
**“PATTERN OF COLONIZATION OF GUT
MICROBIOTA IN PRETERM NEONATES AND
ITS ASSOCIATION WITH NEONATAL SEPSIS- A
ONE YEAR HOSPITAL BASED PROSPECTIVE
OBSERVATIONAL STUDY”**

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LIST OF ABBREVIATIONS

- 1. AEDF – Absent End Diastolic Flow**
- 2. ANC – Absolute Neutrophil Count**
- 3. APGAR – Appearance, Pulse, Grimace, Activity, Respiration**
- 4. ASDR – Age-Standardized Death Rate**
- 5. ASIR – Age-Standardized Incidence Rate**
- 6. BACT/ALERT – Bacterial Alert System for Blood Cultures**
- 7. CIAB – Cried Immediately After Birth**
- 8. CoNS – Coagulase-Negative Staphylococci**
- 9. CPAP – Continuous Positive Airway Pressure**
- 10. CRP – C-Reactive Protein**
- 11. DCDA – Dichorionic Diamniotic Twins**
- 12. DBF – Direct Breastfeeding**
- 13. DAMPs – Damage-Associated Molecular Patterns**
- 14. ELBW – Extremely Low Birth Weight**
- 15. EOS – Early-Onset Sepsis**
- 16. ESR – Erythrocyte Sedimentation Rate**
- 17. ETO – Ethylene Oxide (used for sterilization)**
- 18. FFP – Fresh Frozen Plasma**
- 19. GA – Gastric Aspirate**

- 20. GA-1, GA-2 – Gastric Aspirate Sample 1 and 2**
- 21. GDM – Gestational Diabetes Mellitus**
- 22. GHTN – Gestational Hypertension**
- 23. GNB – Gram-Negative Bacteria**
- 24. HELLP – Hemolysis, Elevated Liver enzymes, and Low Platelets Syndrome**
- 25. HIE – Hypoxic-Ischemic Encephalopathy**
- 26. IFT – Infant Feeding Tube**
- 27. IV – Intravenous**
- 28. IVH – Intraventricular Hemorrhage**
- 29. KMC – Kangaroo Mother Care**
- 30. LBP – Lipopolysaccharide Binding Protein**
- 31. LBW – Low Birth Weight**
- 32. LOS – Late-Onset Sepsis**
- 33. LSCS – Lower Segment Caesarean Section**
- 34. LPS – Lipopolysaccharide**
- 35. LTA – Lipoteichoic Acid**
- 36. MAMPs – Microbial-Associated Molecular Patterns**
- 37. NLRs – Nod-Like Receptors**
- 38. NICU – Neonatal Intensive Care Unit**

- 39. NST – Non-Stress Test**
- 40. PAMPs – Pathogen-Associated Molecular Patterns**
- 41. PBMCs – Peripheral Blood Mononuclear Cells**
- 42. PICC – Peripherally Inserted Central Catheter**
- 43. PMN – Polymorphonuclear Neutrophils**
- 44. PPROM – Preterm Premature Rupture of Membranes**
- 45. PRRs – Pathogen Recognition Receptors**
- 46. PROM – Premature Rupture of Membranes**
- 47. RDP – Random Donor Platelet Transfusion**
- 48. RLRs – Rig-I-Like Receptors**
- 49. RT Feeds – Ryle’s Tube Feeding**
- 50. S-1, S-2 – Stool Sample 1 and 2**
- 51. SGA – Small for Gestational Age**
- 52. SIRS – Systemic Inflammatory Response Syndrome**
- 53. TLR – Toll-Like Receptor**
- 54. TLC – Total Leukocyte Count**
- 55. UTI – Urinary Tract Infection**
- 56. VLBW – Very Low Birth Weight**
- 57. VLOS – Very Late-Onset Sepsis**

ABSTRACT

Background

Neonatal sepsis remains a leading cause of morbidity and mortality in preterm neonates. Emerging evidence suggests that gut microbiota dysbiosis may play a critical role in the pathogenesis of neonatal infections. This study investigates the pattern of gut microbiota colonization in preterm neonates and its association with the incidence and outcomes of neonatal sepsis.

Methods

A one-year prospective observational study was conducted at a tertiary care neonatal unit, enrolling preterm neonates (gestational age **28–37 weeks**). Gastric aspirate and stool samples were collected at **two time points**—within the first few hours of life (Sample-1) and between **day 7-10 of life (Sample-2)**. Bacterial cultures were analysed for colonization patterns, and their association with **clinical and blood culture-positive sepsis** was assessed. Key neonatal risk factors, interventions, and hospital outcomes were also evaluated.

Results

Gut colonization showed a progressive increase over time in both gastric aspirate and stool samples. The most commonly isolated organisms were *Escherichia coli* and *Klebsiella pneumoniae*, with a notable rise in *Enterococcus* in later stool samples. Significant associations were found between bacterial growth in gastric aspirate Sample-1 and clinical sepsis ($p = 0.028$), as well as between stool Sample-2 and clinical sepsis ($p = 0.028$).

No statistically significant correlation was observed between gut colonization and blood culture-positive sepsis, suggesting that direct bacterial translocation may not be the sole mechanism of infection. Risk factors associated with increased gut colonization included prolonged NICU stay, respiratory support, peripherally inserted central catheter (PICC) line use, prolonged antibiotic exposure, and delayed initiation of direct breastfeeding. Neonates receiving Kangaroo Mother Care (KMC) had significantly lower bacterial colonization rates compared to NICU-admitted neonates.

Conclusion

This study highlights the progressive nature of gut colonization in preterm neonates, with a strong association between early bacterial colonization and clinical sepsis. However, blood culture-positive sepsis was not significantly linked to gut microbiota, suggesting that gut-derived infections may be influenced by multiple host and environmental factors. Preventive strategies, including early enteral feeding, minimizing antibiotic overuse, and promoting KMC, may help mitigate gut dysbiosis and reduce sepsis risk in preterm neonates. Future research using metagenomic sequencing and longitudinal microbiome analysis is warranted to better understand the mechanistic link between gut microbiota and neonatal infections.

Keywords: Preterm neonates, gut microbiota, neonatal sepsis, bacterial colonization, risk factors, NICU, Kangaroo Mother Car

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INTRODUCTION

‘Neonatal sepsis’ is a critical disease that impacts neonates and is defined by clinical manifestations and substantiated by laboratory findings, usually requiring microbial culture from blood or other bodily fluids (1). It is a primary factor that contributes to early neonatal ‘mortality’ and neonatal ‘morbidity’, particularly in ‘Neonatal Intensive Care Unit’, where susceptible neonates face an elevated risk of infection (2). Sepsis still presents major problems for healthcare systems despite improvements in care of the neonates, especially in nations with ‘low-income’ and ‘middle income’ where mortality in neonatal population is still high (2). In these areas, the management of newborn infections is frequently hindered by inadequate healthcare infrastructure and a heightened prevalence of antibiotic-resistant pathogens (2).

‘Neonatal sepsis’ is categorized based on the date of onset, which affects both the aetiology and clinical manifestation (3). ‘Early Onset Sepsis’ manifests in a time period of initial 72 hours of birth, frequently due to transmission of infections vertically derived from the organisms in the maternal vaginal tract (3). Conversely, ‘Late Onset Sepsis’ occurs after the initial seventy-two-hour period and is predominantly connected with environmental variables and healthcare-related diseases (3). Irrespective of the time duration of occurrence, sepsis presents significant threat to neonatal health, with sepsis of early onset being more perilous for preterm neonates due to their underdeveloped immune systems (1,2,3).

The worldwide impact of neonatal sepsis is substantial, considerably affecting infant death rates (4). The prevalence of sepsis in preterm neonatal population, especially those having very low weight at birth, might attain 20%, accompanied by a

fatality rate of about 17.6% (4). In 2016, the “World Health Organization” reported that newborn deaths were above 46% of all fatalities among children under five, with 7% of these linked to sepsis (4). Gram-negative sepsis is more prevalent in countries which are developing and is frequently associated with higher death rates due to the aggressive nature of infections and the limited availability of effective antibiotics (4). This underscores the pressing necessity for enhanced infection control measures, prompt diagnosis, and availability of effective antimicrobial treatments (4).

The pathogenesis of neonatal sepsis entails an exaggerated immunological response to infection, resulting in the release of inflammatory mediators and vasoactive agents (5). This may lead to extensive vasodilation, compromised tissue perfusion, and hypoxia, ultimately resulting in multi-organ failure and, in severe instances, mortality (5). The swift advancement of sepsis highlights the necessity for early identification and immediate action to avert irreparable consequences (5).

‘Early Onset Sepsis’ is commonly attributed to infections obtained from the maternal genitourinary system during gestation or parturition (5). ‘*Group B Streptococcus*’ and ‘*Escherichia coli*’ are the predominant disease-causing microbes, with ‘*E. coli*’ posing a significant threat to preterm infants due to its correlation with meningitis as well as septic shock (5). Additional pathogens implicated in EOS comprise ‘*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus species*, and *Listeria monocytogenes*’ (5). Factors that pose a risk for ‘early-onset sepsis (EOS)’ encompass prematurity, prolonged duration of membrane rupture and per vaginal leakage of amniotic fluid, infection of the chorion or amnion, and asphyxia (5).

Neonatal blood stream infections that have occurrence following the initial 72 hours of life, is frequently linked to healthcare-associated illnesses and the horizontal

transfer of pathogens (5). '*Coagulase-negative staphylococci (CoNS)*', an integral part of the normal skin microbiota, are the most prevalent etiological agents of late onset sepsis group (5). Nonetheless, other significant infective agents, such as '*Pseudomonas aeruginosa*', '*Candida albicans*', '*Serratia marcescens*', and '*E. coli*', are also commonly involved (5). GBS may also lead to 'late-onset sepsis', especially in instances of newborn meningitis, but it is less prevalent in 'late-onset sepsis' than in 'early-onset sepsis' (5). The challenge here is to differentiate between actual illnesses and sample contamination, especially with '*Coagulase-Negative Staphylococci*' and '*Staphylococcus epidermidis*', which impairs the diagnosis of LOS (5).

Realizing the risk variables linked to newborn sepsis is essential for enhancing neonatal outcomes (6). Maternal variables, including prolonged duration of rupture of membranes, maternal fever, and chorioamnionitis, heightens the newborn sepsis menace (6). Neonatal characteristics such as preterm, low birth weight, diminished APGAR scores, and the necessity for resuscitation at birth significantly increase the risk (6). Furthermore, intrusive medical procedures, such as mechanical ventilation, prolonged duration of central and peripheral venous access increases the infants' susceptibility to opportunistic infections (6). Enhancing infection control measures and enforcing evidence-based protocols for antibiotic administration are crucial for diminishing the occurrence and severity of newborn sepsis (6).

This study has the objective to investigate the pattern in which colonization of the gastrointestinal tract occurs in neonates who were born prematurely and determine its association with neonatal sepsis. A prospective one year, hospital based observational study will be undertaken to examine the impact of gut microbiota on the onset of newborn illnesses, particularly sepsis, in preterm infants. Comprehending this

link may yield essential insights for novel preventative methods and clinical practices designed to mitigate the burden of sepsis-related morbidity and mortality in preterm newborns. This research aims to discover unique colonization patterns and their correlation with sepsis, perhaps refining infant care procedures and improving early diagnostic and therapeutic approaches.

NEED FOR THE STUDY

‘Neonatal sepsis’ continues to be an important challenge in neonatal care, especially for preterm neonates (7). While established risk factors such as maternal infections, prematurity, and invasive medical interventions contribute to the incidence of sepsis, the part of microbiota in the gut of neonates in sepsis development is an emerging area of research (8).

Understanding how gut microbiota colonization patterns influence neonatal sepsis may help refine existing practices and improve prevention and treatment strategies, ultimately enhancing outcomes for preterm neonates (9). Several studies have implied that gut microbiota disruption may influence neonates to develop sepsis by facilitating ‘bacterial translocation’ and immune dysfunction (10,11).

This study has the aim to further determine these associations, and contribute to the growing body of knowledge that will guide clinical practice and improve neonatal care (12).

AIMS AND OBJECTIVES

Primary Objective:

To study the pattern of colonization of the gut microbiota in preterm neonates (>28 weeks POG and < 37 weeks POG) and its influence on the incidence and outcome of neonatal sepsis

Secondary Objective:

1. To assess the risk factors associated with occurrence of bacteria in the gastric aspirate and stool samples of preterm neonates.
2. To find the similarity in antibiotic sensitivity pattern between isolates from the gut and blood samples in preterm neonates with culture positive sepsis and substantiate with molecular analysis.

REVIEW OF LITERATURE

‘Neonatal sepsis’ is an infection of the organ system in newborns that presents in the initial twenty-eight days after birth (13). It contributes as a significant aetiology to neonatal rates of mortality, particularly in ‘developing’ countries (13). The condition can be divided into two categories based on the timing of symptom onset into ‘early-onset sepsis’, which occurs within 72 hours after birth, and ‘late-onset sepsis’, occurring three days but within the first 28 days of birth (13). EOS is typically transmitted vertically from the mother during birth, while LOS is often acquired horizontally, particularly in healthcare settings (13).

Epidemiology and Global Impact

According to the ‘World Health Organization’, neonatal sepsis is responsible for nearly 8% of deaths among newborns and is a leading contributor to ‘neonatal mortality’ and health complications in the later parts of life, particularly belonging to countries with below average incomes (14). Approximately ‘1.3 million cases’ of neonatal sepsis are known to occur globally each year, leading to around 203,000 deaths (13). The rate of occurrence of EOS has seen a decline as a result of better tools for early screening and methods undertaken for prevention, while LOS remains relatively constant (13). ‘Preterm’ and ‘low-birth-weight’ infants are particularly vulnerable, with mortality rates as high as 17.6% in these groups (13).

A ‘systematic review’ conducted by Fleischmann C and Reichert F, including 26 studies from 14 countries in 2023, shows that from 1990 to 2019, the percentage of incident cases of neonatal with a diagnosis of sepsis and other infections of the systems increased by 12.79%, with cumulative cases rising from ‘5.59 million to 6.31 million’ globally (15). During the same period, the overall mortality rate declined by 12.93%, indicating progress in managing sepsis-related deaths (15).

The study on ‘global incidence and mortality’ of ‘neonatal sepsis’ also showed that age-standardized incidence rates (ASIR) globally rose by approximately 0.46% per year, from 85.21 per 100,000 in 1990 to 97.43 for every 100 thousand population in the year 2019 (15). The death rates which were age standardized (ASDR) decreased by an average of 0.53% annually over the same period, with a drop from 3.97 per 100,000 in 1990 to 3.50 per 100,000 in 2019 (15).

A higher neonatal sepsis rate was observed in regions with lower socio-economic status and healthcare infrastructure (15). For example, in Sub-Saharan Africa, the ASIR and ASDR remain significantly elevated due to poor access to healthcare services and high antimicrobial resistance (15). Conversely, parts of Western Europe showed a decline in ASDR and minimal changes in ASIR (15).

These global statistics highlight the need for continuous improvements in healthcare, timely establishment of the cause, and correct management protocols to mitigate the burden of ‘neonatal sepsis’ worldwide (15).

PATHOPHYSIOLOGY

Infection in neonates occurs through ‘vertical transmission’ of bacterial flora from the mother’s lower genital tract to the uterus (16). This contamination of the ‘amniotic fluid’ results in spread of the infection through the hematogenous route, leading to ‘foetal bacteraemia’ and ‘sepsis’ (16). Both ‘gram-negative’ and ‘gram-positive’ bacteria can be the source of infection (17). Bacterial cell wall of ‘gram-negative’ organisms contains lipopolysaccharide (LPS), an endotoxin, whereas the cell wall of ‘gram-positive’ bacteria is composed of ‘lipoteichoic acid (LTA)’, an exotoxin (18).

LPS signals via ‘Toll-like receptor 4 (TLR-4)’, and ‘lipopolysaccharide-binding protein (LBP)’ plays a critical role as a biomarker (19). As a retort to infection, LBP detects ‘microbial-associated molecular patterns (MAMPs)’ on the bacteria, facilitating the delivery of endotoxin to CD14 immune cells (19). Similarly, ‘lipoteichoic acid’ released from the cell walls of ‘gram-positive’ bacteria triggers signaling through ‘Toll-like receptor 2 (TLR-2)’ (19).

The vascular endothelium and immune cells like macrophages along with monocytes recognize infections by binding pathogen-associated molecular patterns ‘(PAMPs)—including LPS, LTA, DNA, and RNA—to pathogen recognition receptors (PRRs) such as TLRs, Rig-1-like receptors (RLRs), and Nod-like receptors (NLRs)’ (20,21). This leads to signal transduction, where the transcription factor ‘NF- κ B’ is transported inside the nucleus, promoting an immune response (19).

Combining of the ‘PAMPs’ and ‘Damage-associated molecular patterns (DAMPs)’ to receptors on granulocytes, dendritic cells, macrophages, and monocytes activates these immune cells, which then release cytokines, resulting in a cytokine storm (21). This early proinflammatory response results in the release of ‘cytokines, interleukins, chemokines, growth factors, tumour necrosis factors, and interferons’ by the innate immune system (21).

However, in newborns, the lack of mature functioning of the cells of immune system leads to an inflammatory response that is not complete (21).

In response to these proinflammatory factors, vascular endothelial cells become activated, leading to increased polymorphonuclear neutrophil (PMN) and leukocyte migration (22). The immune response triggers coagulation, thrombolysis, and fibrinolysis, leading to a coagulation cascade and enhanced vascular permeability

(22). Proinflammatory cytokines also cause the shedding of the endothelial glycocalyx, which is regulated by 'reactive oxygen species', 'proteoglycans', 'glycosaminoglycans', and 'dermatan sulfate' (22).

Enzymes such as heparinase, hyaluronidase, and metalloproteinases are activated to cleave transmembrane proteins, contributing to the disruption of achieving haemostasis (22).

Thrombosis of the small vessels leads to increased adhesion of white blood cell and platelets, while nitric oxide-mediated dilation of the vessels results in altered tone of the vessels and increased permeability, causing oedema of tissues and leading to microcirculatory dysfunction, hypoperfusion, and eventually multiorgan failure (22).

MICROORGANISMS IN NEONATAL SEPSIS

The microorganisms responsible for 'neonatal sepsis' differ between 'developed' and 'developing' countries (23). In 'developed' countries, 'Group B Streptococcus' is a leading cause of 'neonatal sepsis', whereas countries that are progressing towards development have, 'Gram-negative' bacteria (GNB), including 'Klebsiella, Escherichia coli, Pseudomonas, and Acinetobacter', as a contributor to sepsis (23). In resource-limited settings, hospital-acquired infections are a significant contributor to early-onset sepsis (EOS), with multidrug-resistant organisms playing an increasing role (23).

A study by Basu S highlighted the rising emergence of non-fermenting 'Gram-negative' bacteria in 'neonatal sepsis' (23). 'Early-onset sepsis' is usually transmitted from the mother and tends to have lower rates of antimicrobial resistance compared to 'late-onset sepsis', which is more often linked to factors of the environment (23).

Research conducted by I. Adlerberth in Sweden showed that ‘the gut microbiota in neonates contains a higher proportion of facultative bacteria compared to the microbiota in adults’ (24). The neonatal gut’s elevated oxygen levels create an environment that favors facultative bacteria over obligate anaerobes (24). Studies have identified ‘*Escherichia coli*’ and ‘*Enterococci*’, particularly ‘*Enterococcus faecalis*’ and ‘*Enterococcus faecium*’, as initial colonizers in the gastrointestinal tract of neonates (24). Other members of the ‘*Enterobacteriaceae*’ family, such as ‘*Klebsiella*’ and ‘*Enterobacter*’, are also commonly found in the gut microbiota of newborns (24).

The similarity between the gut microbiota of neonates and isolates from blood cultures in sepsis suggests that gut colonization significantly influences the development of neonatal sepsis (24).

In settings with limited resources, lack of maintenance of hygiene in delivery rooms and poor adherence to ‘aseptic’ practices facilitate pathogen transmission (25). **Zaidi et al.** emphasized that any infection in a neonate born in a hospital in a hospital with limited resources must be presumed to have been acquired in the hospital, even if development of symptoms occurs within the initial days of life (25). This observation is similar to findings from other studies conducted under similar circumstances (25).

GUT MICROBIOTA COLONISATION IN NEONATES

The neonatal gut microbiota begins to develop shortly after birth, with bacteria that are facultative in nature such as ‘*Escherichia coli*’ and ‘*enterococci*’ being the initial colonizers (23). Over time, the environment becomes more favourable for anaerobes such as ‘*Bifidobacterium*’ and ‘*Clostridium*’ (23). However, in neonates who are hospitalized for extended periods, colonization with pathogenic bacteria,

particularly ‘Gram-negative bacteria’, becomes a cause of concern (23). The immaturity of the neonatal gut barrier, especially in ‘preterm’ and ‘low birth weight’ infants, increases their vulnerability to pathogenic colonization (23).

FACTORS INFLUENCING GUT COLONIZATION

Several factors influence gut colonization in neonates (23). Mode of delivery plays a critical role; vaginally delivered infants are colonized by maternal flora, while those born through lower segment caesarean section are more likely to obtain bacteria from the nosocomial environment (23). Prolonged hospital stays and interventions during hospital care, such as feeding tubes, also increase the likelihood of colonization with hospital acquired pathogens (23).

GUT FLORA AND SEPSIS

Research indicates that gut overgrowth with Gram-negative bacteria can suppress ‘mucosal’ and ‘systemic’ immunity, thereby increasing chances of developing sepsis (23). The link between gut colonization and sepsis has been demonstrated both in surgical and non-surgical neonates (23). Overgrowth of ‘*Klebsiella pneumoniae*’ and ‘*Escherichia coli*’ in the gut has been linked to subsequent bloodstream infections (23). Bacterial translocation, where bacteria cross the gut barrier into sterile tissues, is considered as a primary mechanism in the development of sepsis (23).

Widespread antibiotic usage in NICUs has had an influence on the colonization of neonates with bacteria that are resistant to antibiotics (23). These resistant organisms, including strains of *Klebsiella* and *Acinetobacter*, not only pose a direct threat to neonates but can also spread to other infants within the unit, creating a reservoir of multidrug-resistant pathogens (23). Gastric and intestinal environment

acts as a conducive for the transfer of genes causing resistance to antibiotics, further complicating infection control efforts (23).

Mechanism of gut microbiota causing sepsis

Investigations into the connection between sepsis and gut flora have provided valuable insights into how gut microbiota contributes to sepsis. One proposed mechanism is ‘bacterial translocation’ (Fig. 1). "Bacterial translocation" is the term used to denote the travel of bacteria that are viable and original colonizer of the gastrointestinal tract to the mesenteric lymph nodes and internal organs which are otherwise not colonized with these bacteria (26,27). Studies have shown proof to the fact that bacterial translocation from the GI system plays a crucial part in the sepsis development (26,27).

A study conducted by ‘Pierro A et al’, in surgical neonates identified endogenous septicaemias caused by microbial translocation as clinically significant (28). Research using animal models and in vitro methods has also demonstrated that ‘translocation’ of bacteria is particularly prevalent in the immature gut (29).

Certain organisms, particularly Gram-negative bacteria, are known to undergo translocation (28). Pathogens such as *Shigella* species, *Salmonella* species, and enteroinvasive *Escherichia coli* have disease causing factors that allow them to invade the ‘gut epithelial barrier’ and cause disease (30). Emerging evidence further suggests that the entire enteric bacterial community may contribute to bacterial translocation (30).

For example, a study conducted by ‘Kalischuk et al.,’ in 2009 demonstrated that *Campylobacter jejuni* facilitates the movement of commensal bacteria across gut

epithelial barriers through lipid rafts (31). This finding suggests that certain bacteria can exploit host cell structures to enable translocation (31).

DIRECT AND INDIRECT CONTRIBUTION OF GUT FLORA TO SEPSIS

Besides the direct translocation of ‘Gram-negative bacteria’ from the gut to the bloodstream, gut organisms can also indirectly contribute to sepsis through interaction with other body systems (32). One proposed pathway is the transmission of bacterial pathogens to the trachea through the stream of blood. Reduced stomach PH and impaired ‘peristaltic’ movement may allow ‘Gram-negative’ bacteria to migrate to the trachea from the stomach, which increases the rate of sepsis occurrence (32).

Research by Schwartz et al. showed that gut colonization with Enterobacteriaceae often precedes tracheal colonization in neonates, supporting the idea that the gut becomes a reservoir for these pathogens (32). Neonates colonized with Gram-negative bacteria can act as carriers, spreading infections to other patients (33). In ‘neonatal intensive care units’, where neonates continuously excrete large quantities of Gram-negative bacteria, lapses in hygiene—such as staff failing to wash hands—can further facilitate bacterial transmission (34). Although the exact pathways by which gut bacteria enter the bloodstream vary, their role in sepsis development is well established (34).

ANTIBIOTIC RESISTANCE AND ITS IMPACT ON NEONATAL SEPSIS

An emerging issue in managing neonatal sepsis is the increasing prevalence of antibiotic-resistant organisms (35). Intensive care units (ICUs) are hotspots for antimicrobial resistance due to the frequent irrational antibiotic treatment. Intestinal tract is as a reservoir for ‘antibiotic-resistant’ Gram-negative bacteria (35,36).

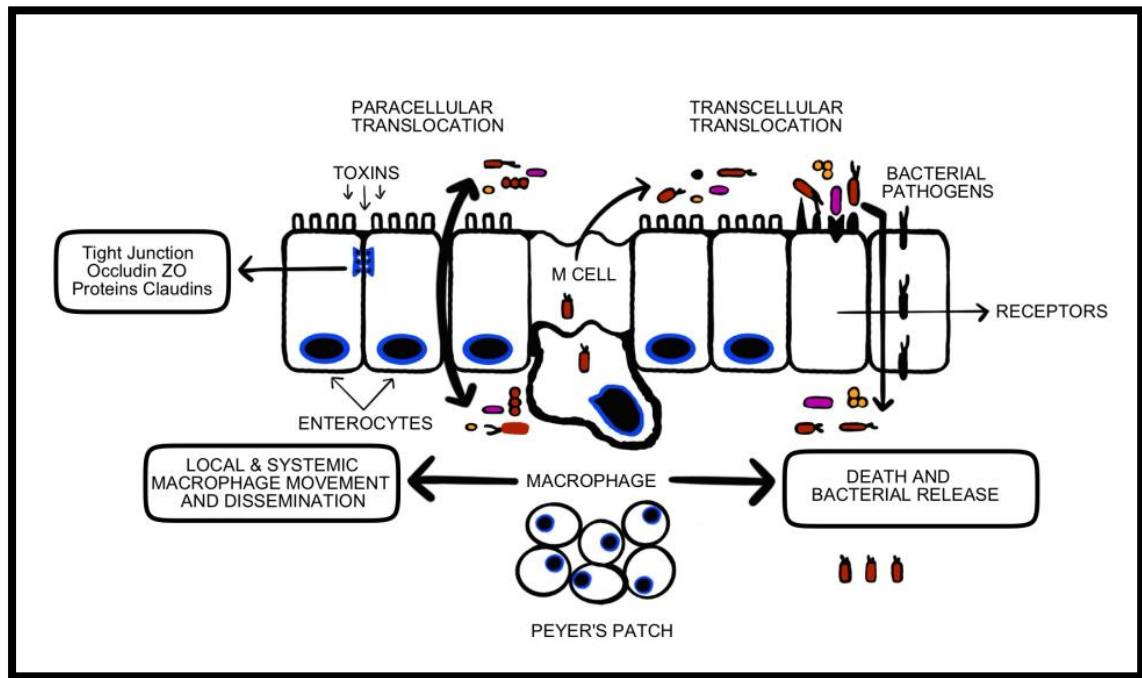
Neonates confined to ICUs are particularly vulnerable to colonization by resistant bacteria (35).

Although some colonized neonates remain asymptomatic, bacterial translocation can lead to bloodstream infections, and colonized neonates may act as carriers, increasing the risk of infections in other neonates (37). The gut also facilitates the transfer of genes conferring antibiotic resistance, allowing even normal flora to acquire resistance and cause infections (38,39).

Many resistance genes are carried on mobile plasmids, allowing for the rapid spread of resistance (40,41). This facilitates the exchange of resistance traits between bacterial species, increasing the likelihood of multidrug-resistant infections (40,41).

Numerous isolated occurrences of sepsis in NICU have been traced back to '*Enterobacteriaceae*' (42,43) with other Gram-negative bacteria such as *Acinetobacter baumannii* (44,45). A study demonstrated that drug-resistant *Enterobacteriaceae* frequently colonized neonates during their hospital stay and persisted even after discharge (46). This creates a reservoir of resistant organisms that increases the risk of recurrent infections (46).

The problem is further compounded by the multidrug resistance of Gram-negative bacteria, with many pathogens resistant to multiple antibiotic classes (47). For example, metallo-beta-lactamases such as 'IMP-1' and 'VIM-1' confer resistance to 'carbapenems, aminoglycosides, third-generation cephalosporins, and ciprofloxacin' (47). Rise of such multidrug-resistant infections underscores the importance of effective antibiotic stewardship and infection control measures in NICUs (47).

Figure 1: Mechanism of gut translocation of bacteria

A study conducted by 'Parijat Das et al.,' at PGI Chandigarh provided significant insights into the 'colonization of the neonatal gut' by 'Gram-negative bacilli (GNB)' ; its implications for 'neonatal sepsis' (48). The study found a high prevalence of GNB, with higher colonization rates in stool samples compared to gastric aspirates, and greater isolation rates in second samples than in the first (48). Among the organisms derived in cultures, '*Klebsiella pneumoniae*' was the common isolate, and the second highest in number was '*Escherichia coli*' (48). Interestingly, '*Klebsiella pneumoniae*' exhibited clonal patterns across different neonates, suggesting possible nosocomial transmission, whereas '*Escherichia coli*' displayed diverse genotypes, indicating varied sources of colonization (48).

Risk factor analysis demonstrated that maternal factors were significantly associated with GNB presence in the first gastric aspirate (48). However, in the second gastric aspirate, NICU-related factors, such as prolonged hospitalization and enteral feeding, emerged as key contributors to GNB colonization (48). This shift

highlights the increasing influence of environmental and medical interventions on gut colonization over time (48).

The study also established a strong association between ‘gut colonization with GNB and clinical sepsis’ (48). Neonates with GNB in their GA-2 or stool samples had a significantly higher incidence of sepsis compared to those without any isolates (48). *Klebsiella pneumoniae* was the predominant pathogen isolated from blood cultures in neonates with sepsis, reinforcing its role as a major contributor to bloodstream infections (48). Thus, findings of the above study emphasize the significance of monitoring gut colonization patterns and implementing strict measures to control infections in NICUs to mitigate the ‘risk of sepsis’ in neonates (48).

‘A prospective longitudinal study’ done by ‘Juliette C. Madan et al.,’ examined the gut microbiota of premature infants (49). ‘Stool samples were obtained weekly, and DNA was extracted for amplification of the V6 hypervariable region of 16S rRNA, followed by pyrosequencing’ (49). Six newborns at 24–27 weeks age of gestation was analyzed, with two developing ‘late-onset culture-positive sepsis’, showing two neonates with ‘culture-negative systemic inflammation’, and two remaining free of sepsis (49). ‘*Lactobacillus*, *Staphylococcus*, and *Enterobacteriales*’ were isolated from the stool sample, which was thus not sterile. (49).

Neonates who were apparently healthy had more diversity and a predominance of ‘*Clostridium*, *Klebsiella*, and *Veillonella*’ whereas those neonates who developed infection had lower diversity of microbial pathogens at birth but during the later times showed the presence of ‘*Staphylococcus*’ (49).

‘Stewart CJ et al.,’ conducted a study showing that “bacteria isolated in diagnostic blood cultures often corresponded with dominant genera in the gut

microbiome (50). Longitudinal changes were tracked using preterm gut community types (PGCTs), revealing that control infants had a greater diversity of PGCTs compared to those with late-onset sepsis (LOS) ($P = 0.011$) (50). PGCT 6, characterized by Bifidobacteria dominance, was present only in control infants” (50).

‘Metabolite profiles also differed between LOS and control infants at diagnosis and seven days after but not seven days prior to diagnosis.’ (50) Thus, we can conclude that, development of sepsis can depend on the microbial residents of the gut and could serve as a potential target for intervention (50).

‘Smith A., Anandan S et al.,’ conducted a study showing that 71% of neonates were colonized by ‘Gram-negative bacteria (GNB)’ at birth, and all of the study cases were colonized by the end of seven days (51). Commonly isolated bacteria included ‘*Escherichia coli*, *Klebsiella*, *non-fermenting Gram-negative bacilli (NFGNB)*, *Pseudomonas*, and *Enterobacter*’ (51).

Percentage of neonates who were colonized with ‘extended-spectrum beta-lactamase (ESBL)-producing organisms’ was sixty eight, while percentage of neonates showing isolation of ‘carbapenem-resistant organisms (CROs)’ was 5 percent (51). ‘An association was also noted between gut colonization and late-onset sepsis, reinforcing the importance of surveillance for resistant organisms in neonatal units’ (51).

‘Lee CC et al.,’ in their study ‘explored the link between gut dysbiosis and neonatal sepsis’ (52). ‘16S rRNA’ sequencing was done on specific days to evaluate microbiota diversity and composition (52).

The results showed that ‘very low birth weight preterm infants’ with sepsis had lower microbial diversity at birth compared to preterm infants without sepsis and

term infants (52). Increasing 'Proteobacteria' and decreasing Firmicutes were observed over time in 'VLBW neonates' (52). PCR confirmed that 40% of causative pathogens in neonates with sepsis were isolated in the GI samples (52).

NEONATAL SEPSIS- RISK FACTORS

Neonatal sepsis remains a critical challenge in neonatal healthcare, particularly in 'neonatal intensive care units' (48). Though we have shown advancement in care of the newborns, especially those born before 37 weeks of gestation, infection has posed as an important determinant of neonatal 'morbidity and mortality' (48). Multiple factors result in the onset of 'neonatal sepsis', including 'maternal, neonatal, and NICU-associated factors' (48).

Maternal Risk Factors

Among the maternal risk factors, 'premature rupture of membranes' is widely recognized as a major contributor to 'neonatal sepsis' (48). Studies have shown that rupture of membranes occurring for a duration of more than 18 hours significantly upsurges the 'neonatal sepsis' risk, particularly 'early-onset sepsis' (48). 'Maternal infections, such as urinary tract infections (UTIs) and sexually transmitted infections (STIs)', are also linked to an increased risk of neonatal sepsis, especially when they occur during the third trimester (48).

Other maternal conditions, including chorioamnionitis and maternal fever during labor, are also factors that lead to 'neonatal sepsis' (48). Chorioamnionitis leads to intra-amniotic inflammation, which can increase the likelihood of ascending infections, predisposing neonates to sepsis (48). Additionally, prolonged labor and maternal colonization with 'Group B Streptococcus' are identified as contributors to sepsis (48).

Neonatal Risk Factors

Both ‘Low birth weight’ and ‘prematurity’ are prominent neonatal contributors to sepsis (48). Infants born at less than thirty seven weeks of pregnancy or with a ‘low birth weight’ are more predisposed to infections as their body defence mechanisms are not fully developed (48). Preterm infants have reduced production of immunoglobulins and impaired neutrophil function, which weakens their ability to combat infections (48).

Additionally, neonates with a ‘low APGAR score’ (below 7 at 5 mins) have an increased tendency to develop sepsis (48). Birth asphyxia, meconium-stained amniotic fluid, and requirement of resuscitation following birth further increase vulnerability to infection (48). Hypoxia and acidosis associated with birth asphyxia impair immune function, increasing susceptibility to opportunistic infections (48).

NICU-Associated Factors

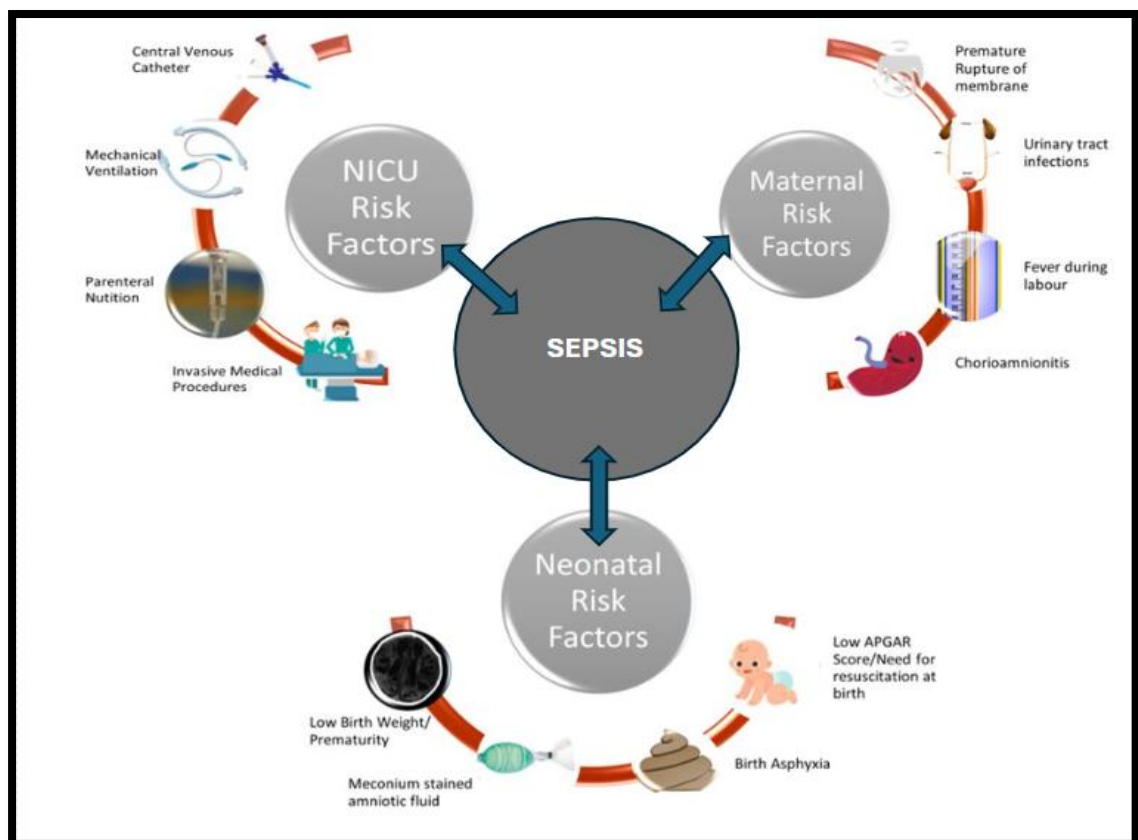
Several NICU-related interventions are ‘risk factors’ for ‘neonatal sepsis’ (48). The use of ‘Centrally inserted venous access’ can lead to infections that are blood borne, particularly when catheterization exceeds 10 days (48). Prolonged use of CVCs provides a surface for growth of microbiome and ‘biofilm formation’, which can cause catheter-associated bloodstream infections (CABSI) (48).

Assisted ventilation is another source of infection, as it predisposes the neonate to ‘ventilator-associated pneumonia (VAP)’, which can progress to sepsis (48). The use of parenteral nutrition and other invasive medical procedures also increases the risk by disrupting the protective barrier of the gut, facilitating bacterial translocation (48).

Moreover, antibiotic overuse in NICUs is an etiology for the emergence of ‘multidrug-resistant organisms’, further increasing complexity in treating neonatal sepsis (48). Inadequate infection control measures and overcrowding in NICUs also play a role in facilitating pathogen transmission (48).

Interplay of ‘maternal, neonatal, and environmental factors’, particularly those related to ‘NICU’ interventions (48). Early identification of these risk factors and implementing targeted interventions can help decrease the occurrence and serious complications of ‘neonatal sepsis (48).

Figure 2: Contributing factors to sepsis



‘Diagnosing neonatal sepsis is challenging due to its nonspecific clinical presentation’ (9). Common presentations include fever, hypothermia, poor feeding, lethargy, tachycardia, and respiratory distress (9). These symptoms may overlap with other neonatal conditions, making early diagnosis difficult (9).

A study conducted by Nyenga AM, Mukuku O, and Wembonyama SO identified abnormal heart and respiratory rates as early indicators of sepsis, often preceding other clinical symptoms by several hours or even days (53). Their findings highlight ‘the importance of monitoring vital signs closely in neonates at risk for sepsis’ (53).

Early recognition of these signs, combined with increased evidence to suspect sepsis is necessary for initiating timely treatment to improve neonatal outcomes (9).

System	Early Onset Sepsis	Late Onset Sepsis
Cardiac	Tachycardia, Low Blood Pressure	Bradycardia, prolonged capillary refill time, poor pulses, poor perfusion
Respiratory	Tachypnoea, subcostal retraction, intercostal retraction, flaring of ala nasi	Grunting, irregular breathing, cessation of breathing, decreased respiratory efforts
Gastrointestinal tract	Feed intolerance, poor latch after established breastfeeding, prolonged feeding times, irritability	Vomiting, distention of the abdomen, metabolic acidosis and hypoglycaemia or hyperglycaemia
Neurological	Inconsolable cry, reduced tone, thermal dysregulations (hypothermia or fever)	Bulging anterior fontanelle, features of meningeal infection, apnoea, lethargy, encephalopathy, seizures
Renal	None	Oliguria
Hepatic/haematological	Hepatomegaly and conjugated hyperbilirubinemia in the absence of other known risk factors	Petechiae or bleeding suggestive of coagulopathy
Dermatological	New-onset rash, joint swelling, vesicles, erythema	Umbilical discharge Mottling

TABLE 1 – SIGNS AND SYMPTOMS OF SEPSIS

DIAGNOSTIC CRITERIA

Current diagnostic approaches for neonatal sepsis include microbiological culture methods, molecular diagnostics, haematological parameters, and inflammatory marker assessments (54).

Screening for sepsis is defined to be positive based on biochemical laboratory parameters if at least two of the below criteria is positive:

1. 'Total leukocyte counts below 5000/mm³ with a band-to-total polymorph ratio exceeding 0.2' (54).
2. 'Absolute neutrophil count less than 1800 cells/mm³' (54).
3. 'C-reactive protein (CRP) levels greater than 1 mg/dL' (54).
4. 'Micro-erythrocyte sedimentation rate exceeding 10 mm in the first hour' (54).

'Gold standard investigation' for diagnosing neonatal sepsis is through 'blood culture' (54). Although it provides definitive confirmation, the time required for culture results often delays treatment decisions, highlighting the need for rapid diagnostic methods (54)

In a study conducted by 'Kurien Anil Kuruvilla et al., from the Department of Neonatology and Microbiology, Christian Medical College Hospital, Vellore' the antibiotic sensitivity patterns of the organisms responsible for 'early onset sepsis (EOS)' and 'late onset sepsis (LOS)' were carefully analyzed (55). The pattern of resistance was notably different between the organisms causing EOS along with those causing LOS (55).

For 'E. coli', the organisms responsible for 'EOS' were generally sensitive to common antibiotics, including cefotaxime, gentamicin, and amikacin, which are

frequently used as the initial option for management of neonatal sepsis (55). However, in LOS cases caused by *E. coli*, resistance was observed in some cases, particularly to ampicillin (50%) and to gentamicin (25%), indicating a higher level of antibiotic resistance in these later infections (55). The organisms showed high sensitivity to ciprofloxacin and imipenem, which were used to treat resistant strains in LOS (55).

For *E. faecalis*, the organisms responsible for both EOS and LOS were generally sensitive to penicillin, cefotaxime, and amikacin (55). Resistance was relatively low, with only a few cases showing resistance to ampicillin (33%) and gentamicin (66%) in LOS. This suggests that *E. faecalis* is more susceptible to common antibiotics but may still present some challenges in treatment, especially in hospital-acquired infections (LOS) (55).

Klebsiella and *Enterobacter*, which were the predominant organisms responsible for LOS in the study, showed significant resistance to multiple antibiotics (55). Both *Klebsiella* and *Enterobacter* strains were resistant to aminoglycosides like gentamicin and amikacin, as well as to cephalosporins like cefotaxime and ceftazidime (55). This resistance pattern suggests that these Gram-negative bacteria were not susceptible to the antibiotics commonly used in neonatal intensive care units (55). These pathogens, however, remained sensitive to more potent antibiotics such as ciprofloxacin and imipenem, which were used as alternative treatments for the resistant strains (55).

The antibiotic resistance observed in the LOS cases caused by *Klebsiella* and *Enterobacter* underline the significance of appropriate ‘antibiotic stewardship’ and close monitoring of resistance patterns (55). In cases of resistance, clinicians were required to shift to broader-spectrum antibiotics, such as imipenem or ciprofloxacin, to effectively manage infections (55). *Staphylococcus aureus* strains, although less

frequently identified in the study, showed resistance to penicillin, but no resistance to vancomycin was reported, highlighting the continued effectiveness of vancomycin in treating *Staphylococcus aureus* infections (55).

The antibiotic sensitivity profile underscores complexities involved in treating ‘neonatal sepsis’ in a setting where multi-drug-resistant organisms are more prevalent, particularly in the cases of ‘*Klebsiella*’ and ‘*Enterobacter*’ (55).

MATERIALS AND METHODS

Data Source: Preterm neonates born between 28 and 37 weeks of gestation at KLE's Dr. Prabakar Kore Hospital, Belagavi

Study Design: Prospective Observational Study

Duration of study: January of 2024–December of 2024 (12 months)

Sample Size: 123 neonates

Sample size for association,

The formula for calculating sample size is:

$$N = (x^2 N p (1-p)) / (d^2 (N-1) + x^2 p (1-p))$$

here,

x denotes the chi-square critical value corresponding to the alpha level and degrees of freedom.

n = estimated population

p = proportion (or prevalence)

d = error (margin of error)

Substituting

n = number of preterm (on an average) babies in a year = 245

p = proportion of 'neonatal sepsis in low-birth-weight neonates' = 20%

d = error (margin of error) = 0.05 or 5% (absolute error)

Since this is a study of association between two factors each of two levels, it forms a 2*2 contingency table

And Chi-square (x^2 critical value for 2*2 table for 1 degree of freedom and alpha = 0,05 is 3.841 i.e, $x^2_{1,0.05} = 3.841$)

Substituting the values we get,

$$N = \frac{3.841 * 245 * 20 * 80}{5^2 (245-1)}$$

$$= \frac{1505672}{12245} = 122.96 \sim 123$$

12245

Sampling technique: Purposive method of sampling

Inclusion Criteria:

- All preterm neonates born at a period of gestation between 28 and 37 weeks, at KLE's Dr. Prabhakar Kore Hospital, Belagavi, within the study time frame of the first of January to the end of December 2024.

Exclusion Criteria:

- Infants with congenital anomalies, cyanotic congenital heart disease
- Neonates with inborn error of metabolism by clinical signs or lab investigations
- Neonates with clinically recognizable genetic syndromes
- Neonates with duration of hospital stay less than 7 days
- Refusal of consent

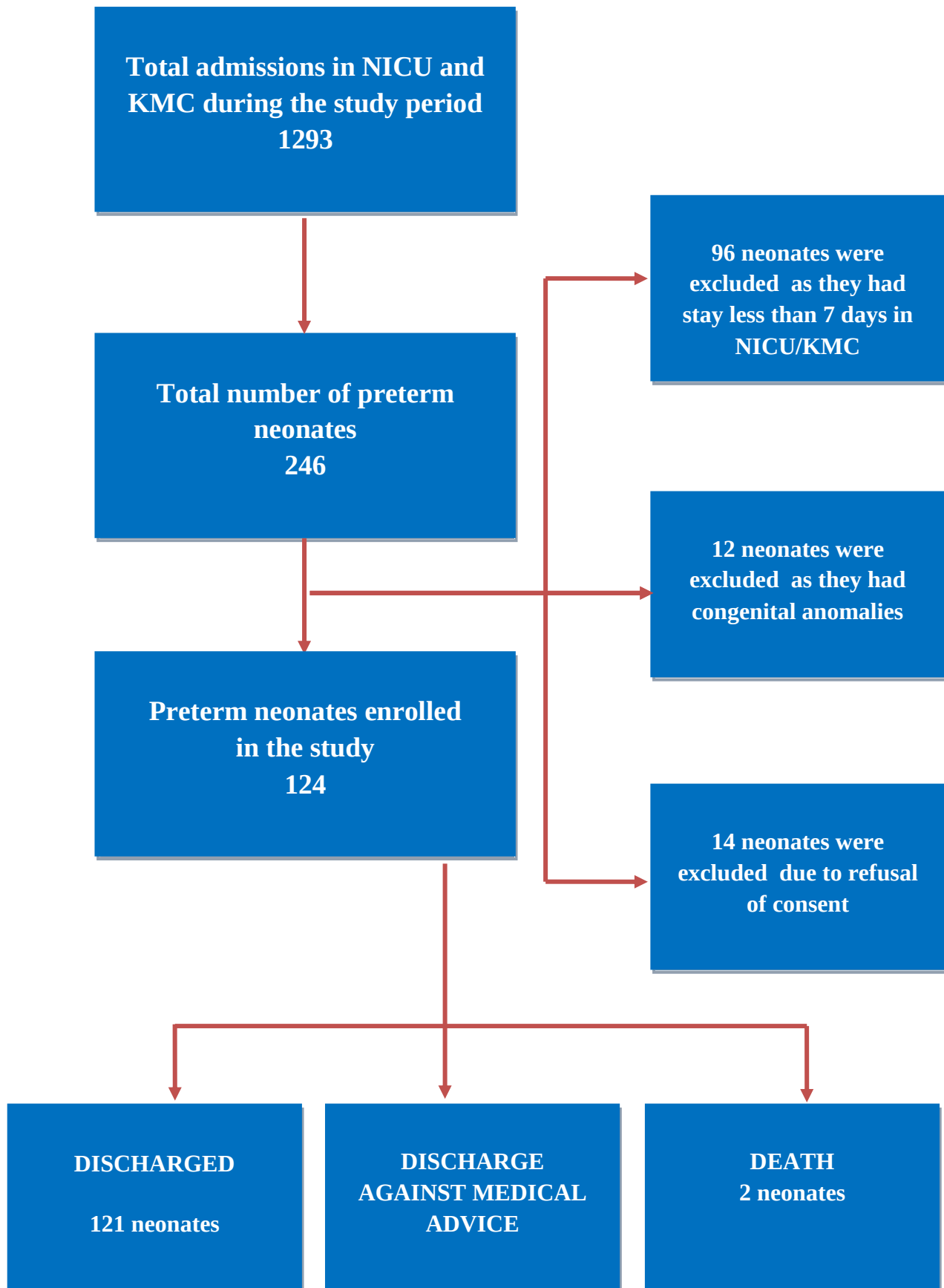


Figure 3: Flow chart of enrolment of neonates in the study

Study protocol:

This one-year prospective observational study took place at KLE's Dr. Prabhakar Kore Hospital located in Belgaum. Ethical approval was secured before commencement, and 'written informed consent' had been taken from those concerned following a detailed elucidation of objective, procedures, benefits, risks, and relevance of the study.

'Preterm infants' delivered between 28 and 37 weeks of gestation who fulfilled the inclusion criteria were incorporated into the study.

The antenatal as well as perinatal histories of the individuals were documented in a structured proforma. This encompassed data included neonatal risk factors, comprising weight at birth, gestational age, delivery mode, antenatal risk factors, premature rupture of membranes (PROM), post-natal adaption characteristics, hospital interventions, and outcomes. Enrolment was limited to newborns hospitalized for a minimum of 7 days whose parents granted consent.

To investigate gut colonization patterns, the initial 'gastric aspirate (GA-1)' and the first 'stool sample (S-1)' were obtained from neonates using ETO-sterilized containers. GA-1 was acquired within the initial four hours post-delivery. Subsequent samples collection, comprising a second 'gastric aspirate (GA-2)' and a second 'stool sample (S-2)', were collected from the same infants between 7 and 10 days of age during their hospitalization, utilizing similar sterilized containers. The occurrence and bacterial colonization patterns in the gastrointestinal tract were subsequently analysed.

Neonates who exhibited clinical signs of sepsis during the research period were evaluated to investigate the relationship between gut colonization and sepsis. Blood samples for culture were drawn prior to initiating antibiotic therapy. Cases

where blood cultures yielded bacterial growth were categorized as "culture-positive sepsis." Neonates displaying one or more clinical indicators, such as lethargy, tachypnoea, tachycardia, temperature instability, hypotension, apnoea, feed intolerance, inadequate perfusion, or distension of the abdomen, were suspected of having sepsis.

Neonates having clinical manifestations of sepsis, were classified as "clinical sepsis only" if blood culture was negative or "clinical sepsis with culture-positive sepsis", if culture results were positive.

‘A septic screen was considered positive if two or more of the following criteria were met:

1. Total leukocyte counts below 5000/mm³
2. Absolute neutrophil count less than 1800 cells/μL or greater than 5400 cells/μL
3. C-reactive protein (CRP) level exceeding 10 mg/dL
4. Micro-erythrocyte sedimentation rate (micro-ESR) above 15 mm in the first hour’

Given the limitations of blood culture, neonates having features indicating sepsis with a septic screen that was positive were classified under "laboratory confirmed sepsis," irrespective of the blood culture results.

Furthermore, antimicrobial susceptibility testing was performed to assess genetic similarities between bacterial strains isolated from gut samples and blood cultures in cases of ‘culture-positive sepsis’

Collection of samples and Methods of microbiological testing

Gastric Aspirate and Stool Samples

To investigate neonatal gut colonization, stool samples as well as gastric aspirate samples were collected under sterile conditions. Gastric aspirates, approximately 2 mL in volume, were obtained using an enteral feeding tube inserted into the neonate's stomach. These samples were then transferred into sterile bottles and immediately kept for storage at a controlled 4°C temperature to preserve their integrity. Stool samples were collected from sterile diapers and subsequently stored at room temperature in sterile containers.

To ensure reliability and accuracy of microbiological analysis, both gastric aspirates and stool samples were delivered to the microbiological laboratory within a duration of four hours of sample collection. Thus, the time-sensitive transfer helped to minimize external contamination and maintain the viability of the microbial flora in the samples.

The collected specimens included the gastric aspirate (GA-1) collected within four hours of birth and first meconium sample (S-1), obtained within initial hours following birth, as well as follow-up samples of the gut (GA-2 and S-2), which were collected between 7 and 10 days of life during the neonate's hospital stay.

Processing of Gastric Aspirates and Stool Samples

Upon arrival at the microbiology laboratory, all collected gastric aspirates and stool specimens were inoculated onto MacConkey agar plates. The plates were then incubated under optimal growth conditions at 37°C for 24 hours. After the incubation period, bacterial growth was examined, and distinct morphological colonies were carefully selected for further identification.

Blood Culture

Blood Sample Collection

To assess the probable relationship between colonization of the gut and neonatal sepsis, blood cultures were collected from neonates who exhibited clinical signs suggestive of sepsis. A total of three millilitre of venous blood was collected using sterile venipuncture techniques to prevent contamination. The collected blood samples were immediately inoculated into paediatric BACT/ALERT culture media bottles to facilitate bacterial growth detection. These bottles were then incubated in the BACT/ALERT 3D automated culture system and at 37°C for up to five days. The automated system continuously monitored the cultures for bacterial growth, enabling detection of bloodstream infections.

Blood Culture Sample processing

Blood culture bottles that exhibited a positive growth signal in the BACT/ALERT system were subjected to further microbiological examination. A portion of the culture was streaked onto '5% sheep blood agar' and 'MacConkey agar plates' to isolate bacterial pathogens. These plates were incubated at 37°C, and bacterial colonies were assessed based on their morphology. Following initial isolation, bacterial species were identified through a series of biochemical tests.

Furthermore, antibiotic susceptibility testing was performed to ascertain the resistance profiles of the detected strains of bacteria. To establish a potential link between gut colonization and bloodstream infections, bacterial strains isolated from gastric aspirates and stool samples were compared with those obtained from positive blood cultures in neonates diagnosed with culture-positive sepsis. This comparison was performed using antimicrobial susceptibility testing with commercially available antibiotic discs.

By following this standardized methodology, the study ensured a comprehensive evaluation of the gut microbial colonization in neonates and its probable significance in the emergence of neonatal sepsis. This step-by-step collection, processing, and analysis of biological samples allowed for a thorough investigation of bacterial colonization patterns and their clinical significance.



Figure 4: BACT/ALERT system

RESULTS

Results of the study: Pattern of colonization of gut microbiota in preterm neonates and its association with neonatal sepsis- A one year hospital based prospective observational study

A one year prospective study which was observational was conducted at KLES Dr. Prabhakar Kore Hospital. All preterm neonates satisfying the inclusion criteria were included. 1,293 neonates were admitted to the 'NICU/KMC' from January 2024 to December 2024, of which 246 were preterm. Among these, 124 neonates were enrolled in the study. Data regarding birth weight, gestation, parity, mode of delivery, bacterial growth in gastric samples, and incidence of sepsis were recorded using a structured proforma.

Statistical methods: Descriptive analysis was conducted using 'standard deviation' and 'mean' for quantitative data, and 'frequency' and 'proportion' for 'categorical variables'. Data was additionally illustrated using suitable diagrams such as 'bar charts', 'pie charts', and 'box plots'.

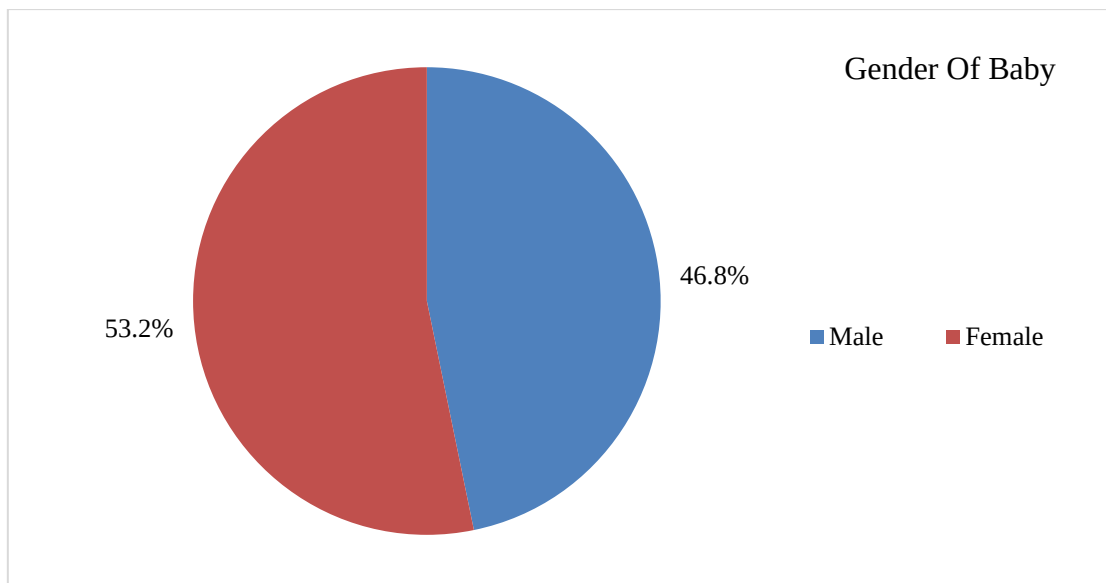
The relationship between 'explanatory variables' and 'categorical outcomes' was evaluated by cross-tabulation and percentage comparison. The chi-square test was employed to assess statistical significance.

The relationship between quantitative explanatory variables and categorical outcomes was evaluated using an independent samples t-test (for two groups) to determine statistical significance.

A P value less than 0.05 was deemed statistically significant. The statistical analysis was conducted using IBM SPSS version 22. (1) 1. IBM Corp. Released in 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corporation.

NEONATAL PARAMETERS IN THE STUDY POPULATION**Table 2: Descriptive analysis of Gender in the study population (N=124)**

Gender Of Baby	Frequency	Percentages
Male	58	46.77%
Female	66	53.23%

Figure 5: Pie chart of gender of baby in the study population (N=124)

Gender: The gender distribution in our study population showed near-equal distribution of 58 male (46.77%) and 66 female (53.23%) neonates.

Table 3.1: Descriptive analysis of gestational age in the study population (N=124)

Neonatal Parameter	Mean $\pm$ SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Gestational Age (Weeks)	33.91 $\pm$ 2.75	34.0	27.1	36.9	33.4	34.4

Table 3.2: Descriptive analysis of Gestational age in the study population (N=124)

Gestational Age	Frequency	Percentages
Extreme Preterm	4	3.23%
Early Preterm	26	20.97%
Moderate Preterm	31	25.00%
Late Preterm	63	50.81%

Gestational Age: The gestational age of neonates in the study ranged from 27.1 to 36.9 weeks, with an average of 33.91 weeks. Majority of the neonates in the study were late preterm (50.81%). Moderate preterm cases were 25%, early preterm cases were 20.97% and extreme preterm cases were 3.23%. The minimum gestational age included in the study was 27.1 weeks.

Table 3.1: Descriptive analysis of weight in the study population (N=124)

Neonatal Anthropometry	Mean $\pm$ SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Birth Weight (Kg)	1.75 $\pm$ 0.52	1.7	0.8	3.6	1.7	1.8
Weight At Discharge (Kg)	1.77 $\pm$ 0.46	1.7	0.9	3.6	1.7	1.9

Table 3.2: Descriptive analysis of Birth weight in the study population (N=124)

Birth Weight (Kg)	Frequency	Percentages
Low Birth Weight	81	65.32%
Very Low Birth Weight	28	22.58%
Extremely Low Birth Weight	9	7.26%
Normal Birth Weight	6	4.84%
SGA/AGA/LGA		
Small for Gestational Age (SGA)	41	33.06%
Appropriate for Gestational Age (AGA)	82	66.13%
Large for Gestational Age (LGA)	1	0.81%

Birth Weight: In the study population, 7.26% of the neonates were ELBW, 22.58% of the neonates were VLBW and 65.32% were LBW. Birth weights varied significantly from 0.8 kg to 3.6 kg, with an average birth weight of 1.75 kg. At discharge, the average weight of the neonates was 1.77 Kg.

Table 4: Descriptive analysis of anthropometry in study population (N=124)

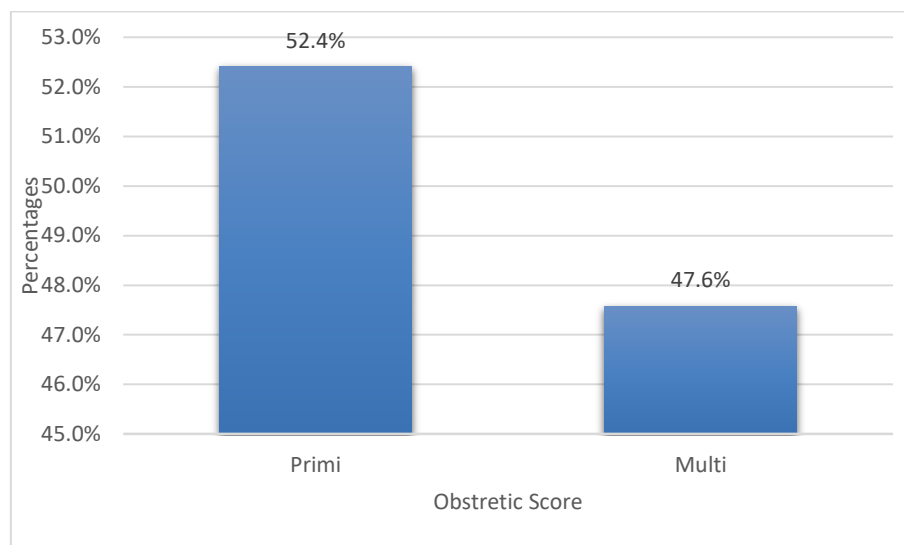
Neonatal Anthropometry	Mean $\pm$ SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Head Circumference (Cm)	31.17 $\pm$ 1.97	31.0	24.0	35.0	30.8	31.5
Length (Cm)	43.9 $\pm$ 3.29	44.0	29.0	50.0	43.3	44.5

Anthropometry: In our study population, average head circumference of the neonates was 31.17 cm and 43.9 cm was the mean length of the neonates which was within the expected ranges for preterm neonates. Maximum head circumference of the neonates was 35 cm and minimum head circumference was 24 cm. Maximum length of the neonates was 50 cm and minimum length was 29 cm.

MATERNAL PARAMETERS IN THE STUDY POPULATION

Table 5: Descriptive analysis of obstetric score in the study population (N=124)

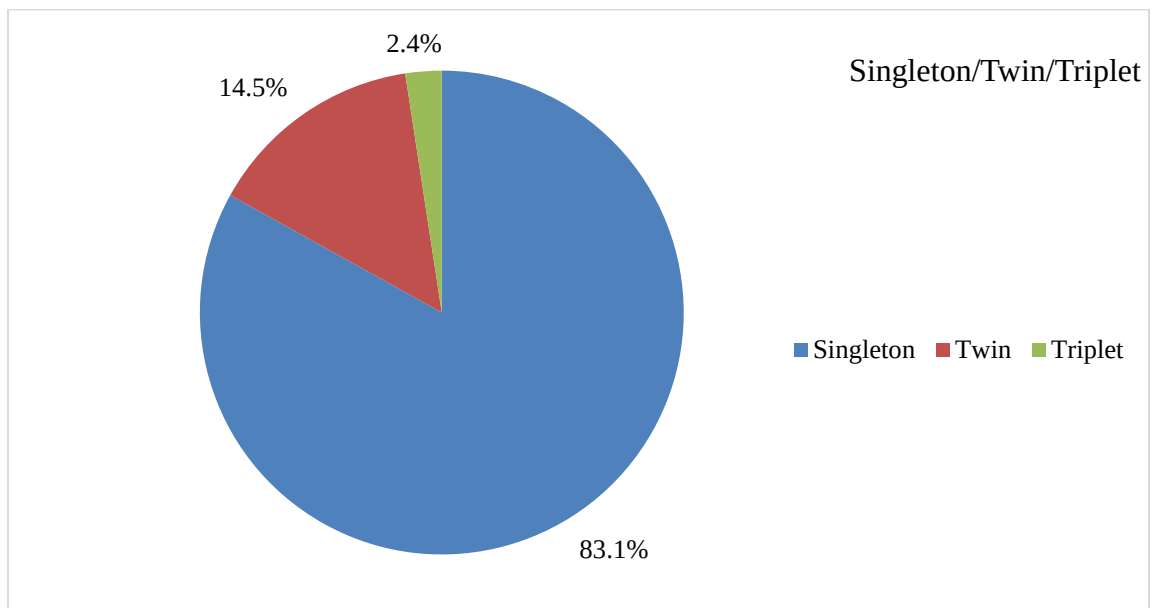
Obstetric Score	Frequency	Percentages
Primigravida	65	52.42%
Multigravida	59	47.58%

Figure 6: Bar chart of obstetric score in the study population (N=124)

Obstetric score: Neonates born to Primigravida mothers (52.42%) were more in number than neonates born to multigravida (47.58%) mothers in the study population.

Table 6: Descriptive analysis of singleton/twin/triplet in the study population (N=124)

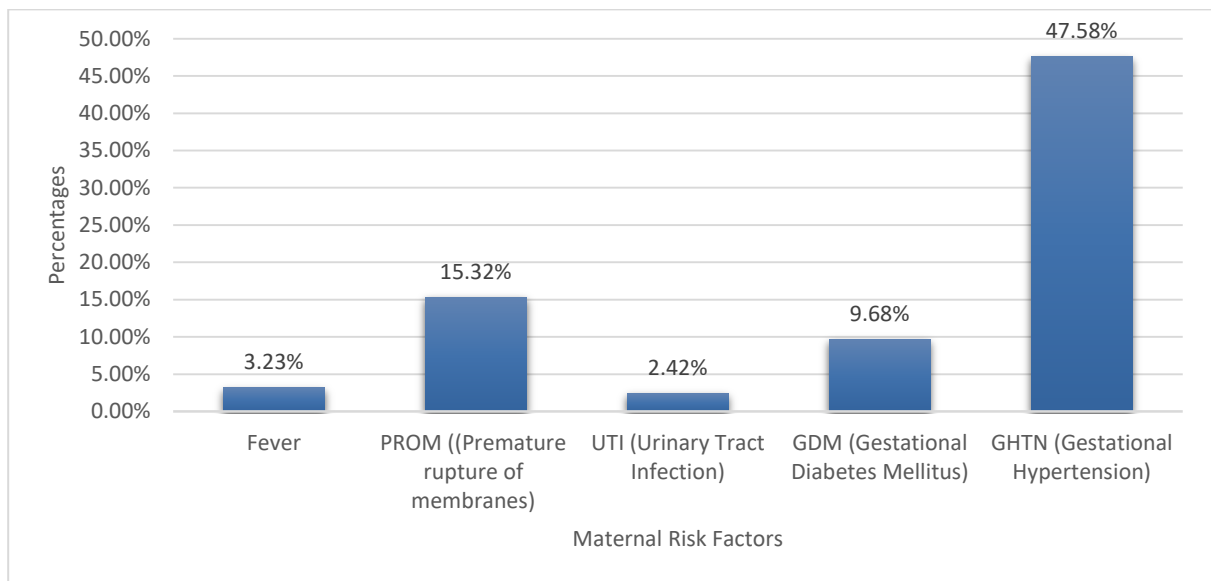
Singleton/Twin/Triplet	Frequency	Percentages
Singleton	103	83.06%
Twin	18	14.52%
Triplet	3	2.42%

Figure 7: Pie chart of singleton/twin/triplet in the study population (N=124)

Gestation Number: In the study population 83.06% of the neonates were born from a singleton pregnancy, 14.52% were born from twin gestation and 2.42% were triplet gestation.

Table 7: Descriptive analysis of Maternal Risk Factors in the study population (N=124)

Maternal Risk Factors	Frequency	Percentages
Fever	4	3.23%
PROM	19	15.32%
UTI	3	2.42%
GDM (Gestational Diabetes Mellitus)	12	9.68%
GHTN (Gestational Hypertension)	59	47.58%

Figure 8: Bar chart of maternal risk factors in the study population (N=124)

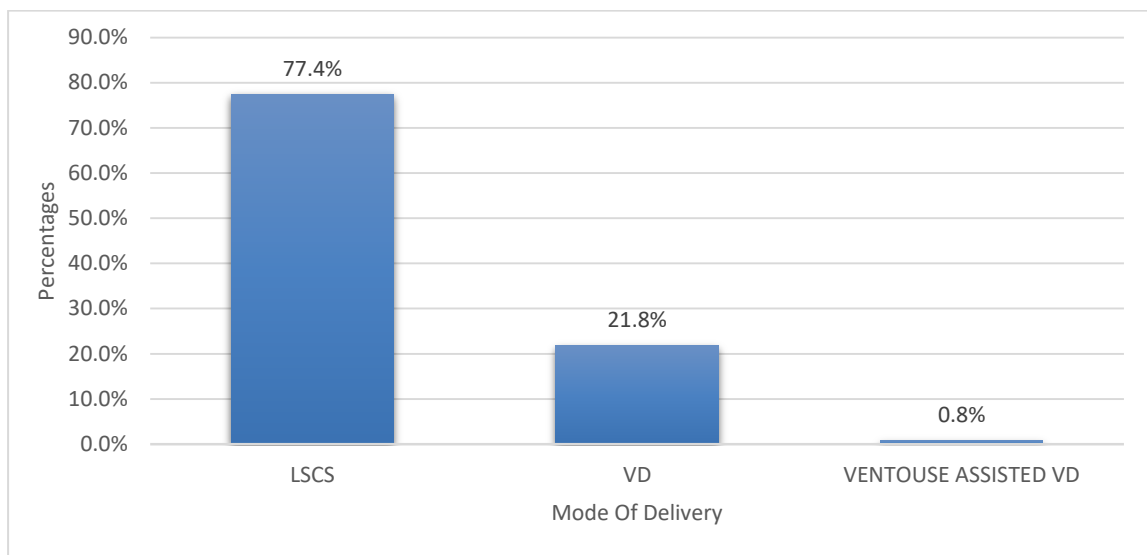
Maternal Risk Factors: Gestational hypertension (GHTN) was the commonest maternal risk factor observed in our study in 47.58% of the mothers followed by PROM (>18 hours) in 15.32% of mothers. GDM was observed in 9.68% of the mothers and UTI was seen in 2.42% of the mothers. 3.23% of mothers had fever, suggesting that maternal infections did not constitute a significant risk factor in our study population.

DELIVERY DETAILS

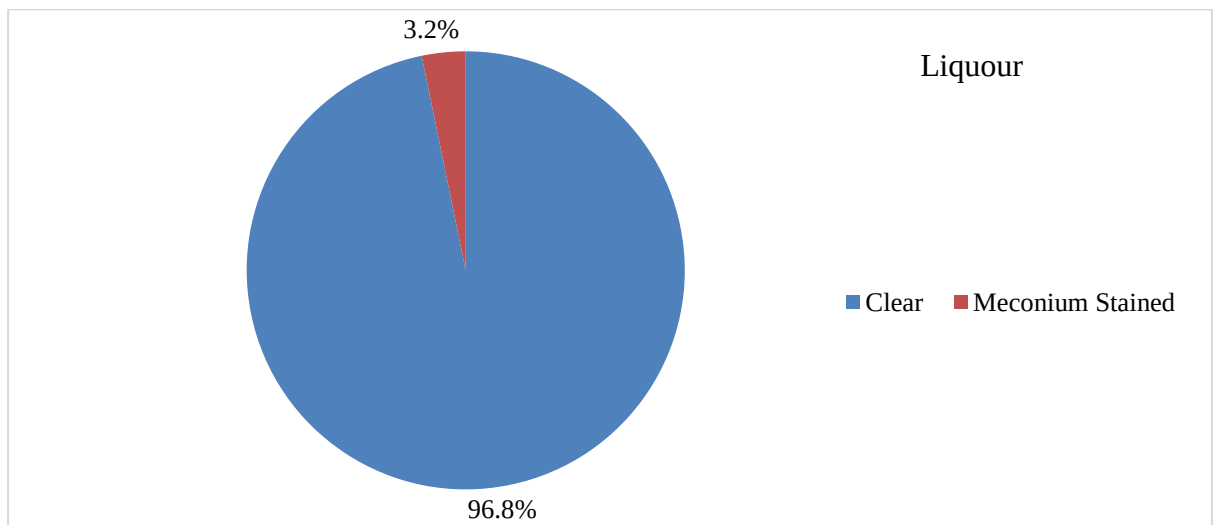
Table 8: Descriptive analysis of Delivery Details in the study population (N=124)

Delivery Details	Frequency	Percentages
Mode Of Delivery		
LSCS	96	77.42%
Vaginal Delivery	27	21.77%
Ventouse Assisted Vaginal Delivery	1	0.81%
Liquor		
Clear	120	96.77%
Meconium Stained	4	3.23%

Figure 9: Bar chart of mode of delivery in the study population (N=124)



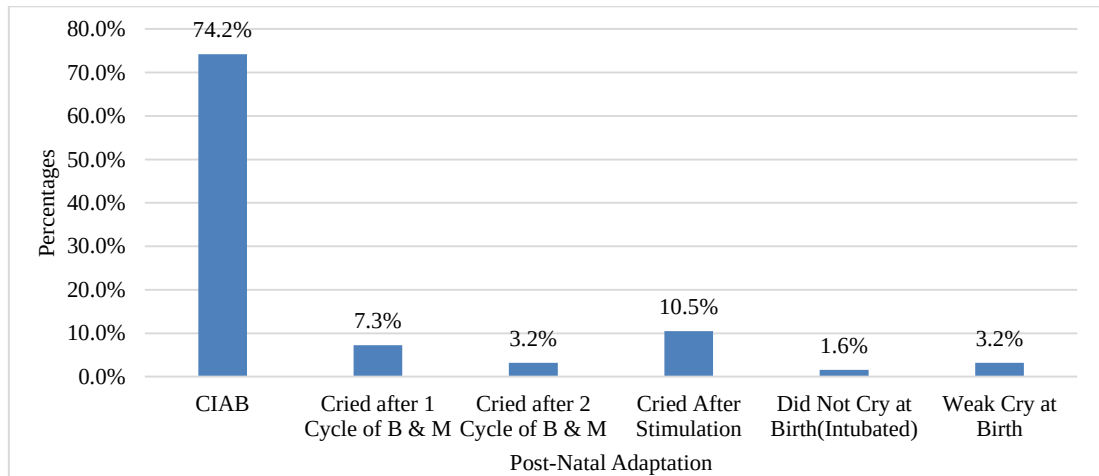
Mode of Delivery: Majority of the deliveries in our study were by LSCS (Lower Segment Caesarean Section) which was observed in 77.42% of cases, followed by Vaginal deliveries which were observed in 21.77% of cases. Only 1 case (0.81%) was delivered by ventouse-assisted vaginal delivery.

Figure 10: Pie chart of liquor in the study population (N=124)

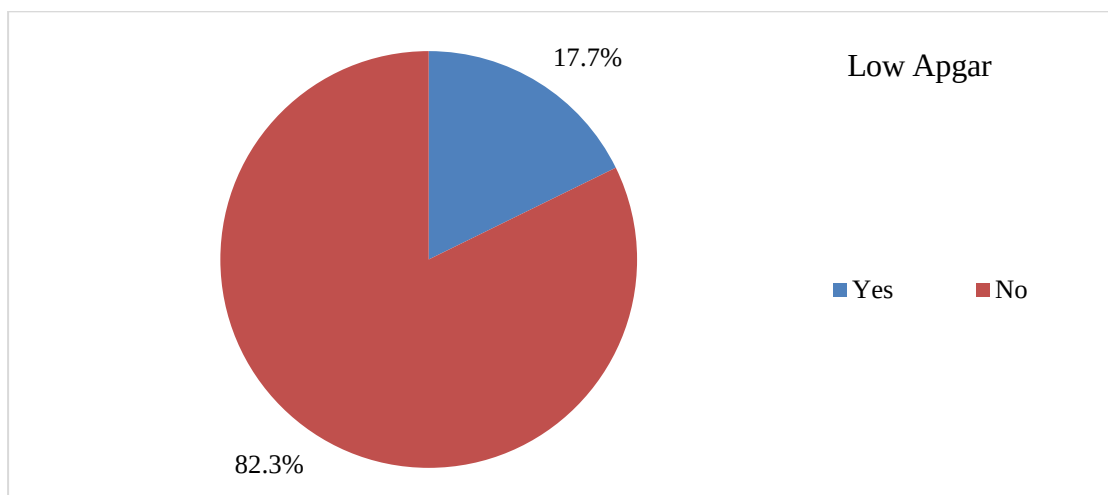
Liquor: Clear liquor was observed in 96.77% of cases. Meconium-stained amniotic fluid was identified in 3.23% of the research group.

Table 9: Descriptive analysis of post-natal adaptation in the study population (N=124)

Post-Natal Adaptation	Frequency	Percentages
Cried immediately after birth (CIAB)	92	74.19%
Cried After Stimulation	13	10.48%
Cried after 1 Cycle of Bag & Mask	9	7.26%
Cried after 2 Cycle of Bag & Mask	4	3.23%
Weak Cry at Birth	4	3.23%
Did Not Cry at Birth (Intubated)	2	1.61%
Low APGAR		
Yes	22	17.74%
No	102	82.26%

Figure 11: Bar chart of post-natal adaptation in the study population (N=124)

Post Natal Adaptation: Majority of Neonates Cried Immediately after birth (CIAB) (74.19%). However, a considerable proportion (about 25%) required some form of resuscitation. 10.48% of neonates cried after stimulation. 7.26% of neonates cried after one cycle of bag & mask ventilation. 3.23% cried after two cycles of bag & mask ventilation, requiring more resuscitation efforts. 3.23% had weak cry at birth, showing poor respiratory effort and higher risk of complications. 1.61% required intubation at birth due to absence of spontaneous cry.

Figure 12: Pie chart of low APGAR in the study population (N=124)

APGAR Score: In our study group, 17.74% of neonates had a low APGAR score (<7), indicating potential perinatal distress or the need for resuscitation. Majority of the neonates (82.26%) had normal APGAR scores.

Table 10: Descriptive analysis of indication for LSCS in the LSCS Delivery population (N=96)

Indication	Frequency	Percentages
Severe Pre-eclampsia	27	28.12%
PPROM	12	12.50%
Absent end diastolic flow (AEDF)	10	10.42%
Previous LSCS In Labour	7	7.29%
Foetal Distress	6	6.24%
DCDA Twins in Labour	4	4.17%
Anamnios	3	3.13%
Severe Oligohydramnios	3	3.13%
Abruptio Placenta	4	4.17%
Breech	2	2.08%
DCDA Twins with Severe PE	2	2.08%
DCDA Twins/GHTN	2	2.08%
Eclampsia	2	2.08%
Meconium-Stained Liquor +Non-Reactive NST	3	3.13%
HELLP Syndrome	2	2.08%
Non-reactive NST	3	3.13%
Others	4	4.17%

Indication for LSCS in the study group

In the study population 77.42% (96/124) of cases were born via LSCS, with severe pre-eclampsia as the most common indication observed in 28.12% of the study population. Maternal hypertensive disorders (pre-eclampsia, HELLP syndrome, eclampsia, and gestational hypertension) were the major indication for LSCS. Other significant indications included PPRM seen in 12.50% of cases, absent end-diastolic

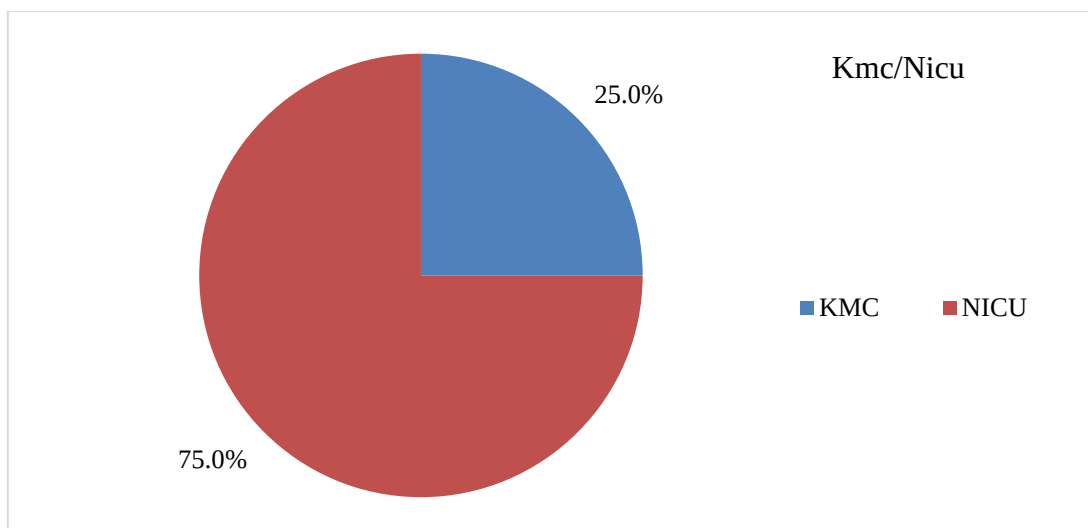
flow (AEDF) observed in 10.42% of cases, and foetal distress in 6.24% of cases. Multiple gestation (DCDA twins) was an indication for LSCS in 4.12% of the study population. Placental and amniotic fluid abnormalities, such as abruptio placenta contributed as an indication in 4.17% of cases, severe oligohydramnios was observed in 3.13% of cases, and anamnios was the indication for LSCS in 3.13% of cases. A history of previous LSCS was an indication in 7.29% of cases and breech presentation was an indication in 2.08% of the cases.

INTERVENTION

Table 11: Descriptive analysis of KMC/NICU in the study population (N=124)

KMC/NICU	Frequency	Percentages
KMC	31	25.00%
NICU	93	75.00%

Figure 13: Pie chart of KMC/NICU in the study population (N=124)

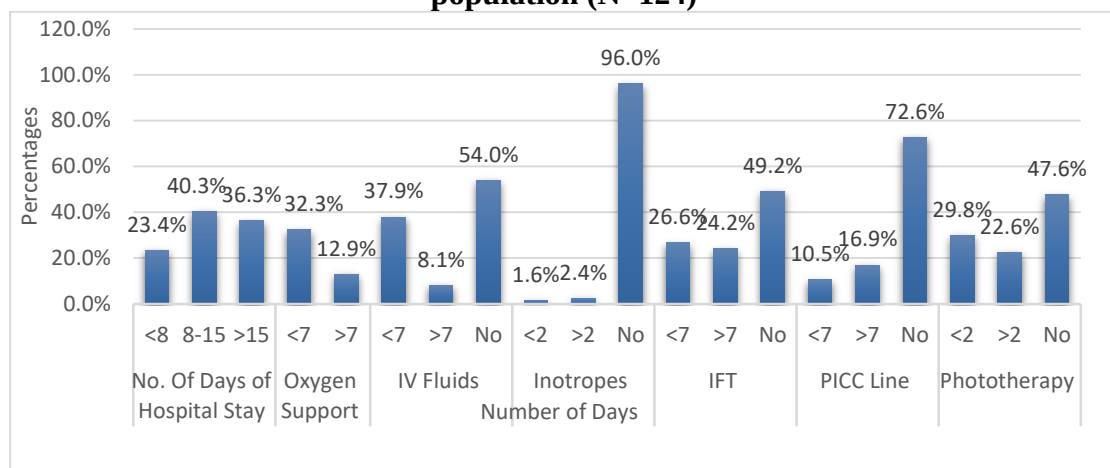


Admitted ward: Neonates were either in ‘Neonatal Intensive Care Unit (NICU)’ or ‘Kangaroo Mother Care (KMC) unit’. The majority (75%) of preterm neonates had NICU admission. Only 25% were managed in KMC, as these neonates were more stable and required minimal intensive care.

Table 12: Descriptive analysis of number of days of interventions during hospital stay in the study population

Parameter	Mean $\pm$ SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
No. Of Days of Hospital Stay (N=124)	16.7 $\pm$ 12.07	12.5	7.0	59.0	14.6	18.9
Oxygen Support (N=56)	6.93 $\pm$ 7.33	4.0	1.0	32.0	5.0	8.9
Mechanical Ventilation (N=8)	3.38 $\pm$ 2.2	3.0	1.0	8.0	1.5	5.2
CPAP (N=39)	4.41 $\pm$ 5.83	3.0	1.0	28.0	2.5	6.3
Nasal Prongs (N=50)	3.48 $\pm$ 3.81	2.0	1.0	25.0	2.4	4.6
Oxygen By Hood (N=5)	3 $\pm$ 1.41	2.0	2.0	5.0	1.2	4.8
IV Fluids (N=57)	4.46 $\pm$ 3.35	3.0	1.0	14.0	3.6	5.3
Inotropes (N=5)	3.4 $\pm$ 2.7	3.0	1.0	8.0	0.1	6.8
IFT (N=63)	9.86 $\pm$ 7.74	7.0	1.0	29.0	7.9	11.8
PICC Line (N=34)	11.53 $\pm$ 5.48	12.0	4.0	24.0	9.6	13.4
Phototherapy (N=65)	2.43 $\pm$ 0.71	2.0	1.0	4.0	2.3	2.6

Figure 14: Bar chart of interventions during hospital stay in the study population (N=124)

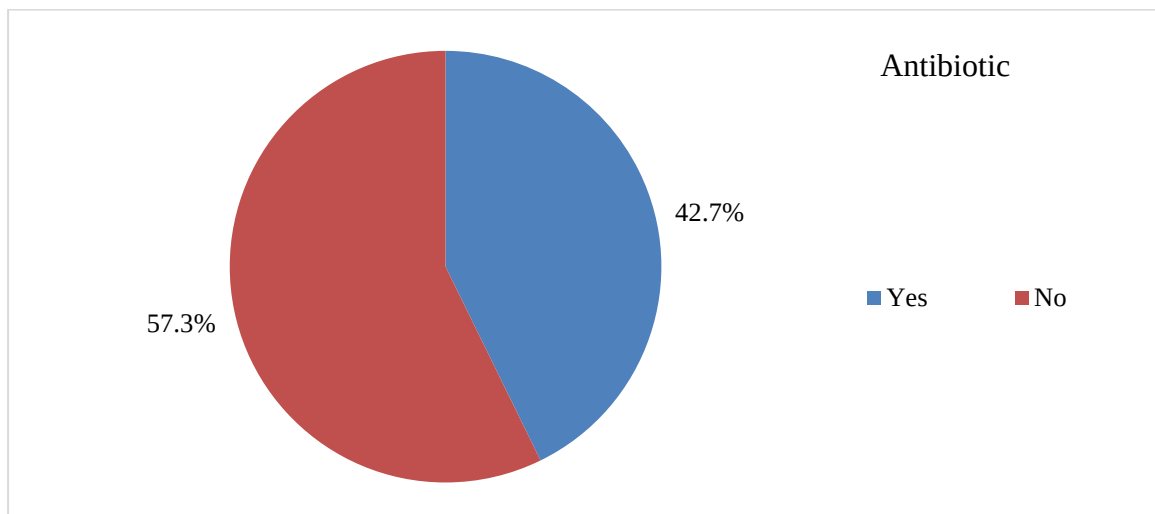


Duration of Hospital Stay: In our study, the preterm neonates had a mean duration of hospital stay for 16.7 days, with a median of 12.5 days. Shortest hospital stay recorded was 7 days, while the longest stay was 59 days.

Duration of intervention during Hospital stay: In our study, preterm newborns received oxygen support for an average of 6.93 ± 7.33 days, with a median period of 4 days. The duration varied from 1 to 32 days. The average length of mechanical ventilation was 3.38 ± 2.2 days, with a median of 3 days. The shortest time period was 1 day, while the maximum duration was 8 days. The mean CPAP duration was 4.41 ± 5.83 days, with a median of 3 days. The minimum period was 1 day, and the maximum term was 28 days. The average duration for nasal prongs was 3.48 ± 3.81 days, with a median of 2 days. The average time for intravenous fluids was 4.46 ± 3.35 days, with a median of 3 days. The minimum time was 1 day, and the maximum duration was 14 days. The mean duration for inotropes was 3.4 ± 2.7 days, with a median of 3 days. The average duration for the infant feeding tube was 9.86 ± 7.74 days, with a median of 7 days. A Peripherally Inserted Central Catheter (PICC) Line was utilized in 34 cases, with a mean duration of 11.53 ± 5.48 days and a median of 12 days. The mean duration of phototherapy was 2.43 ± 0.71 days, with a median of 2 days.

Table 13: Descriptive analysis of antibiotic in the study population (N=124)

Antibiotic	Frequency	Percentages
Yes	53	42.74%
No	71	57.26%

Figure 15: Pie chart of antibiotic in the study population (N=124)

Antibiotics

In our study, 42.74% of neonates received antibiotics for suspected or confirmed sepsis while 57.26% did not require any antibiotic treatment. The high rate of antibiotic usage reflects concerns about sepsis in preterm neonates, particularly given their immature immune system and increased susceptibility to infections.

Table 14: Descriptive analysis of Type of Feed (Days) in study population

Type of Feed (Days)	Mean $\pm$ SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Infant Feeding Tube (N=65)	7.46 $\pm$ 6.57	4.0	1.0	28.0	5.8	9.1
Spoon (N=94)	10.16 $\pm$ 6.45	10.0	1.0	35.0	8.8	11.5
DBF (N=117)	7.2 $\pm$ 3.01	7.0	2.0	20.0	6.7	7.8

Type of Feed:

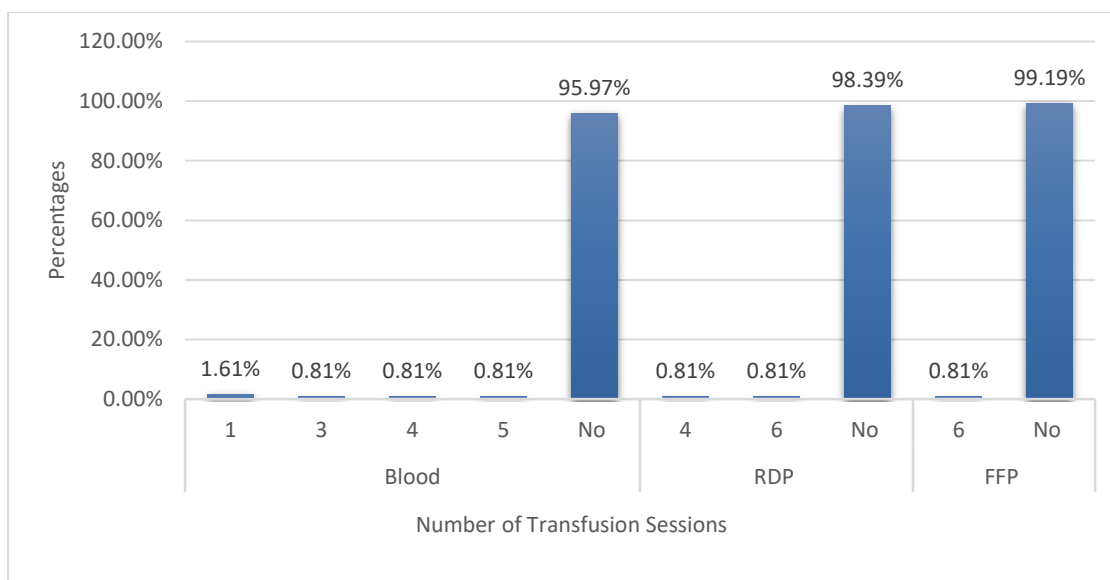
Ryle's tube feeding (RT) was required for an average of 7.46 days, in the study population. Spoon feeding was given for an average of 10.16 days, while direct breastfeeding (DBF) commenced at a mean age of 7.2 days.

Table 15: Descriptive analysis of transfusions in the study population (N=124)

Transfusion	Frequency	Percentages
Blood		
1 Session	2	1.61%
3 Sessions	1	0.81%
4 Sessions	1	0.81%
5 Sessions	1	0.81%
No Session	119	95.97%
RDP		
4 Sessions	1	0.81%

6 Sessions	1	0.81%
No Session	122	98.39%
FFP		
6 Sessions	1	0.81%
No Session	123	99.19%

Figure 16: Bar chart of blood transfusion in the study population (N=124)



Transfusion

Only 4.03% of the neonates in the study population required blood transfusions, with most neonates receiving a single session, suggesting that severe anaemia or hemodynamic instability was relatively uncommon in this study population. Random donor Platelet transfusion (RDP) and Fresh Frozen Plasma (FFP) were required in 0.81% of cases, indicating that most preterm neonates did not develop severe coagulopathies or thrombocytopenia needing intervention. These findings suggest that while some preterm required transfusions due to anaemia or blood loss, the majority had stable haematological parameters.

COLONIZATION OF THE NEONATAL GUT WITH BACTERIA
Table 16: Descriptive analysis of gastric aspirate in the study population (N=124)

Gastric Aspirate	Frequency	Percentages
Sample-1		
No growth in culture	100	80.65%
Positive growth in culture	24	19.35%
<i>Escherichia Coli</i>	7	5.65%
<i>Klebsiella pneumonia</i>	7	5.65%
CONS	2	1.61%
<i>Micrococci</i>	2	1.61%
<i>Acinetobacter</i>	1	0.81%
<i>Citrobacter freundii</i>	1	0.81%
<i>Citrobacter/Enterococcus</i>	1	0.81%
<i>Enterococcus</i>	1	0.81%
<i>Klebsiella oxytoca</i>	1	0.81%
<i>Pseudomonas aeruginosa</i>	1	0.81%
Sample-2		
No growth in culture	90	72.58%
Positive Culture	34	27.42%
<i>Escherichia coli</i>	15	12.10%
<i>Klebsiella pneumonia</i>	6	4.84%
CONS	4	3.23%
<i>Citrobacter freundii</i>	2	1.61%
<i>Enterococcus</i>	2	1.61%

<i>Acinetobacter species</i>	1	0.81%
<i>Enterobacteriaceae</i>	1	0.81%
<i>Klebsiella oxytoca</i>	1	0.81%
<i>Pseudomonas</i>	1	0.81%
<i>Staphylococcus aureus</i>	1	0.81%

**Figure 17: Bar chart of Gastric Aspirate (sample -1) in the study population
(N=124)**

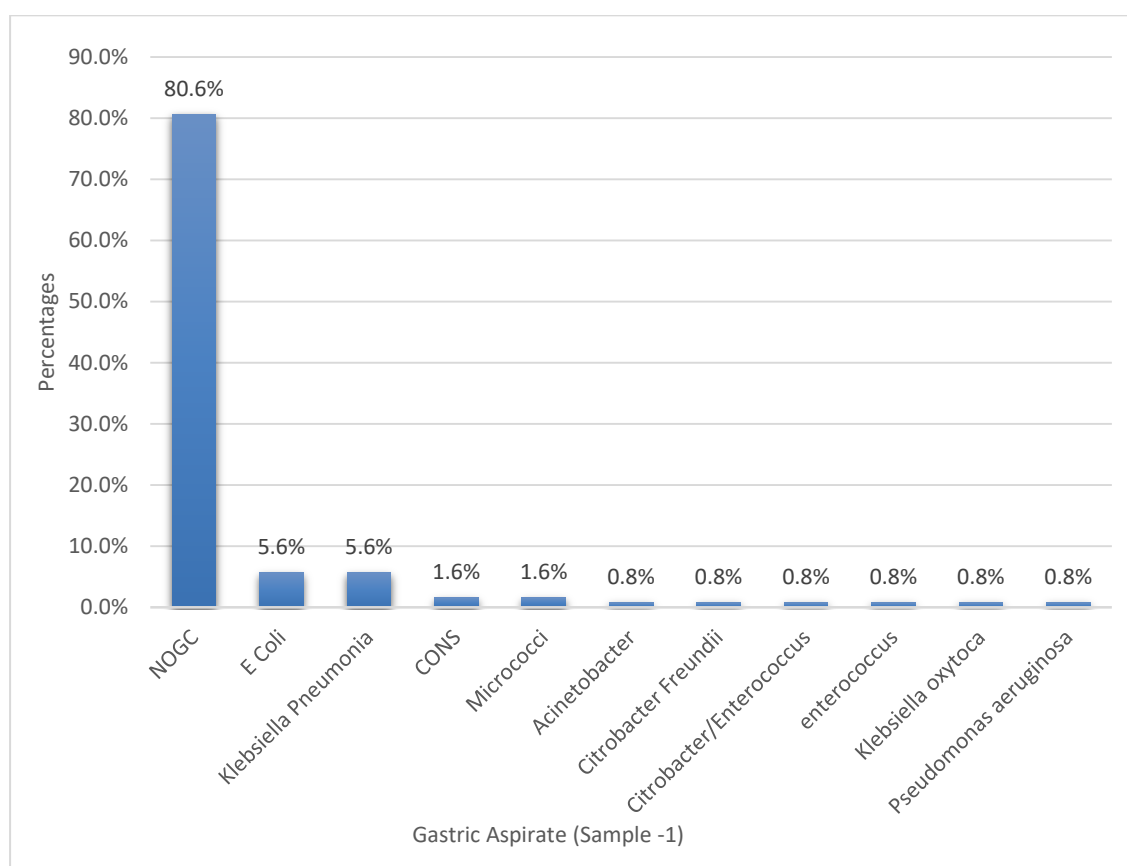
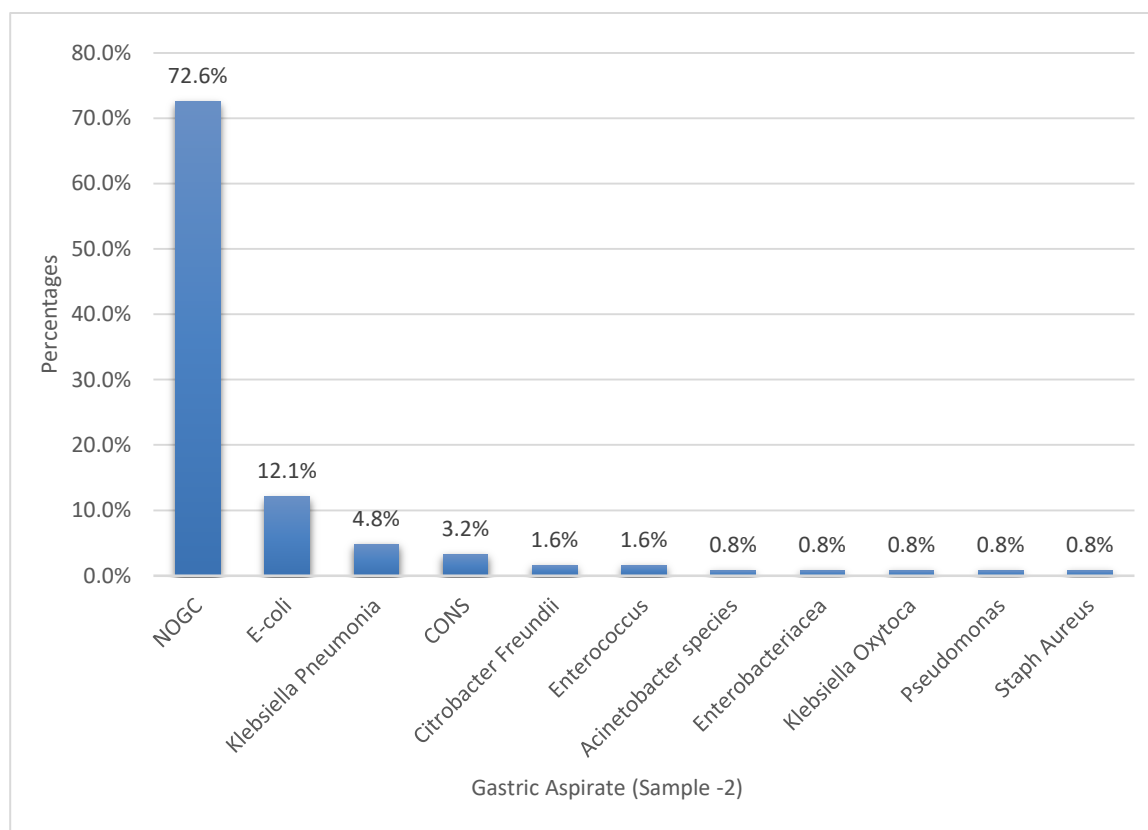


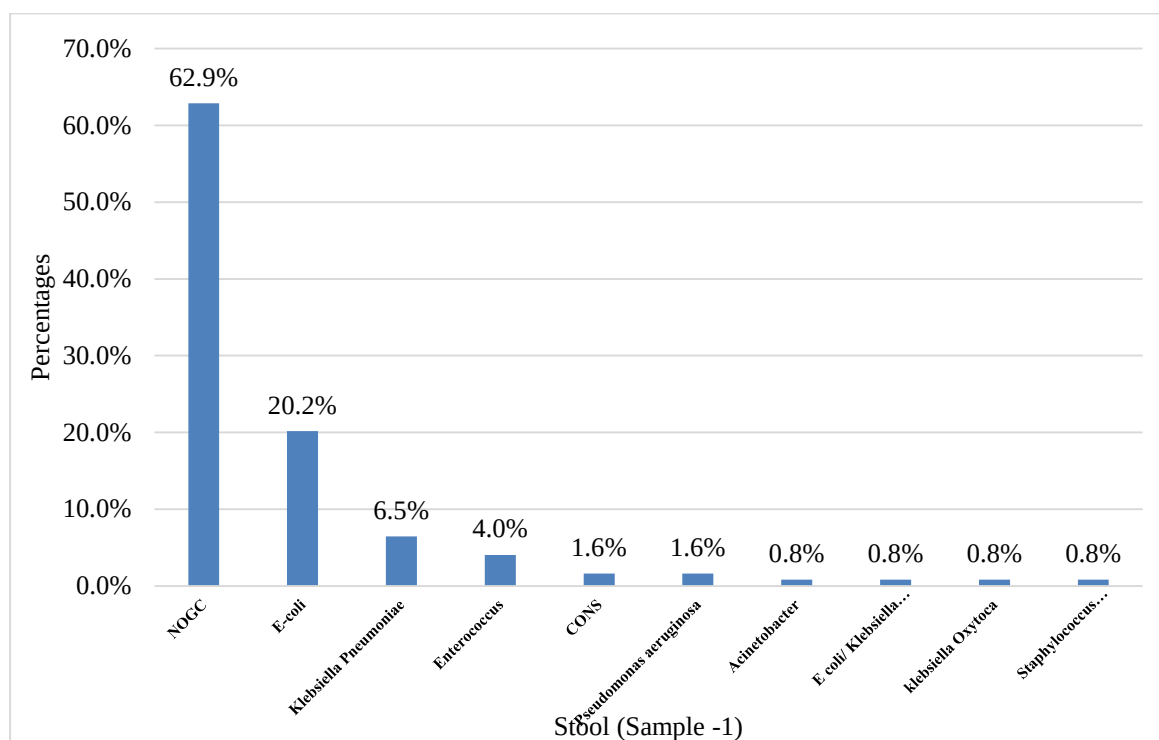
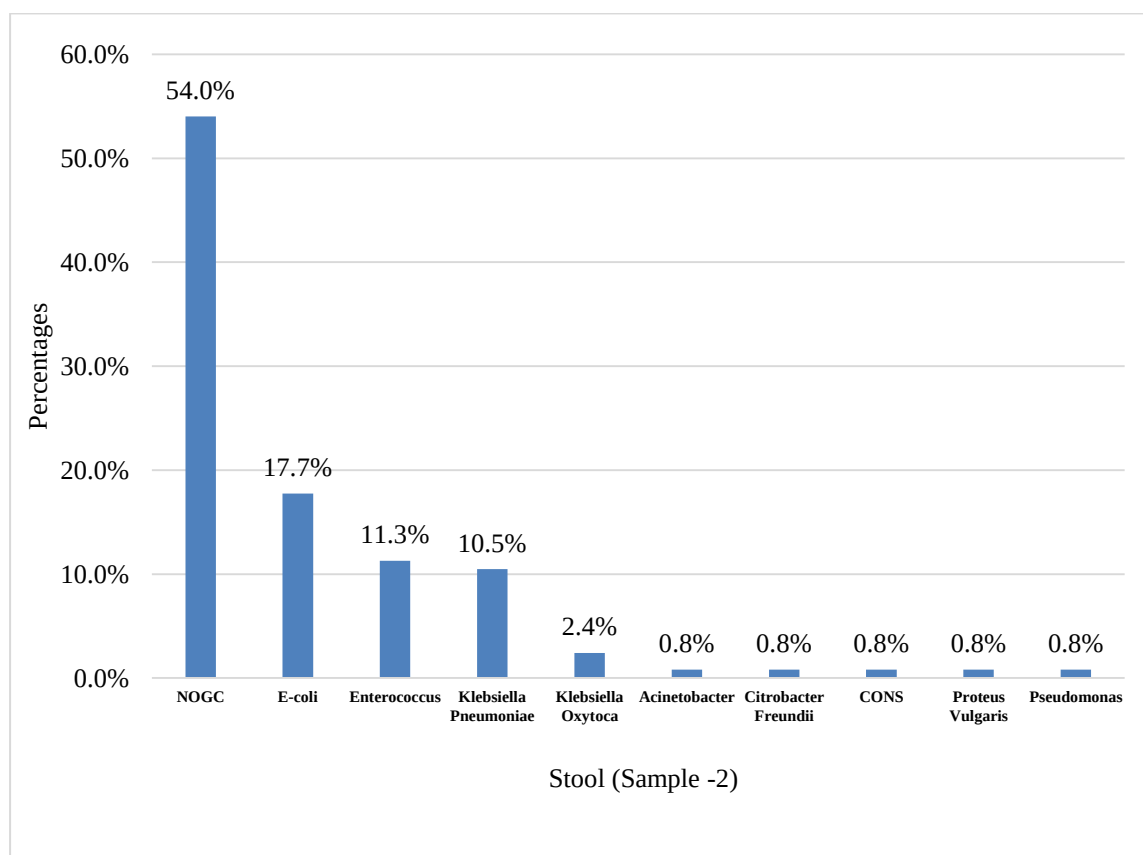
Figure 18: Bar chart of Gastric Aspirate (sample -2) in the study population
(N=124)



Gastric Aspirate Culture: In our study population, 19.35% (24/124) of neonates had bacterial growth in the gastric aspirate sample-1, whereas the gastric aspirate sample-2 taken between day 7-10 of life (Sample 2), showed increased colonization with growth in 27.42% of neonates. The most common organisms grown in Sample-1 were '*E. coli*' (5.65%) and '*Klebsiella pneumoniae*' (5.65%), followed by '*Coagulase-negative staphylococci (CONS)*', '*Micrococci*', and '*Acinetobacter*'. 34 neonates, had bacterial growth in sample-2 with a rise in '*E. coli*' (12.10%), followed by '*Klebsiella pneumoniae*' (4.84%), '*CONS*' (3.23%), and '*Enterococcus*' isolation compared to the gastric aspirate samples-1. The increase in bacterial growth over time suggests progressive gut colonization, with gram-negative bacteria being the dominant organisms.

Table 17: Descriptive analysis of Stool sample in the study population (N=124)

Stool	Frequency	Percentages
Sample-1		
No growth in culture	78	62.90%
Positive Culture	46	37.1%
<i>Escherichia coli</i>	25	20.16%
<i>Klebsiella pneumoniae</i>	8	6.45%
<i>Enterococcus</i>	5	4.03%
CONS	2	1.61%
<i>Pseudomonas aeruginosa</i>	2	1.61%
<i>Acinetobacter</i>	1	0.81%
<i>E coli/ Klebsiella Pneumoniae</i>	1	0.81%
<i>Klebsiella oxytoca</i>	1	0.81%
<i>Staphylococcus saprophyticus</i>	1	0.81%
Sample-2		
No growth in culture	67	54.03%
Positive Culture	57	45.97%
<i>Escherichia coli</i>	22	17.74%
<i>Enterococcus</i>	14	11.29%
<i>Klebsiella pneumoniae</i>	13	10.48%
<i>Klebsiella oxytoca</i>	3	2.42%
<i>Acinetobacter</i>	1	0.81%
<i>Citrobacter freundii</i>	1	0.81%
CONS	1	0.81%
<i>Proteus vulgaris</i>	1	0.81%
<i>Pseudomonas</i>	1	0.81%

Figure 19: Bar chart of stool (sample -1) in the study population (N=124)**Figure 20: Bar chart of stool (sample -1) in the study population (N=124)**

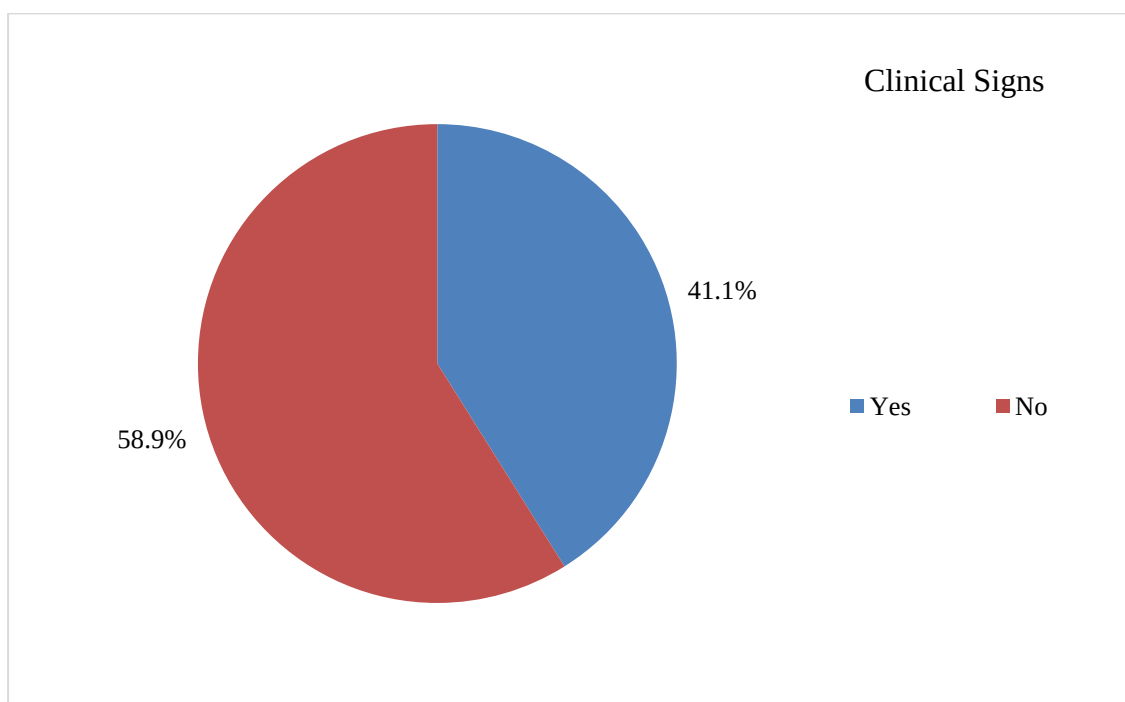
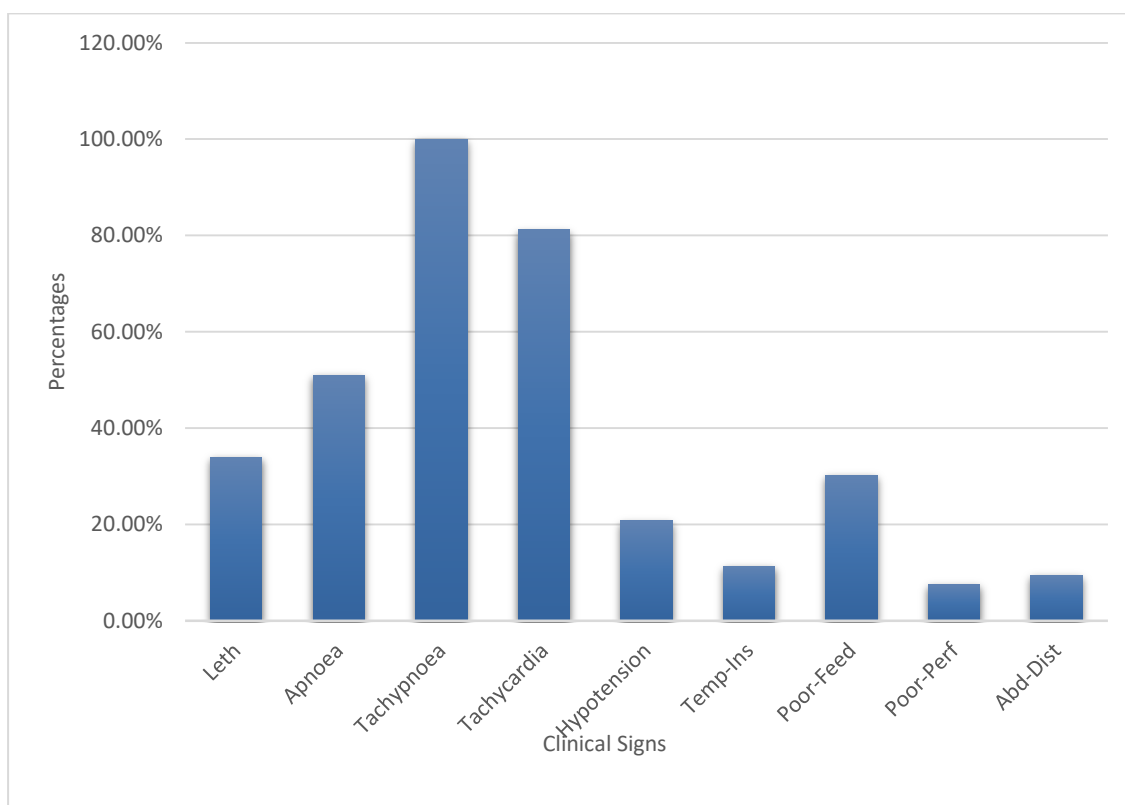
Stool Culture: Stool samples showed a higher prevalence of bacterial growth compared to gastric aspirate samples. In Sample-1, 37.1% (46/124) of neonates had positive stool cultures, increasing to 45.97% (57/124) in Sample-2. The most common organisms in Sample-1 were '*E. coli*' (20.16%) and '*Klebsiella pneumoniae*' (6.45%), followed by '*Enterococcus*', '*Pseudomonas*', and '*Acinetobacter*'. In Sample-2, '*E. coli*' (17.74%) remained the most prevalent, but '*Enterococcus*' (11.29%) and '*Klebsiella pneumoniae*' (10.48%) were also predominant. These findings suggest that gut colonization with pathogenic bacteria increases over time, which may contribute to a higher risk of infections. The predominance of gram-negative bacteria and '*Enterococcus*' is significant, as these organisms are known to be associated with 'neonatal sepsis'.

Common organisms found in the gut samples of Preterm Neonates

The study found progressive bacterial colonization in the gut samples of preterm neonates, with higher bacterial growth in stool samples compared to gastric aspirate samples. In gastric aspirate Sample-1, 19.35% of neonates had bacterial growth, which increased to 27.42% in Sample-2, with '*E. coli*' (5.65%) and '*Klebsiella pneumoniae*' (5.65%) being the most common organisms. In stool Sample-1, 37.1% of neonates had positive cultures, increasing to 45.97% in Sample-2, where '*E. coli*' (20.16%), '*Enterococcus*' (11.29%), and '*Klebsiella pneumoniae*' (10.48%) were predominant. These findings suggest that gram-negative bacteria, particularly '*E. coli*' and '*Klebsiella pneumoniae*', were predominant, with a significant increase in '*Enterococcus*' in later stool samples, indicating the dynamic nature of gut colonization and its potential association with neonatal infections.

Table 18: Descriptive analysis of clinical signs of sepsis in the study population
(N=124)

Clinical Signs	Frequency	Percentages
Clinical signs present	53	41.09%
No clinical signs	76	58.91%
If present,		
Lethargy	18	33.96%
Apnoea	27	50.94%
Tachypnoea	53	100.00%
Tachycardia	43	81.13%
Hypotension	11	20.75%
Temperature-Instability	6	11.32%
Poor-Food	16	30.19%
Poor-Perfusion	4	7.55%
Abdominal-Distension	5	9.43%

Figure 21: Pie chart of clinical signs of sepsis in the study population (N=124)**Figure 22: Bar chart of clinical signs of sepsis in the study population (N=124)**

Clinical sepsis

41.09% of neonates (53/124) exhibited clinical signs suggestive of sepsis, while 58.91% showed no symptoms. Among neonates with sepsis, the most common manifestations were tachypnoea (100%), followed by tachycardia (81.13%), apnoea (50.94%), and lethargy (33.96%). Other notable signs included poor feeding (30.19%), hypotension (20.75%), temperature instability (11.32%), and abdominal distension (9.43%). The findings indicate that respiratory distress (tachypnoea, apnoea) was the predominant clinical sign of sepsis, followed by systemic signs such as tachycardia and lethargy. However, out of the 53 neonates with clinical signs of sepsis, 23 neonates had laboratory evidence of sepsis and only 13 neonates had blood culture positive sepsis.

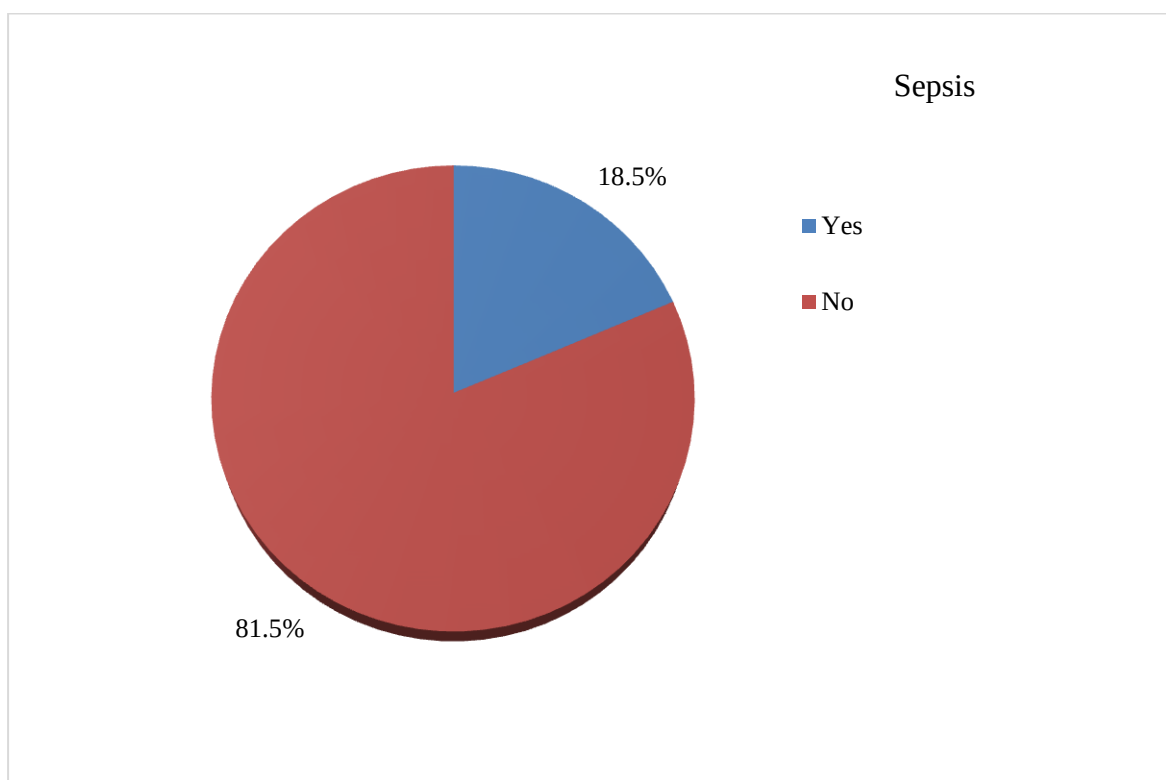
Table 19: Descriptive analysis of Laboratory Evidence of Sepsis in the study population (N=124)

Laboratory Evidence	Frequency	Percentages
TLC <5000	23	18.55%
ANC <1800 Or >5400	22	17.74%
CRP >10	19	15.32%
ESR >15	3	2.42%

Table 20: Descriptive analysis of laboratory evidence of sepsis in the study population (N=124)

Laboratory evidence of Sepsis	Frequency	Percentages
Yes	23	18.55%
No	101	81.45%

Figure 20: Pie chart of laboratory evidence of sepsis in the study population (N=124)

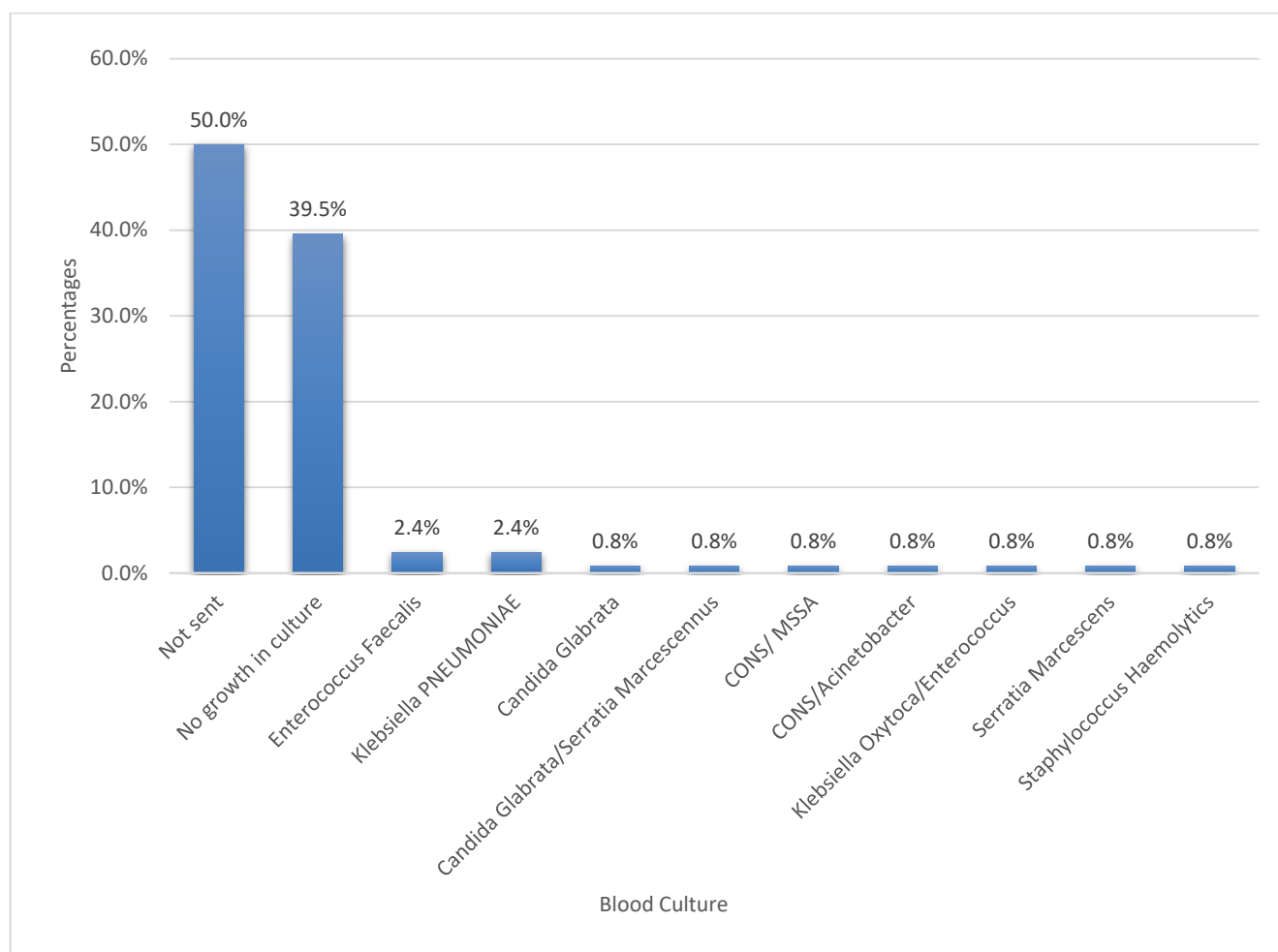


Laboratory evidence of Sepsis

18.55% (23/124) of neonates had lab-confirmed sepsis, while 81.45% had no laboratory evidence of infection. Among those neonates with sepsis, the most frequently abnormal lab parameters were total leukocyte count <5000 in 18.55%, absolute neutrophil count <1800 or >5400 in 17.74%, and elevated C-reactive protein >10 in 15.32% of neonates. Elevated erythrocyte sedimentation rate >15 was observed in 2.42% of neonates. The relatively low percentage of laboratory-confirmed sepsis compared to clinical suspicion (41.09%) suggests that not all neonates with sepsis-like symptoms had confirmed infection, reinforcing the need for combined clinical and laboratory assessments before initiating antibiotics.

Table 21: Descriptive analysis of blood culture in the study population (N=124)

Blood Culture	Frequency	Percentages
Not sent	62	50.00%
No growth in culture	49	39.52%
<i>Enterococcus faecalis</i>	3	2.42%
<i>Klebsiella pneumoniae</i>	3	2.42%
<i>Candida glabrata</i>	1	0.81%
<i>Candida glabrata/Serratia marcescens</i>	1	0.81%
CONS/ MSSA	1	0.81%
CONS/ <i>Acinetobacter</i>	1	0.81%
<i>Klebsiella oxytoca</i> / <i>Enterococcus</i>	1	0.81%
<i>Serratia marcescens</i>	1	0.81%
<i>Staphylococcus haemolytics</i>	1	0.81%

Figure 24: Bar chart of blood culture in the study population (N=124)

Blood Culture Positive Sepsis in the study population

In our study population, 53(41.09%) neonates had clinical signs of sepsis and 23(18.55%) had laboratory evidence of sepsis. Among these, 13 neonates had 'blood cultures positive sepsis'. 'Gram-negative bacteria' were the predominant isolates, with '*Klebsiella pneumoniae*' and '*Klebsiella oxytoca*' detected in four cases. '*Serratia marcescens*' was identified in two cases, including one co-infection with '*Candida glabrata*'. Additionally, '*Acinetobacter*' was isolated in one case, which was co-infected with 'coagulase-negative *Staphylococcus* (CONS)'.

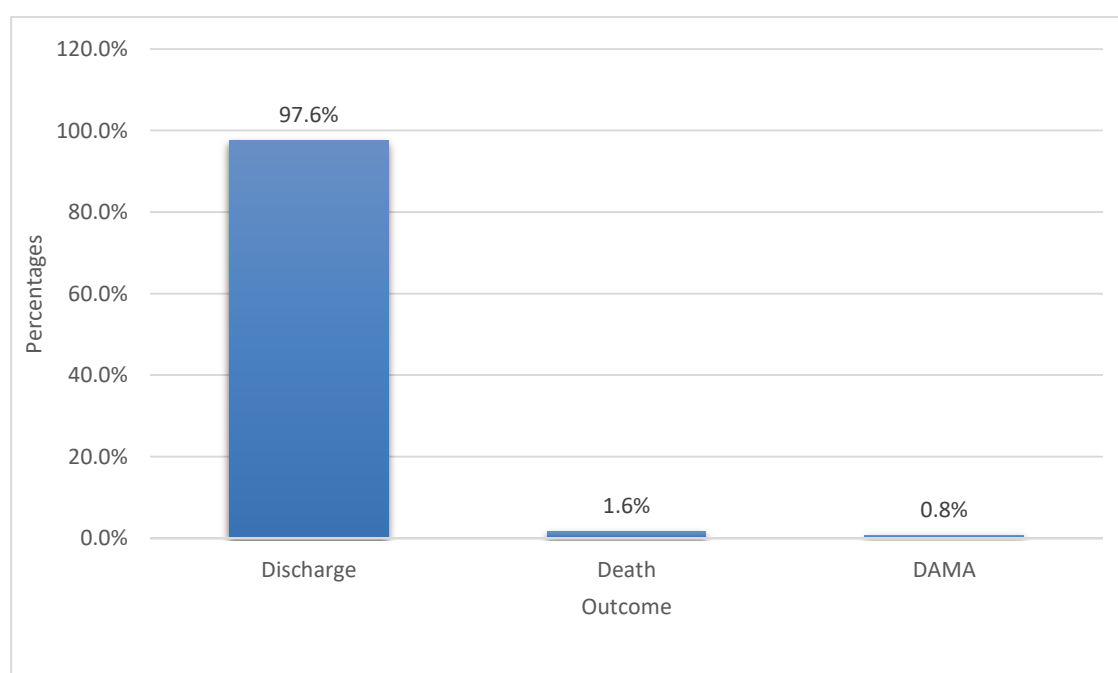
Among the ‘Gram-positive bacteria’, ‘*Enterococcus faecalis*’ was the most frequently identified organism, present in three cases. ‘*Coagulase-negative Staphylococcus* (CONS)’ was isolated in two newborns, while ‘*Staphylococcus haemolyticus*’ was found in one case.

Fungal infections were noted in two cases, both involving ‘*Candida glabrata*’. One of these cases also had a co-infection with ‘*Serratia marcescens*’.

Table 22: Descriptive analysis of outcome in the study population (N=124)

Outcome	Frequency	Percentages
Discharge	121	97.58%
Death	2	1.61%
DAMA	1	0.81%

Figure 25: Bar chart of outcome in the study population (N=124)



OUTCOME

97.58% (121/124) of preterm neonates were successfully discharged. 1.61% (2/124) of neonates died. Cause of death in 1 neonate was due to inotropic refractory shock due to pulmonary haemorrhage on day 10 of life. This neonate showed no bacterial growth in the gut sample or blood culture. The cause of death in the second neonate was inotropic refractory septic shock secondary to late onset sepsis on day 59 of life. This neonate had growth of '*Enterococcus faecalis*' in the blood culture and growth of '*Klebsiella pneumoniae*' in the stool sample-2. 1 neonate (0.81%) was discharged to be taken home against medical advice due to financial constraints. Most preterm neonates had favourable recovery with NICU support.

RISK FACTORS FOR BACTERIAL GROWTH IN THE GUT OF PRETERM NEONATES

Table 23: Comparison of gender and positive bacterial growth in gastric aspirate and stool samples (N=124)

Gender of Baby	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Male (N=58)	14 (24.13%)	0.206	21 (36.20%)	0.848	29 (50.00%)	0.098	29 (50.00%)	0.398
Female (N=66)	10 (15.15%)		25 (37.87%)		28 (42.42%)		38 (56.57%)	

Gender: Above table shows that male sex did not play a role in promoting bacterial growth in both gastric aspirate and stool samples. The table also shows that there is an increase in bacterial growth in gastric aspirate and stool samples in preterm neonates of both groups in the second gut samples. However, these differences were not statistically significant. ($P>0.05$)

Table 24: Comparison of Gestational age and positive bacterial growth in gastric aspirate and stool samples (N=124)

Gestational Age	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Extreme Preterm (N=4)	0 (0%)	0.047	0 (0%)	0.114	0 (0%)	0.101	3 (75.00%)	0.003
Early Preterm (N=26)	7 (26.90%)		10 (38.46%)		11 (42.3%)		19 (73.07%)	
Moderate Preterm (N=31)	10 (26.92%)		16 (51.61%)		10 (32.25%)		15 (48.38%)	
Late Preterm (N=63)	7 (11.11%)		20 (31.74%)		13 (20.63%)		20 (31.74%)	

Gestational Age: According to the above table, early preterm and moderate preterm neonates had more growth in the gastric aspirate-sample 1 and stool sample-2 compared to late preterm neonates, and this difference was statistically significant (gastric aspirate sample-1: $P=0.047$; stool sample-2: $P=0.003$). We only had four preterm neonates who were extremely preterm, who did not have any bacterial colonization in the initial gastric aspirate and stool sample, however three of them developed bacterial colonization in stool sample-2, though this difference was not statistically significant due to small numbers.

Table 25: Comparison of Birth Weight and positive bacterial growth in gastric aspirate and stool samples (N=124)

Neonatal Parameters	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Birth Weight								
Low Birth Weight (N=81)	14 (17.28%)	0.357	34 (41.97%)	0.194	20 (24.69%)	0.183	31 (38.27%)	0.004
Very Low Birth Weight (N=28)	8 (28.57%)		9 (32.14%)		10 (35.71%)		17 (60.71%)	
Extremely Low Birth Weight (N=9)	2 (22.22%)		3 (33.33%)		4(44.44%)		8 (88.88%)	
Normal Birth weight (N=6)	0 (0%)		0 (0%)		0 (0%)		1 (16.6%)	
SGA/AGA/LGA								
Small for Gestational Age (SGA) (N=41)	12 (29.26%)	0.136	21 (45.65%)	0.060	18 (52.94%)	0.014	24 (42.11%)	0.068
Appropriate for Gestational Age (AGA) (N=82)	12 (6.83%)		25 (30.48%)		16 (19.51%)		32 (39.02%)	
Large for Gestational Age (LGA) (N=1)	0 (0%)		0 (0%)		0 (0%)		1 (100.00%)	

Birth Weight: The above table shows that, bacterial growth was more in the gastric aspirate and stool samples for the extremely low birth weight and very low birth weight neonates compared to normal birth weight neonates, however this variation was statistically significant only for stool sample-2 ($P=0.004$). There was a progressive increase in gastric aspirate and stool samples for low-birth-weight babies, though this difference was not statistically significant due to small numbers in the subgroups.

Our study also showed higher bacterial growth in stool sample-1 among SGA babies ($P = 0.014$). However, there was no significant association for other gastric aspirate or stool samples.

Table 26: Comparison of Obstetric score and bacterial growth in gastric aspirate and stool samples (N=124)

Obstetric Score	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool -2 (N=57)	P value
Multigravida (N=59)	13 (22.03%)	0.472	22 (37.28%)	0.966	18 (30.50%)	0.463	28 (47.45%)	0.751
Primigravida (N=65)	11 (16.92%)		24 (36.92%)		16 (24.61%)		29 (44.61%)	

Obstetric score: The above table suggest that the bacterial growth occurred in preterm neonates irrespective of gravida status of mother as progressive increase in bacterial growth was observed in neonates of both multigravida and primigravida mothers. This was true for both gastric and stool samples. However, the differences observed were not statistically significant ($P>0.05$).

Table 27: Comparison of Maternal risk factors and positive bacterial growth in gastric aspirate and stool samples (N=124)

Maternal Risk Factors	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool -2 (N=57)	P value
Fever(N=4)	0 (0%)	0.319	2 (50.00%)	0.627	1 (25.00%)	1.000	2 (50.00%)	0.627
PROM (>18 Hours) (N=19)	5 (3.80%)	0.527	9 (47.36%)	0.314	6 (8.33%)	0.659	10 (52.63%)	0.526
UTI(N=3)	0 (0%)	0.390	0 (0%)	0.178	0 (0%)	0.281	0 (0%)	0.106
GDM(N=12)	2 (16.66%)	1.000	3 (25.00%)	0.532	1 (8.33%)	0.177	5 (41.66%)	0.753
GHTN(N=59)	9 (15.25%)	0.271	22 (37.28%)	0.966	19 (32.20%)	0.255	28 (47.45%)	0.751

Maternal Risk Factors: According to the findings in the above table, Maternal risk factors, including fever, PROM, UTI, GDM, and GHTN, were associated with increased bacterial growth in gastric aspirate sample-2 and stool sample-2. However, these observations were not statistically significant. Hence the findings in our study suggest that maternal risk factors did not have any association with gut microbiota colonization in preterm neonates.

28: Comparison of Mode of Delivery between gastric aspirate and stool samples

(N=124)

Delivery Details	Gastric Aspirate (Sample-1) (N=24)	P value	Gastric Aspirate (Sample-2) (N=46)	P value	Stool (Sample-1) (N=34)	P value	Stool (Sample-2) (N=57)	P value
Mode Of Delivery (N=124)								
LSCS(N=96)	19 (19.79%)	0.876	41 (42.70%)	0.053	26 (27.08%)	0.799	53 (55.20%)	0.527
VD(N=27)	5 (18.51%)		5 (18.51%)		8 (29.62%)		13 (48.14%)	
Ventouse Assisted VD (N=1)	0 (0%)		0 (0%)		0 (0%)		1 (100.00%)	

Mode of Delivery

The above table suggests that LSCS deliveries promoted statistically significant bacterial growth in gastric aspirate sample 2 when compared to preterm neonates born by vaginal delivery ($P=0.053$). Stool sample 2 of preterm neonates born by LSCS also had a higher bacterial growth when compared to those born vaginally though not statistically significant. We had only one preterm neonate born by ventouse assisted vaginal delivery and four preterm neonates had meconium-stained liquor. As the numbers were small, we did not include them for analysis.

Table 29: Comparison of Post Natal Adaptation between gastric aspirate and stool samples (N=124)

Delivery Details	Gastric Aspirate (Sample-1) (N=24)	P value	Gastric Aspirate (Sample-2) (N=46)	P value	Stool (Sample-1) (N=34)	P value	Stool (Sample-2) (N=57)	P value
Post Natal Adaptation(N=124)								
CIAB(N=92)	19 (20.65%)	0.720	33 (35.86%)	0.034	25 (27.17%)	0.331	53 (57.60%)	0.030
Cried After 1 Cycle of B & M(N=9)	1 (11.11%)		1 (11.11%)		0 (0%)		7 (77.77%)	
Cried After 2 Cycle of B & M(N=4)	0 (0%)		2 (50.00%)		1 (25.00%)		3 (75.00%)	
Cried After Stimulation(N=13)	2 (54.16%)		8 (61.53%)		5 (38.46%)		2 (15.38%)	
Did Not Cry at Birth (Intubated)(N=2)	1 (50.00%)		2 (100%)		1 (50.00%)		1 (50.00%)	
Weak Cry at Birth(N=4)	1 (25.00%)		0 (0%)		2 (50.00%)		1 (25.00%)	

Post Natal Adaptation

In the above table, poor postnatal adaptation requiring stimulation at birth, one or two cycles of bag-and-mask ventilation, or intubation were a significant risk factor for increase in bacterial growth in gastric aspirate and stool samples, as indicated by the statistically significant p-values ($p = 0.034$ for gastric aspirate sample-2 and $p = 0.030$ for stool sample-2). Hence, neonates with delayed postnatal adaptation requiring intervention exhibited higher bacterial colonization, particularly in gastric aspirate sample-2 and stool sample-2.

Table 30: Comparison of Low APGAR between gastric aspirate and stool samples (N=124)

Delivery Details	Gastric Aspirate (Sample-1) (N=24)	P value	Gastric Aspirate (Sample-2) (N=46)	P value	Stool (Sample-1) (N=34)	P value	Stool (Sample-2) (N=57)	P value
Low APGAR (N=124)								
Yes(N=22)	5 (22.72%)	0.766	8 (36.36%)	0.937	4 (18.18%)	0.284	12 (54.54%)	0.958
No(N=102)	19 (18.62%)		38 (37.25%)		30 (29.41%)		55 (53.92%)	

Low APGAR

The above table comparing bacterial growth in preterm neonates with low versus normal APGAR scores shows that a greater number of preterm neonates with low APGAR scores had higher bacterial growth in gastric aspirate sample-1 and stool sample-2 compared to those preterm neonates with normal APGAR scores, though these differences were not statistically significant ($P>0.05$).

Admitted ward of the neonate

**Table 31: Comparison of KMC/NICU and gastric aspirate and stool samples
(N=124)**

KMC/NICU	GA-1(N=24)	P value	GA-2(N=46)	P value	Stool-1(N=34)	P value	Stool-2(N=57)	P value
KMC (N=31)	2 (8.33%)	0.036	1 (2.17%)	<0.001	1 (2.94%)	<0.001	1 (1.75%)	<0.001
NICU (N=93)	22 (91.67%)		45 (97.83%)		33 (97.06%)		56 (98.25%)	

From the above table it can be observed that KMC prevents bacterial growth and colonization as the number of preterm neonates receiving KMC with bacterial growth were two in gastric aspirate sample 1 and one each in gastric aspirate sample-2, stool sample 1 and 2 versus preterm neonates admitted to NICU whose comparative figures were 22 for gastric aspirate sample 1, 45 for gastric aspirate sample 2, 33 for stool sample 1 and 56 for stool sample 2. These differences were statistically significant ($P=0.036$ for gastric aspirate sample-1 and $P<0.001$ for gastric aspirate sample 2, stool sample 1 and 2) thus indicating that KMC prevents both early and late gut bacterial colonization.

RISK FACTORS DURING HOSPITAL STAY

Table 32: Comparison of number of days of hospital stay and gut samples

(N=124)

No. Of Days of Hospital Stay(N=124)	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
<8 (N=29)	3 (10.34%)	0.211	7 (24.13%)	0.210	5 (17.24%)	0.352	8 (27.58%)	0.022
8-15(N=50)	9 (18%)		22 (44%)		16 (32.00%)		22 (44%)	
>15(N=45)	12 (26.66%)		17 (37.77%)		13 (28.88%)		27 (60.00%)	

The above table shows that longer the duration of hospital stay, promoted bacterial growth in both gastric aspirates and stool samples in preterm neonates as a greater number of preterm neonates with 8-15 days or >15 days hospital stay had bacterial growth in gastric and stool samples when compared to preterm neonates with <8 days hospital stay. However, statistically significant difference was observed only for stool sample-2 (P-0.022), indicating that prolonged hospital stay promotes gut colonisation. The difference observed of gastric aspirate sample 1 and 2, and stool sample-1 were not statistically significant (P-0.211 for gastric aspirate-1; p-0.210 for gastric aspirate 2 and p-0.352 for stool sample 1).

Table 33: Comparison of number of days of oxygen support and gut samples**(N=124)**

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Oxygen Support (N=124)								
<7(N=40)	9 (22.5%)	0.600	18 (39.13%)	0.422	15 (44.12%)	0.215	20 (52.11%)	0.003
>7(N=16)	4 (25.00%)		6 (37.5%)		4 (25.00%)		11 (68.75%)	
No(N=68)	11 (16.17%)		22 (32.35%)		15 (22.05%)		22 (32.35%)	

The above table shows that oxygen support provided for >7 days for preterm neonates did not promote bacterial growth in gastric aspirates, but it definitely promoted statistically significant bacterial growth in the stool sample-2 (P=0.003), indicating that oxygen support plays a significant role in bacterial colonization of the gut.

Table 34: Comparison of number of days of IV fluids and gut samples (N=124)

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
IV Fluids (Days) (N=124)								
<7(N=47)	11 (23.40%)	0.357	23 (48.93%)	0.081	15 (31.91%)	0.346	27 (57.44%)	0.014
>7(N=10)	3 (30.00%)		2 (20%)		4 (40.00%)		7 (70.00%)	
No(N=67)	10 (6.70%)		21 (31.34%)		15 (22.38%)		23 (34.32%)	

The above table shows that IV fluid provided for >7 days for preterm neonates did not promote bacterial growth in gastric aspirates, but it certainly promoted statistically significant bacterial growth in stool sample-2 (P=0.014), indicating that IV fluid administration for a longer duration plays a significant role in bacterial colonization of the gut.

Table 35: Comparison of number of days of inotropes and gut samples (N=124)

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Inotropes (Days) (N=124))								
Yes(N=5)	1 (20.00%)	0.382	3 (60.00%)	0.518	1 (20.00%)	0.666	4 (80.00%)	0.162
No(N=119)	23 (19.32%)		43 (36.13%)		33 (27.73%)		53 (44.53%)	

Only 5 preterm neonates in the study received inotropes. Bacterial growth was observed in 1 neonate in Gastric aspirate sample 1, 3 neonates in Gastric aspirate sample-2, 1 neonate in stool sample 1 and 4 neonates in stool sample-2. As these numbers were small the difference in bacterial growth in gastric aspirate samples and stool samples were not statistically significant.

Table 36: Comparison of number of days of infant feeding tube and gut samples
(N=124)

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
IFT No. Of Days(N=124)								
<7(N=33)	7 (21.21%)	0.381	15 (45.45%)	0.508	12 (36.36%)	0.158	15 (45.45%)	0.001
>7(N=30)	8 (26.66%)		10 (33.33%)		10 (33.33%)		22 (73.33%)	
No(N=61)	9 (14.75%)		21 (34.42%)		12 (19.67%)		20 (32.78%)	

Prolonged infant feeding tube use (>7 days) was significantly associated with increased bacterial growth in stool sample-2 for 22 neonates (73.33%, $p = 0.001$) compared to those with infant feeding tube use for <7 days having bacterial growth in 15 neonates (45.45%) and those without an infant feeding tube having bacterial growth in 20 neonates (32.78%). Prolonged use of an infant feeding tube (>7 days) was significantly associated with increased bacterial colonization in stool sample-2 ($p = 0.001$), indicating increased gut colonization. However, preterm neonates with infant feeding tube of <7 days were not associated with increased bacterial growth in gastric or stool samples.

Table 37: Comparison of number of days of PICC line and gut samples (N=124)

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
PICC Line(N=124)								
<7(N=13)	5 (38.46%)	0.126	8 (61.53%)	0.155	3 (23.07%)	0.476	7 (53.84%)	0.005
>7(N=21)	5 (23.80%)		7 (33.33%)		8 (38.09%)		16 (76.19%)	
No(N=90)	14 (15.55%)		31 (34.44%)		23 (25.55%)		34 (37.77%)	

The above table shows that presence of PICC line for >7 days for preterm neonates did not promote bacterial growth in gastric aspirates, but it definitely promoted statistically significant bacterial growth in the stool sample-2 (P=0.005), indicating that longer duration of PICC line plays a significant role in bacterial colonization of the gut.

Table 38: Comparison of number of days of phototherapy and gut samples**(N=124)**

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Photo therapy (N=124)								
<2(N=37)	8 (64.86%)	0.233	15 (40.54%)	0.051	15 (40.54%)	0.034	22 (59.45%)	0.033
>2(N=28)	8 (28.57%)		15 (53.57%)		9 (32.14%)		15 (53.57%)	
No(N=59)	8 (13.55%)		16 (27.11%)		10 (16.94%)		20 (33.89%)	

According to the above table, Phototherapy of >2 days was significantly associated with increased bacterial colonization in stool sample-1 ($p = 0.034$) and stool sample-2 ($p = 0.033$). There was no significant association of phototherapy with gut colonization in gastric samples.

Table 39: Comparison of number of days of antibiotics and gut samples (N=124)

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Antibiotics (N=124)								
Yes(N=88)	14 (15.90%)	0.086	22 (25.00%)	0.379	19 (21.59%)	0.069	30 (34.0%)	0.040
No(N=36)	10 (27.77%)		24 (66.66%)		15 (41.66%)		27 (75.00%)	

The about table shows that administration of antibiotics in preterm neonates promotes bacterial growth as a greater number of preterm neonates receiving antibiotics have gastric aspirate and stool samples positive for bacterial growth versus those not receiving antibiotics. The table also shows that administration of antibiotics also promoted bacterial colonisation over time as gastric aspirate sample-2 and stool sample-2 of a greater number of preterm neonates are positive for bacterial growth. However, these differences were significant only for stool sample-2(P-0.04).

Table 40: Comparison of number of days of transfusion and gut samples (N=124)

	GA-1 (N=24)	P value	GA-2 (N=46)	P value	Stool-1 (N=34)	P value	Stool-2 (N=57)	P value
Transfusion (N=124)								
Yes(N=8)	2 (25.00%)	0.248	1 (12.50%)	0.650	1 (12.50%)	1.000	4 (50.00%)	0.179
No(N=116)	22 (18.96%)		45 (38.79%)		33 (28.44%)		53 (45.68%)	

Only 8 preterm neonates in the study, had transfusion of blood components. Bacterial growth was observed in 2 neonates in Gastric aspirate sample 1, 1 neonate in Gastric aspirate sample-2, 1 neonate in stool sample 1 and 4 neonates in stool sample-2. As these numbers were small the difference in bacterial growth in gastric aspirate samples and stool samples were not statistically significant.

Table 41: Comparison of mean of no. of days of intervention and gastric aspirate and stool samples (N=124)

Parameter	GA-1		P value	GA-2		P value	Stool-1		P value	Stool-2		P Value
	Growth (N=24)	No growth (N=100)		Growth (N=46)	No growth (N=78)		Growth (N=34)	No growth (N=90)		Growth (N=57)	No growth (N=67)	
No. Of Days of Hospital Stay (N=124)	18.42 ± 12.23	16.29 ± 12.06	0.441	16.61 ± 9.35	16.76 ± 13.48	0.948	18.32 ± 10.97	16.09 ± 12.47	0.360	20.98 ± 14.15	13.06 ± 8.51	<0.001

It is observed from the about table that the mean duration of hospital stay in days, did not have an effect on the bacterial growth in the gastric aspirate, but it definitely had a role in bacterial growth in stool samples as the mean duration of hospital stay in preterm neonates with positive bacterial growth increased from 18.32 ± 10.97 to 20.98 ± 14.15 and this difference was statistically significant (P<0.01).

Table 42: Comparison of mean of no. of days of respiratory support and gastric aspirate and stool samples (N=124)

Parameter	GA-1		P value	GA-2		P value	Stool-1		P value	Stool-2		P Value
	Growth (N=24)	No growth (N=100)		Growth (N=46)	No growth (N=78)		Growth (N=34)	No growth (N=90)		Growth (N=57)	No growth (N=67)	
Oxygen Support (N=56)	8.08 ± 9.52	6.58 ± 6.63	0.524	5.42 ± 5.2	8.06 ± 8.5	0.184	5.37 ± 5.48	7.73 ± 8.07	0.257	7.6 ± 8.53	5.81 ± 4.68	0.381
Mechanical Ventilation (N=8)	3.5 ± 2.12	3.33 ± 2.42	0.934	3.2 ± 1.1	3.67 ± 3.79	0.796	4 ± 1.41	3.17 ± 2.48	0.678	2.8 ± 1.48	4.33 ± 3.21	0.380
CPAP (N=39)	7.88 ± 10.26	3.52 ± 3.81	0.058	3.8 ± 4.6	4.79 ± 6.55	0.612	4 ± 4.9	4.62 ± 6.33	0.761	5.28 ± 7.12	2.86 ± 1.41	0.218
Nasal Prongs (N=50)	3 ± 1.1	3.62 ± 4.28	0.641	2.38 ± 1.12	4.28 ± 4.79	0.082	2.71 ± 2.27	3.78 ± 4.25	0.381	3.79 ± 4.71	3.05 ± 2.04	0.500
Oxygen By Hood (N=5)	2 ± 0	3.25 ± 1.5	0.510	3.5 ± 2.12	2.67 ± 1.15	0.596	2 ± 0	3.67 ± 1.53	0.239	2.5 ± 1	5 ± 0	0.111

Among neonates requiring respiratory support, the mean duration of oxygen therapy, mechanical ventilation, and CPAP showed no significant association with bacterial colonization in gastric aspirate or stool samples ($p > 0.05$).

Table 43: Comparison of mean of no. of days of circulatory support and gastric aspirate and stool samples (N=124)

Parameter	GA-1		P value	GA-2		P value	Stool-1		P value	Stool-2		P Value
	Growth (N=24)	No growth (N=100)		Growth (N=46)	No growth (N=78)		Growth (N=34)	No growth (N=90)		Growth (N=57)	No growth (N=67)	
IV Fluids (N=57)	4.57 ± 3.48	4.42 ± 3.35	0.884	3.4 ± 2.57	5.28 ± 3.68	0.034	5 ± 3.82	4.18 ± 3.11	0.391	4.79 ± 3.51	3.96 ± 3.11	0.359
Inotropes (N=5)	1 ± 0	4 ± 2.71	0.395	2.33 ± 1.15	5 ± 4.24	0.347	3 ± 0	3.5 ± 3.11	0.895	3.75 ± 2.99	2 ± 0	0.636
IFT (N=63)	9.07 ± 7.76	10.1 ± 7.8	0.654	7.84 ± 5.19	11.18 ± 8.85	0.093	8.86 ± 6.32	10.39 ± 8.43	0.460	11.24 ± 7.75	7.88 ± 7.42	0.090
PICC Line (N=34)	9.8 ± 4.61	12.25 ± 5.74	0.241	9.4 ± 3.79	13.21 ± 6.1	0.042	10.91 ± 3.75	11.83 ± 6.2	0.655	12.43 ± 5.47	9.64 ± 5.26	0.167

Among neonates, prolonged IV fluid administration was significantly associated with bacterial growth in gastric aspirate sample-2, with a mean duration of 3.4 ± 2.57 days in those with bacterial growth compared to 5.28 ± 3.68 days in those without growth ($p = 0.034$). Similarly, longer PICC line duration was significantly associated with bacterial growth in gastric aspirate sample-2, with a mean duration of 9.4 ± 3.79 days in those with bacterial growth compared to 13.21 ± 6.1 days in those without growth ($p = 0.042$). However, the duration of inotropic support and infant feeding tube use showed no significant association with bacterial growth in gastric or stool samples ($p > 0.05$).

Table 44: Comparison of mean of no. of days of feeds and gastric aspirate and stool samples (N=124)

Parameter	GA-1		P value	GA-2		P value	Stool-1		P value	Stool-2		P Value
	Growth (N=24)	No growth (N=100)		Growth (N=46)	No growth (N=78)		Growth (N=34)	No growth (N=90)		Growth (N=57)	No growth (N=67)	
Day Of Initiation of DBF (N=121)	11.3 ± 12.89	9.76 ± 11.91	0.582	9.52 ± 9.47	10.37 ± 13.46	0.708	11.48 ± 10.81	9.51 ± 12.52	0.425	14.07 ± 14.03	6.58 ± 8.79	<0.001
RT (N=65)	7.6 ± 7.54	7.42 ± 6.34	0.927	6.08 ± 4.53	8.38 ± 7.55	0.167	7.13 ± 5.58	7.64 ± 7.12	0.766	9 ± 7.06	5.3 ± 5.21	0.024
Spoon (N=94)	8.92 ± 6.26	10.59 ± 6.5	0.276	8.65 ± 5.19	11.43 ± 7.15	0.037	8.42 ± 4.61	11.1 ± 7.11	0.054	10.54 ± 6.81	9.65 ± 5.97	0.512
DBF (N=117)	7.3 ± 3.05	7.17 ± 3.02	0.849	7.37 ± 3.51	7.08 ± 2.66	0.619	6.97 ± 3.42	7.29 ± 2.85	0.612	7.13 ± 3.41	7.26 ± 2.62	0.807

The above table shows that a longer mean duration of Ryle's tube feeding and a longer mean duration of spoon feeding were associated with increased bacterial growth in gastric aspirate and stool samples. These differences were statistically significant. Among neonates, delayed initiation of direct breastfeeding (DBF) was significantly associated with increased bacterial growth in stool sample-2, with the mean day of initiation of breast feeding at 14.07 ± 14.03 days in those with bacterial growth compared to 6.58 ± 8.79 days in those without bacterial growth ($p < 0.001$). No significant differences were observed in other samples ($p > 0.05$).

Table 45: Comparison of mean of no. of days of intervention and blood culture
(N=124)

Parameter	Blood Culture (Mean± SD)		P value
	Positive (N=13)	No Growth/Not Sent (N=111)	
No. Of Days of Hospital Stay (N=124)	36.92 ± 15.7	14.33 ± 9.06	<0.001
Oxygen Support (N=56)	14.85 ± 10.46	4.53 ± 3.72	<0.001
Mechanical Ventilation (N=8)	4 ± 3.61	3 ± 1.22	0.575
CPAP (N=39)	8.67 ± 9.23	2.52 ± 1.28	0.001
Nasal Prongs (N=50)	5.54 ± 6.23	2.76 ± 2.18	0.022
Oxygen By Hood (N=5)	5 ± 0	2.5 ± 1	0.111
IV Fluids (N=57)	6.38 ± 3.01	3.89 ± 3.26	0.017
Inotropes (N=5)	5.5 ± 3.54	2 ± 1	0.180
IFT (N=63)	17.69 ± 9.58	7.82 ± 5.72	<0.001
PICC Line (N=34)	15.27 ± 5.22	9.74 ± 4.73	0.004
Phototherapy (N=65)	2.9 ± 0.99	2.35 ± 0.62	0.021

The observations in the above table shows that preterm neonates, with longer mean duration of hospital stay, and those requiring longer mean duration of oxygen support, CPAP, oxygen by nasal prongs, IV fluids, IFT, PICC line and phototherapy were statistically significant risk factors associated with positive blood culture. Use of inotropes or oxygen by hood did not show a statistically significant increase in blood culture, probably due to the small number of preterm neonates receiving these interventions.

Table 46: Comparison of mean no. of days of feed and blood culture (N=124)

Parameter	Blood Culture (Mean± SD)		P value
	Positive (N=13)	No Growth/Not Sent (N=111)	
Day Of Initiation of DBF (N=121)	29.69 ± 17.02	7.69 ± 8.84	<0.001
RT (N=65)	15 ± 9.08	5.58 ± 4.08	<0.001
Spoon (N=94)	14.92 ± 7.01	9.4 ± 6.06	0.004
DBF (N=117)	6.77 ± 2.24	7.25 ± 3.1	0.590

This table shows that preterm neonates requiring longer mean duration of Ryles tube feeding or spoon feeds and those who took longer mean days to initiate direct breastfeeding were associated with statistically significant increase in preterm neonates with positive blood culture when compared to preterm neonates who were on direct breastfeeds (P<0.01)

**COLONIZATION OF GUT MICROBIOTA IN PRETERM NEONATES AND
ITS INFLUENCE ON THE INCIDENCE AND OUTCOME OF NEONATAL
SEPSIS**

**Table 47: Comparison between bacterial growth in gut samples and clinical signs
of sepsis (N=124)**

Gastric Aspirate	Clinical Signs of Sepsis		Chi square	P value
	Yes (N=48)	No (N=76)		
Gastric Aspirate (Sample-1) (N=24)	14 (29.17%)	10 (13.16%)	4.830	0.028
Gastric Aspirate (Sample-2) (N=46)	22 (45.83%)	24 (31.58%)	2.562	0.109
Stool (Sample-1) (N=34)	15 (31.25%)	19 (25%)	0.577	0.447
Stool (Sample-2) (N=57)	28 (58.33%)	29 (38.16%)	4.821	0.028

Figure 26: Cluster bar chart of comparison between gastric aspirate (sample-1) and clinical signs of sepsis (N=124)

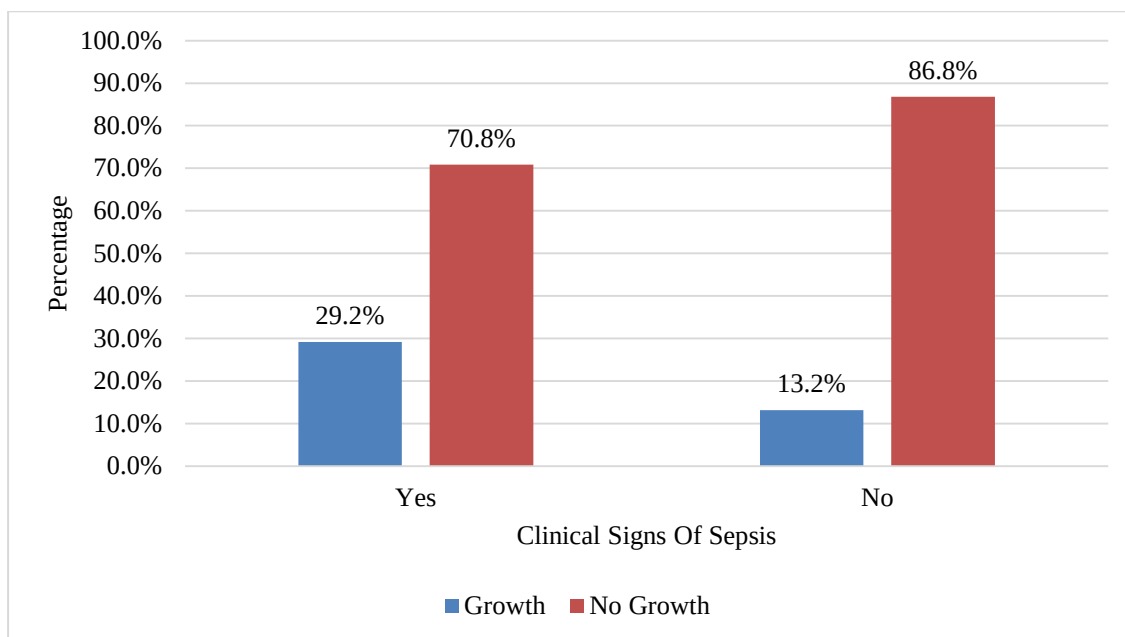


Figure 27: Cluster bar chart of comparison between gastric aspirate (sample-2) and clinical signs of sepsis (N=124)

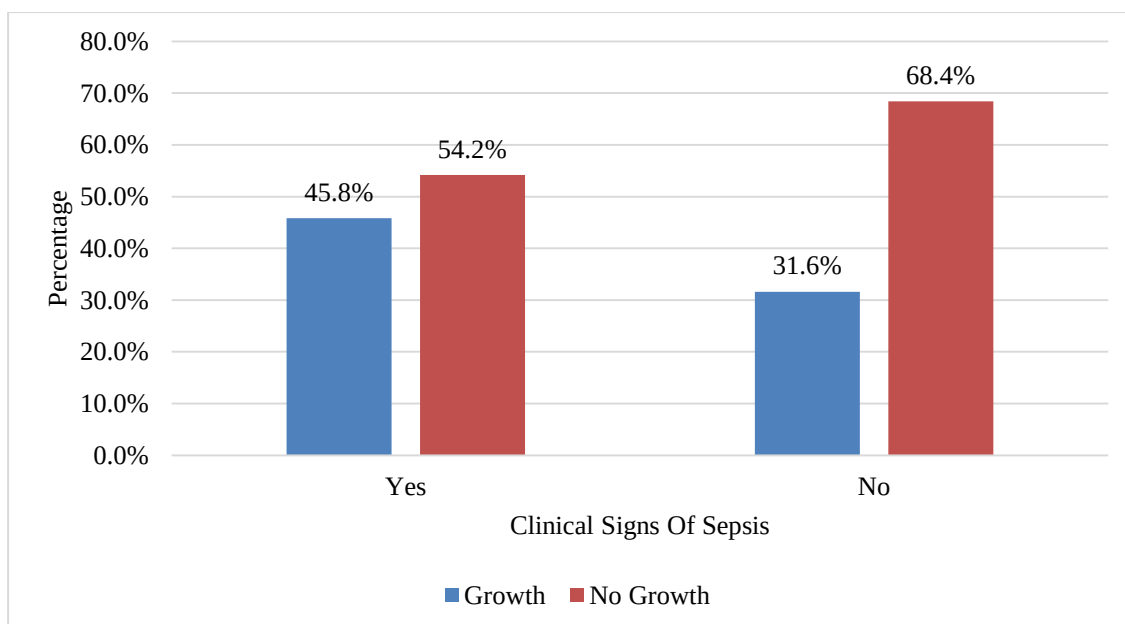


Figure 28: Cluster bar chart of comparison between Stool (sample-1) and clinical signs of sepsis (N=124)

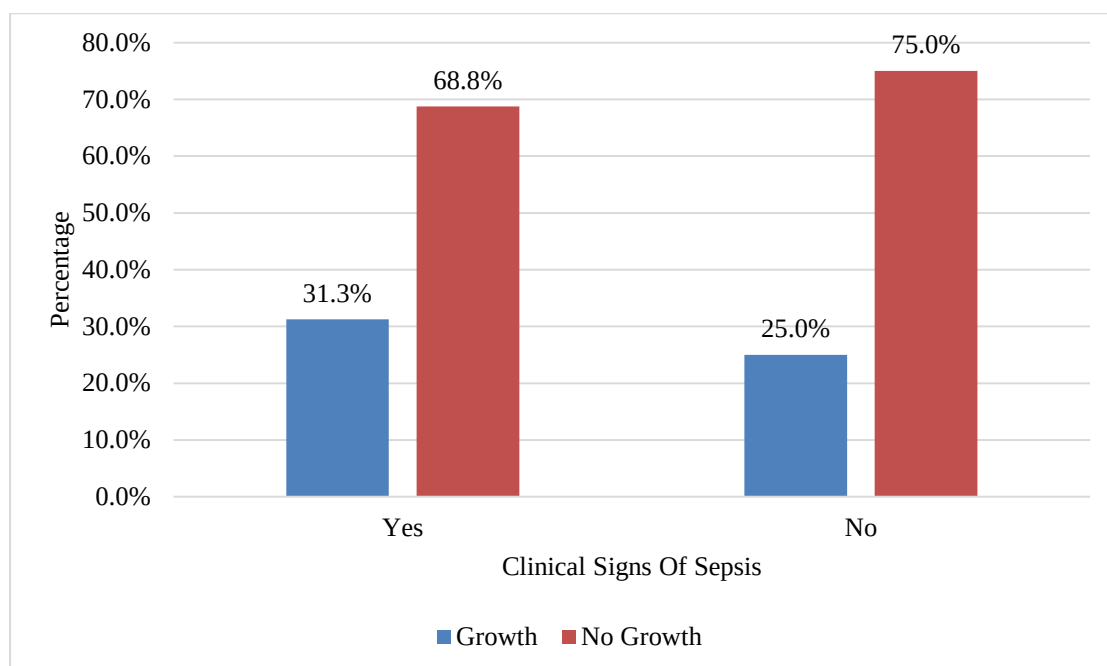
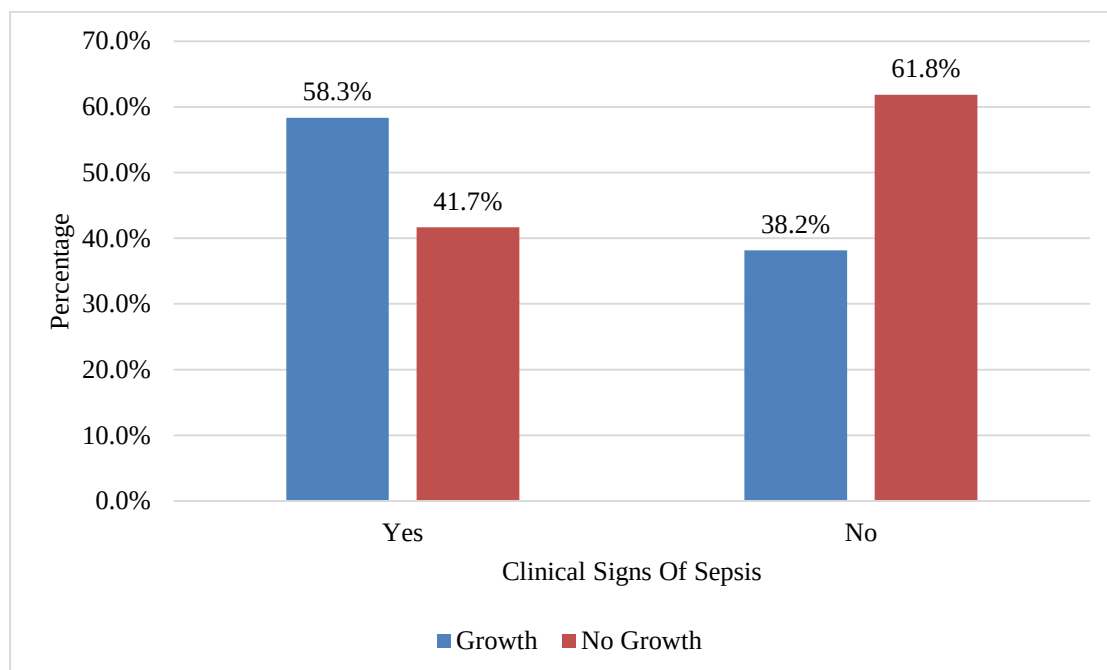


Figure 29: Cluster bar chart of comparison between Stool (sample-2) and clinical signs of sepsis (N=124)



The above table shows that in preterm neonates with clinical sepsis, increase in bacterial growth was observed in gastric aspirate sample 1 and stool sample-2 which was statistically significant ($P=0.028$). Though increase in bacterial growth was also observed in gastric aspirate sample-2 and stool sample-1 the difference was not statistically significant ($P>0.05$). These findings suggest that early bacterial colonization in gastric aspirate sample-1 and stool sample-2 is significantly associated with clinical signs of sepsis.

Table 48: Comparison between bacterial growth in gut samples and Lab Evidence of Sepsis (N=124)

Parameter	Lab Evidence of Sepsis		Chi square	P value
	Yes (N=23)	No (N=101)		
Gastric Aspirate (Sample-1) (N=24)	4 (17.39%)	20 (19.8%)	0.070	1.000
Gastric Aspirate (Sample-2) (N=46)	8 (34.78%)	38 (37.62%)	0.065	0.799
Stool (Sample-1) (N=34)	5 (21.74%)	29 (28.71%)	0.458	0.499
Stool (Sample-2) (N=57)	10 (43.48%)	47 (46.53%)	0.070	0.791

Figure 30: Cluster bar chart of comparison of gastric aspirate (sample-1) and Lab Evidence of Sepsis (N=124)

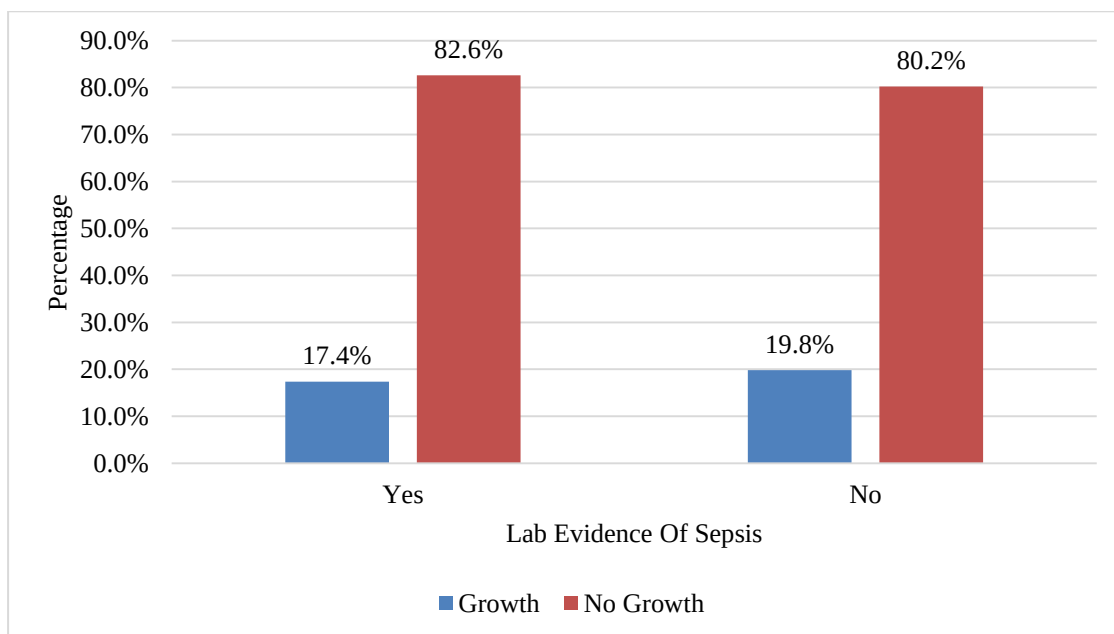


Figure 31: Cluster bar chart of comparison of gastric aspirate (sample-2) and Lab Evidence of Sepsis (N=124)

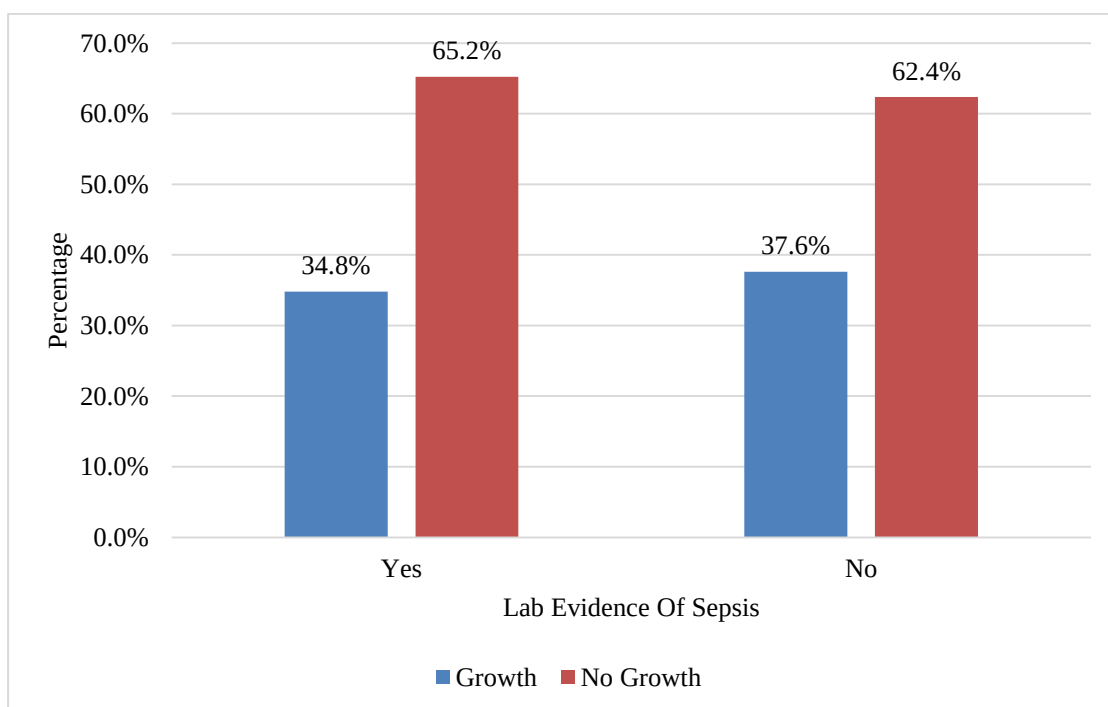
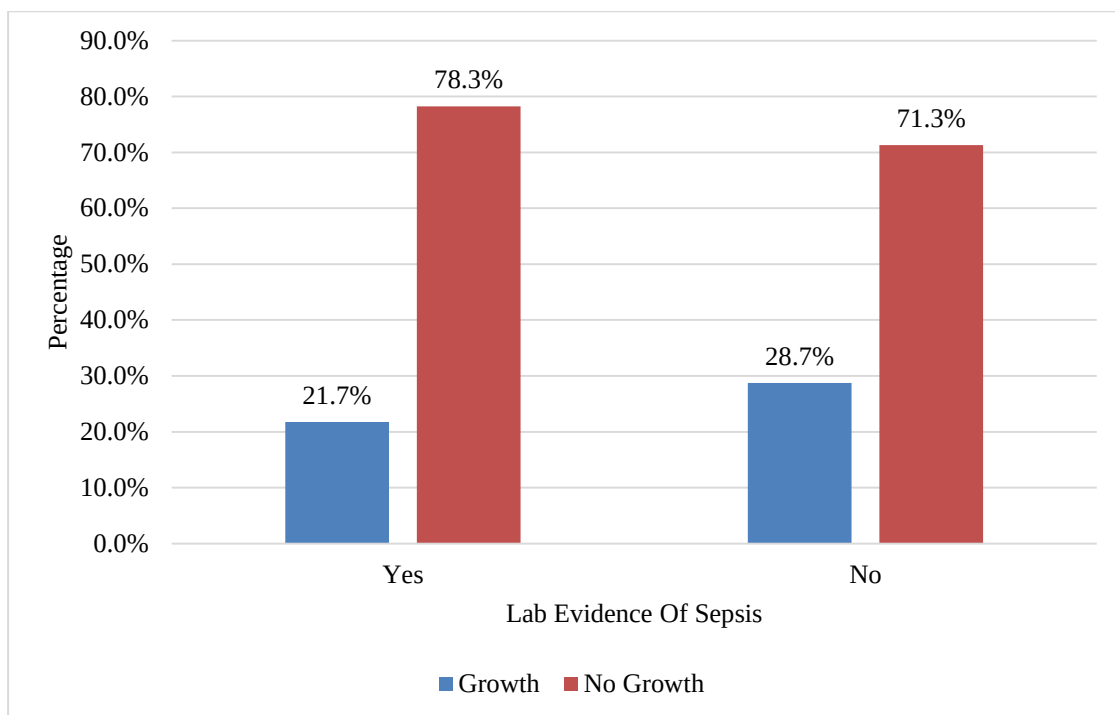
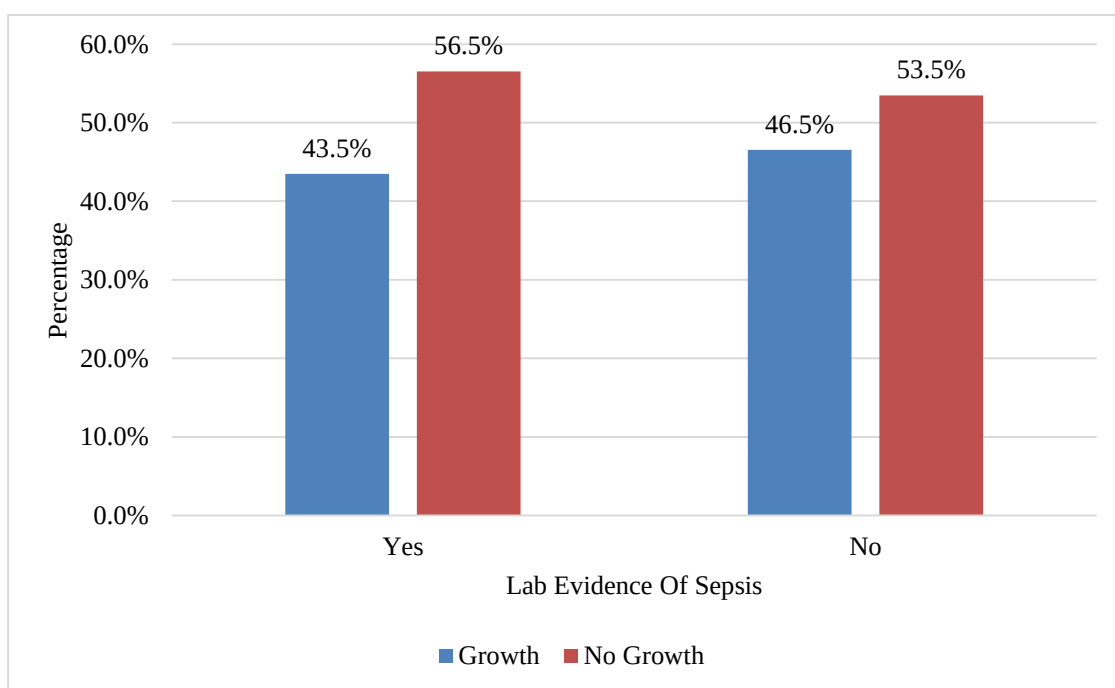


Figure 32: Cluster bar chart of comparison of Stool (sample-1) and Lab**Evidence of Sepsis (N=124)****Figure 33: Cluster bar chart of comparison of Stool (sample-2) and Lab****Evidence of Sepsis (N=124)**

Bacterial growth in gut samples and Lab Evidence of Sepsis

The above table shows that, when sepsis was confirmed through lab parameters (TLC <5000, ANC <1800 or >5400, CRP >10, ESR >15), bacterial growth in gastric aspirate or stool samples was not significantly different between lab confirmed sepsis group and the group of neonates with sepsis not confirmed by lab parameters. Gastric aspirate and stool cultures was not strongly associated with laboratory markers of sepsis, suggesting that sepsis diagnosis should rely on a combination of clinical and lab parameters. (P>0.05)

Table 49: Comparison between bacterial growth in gut samples and blood culture (N=124)

Parameter	Blood Culture		Chi square	P value
	Positive (N=13)	No Growth/ Not Sent (N=111)		
Gastric Aspirate (Sample-1) (N=24)	3 (23.08%)	21 (18.92%)	0.129	0.715
Gastric Aspirate (Sample-2) (N=46)	4 (30.77%)	42 (37.84%)	0.249	0.766
Stool (Sample-1) (N=34)	3 (23.08%)	31 (27.93%)	0.138	1.000
Stool (Sample-2) (N=57)	8 (61.54%)	49 (44.14%)	1.418	0.234

Figure 34: Cluster bar chart of comparison of gastric aspirate (sample-1) and blood culture (N=124)

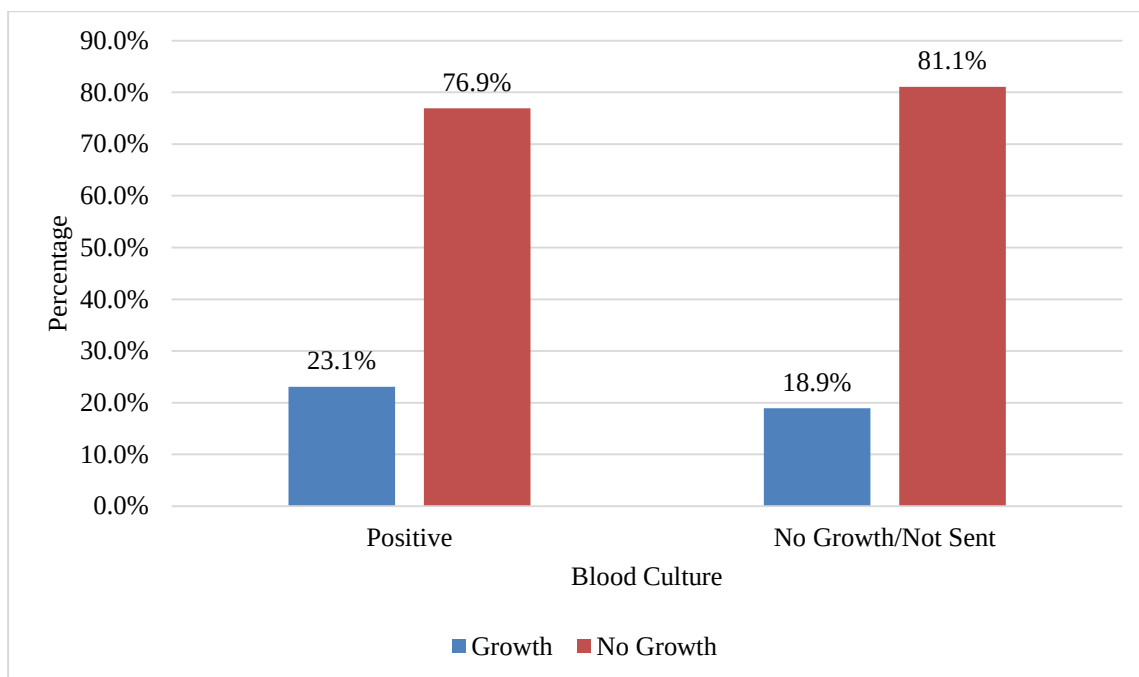


Figure 35: Cluster bar chart of comparison of gastric aspirate (sample-2) and blood culture (N=124)

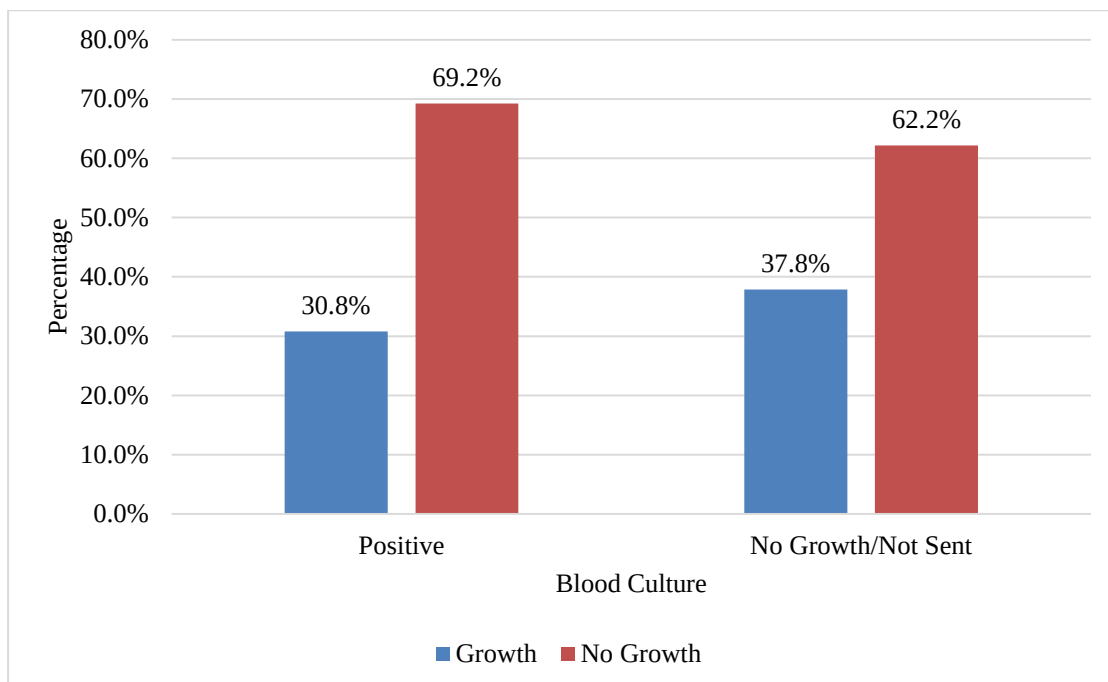
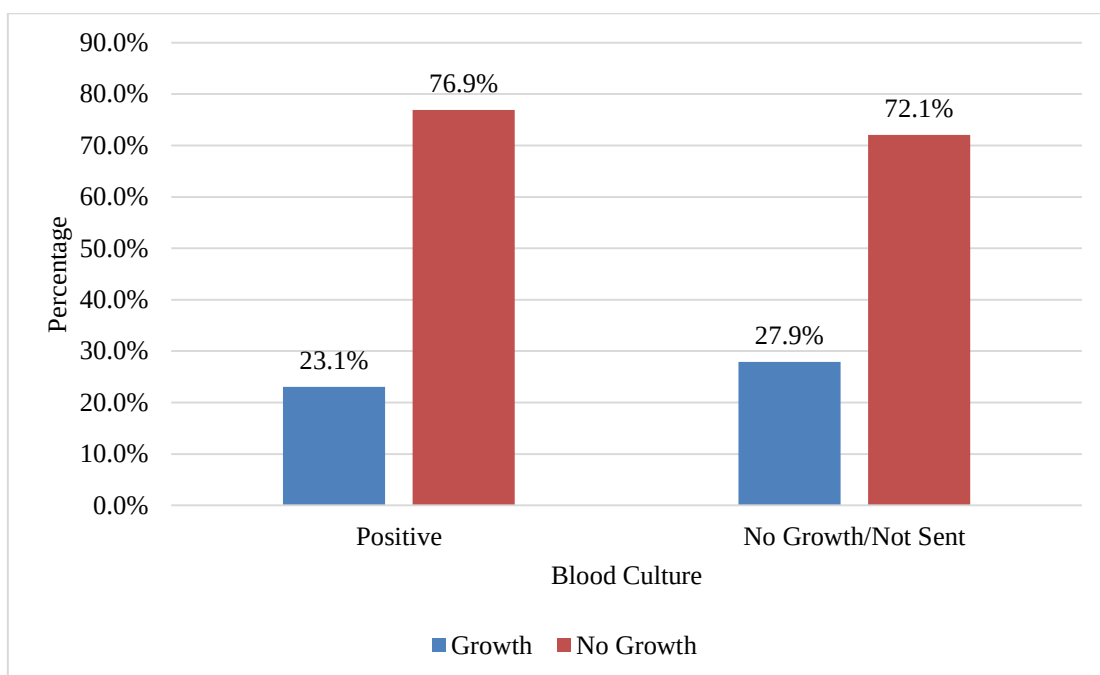
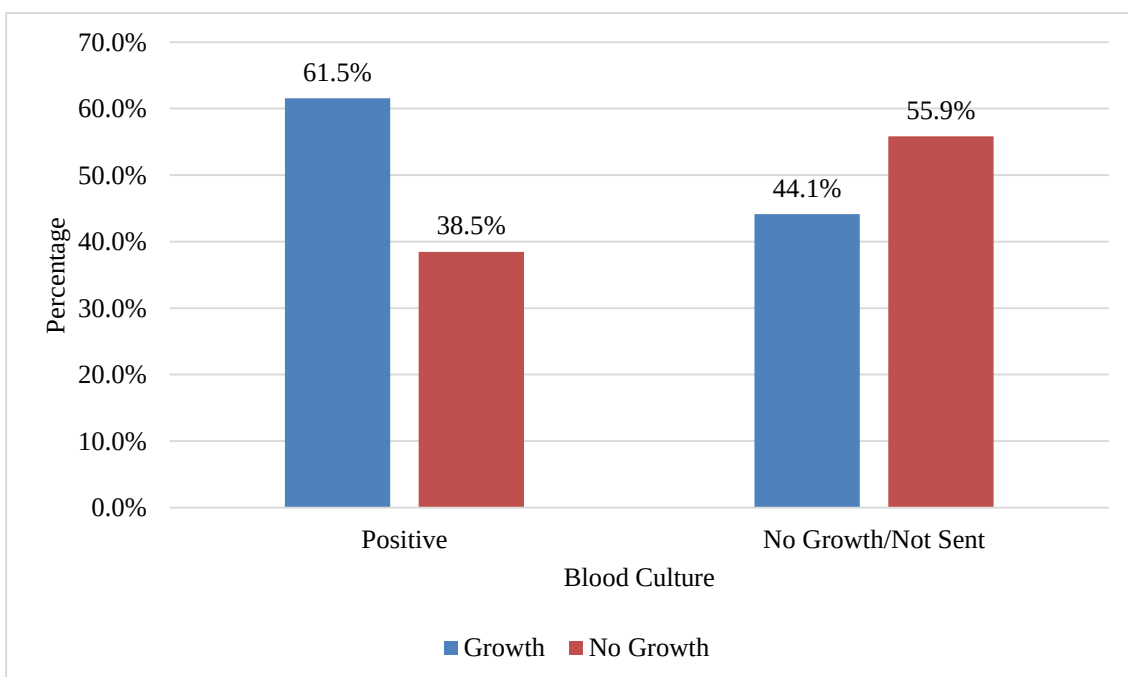


Figure 36: Cluster bar chart of comparison of stool (sample-1) and blood culture**(N=124)****Figure 37: Cluster bar chart of comparison of stool (sample-2) and blood culture****(N=124)**

Bacterial growth in gut samples and blood culture positive Sepsis

These findings indicate that there had been no statistically significant association between bacterial growth in gut specimens and blood culture-positive sepsis ($p > 0.05$). The presence of bacterial colonization in gastric aspirate and stool samples was similar between the neonates with and without blood culture-positive sepsis, with no significant differences. Notably, stool sample-2 showed a higher percentage of bacterial growth (61.54%) in premature babies with blood culture-positive sepsis, but this did not reach statistical significance.

SIMILARITY IN THE ANTIBIOTIC SENSITIVITY PATTERN OF ORGANISMS IN THE GUT WITH CULTURE POSITIVE SEPSIS

Among the 13 neonates with ‘positive blood cultures’, the following organisms were identified: ‘Gram-negative bacteria’ included *Klebsiella pneumoniae* and *Klebsiella oxytoca* in 4 cases, and *Serratia marcescens* in 2 cases. *Acinetobacter* was present in one case, co-infected with coagulase-negative *Staphylococcus* (CONS). In Gram-positive bacteria, *Enterococcus faecalis* was the most frequently isolated organism, found in 3 cases, while CONS was identified in 2 cases and *Staphylococcus haemolyticus* in 1 case. Fungal infection with *Candida glabrata* was present in 2 cases, including one co-infection with *Serratia marcescens*. Out of the bacteria that was isolated in the blood culture only one neonate had co-relation with similar bacteria (*Enterococcus*) being isolated in gut samples and blood culture. However, the antibiotic sensitivity pattern of the *Enterococcus* isolated from the Blood culture and gut samples was different.

Table 50: Organisms isolated in the gastric samples of neonates with Blood

Culture positive sepsis

S. No	Organism in Blood culture	Organism in Gastric Aspirate sample 1	Organism in gastric aspirate sample-2	Organism in stool culture-1	Organism in stool culture-2
1.	<i>CONS/Acinetobacter</i>	NOGC	NOGC	NOGC	NOGC
2.	<i>Klebsiella pneumoniae</i>	NOGC	NOGC	<i>Escherichia coli</i>	NOGC
3.	<i>Klebsiella pneumoniae</i>	NOGC	NOGC	NOGC	NOGC
4.	<i>CONS/ MSSA</i>	NOGC	NOGC	NOGC	NOGC
5.	<i>Staph haemolytics</i>	<i>Klebsiella pneumonia</i>	<i>CONS</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
6.	<i>Klebsiella pneumoniae</i>	NOGC	NOGC	<i>Escherichia coli</i>	<i>Escherichia coli</i>
7.	<i>Klebsiella oxytoca</i> / <i>Enterococcus</i>	<i>Enterococcus</i>	<i>Enterococcus</i>	<i>Enterococcus</i>	<i>Enterococcus</i>
8.	<i>Candida glabrata/Serratia marcescens</i>	NOGC	NOGC	NOGC	<i>Enterococcus</i>
9.	<i>Candida glabrata</i>	NOGC	NOGC	NOGC	NOGC
10.	<i>Serratia marcescens</i>	NOGC	NOGC	NOGC	<i>Klebsiella Pneumoniae</i>
11.	<i>Enterococcus faecalis</i>	NOGC	<i>Citrobacter freundii</i>	NOGC	<i>E Coli</i>
12.	<i>Enterococcus faecalis</i>	<i>Citrobacter freundii</i>	NOGC	NOGC	<i>Klebsiella pneumoniae</i>
13.	<i>Enterococcus faecalis</i>	NOGC	NOGC	NOGC	<i>Klebsiella pneumoniae</i>

DISCUSSION

‘Neonatal sepsis’ continues to be a major cause of ‘morbidity’ and ‘mortality’ among newborns, particularly in preterm infants (56). The global burden of neonatal sepsis remains high, in spite of the various advancements in the medical field, with significant variations in the incidence in addition to outcomes across different regions (57).

‘Established risk factors for neonatal sepsis include prematurity, low birth weight, low Apgar scores, meconium-stained amniotic fluid, birth asphyxia, need for resuscitation, and NICU interventions’ (58). However, a greater number of indicators are pointing towards the notion that microbial diversity in the GI tract plays a critical part in the occurrence of neonatal sepsis (59).

NICU interventions, antibiotic use, and perinatal determinants are recognized influence on the ‘gut microbiota patterns’ (60). While studies in surgical neonates have shown that microbial overgrowth can suppress immunity and increase susceptibility to sepsis (61), data from non-surgical preterm neonates, particularly in developing countries, remain limited (62).

This study aimed to analyze gut microbiota colonization in preterm neonates, identify its association with sepsis, and determine the factors that play a role in the presence of pathogenic bacteria in the gut of preterm neonates. The ultimate goal was to reduce the number of cases of neonatal sepsis and improve long-term outcomes in this vulnerable population (63).

A prospective observational study was conducted at KLES Dr. Prabhakar Kore Hospital for a duration of 1 year. ‘Preterm neonates’ meeting the inclusion criteria during the study period were enrolled (64). Between January 2024 and

December 2024, 1,293 newborns were admitted to the 'NICU/KMC' unit, among which 246 were identified as preterm (64). Out of these, 124 neonates were included in the study after meeting the eligibility criteria and obtaining parental consent (64). Detailed information on parity, mode of delivery, age of gestation at birth, weight at birth, bacterial growth in gastric samples, and incidence of sepsis were systematically recorded using a structured proforma (64).

The formation of 'gut microbiota' in 'preterm neonates' plays a critical role in shaping immune responses and influencing the risk of late-onset sepsis (LOS) (65). Studies conducted by 'Mai et al.' (2013) (47), 'Taft et al.' (2015) (46), and 'Chen et al.' (2024) (44) have shown a strong link between gut dysbiosis and neonatal sepsis.

However, both 'Taft et al.' (46) and 'Mai et al.' (47) highlighted the complexity of gut microbiota's role in predicting LOS. 'Taft et al.' (46) found that microbial disruptions often preceded the onset of LOS but noted variations in bacterial diversity across different NICUs and the absence of a consistent microbial signature. Similarly, 'Mai et al.' (47) observed that a reduction in beneficial bacteria such as '*Bifidobacteria*', rather than an overgrowth of harmful bacteria, was associated with sepsis. Both studies underscore the influence of external factors, such as antibiotic exposure and feeding practices, on microbial composition (66).

Overall, the findings suggest that microbial imbalances may contribute to 'LOS' development, but gut microbiota alone is unlikely to serve as a reliable predictive biomarker for sepsis onset (67). These insights emphasize the need for integrated approaches that combine microbial, genetic, and clinical data to improve the prediction and prevention of neonatal sepsis (67).

Global Incidence and Trends in Neonatal Sepsis

Global data indicates that neonatal sepsis accounts for approximately ‘1.3 million cases’ per year worldwide, leading to an estimated 203,000 deaths annually (68). The incidence of ‘early-onset sepsis’ has decreased following the introduction of antibiotic prophylaxis administered to the mothers during the intrapartum period, whereas ‘late-onset sepsis’ is left behind as a major concern (68).

A meta-analysis conducted by ‘Fleischmann et al.,(2021)’ reported that the global incidence rate of ‘neonatal sepsis’ increased from 85.21 per 100,000 in 1990 to 97.43 per 100,000 in 2019, representing an average annual increase of 0.46% (11). However, the age-standardized death rate (ASDR) showed a gradual decline of 0.53% per year over the same period, decreasing from 3.97 per 100,000 in 1990 to 3.50 per 100,000 in 2019 (11).

Neonatal Sepsis in Developing Countries

The burden of sepsis in neonates is significantly higher in developing countries, where factors such as inadequate healthcare access, insufficient infection control, and antimicrobial resistance play a major role (68).

Sub-Saharan Africa: Studies report an incidence of 38 for every 1,000 live births, with a mortality rate exceeding 40% in some areas (68).

South Asia: The Neonatal Sepsis in South Asia (NSSA) study found that sepsis accounts for nearly 25% of all neonatal deaths (69).

Latin America: Reports indicate a high incidence of hospital-acquired neonatal infections, often linked to prolonged hospital stays and lack of proper hygiene measures (68).

Research conducted by Zaidi et al. (2009) on hospital-acquired newborn illnesses indicated that preterm neonates in underdeveloped nations are three to twenty times more susceptible to sepsis compared to their counterparts in wealthy nations (21).

In high-income countries, the prevalence of early-onset sepsis is roughly 0.5 to 1.0 per 1,000 live newborns, while late-onset sepsis remains at 0.3 to 0.4 per 1,000 live births (68). Countries like the United States and those in Western Europe have seen a decline in newborn sepsis mortality attributed to enhanced infection control and prompt diagnosis (68).

Incidence and Trends of Preterm Neonatal Sepsis in India

India has one of the 'highest neonatal mortality rates' due to sepsis (70). Culture-proven neonatal sepsis cases in Indian in the NICUs has an incidence rate that ranges between 5 to 20 per 1,000 live births, which is significantly higher than in developed countries (70).

A multicentric study by '**Viswanathan et al. (2011)**' reported that 'multidrug-resistant Gram-negative bacilli (MDR-GNB) were responsible for 56% of neonatal sepsis cases in India' (71).

A study conducted by '**Chugh et al. (2018)**' at a tertiary care center in Delhi analyzed blood culture reports from neonatal sepsis cases detected during 2014 and 2015. The study found that 'early-onset sepsis (EOS) was observed in 14.3% of neonates, while late-onset sepsis (LOS) was observed in 20.1% of cases' (72). More than sixty percent of the cases were caused by 'Gram-negative organisms', with '*Klebsiella*, *Acinetobacter*, and *Escherichia coli*' being the most frequently isolated pathogens (72).

In comparison, ‘the prevalence of EOS with positive blood cultures in the United States ranges between 0.77 and 1 per 1,000 live births’ (73). A lot of neonates who are not symptomatic undergo investigations when there are factors which pose a risk or when the clinician suspects infection based on signs and symptoms, as sepsis presents with clinical features that are not specific and also because of increased complication risk in this population (73). Out of 13% of neonates who are treated for sepsis only three percent exhibit growth of microorganisms in the blood culture (73). This can be attributed to maternal antibiotic treatment or inappropriate volume of blood collection required for positive culture isolation (73).

In our study, 41.09% of neonates (53/124) exhibited clinical signs suggestive of sepsis. 18.55% of neonates had lab-confirmed sepsis, and 10.4% had blood culture-positive sepsis.

1. NEONATAL PARAMETERS

1a. GENDER

Our study found an almost equal distribution of preterm neonates by gender (46.77% male, 53.23% female) (75).

In our study, male neonates (9/13) had a greater sepsis prevalence in comparison to females (4/13). Previous studies have had identical findings where male gender is predominance (75). The observed gender disparity in sepsis incidence can be attributed to various immunological and genetic factors (75). Male neonates have higher chances of infections because of their relatively immature immune responses compared to females (75).

‘Rostami-Far et al. (2016)’ conducted research on sepsis which showed that glucose-6-phosphate dehydrogenase (G6PD) impairment, predominantly observed in males, enhances vulnerability to infections and sepsis (76).

‘Boia et al. (2010)’ similarly determined that male neonates exhibited greater risk of developing septicaemia than their female counterparts (77).

Association with Gut Microbiota Composition

Most recent studies have shown that the gut microbiota composition varies between male and female neonates, which may contribute to differential susceptibility to infections (75). Presence of Gram-negative bacterial colonization in stool samples was more frequently observed in male neonates in our study. This finding aligns with research by Shaw ‘AG et al. (2015)’ on late-onset bloodstream infections in neonates, which showed that gender plays a role in gut microbiota, with male neonates exhibiting lower microbial diversity and more pathogenic bacteria (75).

‘Lai et al. (2022)’ reported that gut dysbiosis, particularly low diversity and an overrepresentation of *Proteobacteria*, leads to an increased risk of late-onset sepsis (LOS) in preterm neonates (78). The presence of pathogenic bacteria in male neonates suggests that sex-based differences in gut microbiota could influence susceptibility to sepsis (78).

Association with Neonatal Sepsis

The increased neonatal sepsis incidence in males has been linked to differences in sex hormones and immune system regulation (79). Studies have demonstrated that testosterone suppresses immune responses, making male neonates

more vulnerable to infections, whereas oestrogen exerts protective effects, enhancing immune responses in female neonates (79).

Additionally, X-linked immune regulatory genes contribute to better immune function in females, providing enhanced resistance against bacterial infections (80). The function of Toll-like receptors (TLRs) in innate immunity is substantial, since studies indicate that female neonates demonstrate a more robust TLR-mediated immune response, resulting in enhanced bacterial clearance (80).

1b. GESTATIONAL AGE

The average gestational age in our study cohort was 33.91 weeks, with a range of 27.1 to 36.9 weeks (81). The average gestational age in our study (33.91 weeks) exceeded that documented by ‘Mai et al. (2013)’ in their investigation of alterations in intestinal microbiota linked to late-onset sepsis in preterm neonates (47), as well as by ‘Taft et al. (2015)’ (46) in their analysis of variations in intestinal microbiota preceding late-onset sepsis, both of which predominantly focused on neonates ≤ 32 weeks gestation regarding gut microbiota variations and their correlation with late-onset sepsis (46,47).

Association with Gut Microbiota Composition

Our study revealed that gestational age significantly influences gut microbiota colonization and its correlation with newborn sepsis (81). The average gestational age in our study cohort was 33.91 ± 2.75 weeks, with 50.81% of the neonates classified as late preterm, 25% as moderate preterm, 20.97% as early preterm, and 3.23% as extreme preterm (81). Bacterial colonization was markedly elevated in early preterm and extremely preterm newborns, especially in stool sample-2 ($p = 0.003$), indicating a correlation between prematurity and gut dysbiosis (81).

The gut microbiota of preterm newborns exhibits an overabundance of potentially harmful bacteria, including ‘*Proteobacteria*’ and ‘*Enterobacteriaceae*’ (82). ‘Stewart CJ et al. (2012)’ discovered that neonates delivered prior to 30 weeks of gestation demonstrated markedly elevated incidences of neonatal sepsis (83). This corresponds with the ‘WHO Global Report (2019)’, which suggests that preterm neonates born prior to 32 weeks have an elevated risk of severe morbidity and mortality, consistent with our findings (84).

"Dynamic Changes of the Gut Microbiota in Preterm Infants with Different Gestational Age" by ‘Jia et al. (2022)’ examined 622 faecal samples from infants with diverse gestational ages, spanning from birth to one year (85). Researchers discovered that opportunistic bacteria such as ‘*Klebsiella*’ and ‘*Enterococcus*’ were more abundant in the gut microbiome of extremely preterm newborns (less than 28 weeks) compared to term infants (85). These microbial patterns were linked to an increased risk of infections, including sepsis (85).

Association with Neonatal Sepsis

Neonatal sepsis was observed at higher rates in extremely and early preterm neonates, which correlated with a higher incidence of bacterial growth in stool samples (81). These findings support the hypothesis that gut colonization patterns differ based on gestational age and contribute to sepsis risk (81).

"Intestinal Microbiota Development and Gestational Age in Preterm Neonates" by ‘Korpela K et al. (2014)’ investigated the gut microbiota development in preterm infants and found that lower gestational age was associated with delayed microbial succession and reduced diversity, factors linked to increased susceptibility to late-onset sepsis (86).

"The Premature Infant Gut Microbiome During the First 6 Weeks of Life" by 'Chernikova DA et al. (2017)' highlighted that gestational age at birth significantly affects the gut microbiota composition in premature infants, with those born at earlier gestational ages exhibiting delayed microbial colonization and increased risk of sepsis (87).

1c. BIRTH WEIGHT

'Korpela K et al. (2014)' investigated the gut microbiota development in preterm infants and found that lower gestational age was associated with delayed microbial succession and reduced diversity, factors linked to increased susceptibility to late-onset sepsis (86).

In our study, 7.26% of neonates were 'extremely low birth weight', 22.58% were 'very low birth weight', and 65.32% were 'low birth weight' (88). Second Stool sample showed significantly higher bacterial growth in ELBW (Extremely Low Birth Weight) infants ($P = 0.004$) (88). Our study also showed higher bacterial growth in stool sample-1 among SGA babies ($P = 0.014$) (88). 'Low birth weight babies' had an increased likelihood to show gastric aspirate and stool bacterial growth (88). Higher bacterial growth in SGA and ELBW neonates could indicate immature gut flora or increased susceptibility to infections (88).

These findings are consistent with the work of 'Stewart et al. (2016)', who demonstrated a strong correlation between low birth weight and sepsis risk, particularly among 'ELBW' and 'VLBW' neonates (47). Similarly, 'Lee et al. (2024)' identified a 2.5-fold increase in neonatal sepsis risk among neonates weighing <1.5 kg (89).

The study by ‘Chen et al. (2024)’ demonstrated significant microbiota differences among extremely ‘low birth weight’, ‘very low birth weight’, and ‘low birth weight’ infants, which aligns with our findings that ELBW neonates had more potential pathogens like ‘*Acinetobacter*’ and ‘*Klebsiella*’ (44).

Association with Gut Microbiota Composition

The gut microbiota of LBW infants shows delayed colonization of beneficial bacteria (*Bifidobacterium*, *Lactobacillus*) and dominance of ‘*Proteobacteria*’, a bacterial group linked to inflammation and sepsis (57).

Studies by ‘Shaw et al. (2015)’ (57) and ‘Lee et al. (2021)’ (44) reported that gut microbiota in ELBW infants contained a higher abundance of ‘*Enterobacteriaceae*’, a bacterial family associated with LOS (57).

‘Mai et al. (2013)’ conducted a study that analysed stool samples from preterm infants and found that ‘*Proteobacteria*’ overgrowth preceded the onset of LOS in preterm neonates, reinforcing our study's findings (47).

Association with Neonatal Sepsis

A Taiwanese study examined the prevalence and pathogen distribution of neonatal sepsis in very-low-birth-weight (VLBW) infants at a medical centre in Taiwan, revealing that neonates weighing less than 1,500 g exhibited a significantly elevated incidence of late-onset sepsis compared to those exceeding 1,500 g (32.1% vs. 8.4%) (90).

‘Graham et al. (2006)’ conducted research on Gram-Negative Sepsis in Low-Birth-Weight Infants, identifying risk factors for late-onset gram-negative sepsis in very ‘low birth weight’ infants admitted to neonatal intensive care units (NICUs) (90).

The study determined that gram-negative sepsis was more prevalent in neonates with ‘low birth weight’, particularly in those weighing less than 1,000 grams (90). Our study supports previous findings that lower birth weight is associated with delayed microbiota maturation, increased pathogenic bacteria, and increased risk of sepsis (88).

2. MATERNAL PARAMETERS

2A. Obstetric Score

In our study, 52.42% of neonates were born to primigravida mothers, while 47.58% were born to multigravida mothers. The predominance of primigravida mothers aligns with existing literature indicating that first pregnancies often have a higher risk of obstetric complications, which may contribute to preterm birth (91).

Association with Gut Microbiota:

Maternal obstetric history can influence neonatal gut microbiota composition. Studies suggest that primigravida mothers may have a different vaginal microbiome compared to multigravida mothers, impacting initial neonatal colonization (91). Reduced exposure to maternal commensal bacteria in first pregnancies might lead to an increased risk of gut dysbiosis, favouring colonization by potentially pathogenic organisms like ‘*Escherichia coli*’ and ‘*Klebsiella pneumoniae*’ (91).

Association with Neonatal Sepsis:

A study by ‘Kozuki N et al’. studied the adverse neonatal outcomes in association with parity and found that preterm neonates born to primigravida mothers demonstrated a higher incidence of neonatal sepsis, potentially due to increased maternal inflammatory responses and a higher likelihood of medical interventions

during delivery (91). Our study did not find a statistically significant difference in sepsis rates between primigravida and multigravida mothers, but the trend of increased neonatal infections in primigravida births aligns with prior findings (91).

2B. Gestation Number

In our study, 83.06% of neonates were born from singleton pregnancies, while 14.52% were from twin gestations and 2.42% from triplet gestations (92).

Association with Gut Microbiota:

Multiple gestation pregnancies have been associated with altered gut microbiota colonization in neonates, often due to shared intrauterine conditions and an increased likelihood of premature birth (92). Research indicates that twins and triplets may have reduced microbial diversity and a higher prevalence of ‘*Enterobacteriaceae*’, including ‘*Klebsiella*’ and ‘*Escherichia coli*’, which can increase susceptibility to infections (92).

Association with Sepsis:

Previous literature has reported a greater risk of neonatal sepsis in multiple gestations, primarily due to preterm birth and increased ‘NICU’ interventions (93). ‘Fu M et al’. revealed that twin gestation had a higher incidence of ‘late-onset sepsis’ (LOS) due to shared hospital exposures and delayed gut microbiota maturation (93). While our study did not find a statistically significant association, sepsis rates were slightly higher among multiple gestation neonates, supporting the need for further investigation (93).

2C. Maternal Risk Factors

The most prevalent maternal complication in this study was gestational hypertension (47.58%), followed by premature rupture of membranes (PROM) >18 hours (15.32%) (94).

Association with Gut Microbiota:

We did not find a significant association between these maternal factors and gut bacterial colonization. In contrast, ‘Das et al’. in their study on colonization of gut microbiota in preterm neonates identified maternal sepsis risk factors such as pyrexia, meconium-stained liquor, UTI, and PROM >24 hours as significantly associated with gut colonization in neonates (6). This discrepancy suggests that while maternal factors may play a role in neonatal gut colonization in some populations, other environmental and neonatal factors could also be critical determinants (6).

Association with Neonatal Sepsis:

‘Zaidi et al. (2020)’, in their study on infections acquired in the hospital in developing countries, identified maternal infections and prolonged PROM as the predominant maternal risk factors for neonatal infections (21). However, gestational hypertension was not significantly associated with sepsis incidence in previous studies (21).

The low prevalence of maternal fever (3.23%) in our study suggests effective antenatal care and early treatment, which may have contributed to lower early-onset sepsis (EOS) rates (94).

3. DELIVERY DETAILS

3A. Mode of Delivery

77.42% of neonates in our study were delivered via LSCS, which is higher than the 60% reported in the 'Mai et al. (2013)' study on distortions in development of intestinal microbiota associated with 'late onset sepsis' in preterm neonates (47).

Association with Gut Microbiota:

'Westaway et al. (2021)', in their study on the bacterial gut microbiome in very preterm neonates, demonstrated that C-section delivery alters gut microbiota composition, predisposing neonates to colonization by opportunistic pathogens such as '*Klebsiella*' and '*Enterococcus*' (95).

This is in agreement with our study's finding that C-section-delivered neonates had higher colonization of gastric aspirate-2 samples compared to those delivered via vaginal delivery. However, this was not statistically significant.

Association with Neonatal Sepsis:

'Smith et al. (2020)', in their study on colonization of gut microbiota in preterm neonates, found that vaginally delivered neonates had a more diverse gut microbiome and a lower risk of neonatal sepsis than neonates born by LSCS deliveries (5).

Our study shows that mode of delivery is not a significant contributor to gut dysbiosis.

3B. Postnatal Adaptation

In our study, 74.19% of neonates cried immediately after birth (CIAB), while 25.81% needed active resuscitation, including stimulation, bag-and-mask ventilation, or intubation. Among neonates with poor postnatal adaptation, bacterial growth was

significantly higher in gastric aspirate sample-2 ($p = 0.034$) and stool sample-2 ($p = 0.030$), indicating an increased risk of gut colonization in those requiring resuscitation at birth.

This suggests that neonates experiencing perinatal distress have a higher likelihood of altered gut microbiota composition, which may influence their long-term health outcomes.

Association with Gut Microbiota:

Postnatal adaptation plays a crucial role in establishing a stable gut microbiome (96). Delayed adaptations, as seen in neonates requiring resuscitation, are associated with delayed gut colonization and dysbiosis due to factors such as hypoxic stress, altered gut perfusion, delayed feeding initiation, and increased antibiotic exposure (96).

A study on intestinal microbiota in asphyxiated neonates has shown that neonates with birth asphyxia or requiring resuscitation often develop an altered gut microbial profile, characterized by increased colonization of potentially pathogenic bacteria such as '*Klebsiella pneumoniae*' and '*Escherichia coli*' (96).

In our study, the highest bacterial colonization was observed in stool sample-2, reinforcing the hypothesis that neonates with perinatal distress undergo progressive gut colonization over time.

These findings align with research by 'Stinson et al'. on infant microbiome, which demonstrated that neonates with compromised postnatal adaptation exhibit delayed microbial diversity and are predisposed to colonization by facultative

anaerobes such as '*Enterobacteriaceae*' rather than beneficial '*Bifidobacterium*' species (97).

The observed increase in bacterial growth in stool sample-2 in our study suggests that postnatal adaptation disruptions may contribute to long-term gut microbiota imbalances.

Association with Neonatal Sepsis:

Delayed postnatal adaptation has been linked to an increased risk of neonatal sepsis, primarily due to immune dysregulation and compromised gut barrier integrity. Our study found a significant association between poor postnatal adaptation and bacterial colonization in stool sample-2, suggesting that neonates requiring resuscitation at birth may have a higher risk of developing sepsis.

'Stewart et al'., in their study on preterm gut microbiota, observed that preterm neonates with poor postnatal adaptation were more likely to develop late-onset sepsis, likely due to gut dysbiosis and increased bacterial translocation into the bloodstream (76).

Additionally, research by 'Duess JW et al'. highlighted that neonates who required resuscitative measures had persistent intestinal inflammation, leading to an increased risk of necrotizing enterocolitis (NEC) and sepsis (98).

While our study did not establish a direct link between postnatal adaptation and culture-positive sepsis, the increased bacterial colonization in stool sample-2 supports the hypothesis that perinatal distress may predispose neonates to infection risk. These findings underscore the need for early microbiota-targeted interventions, including early enteral feeding, probiotics, and careful antibiotic stewardship, to mitigate the risk of gut dysbiosis and sepsis in neonates with poor postnatal adaptation.

3C. Low APGAR Score

A low APGAR score (<7) was observed in 17.74% of neonates, indicating potential perinatal distress. There was no association between low APGAR score and gut colonization. Additionally, 74.19% of neonates cried immediately after birth, while 3.23% required intubation.

‘Schwartz et al’. demonstrated that low ‘APGAR’ scores were predictive of increased ‘NICU’ admissions and sepsis risk in their study on gram-negative colonization (28). WHO Sepsis Guidelines (2021) emphasize the role of early ‘CPAP’ and respiratory support in neonates with low ‘APGAR’ scores to mitigate hypoxic injury (64).

4. INTERVENTIONS

4a. Admitted ward

Preterm neonates were admitted either in ‘Neonatal Intensive Care Unit’ (NICU) or Kangaroo Mother Care (KMC) unit. The majority (75%) of preterm neonates in the study population were admitted to the ‘NICU’. Only 25% were managed in ‘KMC’, suggesting that these neonates were more stable and required minimal intensive care. Neonates admitted in ‘NICU’ had higher bacterial growth in the first and later gastric aspirate sample compared to those admitted in the Kangaroo Mother Care (KMC) unit. Bacterial growth in neonates admitted in KMC ‘Kangaroo Mother Care’ unit was significantly lower ($P < 0.001$). Neonates admitted in ‘NICU’ had higher bacterial growth in the first and later stool sample compared to those admitted in the ‘Kangaroo Mother Care’ (KMC) unit. Bacterial growth in neonates admitted in KMC (Kangaroo Mother Care) unit was significantly lower ($P < 0.001$) and showed progressive decrease. This shows that ‘NICU’ admission was a risk factor for gut dysbiosis.

4b. Duration of hospital stay

In our study, preterm neonates had a mean hospital stay of 16.7 days, ranging from 7 to 59 days. Prolonged hospitalization (>15 days) was significantly associated with increased bacterial colonization in stool sample-2 ($p = 0.022$), with 60% of neonates in this group showing bacterial growth compared to 44% in the 8–15-day group and 27.58% in the <8-day group.

Association with Gut Microbiota:

Extended hospital stay has been linked to altered gut microbiota composition due to increased exposure to hospital-acquired bacteria, frequent antibiotic use, delayed enteral feeding, and prolonged invasive interventions. Our findings showed a progressive increase in bacterial colonization from gastric aspirate sample-1 to stool sample-2, with the highest colonization in stool sample-2 among neonates hospitalized for >15 days.

Similar studies have reported higher prevalence of gut dysbiosis in neonates with prolonged NICU stays, with increased colonization by '*Klebsiella pneumoniae*', '*Escherichia coli*', and '*Enterococcus*' species (102). A study by 'Devarajalu et al', on preterm gut microbiota observed reduced microbial diversity and overgrowth of pathogenic organisms in neonates with longer hospitalization (102). These findings support our observation that prolonged hospital stays contribute to gut microbiota dysbiosis in preterm neonates.

Association with neonatal sepsis:

A longer duration of hospital stay was significantly associated with bloodstream infections ($p < 0.001$). Neonates with positive blood cultures had a mean

hospital stay of 36.92 ± 15.7 days, which was significantly longer than 14.33 ± 9.06 days in those with negative cultures. This aligns with previous research that found higher rates of late-onset sepsis (LOS) in preterm neonates with prolonged hospitalization. ‘Chernikova et al.’, reported that gut dysbiosis in long-stay neonates contributes to systemic infections through bacterial translocation (103).

Our study reinforces the need for infection control strategies, early enteral feeding, and gut microbiota modulation in neonates with extended hospital stays to reduce the risk of gut dysbiosis and sepsis.

4c. Interventions during hospital stay

In our study, various interventions, including oxygen therapy, mechanical ventilation, ‘CPAP’, intravenous fluids, peripherally inserted central catheters (PICC lines), infant feeding tubes, blood transfusions, and phototherapy, were administered based on clinical indications.

- Prolonged PICC line use (>7 days) was significantly associated with increased bacterial growth in stool sample-2 ($p = 0.005$), with 76.19% of neonates in this group exhibiting bacterial colonization, compared to 53.84% in those with a ‘PICC’ line for ≤ 7 days and 37.77% in those without a ‘PICC’ line.
- Prolonged infant feeding tube use (>7 days) was significantly associated with bacterial colonization in stool sample-2 ($p = 0.001$), with 73.33% of these neonates showing bacterial growth.
- Phototherapy for >2 days was significantly associated with increased bacterial colonization in stool sample-2 ($p = 0.033$).

- Oxygen support for >7 days was significantly associated with increased bacterial colonization in stool sample-2 ($p = 0.003$).
- Neonates requiring blood transfusions had higher bacterial colonization in stool sample-2 (50%), though this was not statistically significant ($p = 0.179$) as sample sizes were small.

These findings suggest that prolonged 'NICU' interventions contribute to increased gut bacterial colonization, particularly in stool sample-2, which represents later-stage colonization.

Association with Gut Microbiota:

Prolonged medical interventions, especially long-term use of invasive devices such as PICC lines and infant feeding tubes, are known to influence gut microbiota composition and contribute to dysbiosis. A study by 'Cuna et al'., on preterm gut microbiome has reported increased colonization of '*Klebsiella pneumoniae*', '*Escherichia coli*', and '*Enterococcus*' species in neonates subjected to prolonged interventions (104).

'Cuna et al'., observed reduced microbial diversity and overgrowth of pathogenic organisms in neonates with prolonged hospitalization, supporting our observation that prolonged 'NICU' stay and invasive interventions promote gut dysbiosis (104). 'Pammi et al'., in their study on intestinal dysbiosis reported that intestinal dysbiosis in preterm neonates increases the risk of systemic infections due to bacterial translocation (105).

Furthermore, oxygen therapy and mechanical ventilation have been implicated in altering gut microbiota composition through increased oxidative stress and

inflammatory responses as reported by ‘Taft et al’. in their study on variation in intestinal microbiota prior to late-onset sepsis (46). ‘CPAP’ support has also been associated with higher rates of gram-negative bacterial colonization, including ‘*Klebsiella pneumoniae*’ and ‘*Escherichia coli*’ (107).

Association with neonatal sepsis:

Our study found that prolonged use of ‘NICU’ interventions, including ‘PICC’ lines, infant feeding tubes, and phototherapy, was significantly associated with increased bacterial colonization, which may contribute to the risk of neonatal sepsis. Although a direct correlation between interventions and bloodstream infections was not observed, previous studies have demonstrated that prolonged use of invasive devices increases the risk of nosocomial infections and late-onset sepsis (LOS).

A study by ‘Stewart et al’., found that neonates on prolonged IV support and ‘PICC’ lines had higher rates of bacterial translocation and sepsis, primarily due to gut dysbiosis (106). Similarly, ‘Pammi et al’., in their study on intestinal dysbiosis in preterm neonates demonstrated that neonates requiring prolonged enteral support, particularly via infant feeding tubes, had an increased risk of gut-derived sepsis due to delayed microbial maturation and altered gut barrier integrity (105).

Our findings reinforce existing evidence that minimizing unnecessary prolonged interventions, promoting early enteral feeding, may reduce the risk of gut dysbiosis and subsequent infections in preterm neonates.

5. COLONIZATION OF THE NEONATAL GUT WITH BACTERIA

5a. Gastric Aspirate

In our study, bacterial colonization was detected in 19.35% (24/124) of neonates in the initial gastric aspirate sample (GA-1), with an increase to 27.42% in the second sample (GA-2), collected between days 7–10 of life. The most frequently identified bacteria in GA-1 were '*Escherichia coli*' (5.65%) and '*Klebsiella pneumoniae*' (5.65%), along with '*Coagulase-negative staphylococci*' (CONS), '*Micrococci*', and '*Acinetobacter*'. By the time GA-2 was obtained, '*E. coli*' prevalence had risen to 12.10%, followed by '*Klebsiella pneumoniae*' (4.84%), CONS (3.23%), and '*Enterococcus*'. The observed increase in bacterial growth, particularly among gram-negative organisms such as '*E. coli*' and '*Klebsiella pneumoniae*', indicates a gradual establishment of gut microbiota over time.

Comparison with Other Studies

Similar trends have been reported in other studies analysing bacterial colonization in neonatal gastric aspirates:

- 'Basu et al'., in their study on neonatal gut microbiota, found that '*Klebsiella pneumoniae*' was the predominant gram-negative bacillus (GNB) in gastric aspirates and fecal specimens of hospitalized neonates, with a significant increase in colonization over time. Their study emphasized that the progressive rise in '*E. coli*' and '*Klebsiella pneumoniae*' colonization was associated with prolonged 'NICU' stay and enteral feeding tube use (108).
- 'Das et al'., in their study on colonization of neonatal gut microbiota, reported that bacterial growth in neonatal gastric aspirates increased from 42% (GA-1)

to 69% (GA-2), taken between days 3–7 of life. In their study, '*Klebsiella pneumoniae*' was present in 19% of GA-1 samples but increased significantly to 57% in GA-2 ($p < 0.001$), further confirming that it is a dominant pathogen in neonatal gut colonization (109).

- 'Fleischmann et al'., noted that gram-negative bacteria, particularly '*Klebsiella pneumoniae*', were the most frequently isolated organisms in neonatal sepsis cases and emphasized that their presence in gastric aspirates could be an early predictor of bloodstream infections (110).
- 'Schwartz et al'., documented a similar progressive increase in '*E. coli*' and '*Klebsiella pneumoniae*' colonization in gastric aspirates, highlighting that these bacteria are major contributors to neonatal sepsis due to their ability to translocate from the gut to the bloodstream (111).

5b. Stool Sample

In our study, stool cultures demonstrated a higher prevalence of bacterial growth compared to gastric aspirate samples. In the first stool sample (S-1), 37.1% of neonates had positive cultures, which increased to 45.97% in the second stool sample (S-2). The predominant organisms in S-1 were '*Escherichia coli*' (20.16%) and '*Klebsiella pneumoniae*' (6.45%), followed by '*Enterococcus*', '*Pseudomonas*', and '*Acinetobacter*'. In S-2, '*E. coli*' remained the most frequently isolated organism (17.74%), but '*Enterococcus*' (11.29%) and '*Klebsiella pneumoniae*' (10.48%) showed an increasing trend. This progressive colonization suggests a higher bacterial load over time, which could contribute to an increased risk of infections, particularly in preterm neonates.

Comparison with Other Studies:

Several studies have reported similar patterns of progressive bacterial colonization in neonatal stool samples:

1. 'Das et al.', observed that bacterial colonization in neonatal stool samples increased significantly over time, with '*Klebsiella pneumoniae*' and '*E. coli*' being the most frequently isolated organisms. Their study emphasized that neonates with prolonged 'NICU' stays and enteral feeding tubes exhibited a higher bacterial load (109).
2. 'Basu et al.', highlighted that '*Klebsiella pneumoniae*' was the predominant gram-negative bacillus in both stool and gastric aspirate samples of hospitalized neonates. Their findings indicated that neonates who remained in the 'NICU' for longer durations had significantly increased bacterial colonization, particularly with '*E. coli*' and '*Klebsiella pneumoniae*' (108).
3. 'Schwartz et al.', documented a similar trend of increased '*E. coli*' and '*Klebsiella pneumoniae*' colonization over time, particularly in preterm neonates. They suggested that these organisms could translocate from the gut into the bloodstream, contributing to late-onset sepsis (LOS) (111).
4. 'Law et al.', conducted a systematic review that found a strong association between gut colonization with '*Enterobacteriaceae*' (including '*E. coli*' and '*Klebsiella pneumoniae*') and the subsequent development of neonatal sepsis. Their study emphasized that stool samples often contained potential pathogens before the onset of bloodstream infections (112).

Neonatal Sepsis

In our study, 41.09% of neonates (53/124) exhibited clinical signs of sepsis, with tachypnoea (100%) and tachycardia (81.13%) being the most common symptoms, followed by apnoea (50.94%), lethargy (33.96%), and poor feeding (30.19%). These findings align with Mahmoud et al., who reported tachypnoea and tachycardia as early indicators of neonatal sepsis, while Popescu et al. highlighted respiratory distress as the earliest sign (113,114). Laboratory-confirmed sepsis was observed in 18.55% (23/124) of neonates, with leukopenia (TLC <5000 in 18.55%) and ANC abnormalities (17.74%) being common. CRP >10 was found in 15.32% of cases, supporting prior studies by 'Sharma et al'. and 'Newman et al'. , which noted low sensitivity but high specificity for leukopenia in sepsis diagnosis (115,116). 'Benitz et al'. emphasized that 'CRP' alone has limited early diagnostic value, requiring serial measurements (117), while 'Pourcyrus et al'. suggested its predictive accuracy improves when combined with clinical signs (118).

Blood cultures were positive in 13 neonates, predominantly isolating gram-negative bacteria, including 'Klebsiella pneumoniae' (four cases), 'Klebsiella oxytoca', 'Serratia marcescens', and 'Acinetobacter', with fungal co-infections in two cases. Gram-positive isolates included Enterococcus faecalis (three cases) and Coagulase-negative Staphylococcus (two cases). These findings align with 'Das et al'. , who found 'Klebsiella pneumoniae' to be the most frequently isolated pathogen in septic neonates, reinforcing the gut-sepsis link (109). 'Fleischmann et al'. similarly reported Klebsiella and Staphylococcus aureus as the leading causes of neonatal sepsis, while Mahmoud et al. highlighted Acinetobacter as a major hospital-acquired pathogen (110,113). 'Schwartz et al'. also documented 'Klebsiella' and 'Serratia' as leading causes of neonatal bloodstream infections, often linked to multi-organ

dysfunction (111). The discrepancy between clinical and laboratory-confirmed sepsis in our study (41.09% vs. 18.55%) reflects the challenges in neonatal sepsis diagnosis, as emphasized by ‘Cantey et al’., who noted overuse of antibiotics in culture-negative sepsis, and ‘Klingenberg et al’., who stressed the need for improved diagnostic markers (119,120).

7. Colonization of the Gut Microbiota in Preterm Neonates and Its Association with Neonatal Sepsis

Preterm infants often experience gut dysbiosis, characterized by reduced microbial diversity and an overrepresentation of pathogenic bacteria such as Proteobacteria (121). This imbalance can lead to bacterial translocation across the immature intestinal barrier, increasing the risk of systemic infections, including sepsis (121). ‘Pammi et al’., in their study on intestinal dysbiosis, highlighted that pathogenic bacteria can colonize the gut prior to sepsis and cause sepsis through translocation (80).

In our study, bacterial colonization in gastric aspirate and stool samples was assessed in relation to clinical sepsis, laboratory-confirmed sepsis, and blood culture-positive sepsis. Early gastric aspirate colonization (sample-1) was significantly higher in neonates with clinical sepsis (29.17%) compared to those without (13.16%) ($p = 0.028$), indicating a possible association. Late stool sample colonization (sample-2) also showed a significant increase in bacterial growth in neonates with clinical sepsis (58.33%) compared to those without (38.16%) ($p = 0.028$). However, no statistically significant association was observed between bacterial growth in gut samples and laboratory-confirmed or blood culture-positive sepsis.

Comparison with Other Studies

Several studies have reported findings that align with or contrast our results:

7a. Neonatal Gut Colonization and Clinical Sepsis

- A study by ‘Schwartz et al’., found that colonization with ‘*Klebsiella pneumoniae*’ and ‘*Escherichia coli*’ in gastric aspirate and stool samples preceded clinical sepsis in preterm neonates (111). This supports our finding that early colonization in gastric aspirate (sample-1) and stool sample-2 were significantly associated with clinical signs of sepsis.
- ‘Das et al’., reported that ‘*Klebsiella pneumoniae*’ colonization in gastric aspirate increased from 19% (sample-1) to 57% (sample-2), with a significant correlation to sepsis onset ($p < 0.001$) (6). Our study also found increased colonization over time but without a strong correlation to blood culture-positive sepsis.
- ‘Law et al’., conducted a systematic review demonstrating that ‘*Enterobacteriaceae*’ colonization in the gut was associated with late-onset sepsis (LOS) in neonates (45). Our study found that later stool colonization correlated with clinical sepsis but not with laboratory-confirmed or blood culture-positive sepsis.

7b. Gut Microbiota and Laboratory-Confirmed Sepsis

- A study by ‘Fleischmann et al’., found no strong correlation between gut bacterial colonization and laboratory markers such as ‘CRP’ and leukocyte counts, indicating that clinical assessment remains essential for sepsis

diagnosis (110). This aligns with our findings where gastric aspirate and stool cultures did not significantly correlate with lab-confirmed sepsis.

- ‘Mai et al’., showed that neonates with positive gut colonization had a higher incidence of abnormal leukocyte counts and ‘CRP’ elevations, but the predictive value was still limited (47).

7c. Gut Microbiota and Blood Culture-Positive Sepsis

- A study by ‘Pierro et al’., found that ‘*Klebsiella pneumoniae*’ and ‘*E. coli*’ were frequently isolated from both gut samples and blood cultures, suggesting bacterial translocation from the gut to the bloodstream (24). In contrast, our study did not find a statistically significant association between gut colonization and blood culture positivity.

Our study found no statistically significant correlation between gut bacterial colonization and subsequent culture-positive neonatal sepsis. Similarly, several other investigations have reported a lack of clear association between neonatal gut microbiome “dysbiosis” and bloodstream infections.

- ‘Wandro et al’. (2018) conducted a prospective observational study on 32 very-low-birth-weight preterm infants in the ‘NICU’, analysing 77 stool samples over the first six weeks of life using 16S rRNA gene sequencing and untargeted metabolomics (GC-MS) (123). The study categorized neonates into groups based on health outcomes, including healthy, late-onset sepsis (LOS), and necrotizing enterocolitis (NEC) (123). Their findings indicated that gut microbiota composition was highly individualized, with antibiotic exposure and infant-specific factors playing a more significant role than sepsis status (123). No distinct microbial “sepsis signature” was identified, as the variation

in gut microbiome and metabolite patterns was not linked to sepsis outcomes (123). Instead of finding universal microbial shifts preceding sepsis, the study revealed that preterm infant microbiomes are personalized and do not consistently predict sepsis (123). These findings are similar to the observations of our study, which also found no significant association between gut bacterial colonization and culture-positive neonatal sepsis, emphasizing the complexity of microbiome dynamics in preterm neonates (123). Both ‘Wandro et al’., and our study conclude that there is no clear, predictable shift in gut bacterial diversity or composition preceding neonatal sepsis (123). Gut dysbiosis (as measured by 16S profiles or metabolite changes) did not correlate significantly with sepsis onset in this cohort (123). Thus, this 2018 study notably emphasizes that any microbial differences were overshadowed by individual variability and antibiotic use – a finding consistent with our results showing no robust microbiome–sepsis association (123).

- ‘Taft et al. (2015)’ conducted a prospective cohort study across two ‘NICUs’ (Cincinnati and Birmingham), examining preterm infants with late-onset sepsis (LOS) and matched controls. Stool samples were collected 1–3 weeks before sepsis onset and analysed using 16S rDNA sequencing, with whole-genome sequencing for further validation in a subset (46). The study included 38 LOS cases and 38 matched controls (46). Their findings revealed that 82% of sepsis cases had the causative organism present in stool samples before illness, but there was no uniform dysbiosis pattern to distinguish sepsis cases (46). Changes in gut flora, such as blooms of specific taxa, were inconsistent across hospitals and time points (46). For instance, lower ‘*Actinobacteria*’ levels were noted before sepsis at one hospital, but no differences were seen at

the other (46). Likewise, taxa such as ‘*Lactobacillales*’ and ‘*Pseudomonadales*’ fluctuated differently across sites, showing no generalizable microbiome signature predicting sepsis onset (46). The authors concluded that gut microbial distortions occur before LOS, but their form depends on timing and location, making them unreliable for sepsis prediction (46). These findings align with our study, where out of 68 neonates with bacterial growth in gut samples only 9 neonates (13.2%) developed blood culture-positive sepsis, which was not statistically significant, reinforcing the idea that mere bacterial colonization does not necessarily translate to neonatal sepsis risk (46).

High Colonization vs. Low Sepsis Rate

Large cohort studies using traditional cultures have shown that gut colonization is far more common than sepsis, highlighting the weak positive predictive value of colonization.

- For instance, a ‘NICU’ surveillance study of 698 VLBW infants conducted by Smith ‘A et al’., reported 90% were colonized with Gram-negative bacilli by 6 weeks of age, yet only ~7% developed a Gram-negative bloodstream infection (124). Thus, most infants with gut colonization never progress to sepsis, consistent with our finding of no statistical correlation (124). Even when the same strain is present in gut and blood during sepsis, it’s the exception rather than the rule that colonization leads to disease in a predictable way (124).

Mixed Findings on Microbial Diversity

Some smaller studies have reported lower gut microbial diversity preceding sepsis (e.g., ‘Madan et al. (2012)’ noted less diverse flora at birth in infants who later

got sepsis (8)), or specific taxa growth (e.g., Shaw et al. (2015) observed increased Enterobacteriaceae before sepsis (57)). However, these patterns have not held true across all studies. For example, another analysis of preterm infants by ‘Diana et al’., found an increase in Staphylococcus prior to sepsis but no significant difference in overall diversity between sepsis and control groups (46).

Our study aligns with those reporting no significant diversity gap between septic and non-septic neonates.

Surveillance Culture Studies

Older cohort studies using serial stool cultures similarly show an equivocal link between gut colonization and sepsis. ‘Parm et al. (2011)’ followed 27 preterm infants with weekly mucosal cultures and noted that while certain Gram-negative colonization preceded some late-onset sepsis cases, there was no broad predictive relationship—many colonized infants did not become septic, and not all sepsis cases had been detected by prior cultures (125).

Likewise, a 2010 study by ‘Smith et al’. found that although virtually all infants were colonized with Gram-negatives, only half of the infants who developed Gram-negative sepsis had an identical strain in the gut by genetic fingerprinting, indicating colonization did not always translate into infection (124). These outcomes reinforce the lack of a one-to-one association, echoing the results of our study.

In conclusion, across different methodologies—from 16S sequencing and metagenomics to conventional culture surveillance—the presence or colonization of gut bacteria alone has not shown a consistent, significant association with neonatal sepsis in several studies. ‘Wandro et al’., demonstrated that preterm gut dysbiosis is highly individualized rather than sepsis-driven (123), and ‘Taft et al’. reported that

microbiome changes prior to sepsis are variable and non-predictive (46). Thus, while pathogenic bacteria often reside in the infant gut before causing infection, simply having an “abnormal” gut microbiota or colonization is not reliably associated with sepsis in neonates, a conclusion in line with our study’s observations.

A comparison with previous studies indicates that our findings align with existing research, particularly in relation to gut colonization patterns, the predominance of Gram-negative bacteria, and the connection between dysbiosis and neonatal sepsis. However, variations in the association of maternal risk factors and gut-blood concordance rates highlight the role of multiple host, perinatal, and environmental influences in shaping neonatal gut microbiota and its clinical impact.

Gut dysbiosis in preterm neonates is a dynamic and evolving process driven by a complex interaction of host factors, environmental exposures, and clinical interventions. While our study identifies no association between gut microbiota composition and neonatal sepsis, further investigations utilizing metagenomic sequencing and longitudinal microbiome assessments are essential to elucidate the mechanisms underlying gut-derived infections. Developing approaches to optimize early gut colonization, reduce unnecessary antibiotic use, and encourage beneficial microbial diversity could potentially improve neonatal health outcomes and advance sepsis prevention strategies in preterm populations.

Limitations of the Study**1. Small Sample Size**

- Limited number of preterm neonates enrolled in the study may affect the generalizability of the findings to a larger neonatal population.

2. Single-Centre Study

- The study was carried out in a single ‘neonatal intensive care unit’, which may not reflect variations in microbiota colonization and sepsis patterns observed in different hospitals or geographic regions.

3. Short Follow-Up Duration

- The study only followed bacterial colonization up to 7-10 days of life, limiting insights into long-term gut microbiota development and its potential impact on late-onset sepsis (LOS) beyond the neonatal period.

4. Lack of Advanced Microbiome Analysis

- The study primarily used culture-based methods to detect bacterial growth, which may have missed key microbial communities detectable by advanced techniques including 16S rRNA sequencing or metagenomic analysis.

However, this study has several strengths that enhance its scientific validity and clinical relevance. The prospective design ensured systematic data collection on gut colonization and neonatal sepsis over time. Sequential microbiota assessment, with samples collected at two time points – Gastric aspirate sample-1(within 4 hours

of birth) , Stool sample 1 (First sample) & follow up samples of Gastric aspirate and Stool sample-2(7—10 days of life), captured the progression of bacterial colonization in preterm neonates. The exclusive focus on preterm neonates, a high-risk group for gut dysbiosis and sepsis, makes the findings directly applicable to NICU settings.

A comprehensive risk factor analysis assessed gestational age, mode of delivery, maternal infections, NICU interventions (mechanical ventilation, enteral feeding, PICC lines), and hospital stay duration, providing insights into factors influencing gut colonization and sepsis risk. The study linked gut colonization to sepsis markers, identifying prolonged oxygen therapy, enteral feeding tube use, and extended PICC line duration as significant risk factors for higher bacterial colonization in stool sample-2.

The integration of clinical signs, laboratory markers (TLC, ANC, CRP, ESR), and blood cultures strengthened the diagnostic approach to neonatal sepsis.

Recommendations for Future Research

- Conducting multi-centre studies with a larger sample size to improve external validity.
- Utilization of next-generation sequencing (NGS) methods for a comprehensive microbiome analysis.
- Performing longitudinal follow-up studies to assess microbiota changes beyond the neonatal period.
- To study targeted interventions such as probiotics, early enteral feeding, and antibiotic stewardship programs to optimize gut microbiota and reduce neonatal infections

CONCLUSION

‘Neonatal sepsis’ is an important determinant of ‘morbidity’ and ‘mortality’ in preterm infants. Recent research indicates that dysbiosis of gastric and intestinal microbiota may significantly influence the development of neonatal infections. This study examined the pattern of gastrointestinal microbiota colonization in preterm neonates and its association with the incidence and consequences of ‘neonatal sepsis’. A one-year prospective observational study was performed at a tertiary care newborn facility, enrolling preterm neonates with a gestational age of 28 to 37 weeks. Gastric aspirate and stool specimens were obtained at two intervals—within the first four hours of life (Sample-1) and between day 7-10 of life (Sample-2). Bacterial cultures were analyzed for colonization patterns, and their association with clinical and blood culture-positive sepsis was assessed. Key neonatal risk factors, interventions, and hospital outcomes were also evaluated.

Our findings indicate that bacterial colonization in the neonatal gut follows a progressive trajectory, with increasing colonization rates observed over time in both gastric aspirate and stool samples. The predominant organisms isolated were *Escherichia coli* and *Klebsiella pneumoniae*, which aligns with existing literature on gut microbiota composition in preterm neonates.

Association was identified between bacterial growth in early gastric aspirate samples and the later development of clinical sepsis ($P = 0.028$), as well as between bacterial colonization in late stool samples and clinical sepsis ($P = 0.028$). However, statistically significant correlation did not exist between gut colonization and blood culture-positive sepsis ($P > 0.05$), suggesting that while gut microbiota dysbiosis may

contribute to sepsis pathogenesis, the direct translocation of gut bacteria into the bloodstream may not be the sole mechanism of infection.

Our study further highlights that prolonged NICU stay, the need for respiratory support, enteral feeding via an infant feeding tube (IFT), peripherally inserted central catheter (PICC) line usage, and extended antibiotic exposure are key risk factors for gut dysbiosis in preterm neonates. Additionally, neonates with delayed initiation of direct breastfeeding exhibited a higher burden of bacterial colonization, reinforcing the importance of early enteral feeding strategies in promoting a healthy gut microbiome.

Comparison with previous studies demonstrates that our findings are largely consistent with existing literature, particularly regarding gut colonization trends, the predominance of Gram-negative organisms, and the association between dysbiosis and neonatal sepsis. However, differences in maternal risk factor associations and gut-blood concordance rates suggest that multiple host and external factors of environment had an impact on colonization of the gastrointestinal tract and its clinical implications.

To conclude, gut dysbiosis in preterm neonates is an evolving process predisposed by a complex interaction of host, environmental, and clinical factors. While our study establishes a substantial association between gastrointestinal microbiota composition and clinical sepsis, further research using metagenomic sequencing and longitudinal microbiome analysis is necessary to determine the mechanisms underlying gut-derived neonatal infections. Identifying strategies to optimize early gut colonization, minimize antibiotic overuse, and promote beneficial microbial diversity may contribute to improved neonatal outcomes and sepsis prevention strategies in preterm populations.

SUMMARY

- This study was conducted with the objective to evaluate the pattern of microbiota colonization in the gastrointestinal tract of preterm neonates and its association with neonatal sepsis.
- Conducted at KLES Dr. Prabhakar Kore Hospital over one year (Jan-Dec 2024), the study included 124 preterm neonates out of 1,293 NICU/KMC admissions.
- The study analysed maternal, neonatal, and hospital-related factors that influence gut microbiota and sepsis.

Neonatal Characteristics

- Gender: The distribution was nearly equal (Male - 46.77%, Female - 53.23%), indicating no significant gender predisposition for gut colonization or sepsis.
- Gestational Age:
 - Majority were late preterm (50.81%), followed by moderate (25%), early (20.97%), and extreme preterm (3.23%).
- Birth Weight:
 - ‘Low Birth Weight’ <2.5kg: 65.32%, ‘Very Low Birth Weight’, <1.5kg: 22.58%, and ‘Extremely Low Birth Weight’ <1kg: 7.26%.

Maternal and Delivery Factors

- Maternal Risk Factors:

- Gestational Hypertension (GHTN) was the most common (47.58%), followed by Premature Rupture of Membranes (PROM, 15.32%). Other risk factors include fever, GDM and UTI
- Mode of Delivery:
 - LSCS (77.42%) was the predominant mode, followed by vaginal delivery (21.77%).

Postnatal Adaptation and Hospital Stay

- Neonatal Adaptation:
 - 74.19% cried immediately, while 25.81% required resuscitation, suggesting perinatal distress in some cases.
 - 17.74% had a low APGAR score (<7)
- NICU vs KMC Admission:
 - 75% were admitted to NICU, while 25% were managed in Kangaroo Mother Care (KMC). NICU admission, while necessary for critical cases, increases the risk of hospital-acquired infections, whereas KMC was protective against bacterial colonization.
- Hospital Stay & Interventions:
 - Mean hospital stay was 16.7 days (range: 7-59 days), with longer stays associated with higher bacterial colonization rates.
 - Interpretation: Prolonged hospitalization and interventions like oxygen therapy, IV fluids, PICC line and feeding tubes may contribute to increased gut colonization and increased infection risks.

Gastric Aspirate Culture

- Early gut colonization was limited, with 19.35% (24/124) of neonates showing bacterial growth in Sample-1 (Day 1-3).
- Colonization increased by Sample-2 (Day 7-10), with 27.42% (34/124) of neonates showing bacterial growth.
- Common organisms in Sample-1:
 - *E. coli* (5.65%) and *Klebsiella pneumoniae* (5.65%) were predominant.
 - Other bacteria included Coagulase-negative Staphylococci (CONS), Micrococci, and Acinetobacter.
- In Sample-2, bacterial growth increased, with a notable rise in *E. coli* (12.10%) and *Klebsiella pneumoniae* (4.84%), along with CONS (3.23%) and Enterococcus.
 - The progressive increase in bacterial colonization indicates delayed gut microbiota development, with a dominance of gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*).
 - This suggests an increased risk of gut dysbiosis, which may contribute to sepsis in preterm neonates.

Stool Culture

- Stool cultures showed a higher prevalence of bacterial growth than gastric aspirates.
- Sample-1 (Day 3-5): 37.1% (46/124) of neonates had positive stool cultures.

- Sample-2 (Day 7-10): 45.97% (57/124) of neonates had positive cultures, indicating increased gut colonization over time.
- Common organisms in Sample-1:
 - *E. coli* (20.16%) and *Klebsiella pneumoniae* (6.45%) were the most common.
 - Other bacteria included *Enterococcus*, *Pseudomonas*, and *Acinetobacter*.
- In Sample-2, *E. coli* (17.74%) remained the most common, but there was a notable increase in *Enterococcus* (11.29%) and *Klebsiella pneumoniae* (10.48%).
 - Stool samples had higher colonization than gastric aspirates, indicating that lower gut flora develops earlier.
 - An alteration in gut microbiota was detected, with increasing *Enterococcus* in later samples, possibly linked to antibiotic use or NICU exposure.

Sepsis - Blood Culture Findings

- Clinical sepsis was observed in 41.09% (53/124) of neonates, but only 18.55% (23/124) had laboratory-confirmed sepsis, and 10.48% (13/124) had positive blood cultures.
- Common clinical signs of sepsis:
 - Tachypnea (100%), tachycardia (81.13%), apnea (50.94%), lethargy (33.96%).

- Poor feeding (30.19%), hypotension (20.75%), and temperature instability (11.32%) were also observed.
- Laboratory markers of sepsis:
 - Total leukocyte count (TLC) <5000 in 18.55%.
 - Elevated C-reactive protein (CRP >10) in 15.32%.
 - Absolute neutrophil count (ANC) abnormalities in 17.74%.
- Blood culture-positive cases (10.48%):
 - Gram-negative bacteria were predominant, including *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Serratia marcescens*.
 - Gram-positive bacteria included *Enterococcus faecalis* (most common, 3 cases) and *Staphylococcus* species.
 - Fungal infections (*Candida glabrata*) were identified in two cases.

Clinical Outcome

- 97.58% (121/124) of neonates were successfully discharged.
- 1.61% (2/124) mortality:
 - One case due to pulmonary haemorrhage (Day 10, no bacterial growth in samples).
 - One case due to septic shock (Day 59, blood culture: *Enterococcus faecalis*, stool culture: *Klebsiella pneumoniae*).
- 0.81% (1 neonate) was discharged against medical advice

Risk Factors for Bacterial Growth in the Gut of Preterm Neonates

This study analysed multiple risk factors influencing bacterial colonization in preterm neonates, including gender, gestational age, birth weight, obstetric history, maternal risk factors, mode of delivery, postnatal adaptation, and APGAR scores. The findings suggest that gestational age, birth weight, mode of delivery, and postnatal adaptation play key roles in determining gut bacterial colonization, while some other factors showed trends without reaching statistical significance.

1. Gender and Bacterial Colonization

- No significant association was found between gender and bacterial growth in either gastric aspirate or stool samples ($p > 0.05$).
- Both male and female neonates exhibited increased bacterial growth in later gut samples, suggesting progressive colonization over time, though not statistically significant.
- Gender was not a significant risk factor for gut colonization in preterm neonates.

2. Gestational Age and Bacterial Colonization

- Early and moderate preterm neonates had significantly higher bacterial growth in gastric aspirate Sample-1 ($p = 0.047$) and stool Sample-2 ($p = 0.003$) compared to late preterm neonates.
- Extremely preterm neonates initially had no bacterial colonization, but 75% of them developed bacterial growth in stool Sample-2, though this was not statistically significant due to small sample size.

- Conclusion: Lower gestational age was a significant risk factor for bacterial colonization, particularly in stool Sample-2, indicating that more immature neonates are at higher risk for gut dysbiosis.

3. Birth Weight and Bacterial Colonization

- Bacterial growth was higher in extremely low birth weight (ELBW) and very low birth weight (VLBW) neonates compared to normal birth weight neonates.
- Significant association was found only in stool Sample-2 ($p = 0.004$).
- SGA neonates had significantly higher bacterial growth in stool Sample-1 ($p = 0.014$), but no significant association was found in other samples.
- Conclusion: Lower birth weight was a strong predictor of increased bacterial colonization, particularly in stool samples.

4. Obstetric Score and Maternal Risk Factors

- Bacterial growth occurred in neonates born to both primigravida and multigravida mothers, showing progressive colonization over time.
- No statistically significant difference was observed between gravida groups ($p > 0.05$).
- Maternal risk factors such as fever, PROM, UTI, GDM, and GHTN were associated with increased bacterial growth in gastric aspirate Sample-2 and stool Sample-2, but these findings were not statistically significant.
- Conclusion: Maternal risk factors and gravida status were not significant determinants of gut bacterial colonization in preterm neonates.

5. Mode of Delivery and Bacterial Colonization

- LSCS was associated with significantly higher bacterial growth in gastric aspirate Sample-2 ($p = 0.053$) compared to vaginal delivery.
- Stool Sample-2 also showed higher bacterial growth in LSCS neonates compared to vaginal delivery, though this was not statistically significant.
- Neonates born by ventouse-assisted delivery and those with meconium-stained liquor were too few for statistical analysis.
- Conclusion: LSCS delivery was associated with increased bacterial colonization, particularly in gastric aspirate Sample-2, but mode of delivery was not a statistically significant risk factor overall.

6. Postnatal Adaptation and Bacterial Colonization

- Neonates with poor postnatal adaptation requiring stimulation, bag-and-mask ventilation, or intubation had significantly higher bacterial growth in:
 - Gastric aspirate Sample-2 ($p = 0.034$).
 - Stool Sample-2 ($p = 0.030$).
- Conclusion: Delayed postnatal adaptation requiring resuscitation was a statistically significant risk factor for increased bacterial colonization, indicating a potential link between perinatal distress and altered gut microbiota.

7. APGAR Score and Bacterial Colonization

- Neonates with lower APGAR scores had higher bacterial growth in gastric aspirate Sample-1 and stool Sample-2 compared to those with normal APGAR scores.
- However, these differences were not statistically significant ($p > 0.05$).
- Conclusion: While a trend was observed linking low APGAR scores to increased bacterial colonization, the association was not statistically significant.

8. Ward of Admission: KMC vs NICU

- KMC significantly reduced bacterial colonization, with only 2 neonates showing growth in gastric aspirate Sample-1 and 1 neonate each in gastric aspirate Sample-2, stool Sample-1, and stool Sample-2.
- In contrast, NICU-admitted neonates had much higher bacterial growth:
 - Gastric aspirate Sample-1: 22 cases($p = 0.036$); Gastric aspirate Sample-2: 45 cases ($p < 0.001$); Stool Sample-1: 33 cases($p < 0.001$); Stool Sample-2: 56 cases($p < 0.001$).
- Statistical significance:
 - Gastric aspirate Sample-1:
 - Gastric aspirate Sample-2, stool Sample-1, and stool Sample-2: $p < 0.001$.
- KMC was highly protective against bacterial colonization, significantly reducing both early and late gut colonization.

2. Hospital Stay Duration and Gut Colonization

- Longer hospital stays were associated with increased bacterial growth in both gastric aspirate and stool samples, with more neonates showing bacterial growth after 8-15 days or >15 days of hospitalization.
- Significant association was found only in stool Sample-2 ($p = 0.022$), suggesting that prolonged hospitalization promotes gut colonization.
- No statistically significant differences were observed for gastric aspirate Sample-1 ($p = 0.211$), gastric aspirate Sample-2 ($p = 0.210$), or stool Sample-1 ($p = 0.352$).
- Longer hospital stays contribute to increased gut bacterial colonization, with the most significant impact observed in stool Sample-2.

3. Impact of Oxygen Support on Gut Colonization

- Oxygen support for >7 days did not increase bacterial growth in gastric aspirates.
- However, it significantly increased bacterial growth in stool Sample-2 ($p = 0.003$).
- Prolonged oxygen therapy plays a significant role in gut colonization, particularly in stool samples.

4. IV Fluids and Gut Colonization

- IV fluid administration for >7 days did not increase bacterial growth in gastric aspirates.

- However, it significantly increased bacterial growth in stool Sample-2 ($p = 0.014$).
- Longer IV fluid administration promotes gut bacterial colonization, particularly in stool samples.

5. Inotropes and Gut Colonization

- Only 5 neonates received inotropes, and bacterial growth was observed in:

Gastric aspirate Sample-1: 1 neonate; Gastric aspirate Sample-2: 3 neonates; Stool Sample-1: 1 neonate.; Stool Sample-2: 4 neonates.
- Due to small numbers, statistical significance could not be established.
- No significant association was found between inotrope use and bacterial colonization.

6. Infant Feeding Tube and Gut Colonization

- Prolonged infant feeding tube use (>7 days) was significantly associated with increased bacterial growth in stool Sample-2 (73.33%, $p = 0.001$).
- Comparatively, neonates with feeding tube use <7 days had bacterial growth in stool Sample-2 (45.45%) and those without a feeding tube had bacterial growth in stool Sample-2 (32.78%).
- No significant association was found in other gastric or stool samples.
- Prolonged use of an infant feeding tube (>7 days) significantly increases gut colonization.

7. PICC Line and Gut Colonization

- PICC line use for >7 days did not increase bacterial growth in gastric aspirates.
- However, it significantly increased bacterial growth in stool Sample-2 ($p = 0.005$).
- Longer PICC line duration is a significant risk factor for gut bacterial colonization in stool samples.

8. Phototherapy and Gut Colonization

- Phototherapy for >2 days was significantly associated with increased bacterial growth in: Stool Sample-1 ($p = 0.034$); Stool Sample-2 ($p = 0.033$).
- No significant association was found with gastric aspirates.
- Prolonged phototherapy is associated with increased gut bacterial colonization, particularly in stool samples.

9. Antibiotic Use and Gut Colonization

- Preterm neonates receiving antibiotics had higher bacterial growth in both gastric aspirate and stool samples compared to those not receiving antibiotics.
- Antibiotics also promoted bacterial colonization over time, with more neonates showing bacterial growth in gastric aspirate Sample-2 and stool Sample-2.
- Statistical significance was observed only in stool Sample-2 ($p = 0.04$).

- Antibiotic use is associated with increased bacterial colonization, particularly in stool Sample-2.

10. Blood Component Transfusion and Gut Colonization

- Only 8 neonates received transfusions.
- Bacterial growth was observed in:
 - Gastric aspirate Sample-1: 2 neonates.
 - Gastric aspirate Sample-2: 1 neonate.
 - Stool Sample-1: 1 neonate.
 - Stool Sample-2: 4 neonates.
- Due to small numbers, statistical significance could not be established.
- No significant association was found between transfusions and bacterial colonization.

11. Duration of Hospital Stay and Gut Colonization

- Mean duration of hospital stay in neonates with bacterial growth increased from 18.32 ± 10.97 days to 20.98 ± 14.15 days ($p < 0.01$).
- Longer hospital stays significantly contribute to gut bacterial colonization, particularly in stool samples.

12. Feeding Methods and Gut Colonization

- Longer Ryle's tube feeding and spoon-feeding durations were associated with increased bacterial growth in gastric aspirate and stool samples.

- Delayed initiation of direct breastfeeding was significantly associated with increased bacterial growth in stool Sample-2 ($p < 0.001$).
- Delaying direct breastfeeding increases gut bacterial colonization, emphasizing the importance of early enteral feeding.

13. Blood Culture and Gut Colonization

- Longer hospital stays, oxygen support, CPAP, IV fluids, PICC line, phototherapy, and delayed feeding were significant risk factors for positive blood culture ($p < 0.01$).
- Prolonged medical interventions increase the risk of bloodstream infections in preterm neonates.

Gut Microbiota Colonization and Its Influence on Neonatal Sepsis

This study examined the relationship between gut microbiota colonization in preterm neonates and the incidence and outcomes of neonatal sepsis. Key findings highlight a significant association between early bacterial colonization and clinical sepsis, while laboratory markers and blood culture-positive sepsis did not show strong correlations with gut colonization.

1. Association Between Gut Colonization and Clinical Sepsis

- Neonates with clinical signs of sepsis showed a significant increase in bacterial growth in:
 - Gastric aspirate Sample-1 ($p = 0.028$).
 - Stool Sample-2 ($p = 0.028$).

- Bacterial growth also increased in gastric aspirate Sample-2 and stool Sample-1, but these differences were not statistically significant ($p > 0.05$).

Early bacterial colonization in gastric aspirate Sample-1 and stool Sample-2 is significantly associated with clinical signs of sepsis, suggesting that early gut colonization may play a role in predisposing neonates to sepsis.

2. Gut Colonization and Laboratory-Confirmed Sepsis

- No statistically significant difference in bacterial growth between neonates with lab-confirmed sepsis (TLC <5000 , ANC abnormalities, CRP >10 , ESR >15) and those with clinical signs of sepsis but without lab confirmation.
- Gastric aspirate and stool cultures were not strongly associated with laboratory markers of sepsis ($p > 0.05$).

Sepsis diagnosis should rely on a combination of clinical and laboratory markers rather than gut colonization alone.

3. Gut Colonization and Blood Culture-Positive Sepsis

- No statistically significant association was found between bacterial growth in gastric aspirate/stool samples and blood culture-positive sepsis ($p > 0.05$).
- However, stool Sample-2 showed a higher bacterial growth rate (61.54%) in neonates with blood culture-positive sepsis, though this was not statistically significant.

While gut colonization is common in preterm neonates, it does not directly predict blood culture-positive sepsis, suggesting that other factors (e.g.,

immune response, hospital-acquired infections) contribute to bloodstream infections.

4. Antibiotic Sensitivity Patterns: Gut Microbiota vs Blood Culture-Positive Sepsis

- Among 13 blood culture-positive neonates, the following organisms were isolated:
 - Gram-negative bacteria:
 - *Klebsiella pneumoniae* and *Klebsiella oxytoca* (4 cases).
 - *Serratia marcescens* (2 cases).
 - *Acinetobacter* (1 case, co-infected with CONS).
 - Gram-positive bacteria:
 - *Enterococcus faecalis* (3 cases, most frequently isolated gram-positive organism).

The antibiotic resistance patterns of gut microbiota and sepsis-causing organisms in blood cultures should be further explored to determine if gut colonization contributes to bloodstream infections.

Comparison with Other Studies

- Taft et al. (2015) and Wandro et al. (2018) reported no uniform dysbiosis pattern preceding sepsis, similar to our findings.
- Parm et al. (2011) found that many colonized infants did not develop sepsis, reinforcing that gut dysbiosis is not a definitive predictor of bloodstream infections.

- Basu et al. and Das et al. confirmed progressive colonization trends in NICU neonates but did not establish a direct correlation with sepsis.

Bacterial colonization in the gut followed a progressive trajectory, with higher colonization rates observed over time in both gastric aspirate and stool samples.

Predominant organisms isolated:

- *Escherichia coli* and *Klebsiella pneumoniae* were the most common, aligning with previous literature on preterm gut microbiota.

Gut dysbiosis contributes to sepsis pathogenesis, direct bacterial translocation from the gut into the bloodstream may not be the sole mechanism of infection.

Multiple intrinsic (host) and extrinsic (environmental, medical) factors shape neonatal gut microbiota, influencing its clinical implications.

Optimizing gut microbiota through early enteral feeding, reducing antibiotic overuse, and promoting beneficial microbial diversity can enhance neonatal outcomes and reduce sepsis risk.

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ANNEXURE – I - INFORMED CONSENT FORM

“Pattern of colonization of gut microbiota in preterm neonates and its association with neonatal sepsis- A one year hospital based prospective observational study”

Chief Guide : -

Investigator: -

You have been asked to involve your child in the said study to be conducted at department of paediatrics KLE University's Dr Prabhakar Kore Charitable Hospital, Belagavi by

PG student in department of paediatrics at Jawaharlal Nehru Medical College , Belagavi.

PURPOSE OF THE STUDY:

Participation of your child will help us to assess the association between the gut microbiome and sepsis. This study will help identify ways for early detection of sepsis in neonates and improve the outcome. It may form the foundation to reduce the incidence of neonatal sepsis and neonatal mortality, thus helping the community at large.

EXPLANATION OF THE PROCEDURE:

Once your child is enrolled in the study, first day sample of the gastric aspirate will be collected within 4 hours of life. This involves the insertion of infant feeding tube, and drawing of a small quantity of fluid from the gut of your child. This procedure carries minimal to no risk for the child. The sample will be sent to KLE Dr. Prabhakar Kore's microbiology department for further processing. The first stool sample of your child will be collected from the sterile nappy provided at birth. This sample will be sent for further processing to KLE Dr. Prabhakar Kore's microbiology department. Similarly, the gut aspirate and stool sample will be collected at day 7 to day 10 of life by the same procedure. Your child's blood samples will be sent for investigations if found to develop clinical sepsis during the course of hospital stay. Further investigations as per requirement to the research protocol will be conducted on the samples collected. Your child's course of stay in the hospital will be followed up on a daily basis and evaluated.

Risk and benefits

There are no risks or complications involved by participation in this research. However complications can occur due to the underlying condition of the baby during the course of participation in the study.

Withdrawal from the study

You are free to discontinue the participation in the study at any time for any reasons and you will not be paid any reimbursement for participation in the research. Hence involving your child in the study is your voluntary decision. Your decision to participate or not to participate in this study will not affect the child's course or quality of treatment, your current or future relationship with KLEs Dr. Prabhakar Kore Hospital & Medical research centre, Belagavi.

Privacy and Confidentiality

The only people who will know that you are a research participant are members of the research team. No information about you or provided by you, during research will be disclosed to others without your written consent. When the results of the research are published or discussed in the conferences, no information will be disclosed that would reveal your identity. Any information obtained in connections with this study and that can be identified with you remain confidential and will be disclosed only with your permission.

Financial incentives: You will not receive any payment for participating in this study.

Cost of investigations : Cost for additional investigation will be boned by the investigator

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

Queries

If you have any queries you may contact

STATEMENT OF CONSENT

I hereby voluntarily agree for my participation in this study. I understand that even I have the liberty to withdraw at any time. My signature below indicates that I have read or have been told in the language I understand, about this entire consent form including the risks and benefits and have had all my questions answered. I will be given a copy of this consent form.

Signature of the authorized representative/ parent: _____

Date: _____

Name: _____

Relation to the Subject: _____

Signature of the witness: _____

Date: _____

Name: _____

Signature of investigator: _____

Date: _____

Name: _____

If you have any question or complaints with regard to your right as study participant you may contact DR HARSHA HEGDE, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

ANNEXURE – II - PROFORMA**PATIENT PARTICULAR'S: -**

Baby of _____ (Name of Mother) _____
 In Patient Number _____ Mother's IP Number _____
 Name of Father _____
 Date and Time of Birth: _____ Age (minutes/hours/days) _____
 Sex (M/F/Ambiguous) _____ Date and time of Admission _____
 Address with Phone Number _____

Maternal / Family History:

Age _____ years Blood Group _____ Rh type _____

Gravida _____ Living children _____

Abortion Y/N Trimester Reason _____ Previous LSCS Y/N Indication _____

Sibling deaths Y/N Age _____ Cause _____

Clinical details _____

Preterm delivery developmental delay / seizure disorder / congenital anomaly If yes, details. _____

Consanguinity Y/S _____ Degree of consanguinity _____

Maternal long term medications _____

Present Pregnancy _____

LMP _____ EDD _____ VDRL _____ HIV _____

HBsAg _____ Any other relevant tests _____

Perconceptional Folic Acid Y/N _____

First Trimester-UTI / Vaginal bleeds / any medication / fever / rash If Yes Details _____

Second Trimester-Fever and / or rash / PIH / anemia / GDM If Yes Details _____

Third trimester - PIH / GDM / Abnormal presentation / medications If Yes Details _____

USG 1st Trimester _____
 2nd Trimester _____
 3rd Trimester _____

Other Significant Investigations

LABOUR and Delivery

Hospital / Home / Any other place

PROM/PROM

H/o. Fetal distress Yes/No

Liquour clear / meconium stained / foul smelling

Number of per vaginal examinations done after rupture of Membranes

Fever just before or after deliver Y/N, reason _____ Mother on any antibiotics

Mode of Delivery - Normal/Vacuum/Forceps/LSCS elective / emergency

Indication

Anesthesia spinal / epidural / general out come _____

POST NATAL PERIOD

Breast feeding initiated at what time _____ Problems _____

Urine passed at what time _____ Stream _____ Meconium passed at what time _____

Jaundice Y/N hour of life _____ Treatment _____

SYMPTOM NOTICED (To be completed at admission to NICU/at discharge)

Respiratory distress / breathing difficulty / apnoes Y/N Feeding problem / poor feeding Y/N

Poor activity Y/N Seizure / Seizure like episodes Y/N

Any congenital anomalies / dysmorphism observed Y/N

Jaundice Y/N Bleeding Y/N

Fever / Cold to Touch Y/N

Abdominal distension / constipation / vomiting Y/N

If Yes give details

On Examination:

Alert/Active/Lethargic/ Sick

Sick

Lethargic/ Sick

Vital signs: Heart rate _____ Respiratory rate _____ Temperature _____

Capillary Refill Time _____

Anthropometry: Weight _____ Length _____ Head circumference _____

US: LS Ratio _____ Chest circumference _____

Weight gain / Weight loss _____

HEAD TO FOOT EXAMINATION:

Head moulding / fontanelle / caput / cephalhaematoma

Eyes-haemorrhage / red reflex / any abnormality

Ears-position / Shape

Nose abnormal shape / size /

hypertelorism

Mouth Lips / gums / palate / natal teeth

Neck:

Skin: Jaundice / cyanosis / rashes / haemangioma

Umbilicus

Genitalia: Testes descended/Y/N, penis size _____ hypospadias Y/N ambiguity Y/N pigmentation _____

Hernia/hydrocele _____ Hip DDH present / No _____

Extremities _____ Back and spine myelomeningocele/spina bifida

Femoral artery felt Right _____ Left _____

Any other finding _____

Systemic Examination

CVS

PA

RS

CNS

FINAL DIAGNOSIS

Gestational age-term/preterm AGA/LGA/SGA
Single/First/Second of

Male/Female

INVESTIGATIONS

DATE									
Blood group									
<u>Haematology</u>									
<u>Haemoglobin</u>									
P.C.V.									
Red cell count									
Platelets									
Reticulocyte count									
Total leucocytes(differential count)									
Neutrophils									
Lymphocytes									
Eosinophils									
E.S.R									
HSCRp									
Others									
BIOCHEMISTRY									
Urea									
Creatinine									
Total Bilirubin									
Direct Bilirubin									
Indirect Bilirubin									
S.G.O.T									
S.G.P.T									
Alk. Phosphatase									
Ser. Albumin									
Ser. Globulin									
Total protein									
A:G ratio									
Electrolytes									
Sodium									
Potassium									
Bicarb									
Chloride									
COAGULATION									
P.T(control/test)									
INR									
aPTT									
Thyroid function test									
T3/T4/TSH									
MISCELLANEOUS									

Sample collected	Gastric aspirate-1	Stool sample-1	Gastric aspirate-2	Stool sample-2
Date of collection/ day of life				
48 hours report				
Final report				
Organism isolated				
Antibiotic sensitivity				

Blood Culture

Date/ day of life				
48 h report				
Final report				
Organism isolated				
Antibiotic sensitivity				

Others:

INTERVENTION CHART

DATE/DAY OF LIFE									
OXYGEN SUPPORT									
MECHANICAL VENTILATION									
IV FLUIDS									
INOTROPES									
FEED/ TYPE OF FEED									
WEIGHT GAIN IN GRAMS									
INFANT FEEDING TUBE									
TPN									
ANTIBIOTICS									
LONG LINE CATHETERIZATION									
SEPSIS/NEC/RDS									
Other COMPLICATIONS									
IVIG									
IVH/PHTN									
RDP TRANSFUSION									
PCV TRANSFUSION									
FFP TRANSFUSION									

INTERVENTION CHART

DATE/DAY OF LIFE									
OXYGEN SUPPORT									
MECHANICAL VENTILATION									
IV FLUIDS									
INOTROPES									
FEED/ TYPE OF FEED									
WEIGHT GAIN IN GRAMS									
INFANT FEEDING TUBE									
TPN									
ANTIBIOTICS									
LONG LINE CATHETERIZATION									
SEPSIS/NEC/RDS									
Other COMPLICATIONS									
IVIG									
IVH/PHTN									
RDP TRANSFUSION									
PCV TRANSFUSION									
FFP TRANSFUSION									

INTERVENTION CHART

DATE/DAY OF LIFE									
OXYGEN SUPPORT									
MECHANICAL VENTILATION									
IV FLUIDS									
INOTROPES									
FEED/ TYPE OF FEED									
WEIGHT GAIN IN GRAMS									
INFANT FEEDING TUBE									
TPN									
ANTIBIOTICS									
LONG LINE CATHETERIZATION									
SEPSIS/NEC/RDS									
Other COMPLICATIONS									
IVIG									
IVH/PHTN									
RDP TRANSFUSION									
PCV TRANSFUSION									
FFP TRANSFUSION									

TREATMENT CHART

MEDICATION	NO OF DAYS	DOSAGE	MEDICATION	NO OF DAYS	DOSAGE

IN CASE OF SEPSIS (To be FILLED)**CLINICAL SIGNS****INVESTIGATION (DIAGNOSTIC CRITERIA):****Blood Culture****COMPLICATIONS:**

MATERNAL RISK FACTORS

PROM (>18 hrs)	
Fever	
UTI	
GDM	
Gestational Hypertension	

NEONATAL/ NICU RISK FACTORS

Gestational age	
Birth weight	
Ventilation(>7 days)	
Intubation(>2 times)	
Long line insertion(>7 days)	
Total parenteral nutrition	
Duration of stay(>7 days)	
Broad spectrum antibiotics(>7 days)	

OUTCOME:

**IMPROVED/RECOVERED/DISCHARGED/ DEATH/ AGAINST MEDICAL
ADVICE:**

COMPLICATIONS:

ANNEXURE – III -PHOTOGRAPHS

Fig 1: Neonate in Kangaroo Mother Care Unit



Fig 2: Neonate in NICU



Fig 3: Collection of gastric aspirate sample from a preterm neonate with sepsis in NICU



Figure 4: Blood culture

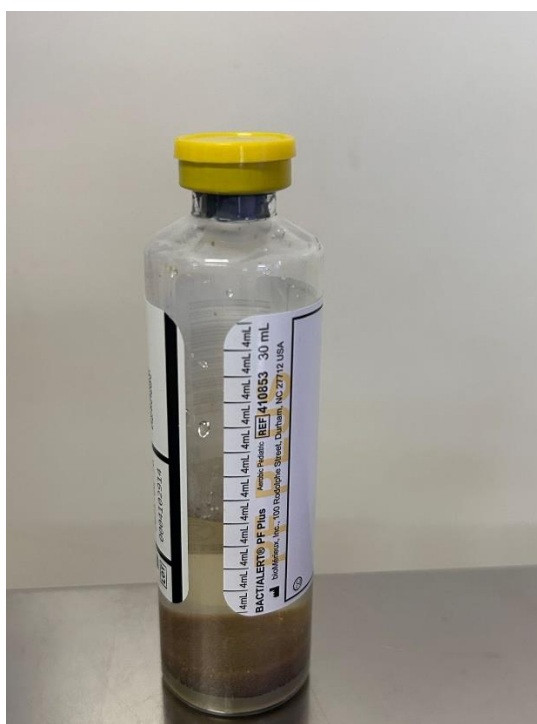


Figure 5: Sterile containers for collection of gastric aspirate and stool sample



Figure 6: Inoculated culture medium showing growth

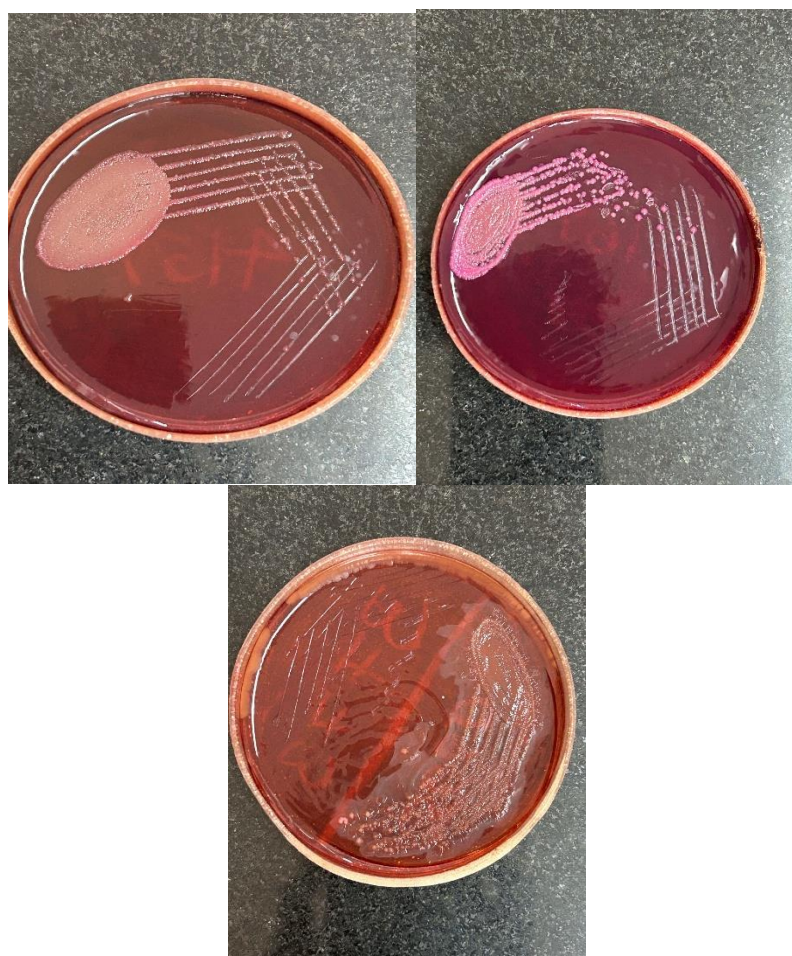
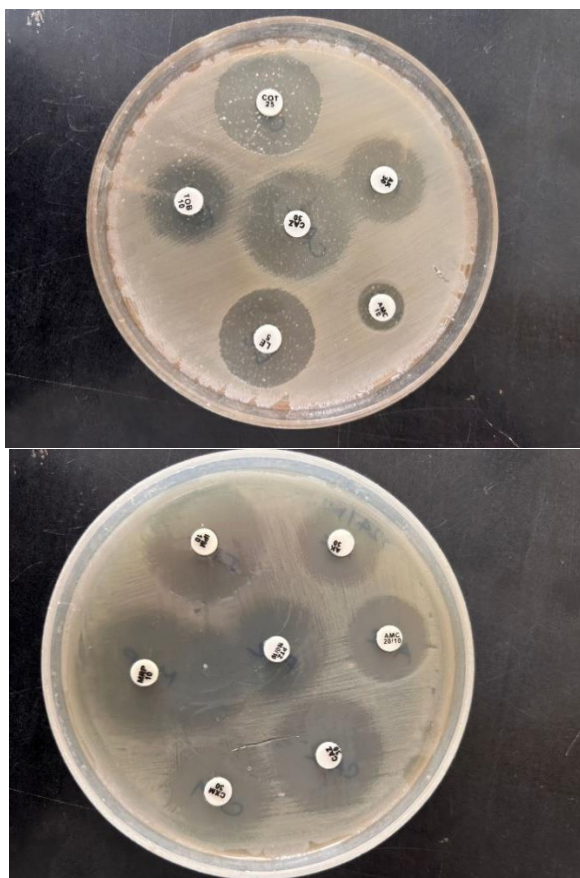
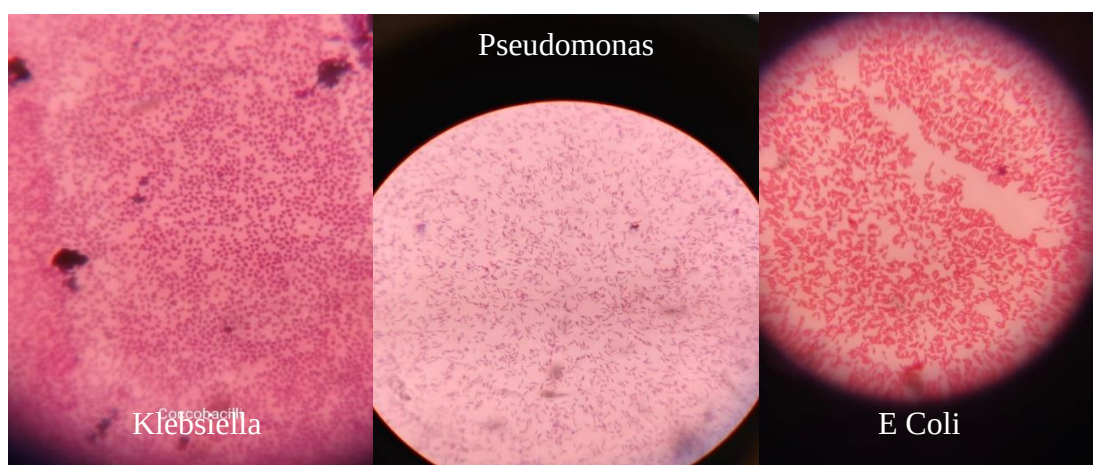


Figure 7: Antibiotic sensitivity test**Figure 8- Organisms Isolated**

ANNEXURE – IV

MASTER CHART

[illegible]

