the term neonates and this finding is statistically significant at p<0.05.

Table 8. Association of birth weight with blood culture-positive neonatal sepsis (n=262)

Birth weight	Blood culture		Inferential statistics
	Positive	Negative	
Very low birth weight (n=20)	11 (55.0%)	9 (45.0%)	**Fischer exact=30.427; df=2; p<0.001**
Low birth weight (n=111)	31 (27.9%)	80 (72.1%)	
Normal birth weight (n=131)	11 (8.4%)	120 (91.6%)	
Total	53 (20.2%)	209 (79.8%)	

Comment: The above table shows the association of birth weight with blood culture-positive neonatal sepsis. Among twenty- very low birth weight neonates, 55% showed growth of pathogenic bacteria in their blood cultures. Among 111 low birth weight neonates, bacteria were isolated from 31 (27.9%) cases. Whereas among 131 neonates who had normal birth weight, 8.4% showed growth of pathogenic bacteria. This suggests that very low birth weight and low birth weight neonates had higher chances of developing neonatal sepsis than those having normal birth weight. The Fischer exact test showed this association was statistically significant at $p<0.05$.

Table 9. Association of mode of delivery with blood culture positive neonatal sepsis (n=262)

Mode of delivery	Blood culture		Inferential statistics
	Positive	Negative	
Normal vaginal delivery (n=131)	30 (22.9%)	101 (77.1%)	χ^2=1.159; df=1; p=0.356
LUCS (n=131)	23 (17.6%)	108 (82.4%)	
Total (n=262)	53 (20.2%)	209 (79.8%)	

Comment: The above table showed the association of the mode of delivery with blood culture- positive neonatal sepsis cases. Among the neonates who took birth by normal vaginal delivery, 30 (22.9%) had positive blood culture reports, whereas 23 (17.6%) had positive blood culture reports of those who were delivered by LUCS.

Table 10. Association of place of delivery with blood culture-positive neonatal sepsis (n=262)

Place of delivery	Blood culture		Inferential statistics
	Positive	Negative	
At hospital (n=257)	48 (18.7%)	209 (81.3%)	**Fischer exact=20.101; df=1; p<0.001**
At home (n=5)	5 (100%)	0 (0%)	
Total (n=262)	53 (20.2%)	209 (79.8%)	

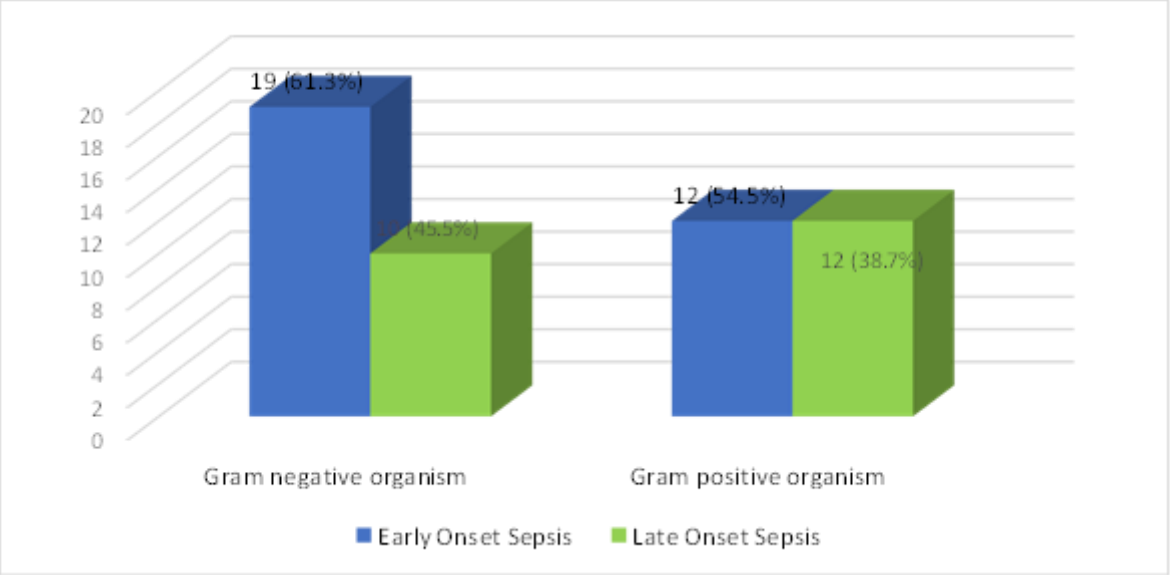
Comment: The above table shows the association between blood culture-positive sepsis and the place of delivery. All of the neonates who were delivered at home had blood culture- positive result, whereas 18.7% cases of hospital delivery cases had blood culture-positive sepsis. This finding showed a statistically significant association by a Fischer exact test at $p<0.05$.

Table 11. Association of days of onset of sepsis with blood culture positive result (n=262)

Variable	Blood culture		Inferential statistics
	Positive	Negative	
Early onset sepsis (n=57)	31 (54.4%)	26 (45.6%)	**χ^2=52.670; df=1; p<0.001**
Late-onset sepsis (n=205)	22 (10.7%)	183 (89.3%)	
Total (n=262)	53 (20.2%)	209 (79.8%)	

Comment: The above table shows the association of blood culture-positive results with days of commencement of sepsis. Among 57 EOS cases, 54.4% had blood culture-positive sepsis, whereas among 205 LOS cases, 10.7% had positive blood culture reports. This finding showed early onset sepsis cases had higher blood culture-positive sepsis than late-onset sepsis. The association was statistically significant at $p<0.05$ by the Chi-square test.

Figure 7. Percentage of growth of gram-positive and gram-negative organisms in Early onset and Late-onset sepsis in blood culture-positive neonatal sepsis (n=53)



Comment: The above clustered bar diagram shows that among all gram-negative organisms isolated in blood cultures, 61.3% were from EOS cases and 45.5% were from LOS cases. Conversely, among all gram-positive sepsis cases, 54.5% were EOS cases and 38.7% were LOS cases.

Table12. Antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from blood cultures of neonates (n=16)

Staphylococcus aureus	Resistant	Sensitive
Cefoxitin	12 (75%)	4 (25%)
Clindamycin	6 (37.5%)	10 (62.5%)
Trimethoprim-Sulfamethoxazole	7 (43.8%)	9 (56.3%)
Gentamicin	10 (62.5%)	6 (37.5%)
Vancomycin	0 (0%)	16 (100%)
Linezolid	0 (0%)	16 (100%)
Ciprofloxacin	10 (62.5%)	6 (37.5%)
Levofloxacin	8 (50%)	8 (50%)

Comment: Among all *Staphylococcus aureus* isolated from blood cultures, 62.5% resistance was seen to gentamicin as well as ciprofloxacin. Half of them were sensitive to levofloxacin. Maximum resistance was seen to cefoxitin. Resistance to trimethoprim-sulfamethoxazole was seen in 43.8% of staphylococcus aureus. Resistance to clindamycin was observed in 37.5% of cases. No resistance to vancomycin and linezolid was noted.

Table 13. Distribution of Methicillin resistant Staphylococcus aureus (MRSA) among the blood cultures came positive for Staphylococcus aureus (n=16)

MRSA	Frequency	Percentage
Yes	12	75%
No	4	25%
Total	16	100%

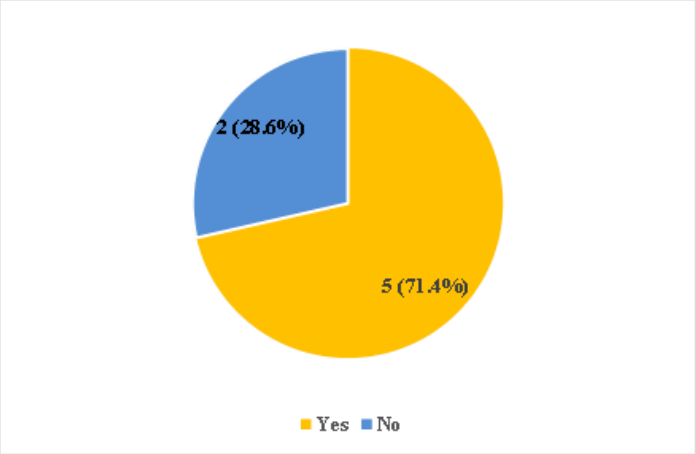
Comment: Among a total of 16 cases of *Staphylococcus aureus*, 12 (75%) were identified as Methicillin-resistant.

Table 14. Antibiotic sensitivity pattern of coagulase-negative Staphylococcus (CONS) isolated from blood cultures of neonates (n=7)

Coagulase Negative Staphylococcus	Resistant	Sensitive
Cefoxitin	5 (71.4%)	2 (28.6%)
Clindamycin	3 (42.9%)	4 (57.1%)
Trimethoprim-Sulfamethoxazole	2 (28.6%)	5 (71.4%)
Gentamicin	4 (57.1%)	3 (42.9%)
Vancomycin	0 (0%)	7 (100%)
Linezolid	0 (0%)	7 (100%)
Ciprofloxacin	5 (71.4%)	2 (28.6%)
Levofloxacin	4 (57.1%)	3 (42.9%)

Comment: The isolated CONS showed maximum resistance to cefoxitin and ciprofloxacin (71.4%), followed by levofloxacin and gentamicin (57.1%), clindamycin (42.9%), trimethoprim-sulfamethoxazole (28.6%). However, no resistance was found for linezolid and vancomycin.

Figure 8. Distribution of Methicillin resistant Coagulase negative Staphylococcus (MRCONS) among all CONS isolated in blood culture (n=7)



Comment: Among a total of seven cases of CONS, 5 (71.4%) were MRCONS, while the remaining 2 (28.6%) were not.

Table15. Antibiotic sensitivity pattern of Group B Streptococci isolated from blood cultures of neonates (n=1)

Group B streptococci	Sensitive
Ampicillin	Yes
Vancomycin	Yes
Linezolid	Yes
Cefotaxime	Yes
Ceftriaxone	Yes
Cefepime	Yes
Levofloxacin	Yes

Comment: The above table shows that the only Group B streptococci isolated from blood culture was sensitive to ampicillin, cefotaxime, ceftriaxone, cefepime, levofloxacin, vancomycin, and linezolid.

Table 16. Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolated from blood cultures of neonates (n=2)

Pseudomonas aeruginosa	Resistant	Sensitive
Ceftazidime	1 (50%)	1 (50%)
Cefepime	1 (50%)	1 (50%)
Piperacillin- tazobactam	1 (50%)	1 (50%)
Tobramycin	0 (0%)	2 (100%)
Ciprofloxacin	1 (50%)	1 (50%)

Levofloxacin	1 (50%)	1 (50%)
Imipenem	0 (0%)	2 (100%)
Meropenem	1 (50%)	1 (50%)
Ceftriaxone	2 (100%)	0 (0%)
Cefotaxime	2 (100%)	0 (0%)
Ampicillin	2 (100%)	0 (0%)
Gentamicin	1 (50%)	1 (50%)
Amikacin	0 (0%)	2 (100%)

Comment: The above table shows that the isolated two *Pseudomonas aeruginosa* bacteria exhibited 100% resistance to ceftriaxone, cefotaxime, and ampicillin. Fifty percent of them exhibited resistance to ceftazidime, cefepime, piperacillin-tazobactam, ciprofloxacin, levofloxacin, meropenem, and gentamicin. However, no resistance was noted for tobramycin, imipenem, and amikacin.

Table 17. Antibiotic sensitivity pattern of *Burkholderia cepacia* isolated from blood cultures of neonates (n=11)

Burkholderia cepacian	**Resistant**	**Sensitive**
Cefepime	11 (100%)	0 (0%)
Piperacillin-tazobactam	11 (100%)	0 (0%)
Ceftazidime	6 (54.5%)	5 (45.5%)
Minocycline	9 (81.8%)	2 (18.2%)
Levofloxacin	9 (81.8%)	2 (18.2%)
Ciprofloxacin	9 (81.8%)	2 (18.2%)
Imipenem	11 (100%)	0 (0%)
Meropenem	0 (0%)	11 (100%)
Ceftriaxone	10 (90.9%)	1 (9.1%)
Tigecycline	10 (90.9%)	1 (9.1%)
Cefoperazone-sulbactam	8 (72.7%)	3 (27.3%)
Gentamicin	11 (100%)	0 (0%)
Amikacin	11 (100%)	0 (0%)
Trimethoprim-sulfamethoxazole	1 (9.1%)	10 (90.9%)

Comment: The above table shows that among the *Burkholderia cepacia* isolated from blood cultures, 100% resistance was observed for piperacillin-tazobactam, imipenem, gentamicin, and amikacin. About 91% resistance was noted for ceftriaxone and tigecycline followed by nearly 82% resistance for minocycline, levofloxacin, and ciprofloxacin. For trimethoprim-sulfamethoxazole, 9.1% resistance was noted. However, no resistance was identified for meropenem.

Table18. Antibiotic sensitivity pattern of *Acinetobacter baumannii* isolated from blood cultures of neonates (n=6)

Acinetobacter baumannii	**Resistant**	**Sensitive**
Ampicillin-sulbactam	3 (50%)	3 (50%)
Ceftazidime	2 (33.3%)	4 (66.7%)
Cefepime	2 (33.3%)	4 (66.7%)
Ciprofloxacin	5 (83.3%)	1 (16.7%)
Levofloxacin	4 (66.7%)	2 (33.3%)
Gentamicin	6 (100%)	0 (0%)
Tobramycin	6 (100%)	0 (0%)
Imipenem	2 (33.3%)	4 (66.7%)
Meropenem	2 (33.3%)	4 (66.7%)
Amikacin	6 (100%)	0 (0%)
Piperacillin-tazobactam	2 (33.3%)	4 (66.7%)
Trimethoprim-sulfamethoxazole	2 (33.3%)	4 (66.7%)
Minocycline	4 (66.7%)	2 (33.3%)
Colistin	1 (16.7%)	5 (83.3%)
Ceftriaxone	5 (83.3%)	1 (16.7%)
Cefotaxime	5 (83.3%)	1 (16.7%)

Tigecycline	2 (33.3%)	4 (66.7%)

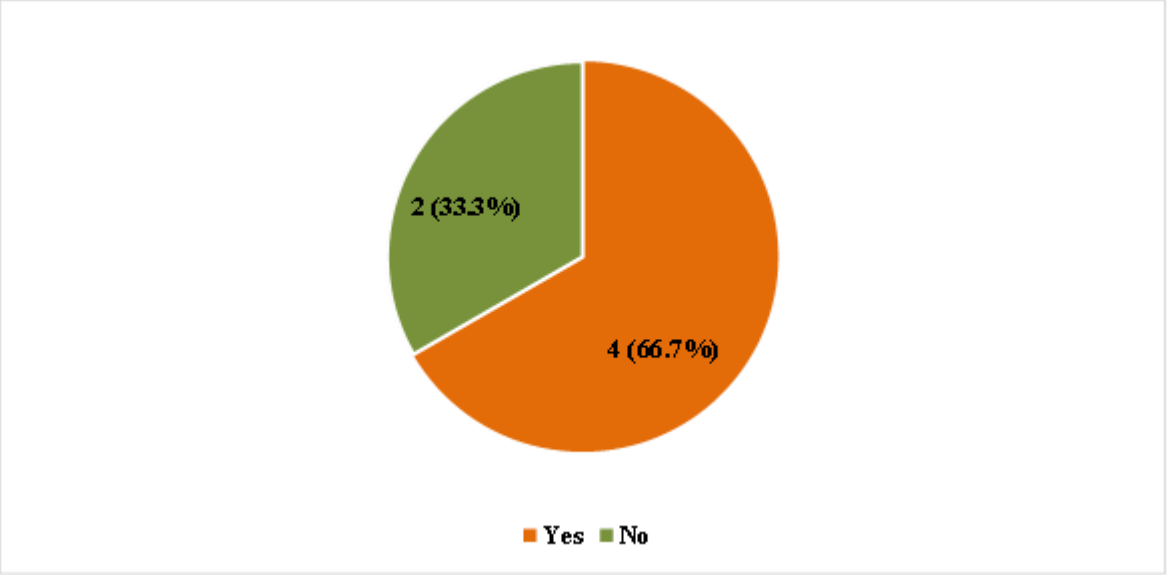
Comment: The isolated *Acinetobacter baumannii* exhibited 100% resistance to gentamicin, tobramycin, and amikacin, followed by 83.3% resistance to ceftriaxone, cefotaxime and ciprofloxacin, 66.7% to minocycline, 50% to ampicillin-sulbactam, 33.3% to ceftazidime, cefepime, imipenem, meropenem, piperacillin-tazobactam, trimethoprim- sulfamethoxazole, and tigecycline. Relatively less resistance was observed for colistin (16.7%).

Table 19. Antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolated from blood cultures of neonates (n=6)

Klebsiella pneumoniae	**Resistant**	**Sensitive**
Ceftazidime	5 (83.3%)	1 (16.7%)
Cefepime	4 (66.7%)	2 (33.3%)
Piperacillin-tazobactam	4 (66.7%)	2 (33.3%)
Tobramycin	3 (50%)	3 (50%)
Ciprofloxacin	4 (66.7%)	2 (33.3%)
Levofloxacin	4 (66.7%)	2 (33.3%)
Imipenem	1 (16.7%)	5 (83.3%)
Meropenem	1 (16.7%)	5 (83.3%)
Ceftriaxone	5 (83.3%)	1 (16.7%)
Cefotaxime	5 (83.3%)	1 (16.7%)
Ampicillin	6 (100%)	0 (0%)
Ampicillin-sulbactam	4 (66.7%)	2 (33.3%)
Gentamicin	5 (83.3%)	1 (16.7%)
Amikacin	4 (66.7%)	2 (33.3%)
Colistin	6 (100%)	0 (0%)
Trimethoprim-sulfamethoxazole	2 (33.3%)	4 (66.7%)

Comment: The isolated *Klebsiella pneumoniae* exhibited 100% resistance to ampicillin and colistin, followed by 83.3% resistance to ceftazidime, ceftriaxone, cefotaxime, and gentamicin, 66.7% resistance to ampicillin-sulbactam, amikacin, cefepime, piperacillin- tazobactam, ciprofloxacin, and levofloxacin, and 50% resistance to tobramycin. For trimethoprim-sulfamethoxazole, resistance was observed in 33.3% of cases. Greater sensitivity was observed for imipenem (83.3%) and meropenem (83.3%).

Figure 9. Distribution of Extended-spectrum beta-lactamase (ESBL) producing Klebsiella pneumoniae among all *Klebsiella pneumoniae* isolated in blood cultures (n=6)



Comment: Among a total of six *klebsiella pneumoniae* isolated from blood cultures, 4 (66.7%) were ESBL Klebsiella spp. while remaining 2 (33.3%) were not.

Table 20. Antibiotic sensitivity pattern of *Escherichia coli* isolated from blood cultures of neonates (n=3)

Escherichia coli	Resistant	Sensitive
Ceftazidime	3 (100%)	0 (0%)
Cefepime	3 (100%)	0 (0%)
Piperacillin-tazobactam	3 (100%)	0 (0%)
Tobramycin	2 (66.7%)	1 (33.3%)
Ciprofloxacin	1 (33.3%)	2 (66.7%)
Levofloxacin	0 (0%)	3 (100%)
Imipenem	0 (0%)	3 (100%)
Meropenem	0 (0%)	3 (100%)
Cefotaxime	3 (100%)	0 (0%)
Ceftriaxone	3 (100%)	0 (0%)
Ampicillin	3 (100%)	0 (0%)
Ampicillin-sulbactam	3 (100%)	0 (0%)
Gentamicin	1 (33.3%)	2 (66.7%)
Amikacin	1 (33.3%)	2 (66.7%)
Colistin	0 (0%)	3 (100%)
Trimethoprim-sulfamethoxazole	2 (66.7%)	1 (33.3%)

Comment: The isolated *Escherichia coli* showed 100% resistance to ceftazidime, cefepime, piperacillin-tazobactam, cefotaxime, ceftriaxone, ampicillin, and ampicillin- sulbactam, followed by tobramycin (66.7%) and trimethoprim-sulfamethoxazole (66.7%), and gentamicin (33.3%), amikacin (33.3%). However, no resistance was noted for meropenem, imipenem, levofloxacin, and colistin.

***All 3 isolated Escherichia coli bacteria were found to be Extended spectrum beta- lactamase producers.**

Table 21. Antibiotic sensitivity pattern of *Serratia marcescens* isolated from blood cultures of neonates (n=2)

Serratia marcescens	Sensitive
Ceftazidime	2 (100%)
Cefepime	2 (100%)
Piperacillin-tazobactam	2 (100%)
Tobramycin	2 (100%)
Ciprofloxacin	2 (100%)
Levofloxacin	2 (100%)
Imipenem	2 (100%)
Meropenem	2 (100%)
Cefotaxime	2 (100%)
Ceftriaxone	2 (100%)
Ampicillin	2 (100%)
Ampicillin-sulbactam	2 (100%)
Gentamicin	2 (100%)
Amikacin	2 (100%)
Colistin	2 (100%)
Trimethoprim-sulfamethoxazole	2 (100%)

Comment: Isolated two *Serratia marcescens* had 100% sensitivity for cefotaxime, ceftriaxone, cefepime, ampicillin, ampicillin-sulbactam, gentamicin, amikacin, tobramycin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, piperacillin-tazobactam, meropenem, imipenem, and colistin.

Outcome: Among the total 262 neonates, one neonate with culture-positive sepsis died. The neonate was a very low birth category and was delivered preterm by LUCS. The rest of the neonates had been discharged after treatment.

V. Discussion

The first 28 days of life are very important as there is a higher risk of mortality in this period due to several causes and neonatal sepsis is an important cause of neonatal mortality. The emergence of multidrug-resistant bacteria has raised the concern further leading to poor outcomes among neonatal sepsis cases. For effective management of neonatal sepsis cases, identification of a common clinical profile is important for identifying the sepsis cases early, and exploring the bacteriological profile will further guide the clinician about the commonly prevalent organisms. This finding can help to develop antibiotic guidelines for the treatment of

neonatal sepsis cases. The current study has been conducted to estimate the prevalence of neonatal sepsis and also the bacteriological profile of culture-positive sepsis cases. The resistance pattern of isolated organisms has also been identified by performing an antibiogram.

In the current study, 262 neonates who were admitted to the SNCU of the College of Medicine & JNM Hospital, Kalyani with possible neonatal sepsis and met our inclusion & exclusion criteria have been included as study participants. Among them, about 53% were boys and 47% were girls. Most of them (62.6%) were delivered at term, while 35.5% were preterm, and about 2% were delivered post-term. About half of them had normal birth weight (≥2500gm), while the remaining 50% had birth weight <2500gm. Twenty of the neonates (7.6%) had even very low birth weight. As per National Family and Health Survey-5 (2019- 21), in West Bengal, the female-to-male ratio for the year 2018-19 is 928 females per 1000 males. This finding is close to the male-female ratio in the present study.

In the current study, among 262 possible neonatal sepsis cases, blood culture shows the growth of pathogenic organisms in 53 neonates owing to a proportion of 20.73% of culture- positive neonatal sepsis. A similar prevalence has been seen in a study conducted by Jyothi et al.[36] About 19.2% prevalence was noted by Jyothi et al.[36] Muley et al.[37] and Mohamud et al.[29] also reported a culture-positive neonatal sepsis prevalence of 26.6% and 25% respectively.

Jatsho et al.[21] conducted a similar study in Bhutan and they found the prevalence of blood culture-positive neonatal sepsis as 14%. Ibrahim et al[19] reported a relatively lower prevalence i.e., 8.95%. However, a higher prevalence of culture-positive neonatal sepsis has been observed with a range of 30 to 48%. [23-,27, 31] The variation in culture-positive neonatal sepsis might be due to sample size variation and also varied antibiotic policies in different parts of India and even across different hospitals in the same region. A relatively lower prevalence of culture- positive sepsis in the present study might be due to the prior administration of antibiotics, and sepsis due to other causes, like fungal, anaerobic pathogens, or viral.

The current study identified that 21.8% were Early onset sepsis (≤72 hours), while the remaining 78.2% were Late-onset sepsis (>72 hours). Mohamud et al.[29], in their study, reported the prevalence of EOS as 27.8% and LOS as 72.2%. Wani et al.[38] reported the prevalence of EOS and LOS as 37% and 63% respectively. However, Sharma et al.[39] reported a higher prevalence of EOS (67.7%) than LOS (32.3%). Similarly, Jatsho et al.[21], Gamit et al.[23], Jyothi et al.[36], Zakariya et al.[26], Jaybhay et al.[27] reported a higher prevalence of EOS. Other studies[21,24] reported almost equal prevalence of EOS and LOS. In our study, the incidence of late onset sepsis was higher than early onset sepsis, possibly due to improved maternal screening and intrapartum antibiotic use, reducing EOS cases. However, prolonged hospital stays, invasive procedures and nosocomial infections likely contributed to the increased LOS incidence.

Neonates having sepsis present with varied and non-specific symptoms and it makes it difficult to identify the cases early. Among the culture-positive sepsis cases, the most common presentation was refusal to feed, followed by respiratory distress, poor cry, birth asphyxia, neonatal jaundice, abdominal distension, vomiting, and neonatal seizure. Kurma et al.[18] reported a similar finding and the predominant symptoms of neonatal sepsis in their study were respiratory distress, refusal of feeds, neonatal seizures, absent cry, lethargy, neonatal jaundice, and abdominal distension. Sharma et al.[39] also identified respiratory distress as the commonest presenting symptom, followed by lethargy, jaundice, neonatal seizure, and hypoglycaemia.

In the present study, the growth of gram-negative organisms was seen in 54.7% of culture-positive samples, whereas in 45.3% of cases, gram-positive organisms were isolated. Jyothi et al.[36] also found a 55.7% prevalence of gram-negative organisms in blood culture and a 44.3% prevalence of gram-positive organisms in culture-positive blood samples. A higher proportion of gram-negative sepsis has been reported by Zakariya et al.[26] and Gupta et al.[31] Another important finding of our study is more gram-negative organisms were isolated in EOS cases (61.3%) than LOS (38.7%) cases. Gram-positive organisms were found predominantly in LOS cases (54.5%) than EOS (45.5%). Among the total 53 culture-positive cases, the prevalence of gram-negative organisms was higher than gram-positive organisms (54.72% vs 45.28%) in our study. This finding aligns with other studies where a higher prevalence of gram- negative organisms has been reported.[18,31,36,39] Gram-negative bacteria are becoming a growing concern, especially in low-and middle-income countries, with the added concern of multidrug resistance. Increased cases of LOS are also of high concern as the organisms in these NS are usually acquired from the surrounding hospital environment.

Among the gram-positive organisms, *Staphylococcus aureus* had the highest prevalence in our study. The prevalence of *Staphylococcus aureus* is approximately 30.2% among all organisms isolated from blood cultures, followed by *Burkholderia cepacia* (18.9%), Coagulase-negative Staphylococci (13.2%), *Klebsiella pneumoniae* (11.3%), *Acinetobacter baumannii* (11.3%), *Escherichia coli* (5.7%), *Pseudomonas aeruginosa* (3.8%), *Serratia marcescens* (3.8%). Only one case of Group B Streptococci has been identified from blood culture. Jyothi et al.[36] mentioned that among the gram-negative organisms Klebsiella spp. and Acinetobacter spp. were the two most frequently isolated organisms and CONS & *Staphylococcus aureus* were in highest prevalence among the gram-positive bacteria. The findings from the study by Yadav et al.[22] are quite similar

to us as they reported the highest prevalence of *Staphylococcus aureus* (35.6%), followed by *Klebsiella pneumoniae* (15.3%), *Acinetobacter spp.* (11.9%), CONS (10.2%), *Pseudomonas aeruginosa* and *E. coli* (both 6.8%). Most of the studies have reported *Klebsiella pneumoniae* as the most commonly isolated organism in neonatal sepsis cases.[21,23,25,26,27] Many studies identified *Staphylococcus aureus* as the most common causative organism of neonatal sepsis among all gram-positive sepsis cases.[24,25,27,29,31] In our study , while Gram-negative pathogens still dominate EOS, our SNCU is experiencing a rise in Gram-positive LOS cases, likely driven by hospital-acquired infections and infection control gaps.

The current study showed a higher prevalence of multidrug-resistant sepsis. Most of the gram-negative bacteria exhibited higher resistance to ampicillin, aminoglycosides, fluroquinolones, and third-generation cephalosporins. The gram-negative bacteria had lower resistance to carbapenems. However, E. coli showed 100% resistance to meropenem and imipenem. About 67% of *Klebsiella pneumoniae* were identified as extended-spectrum beta- lactamase (ESBL) producers, whereas 100% of *E. coli* were ESBL producers. Isolated *Klebsiella pneumoniae* shows 100% resistance to colistin. The gram-positive organisms were most sensitive to vancomycin and linezolid. A similar picture was also reported by several other studies.[24,25,29,30,39] Zakariya et al.[26,] in their study found 32% cases of ESBL *Klebsiella spp.* Mohamud et al.[29] also reported 40% cases of ESBL bacteria. We found 75% of *Staphylococcus aureus* resistant to methicillin which constituted about 22% of a total number of culture-positive cases. Mohamud et al.[29] reported 18% MRSA cases in their study. Only one Group B Streptococci has been isolated in blood culture and it exhibited 100% sensitivity to ampicillin, 3rd generation cephalosporins, levofloxacin, clindamycin, vancomycin, and linezolid.

In our study, *Burkholderia cepacia* was isolated in 18.9% of neonatal sepsis cases, highlighting its significance as an emerging nosocomial pathogen. Compared to previous studies, which reported varying susceptibility patterns, our isolates exhibited alarming levels of multidrug resistance. Unlike earlier reports by Patra et al.[40] and Kochar et al.[41], where *B. cepacia* showed susceptibility to piperacillin-tazobactam, imipenem, and aminoglycosides, we observed 100% resistance to these antibiotics. Similarly, resistance to ceftriaxone (91%), tigecycline (91%), fluoroquinolones (~82%), and minocycline (82%) suggests a worsening resistance trend, as also noted by Bennaoui et al.[42] However, in contrast to prior studies by Bennaoui et al., our isolates remained susceptible to meropenem, which could serve as a last- resort option. The relatively lower resistance (9.1%) to trimethoprim-sulfamethoxazole aligns with previous findings by Patra et al. and reinforces its potential role in therapy. These findings emphasize the urgent need for hospital-specific antibiotic stewardship programs, stringent infection control measures, and continuous surveillance to curb the spread of highly resistant

B. cepacia strains in NICU settings.

Preterm births, low birth weight, and home delivery are found to be associated with a higher risk of having neonatal sepsis in the present study. These risk factors have been also identified in several studies.[18,21,22,29,38]

One neonate with culture-positive sepsis who was preterm and had a very low birth weight died. Other neonates were discharged after recovery. Ibrahim et al.[19] reported 22.4% mortality in their study. A large sample size (n=6090) might be one reason.

Limitations:

- The neonates discharged from the SNCU after recovery were not followed up and a relatively better outcome of neonatal sepsis cases with one mortality can be explained from this.
- Prior antibiotic therapy before admission and the history of readmissions were not considered in our study.

Summary:

Key findings:

- Among a total of 262 cases of possible neonatal sepsis, 53 (20.73%) were confirmed as culture-positive sepsis.
- Among 262 cases, EOS was seen in 21.8% and the remaining 78.2% were LOS. However, among 53 culture-positive sepsis cases, 31 (58.5%) had EOS and the rest had LOS.
- About 45.3% had gram-positive sepsis and 54.7% had gram-negative sepsis.
- Among all isolated bacteria, the most commonly isolated organism was *Staphylococcus aureus* which constituted 30.2% of total culture-positive sepsis, followed by *Burkholderia cepacia* (18.9%), CONS (13.2%), *Klebsiella pneumoniae* (11.3%), *Acinetobacter baumannii* (11.3%), *Escherichia coli* (5.7%), *Pseudomonas aeruginosa* and *Serratia marcescens* (both 3.8%). One GBS was identified.
- All isolated E. coli were ESBL producers, whereas 66.7% of Klebsiella pneumoniae were ESBL producers.
- Twelve cases of MRSA (22.6% of all culture-positive sepsis) had been isolated.
- Five cases of MRCONS were isolated from blood culture which constituted 9.4% of total culture-positive NS.
- Gram-negative organisms showed higher resistance to ampicillin, aminoglycosides, fluoroquinolones, and third-generation cephalosporins. Gram-negative organisms exhibited good sensitivity to carbapenems. Gram-

positive organisms were 100% sensitive to vancomycin and linezolid.
- Preterm birth, low birth weight, and home delivery have been identified as significant risk factors for having neonatal sepsis.
- One neonate having culture-positive sepsis who was preterm and had very low birth weight died.

VI. Conclusion:

This study highlights *Staphylococcus spp.*, *Burkholderia cepacia*, coagulase-negative *Staphylococci* (CONS), and *Klebsiella pneumoniae* as the predominant pathogens causing neonatal sepsis, with a concerning trend of multidrug resistance. The emergence of antimicrobial resistance underscores the critical need for continuous surveillance and stringent infection control measures. Hospitals must formulate and periodically update their antibiotic policies based on local epidemiological and susceptibility data to ensure effective treatment. Implementing robust antibiotic stewardship programs, promoting rational antibiotic use, and adhering to strict hygiene protocols are essential to curb the rising burden of hospital-acquired infections and prevent the spread of resistant pathogens. Regular review of antibiotic guidelines, aligned with regional resistance patterns, is imperative to optimize patient outcomes and combat the growing threat of antimicrobial resistance.

Recommendations For Clinical Practice
Optimization of Empirical Therapy:
- Given the observed resistance trends, empirical therapy for neonatal sepsis should be guided by **local antibiogram data** and updated regularly to ensure effective treatment.
- The increasing resistance to aminoglycosides and fluoroquinolones necessitates careful **antibiotic selection** to preserve efficacy and minimize further resistance development.

Strengthening Infection Control Measures:
- Adherence to **strict hand hygiene protocols, aseptic techniques, and sterilization protocols** in SNCUs should be reinforced to minimize nosocomial transmission.
- Regular **environmental surveillance** of medical devices, disinfectant solutions, and healthcare equipment should be conducted to detect potential sources of contamination.

Enhanced Surveillance & Monitoring:
- Routine **antibiogram reviews** should be implemented to track evolving resistance patterns and optimize treatment guidelines accordingly.
- A **neonatal sepsis registry** should be established to monitor pathogen prevalence, resistance trends, and clinical outcomes over time.

For future research:
- Genetic mechanisms of resistance among common neonatal sepsis pathogens should be investigated to understand resistance evolution and potential therapeutic targets.
- Regional and national studies should be conducted to compare pathogen distribution and resistance patterns, aiding in the development of standardized treatment protocols.
- Neonatal outcomes following MDR infections should be assessed, with evaluations of different treatment regimens and preventive strategies to determine their effectiveness in improving survival and reducing complications.

List Of Additional Figures:

FIG 1: BacT/ALERT PF Plus bottle

FIG 2: BacT/ALERT 3D

FIG 3: VITEK 2

FIG 4: Staphylococcus aureus in Nutrient Agar

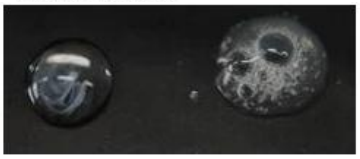


FIG 5: Catalase Test Positive(right)

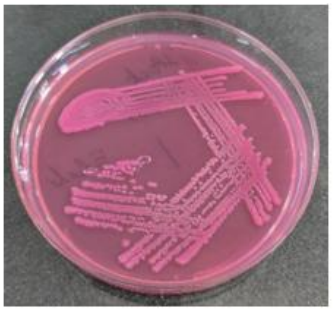


FIG 8: Escherichia coli in MacConkey agar

FIG 6: Tube coagulase positive

FIG 6: Slide coagulase positive(left)

FIG 10: Oxidase test positive (purple color)

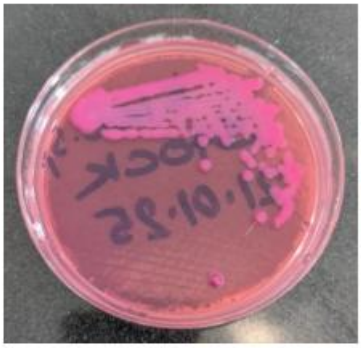


FIG 7: Klebsiella pneumoniae in MacConkey agar

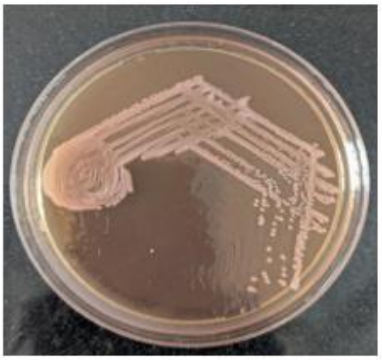


FIG 11: Growth of Acinetobacter baumannii in MacConkey agar

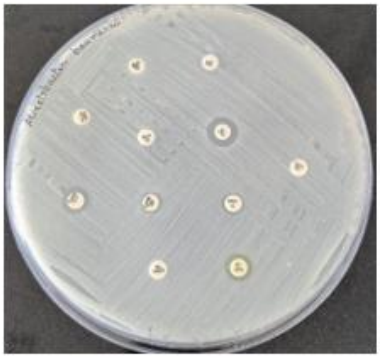


FIG 12: AST plate

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