Finding Etiological Factors for the Onset of Neonatal Sepsis Diagnosed in Tertiary Care Hospitals in Dhaka, Bangladesh

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfilment of the requirements for the degree of MS in Biotechnology

Department of Mathematics and Natural Sciences, Biotechnology Program

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing my degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except

where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted, or submitted, for any other

degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

This research is done under proper supervision and it is the author's original work. No animal was harmed during experiments. The article is written in a manner that the write-up does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing. The experiment was done by maintaining all the rules and regulations of the Biotechnology laboratory of the Department of Mathematics and Natural Sciences, BRAC University.

Abstract:

With an incidence rate of 76.8/1000 live births, or 36% of all neonatal fatalities, neonatal sepsis is one of the main causes of infant mortality in Bangladesh. Research from a few lower-middle-income (LMI) countries has shown that maternal and neonatal features are among the factors determining the incidence of sepsis in newborns. Thus, the purpose of this research is to determine whether maternal and newborn characteristics are associated with the incidence of neonatal sepsis in patients who are admitted to various Bangladeshi tertiary care facilities in Dhaka, Bangladesh. Finding the causative agents responsible for newborn sepsis along with their antibiogram patterns is the second objective. This study collected blood samples and information on maternal and neonatal risk factors from suspected neonatal sepsis patients admitted at Ad-Din Medical College and Hospital, BIRDEM Women and Child Hospital, and Kurmitola General Hospital from Dhaka City. Maternal and neonatal risk variables have been identified using the Chi-square test with the SPSS analysis tool. A total of sixty questionnaire forms and 40 blood samples have been gathered among which *E. Coli* (19 isolates), *Klebsiella pneumonia* (16 isolates), and *Pseudomonas aeruginosa* (16 isolates) have been identified as the primary organisms causing neonatal sepsis. While *E. coli* isolates showed the most resistance only against ceftazidime antibiotic and had three beta-lactamase-resistant genes (blaCTX, blaSPM, and blaTEM), *Klebsiella pneumonia* showed the most resistance towards six classes of antibiotic drugs and had two beta-lactamases resistant and one carbapenem gene (blaSPM and blaSHV, blaNMD). Nonetheless, most of the *Pseudomonas aeruginosa* exhibited reduced susceptibility to the antibiotic drug classes beta-lactamase, macrolide, and penicillin, as well as one gene that was resistant to beta-lactamase (blaVIM). Neonate gender, Apgar score, premature birth, low birth weight, maternal history of UTI, preeclampsia, gestational diabetes, parity, antepartum haemorrhage, and meconium-stained amniotic fluid were found to be linked with neonatal sepsis. The findings of this study will contribute to better treatment, early detection, and prevention, which could aid in reducing Bangladesh's neonatal sepsis death and prevalence rates.

Key Words: Neonatal Sepsis, Risk Factors, Maternal Risk Factors, Neonatal Risk Factors, Causative organisms, Antibiogram profile, Pregnancy history

Dedication

I dedicate this Thesis work to my family and the newborn babies and mothers who participated in this study.

Acknowledgment

I am very much thankful to certain people for supporting me in this journey. First, I want to congratulate myself for completing the thesis successfully by abiding by the rules as much as possible. I am thankful to my parents, who have never stopped supporting me and were beside me in every moment of my life. I am very humbly indebted to my thesis supervisor, Associate professor, and Program Director Dr. Munima Haque, Biotechnology Program, Department of Mathematics and Natural Sciences, who guided me efficiently in this journey and without whom, the research would not be possible and helped me to find my true potential as a researcher. I would like to thank Professor Dr. A F M Yusuf Haider, Chairperson, MNS department for allowing me to research this topic. I would like to thank Md. Hasanuzzaman, Senior Lecturer, Microbiology program, MNS department for motivating and supporting me throughout the journey. I would like to thank some of my lab colleagues, Iftikhar Zaman, Research Assistant; Tamanna Islam Toma, Research Assistant; for having my back and supporting me. I would like to also thank the lab officers and assistants who made the lab work so much easier. I would like to show my gratitude to Ad-Din Medical College and Hospital, BIRDEM Women and Child Hospital, and Kurmitola General Hospital for all the support in collecting the samples.

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List of Acronyms

EONS: Early-Onset Neonatal Sepsis

LONS: Late-Onset Neonatal Sepsis

LMIC: Lower Middle-Income Country

UTI: Urinary Tract Infection

CRP: C-Reactive Protein

NA: Nutrient Agar

Chapter-1

Introduction

1.1 Background:

Neonatal sepsis is one of the leading causes of newborn death worldwide. In newborns (0–28 days of age), it is a potentially fatal bacterial infection that triggers an inflammatory reaction throughout the body [1]. Based on the timing of occurrence, neonatal sepsis is divided into two categories: Early Onset Sepsis (EOS) and Late Onset Sepsis (LOS). Neonatal sepsis is classified as EOS if clinical symptoms appear within 7 days of birth, while clinical signs of LOS must be seen between 7 and 28 days of delivery [2]. Neonatal sepsis appears to have different causes in different places, and resistance to antibiotics causes fluctuation over time within a given region. Three types of agents are responsible: fungi, bacteria, and parasites. Globally, bacteria are the most frequent source of illness. *Salmonella, Pseudomonas, Acinetobacter baumannii, Escherichia coli, Staphylococcus aureus, coagulase-negative staphylococci (CONS), Streptococcus pneumoniae, and Streptococcus pyogenes* are the most common causing microorganisms [3].
Sepsis affects 48 children per 1,000 people worldwide, and the mortality rate ranges from 11 to 19% [4]. The global incidence rate of neonatal sepsis was 2202 per 100,000 live births in 2018, according to a meta-analysis. By 2022, that rate had grown to 2824 per 100,000 live births [5]. Neonatal sepsis is more common in lower-middle-income countries (LMICs) such as Africa, Bangladesh, Ethiopia, and India. In LMICs, there were 46.9 confirmed cases of neonatal sepsis for every 1,000 live births between 2015 and 2018, and 30–50% of these cases resulted in newborn fatalities [6] The incidence rate in Bangladesh is 76.8/1000 live births, accounting for 36% of all neonatal fatalities [7].

Lower-middle-income nations have a much higher frequency of EOS than LOS prevalence [6]. Since the infection is brought on by Early Onset Sepsis during the first week of the neonate's life, the mother's comorbidities are largely responsible for the infection [6]. Numerous countries on the LMIC list have focused on a few risk factors that mothers face during pregnancy and which could lead to neonatal sepsis. According to research, several LMI countries, such as Ethiopia, Syria, and Ghana, have higher rates of sepsis among newborns due to characteristics such as gestational age, mother's age, number of pregnancies, and urinary tract infection (UTI) [8–10].

In Bangladesh, neonatal intensive care units (NICUs) account for most newborn infections, and here is where neonatal sepsis is most common [7]. Bangladesh is a developing nation where many women live relatively disadvantaged lives, increasing their vulnerability to numerous ailments. Because most of our women lack understanding and education, there is a higher risk of pregnancy complications that may be prevented, which can result in sepsis in

the fetus. Numerous research shows the etiological components of neonatal sepsis, despite noteworthy evidence revealing the mortality rate of the illness in Bangladesh. Furthermore, despite substantial global research on the bacteriological nature of the sickness, Bangladesh still lacks the knowledge and comprehension required. This study aims to determine the factors that lead to infant sepsis in patients admitted to Bangladesh's tertiary care hospitals. The information might be utilized to detect patterns of risk among mothers and newborns and develop a preventive program in which pregnant women can take part to lower the incidence of neonatal sepsis.

1.2 Neonatal Sepsis:

Neonatal Sepsis is a systemic illness mainly caused by bacteria, viruses, or fungi having a high morbidity and fatality rate. The prevalence rate is between 1 and 5 per 1000 live births, it can grow easily from a small illness to a major infection. The main etiological factors depend on the mothers, environment, and the hospital they are born. Neonatal sepsis is classified between Early onset and Late onset depending on the occurrence of clinical symptoms. Early onset neonatal sepsis (EONS) is referring when the symptoms occur within the first three days of the neonate's life whereas late onset is said to be defined when the onset starts between the 4th to 30th days. Neonatal sepsis can further be defined in three sub-classes which include Suspected Sepsis when clinical symptoms are absent, but sepsis risk factors are prominent in the infant [13] then comes clinical sepsis where laboratory and clinical symptoms are present, however, the causative pathogen is unable to recognize. Finally, proven sepsis where the causative organisms are identified through blood cultures [13].

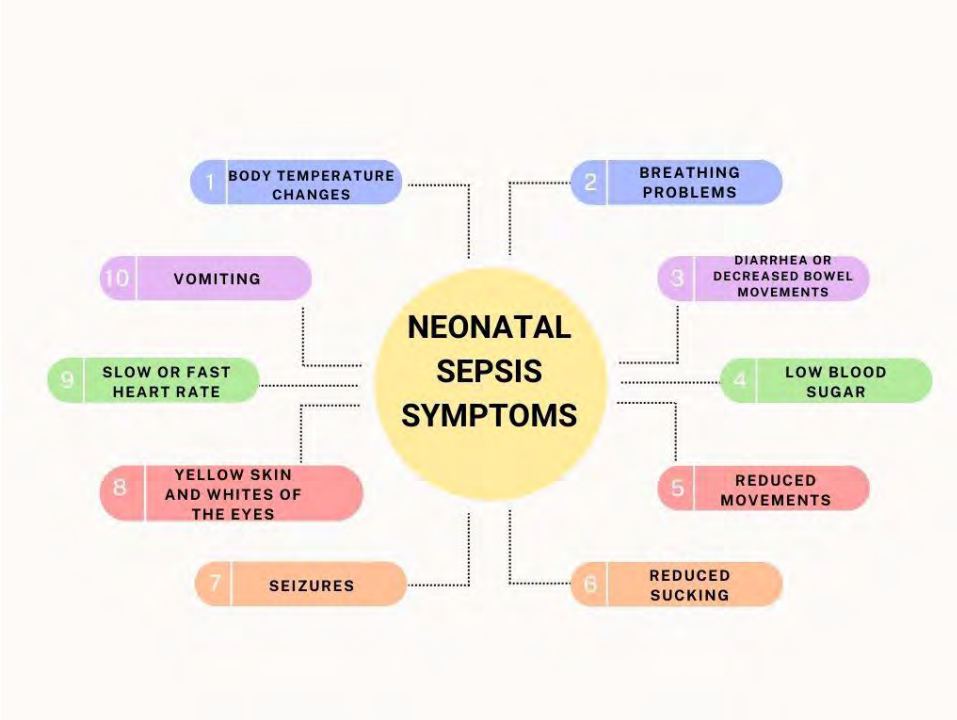


Figure 1: Common Symptoms of Neonatal Sepsis [14]

1.2.2 Early Onset Neonatal Sepsis (EONS):

Early-onset neonatal sepsis (EONS) is a severe deficiency that occurs when an infant develops a systemic infection during the early days of its life, usually within the first 72 hours after delivery. This deficiency is typically caused by bacteria, that a child may have received from its mother at delivery [14]. The means of transmission in this scenario may be the birth canal, but it could also occur from intrauterine infection or maternal colonization of the genital tract. Further, the risk factors of EONS include premature rupture of the membranes, infection of the mother by harmful germs such as group B streptococci, or Group B streptococci including E. coli, maternally acquired fever during labour, protracted labour, and chorioamnionitis, an inflammation of the fatal membranes brought on by a mother's Urinary tract infection [12-14]. To cause EONS Bacterial pathogens need to enter the bloodstream and trigger an immune response that will result in inflammation [15]. Therefore, the immune system of the infant is undeveloped and unable to fight off the infection, hence developing sepsis. To test the causative pathogen, the most common method is blood although the results may not be available right away. The infection can be further confirmed by laboratory tests that can be used to determine the blood count (CBC), levels of C-reactive protein (CRP), and markers of coagulation of the baby. The symptoms include seizures, fever or hypothermia, lethargy, poor feeding, respiratory distress, tachycardia), jaundice, and signs of shock such as hypotension (low blood pressure) or poor perfusion [16]. To manage the consequences of EONS, treatment should be started right away and provide immediate breathing support, intravenous fluids, and tracking of vital signs [16].

According to WHO, around the world about 15% of neonatal sepsis is EONS and it is a major cause of neonatal death [17]. Especially in low-middle-income countries like sub-Saharan Africa and South Asia where malaria, HIV, and STD infections are common scenarios, the mothers infecting the baby have a greater chance. Apart from that, factors like malnutrition, poverty, overpopulation, and illiteracy are major influencing causes of mothers being responsible for neonatal sepsis. [18]. To avoid these unprecedented situations, screening for Group B Streptococcus (GBS) in mothers and administration of subsequent antibiotics to mothers during pregnancy, making the mothers immunized against flu and tetanus can reduce the risk of EONS among babies [18].

1.2.3 Late-Onset Neonatal Sepsis (LONS):

To the contrary of Early Onset Neonatal Sepsis (EONS), Late-onset neonatal sepsis (LONS) is an infection that babies acquire after the first week of birth and will continue to have up to three months after that [19]. The conditions and symptoms of both classes of neonatal sepsis are alike but they are not the same. This is because the causative organisms of the two classes are different hence LONS have different risk factors, clinical presentations, and treatment plans than EONS. LONS is mostly caused by hospital-acquired bacterial infections whereas EONS is caused by organisms passed on from the mothers [18-20]. The organisms include coagulase-negative *staphylococci (CoNS), Staphylococcus aureus, Escherichia coli, Klebsiella species,* Enterobacter species, and other gram-negative bacilli [20]. These organisms mostly infect babies who are born before their due time and are admitted to the hospital for an exceeding period. The major influencing factors for LONS quite differ from LONS as it includes babies having mechanical ventilation, central venous catheterization, or umbilical catheterization, taking broad-spectrum antibiotics for a long time, and being in a dirty healthcare environment

[21]. The symptoms of LONS are fever, hypothermia, sleepiness, trouble feeding, breathing problems, apnea, bradycardia, low blood pressure, irritability, jaundice, and a swollen abdomen. In terms of diagnosis, the procedure is the same as EONS diagnosis which includes blood culture tests [22].

As LONS is caused primarily by environmental factors rather than maternal obstetric characteristics, the frequency of LONS differs based on geographical location, socioeconomic position, healthcare infrastructure, and adherence to infection control methods [23]. The contributing factors further include restricted healthcare resources and inadequate sanitary situations, insufficient prenatal care, absence of proficient birth attendants, and inappropriate hygiene practices [24]. Further, Lower-Middle Income Countries (LMIC) have a heightened prevalence of LONS because of inadequate nutrition, overcrowded living circumstances, and limited availability of clean water and sanitation facilities as well as the accessibility of antibiotics and proficient healthcare providers [25]. Therefore, to reduce the frequency of LONS, highly developed healthcare systems including extensive prenatal care, proficient birth attendants, and the availability of neonatal intensive care units (NICUs) need to be in progress. Moreover, improper hygiene maintenance of medical equipment or overcrowding of healthcare facilities should be limited in addition to building an implementable infection prevention and control program which should have maintained hand hygiene, sterile techniques during operations, and proper antibiotic usage to limit the spread of hospital-acquired microorganisms [26].

In a nutshell, LONS will continue to be an alarming worldwide health issue, especially in LMIC nations. Therefore, it has become crucial to address and overcome the key reasons behind the occurrence which include socioeconomic inequities, poor healthcare infrastructure, and antimicrobial resistance.

1.3 Limitations in Reducing the Prevalence of Neonatal Sepsis:

Declining the frequency of neonatal sepsis is not an easy task as it is a serious infection that affects infants and is associated with several variables. Despite advances in medical research and healthcare infrastructure, there is a huge number of restrictions in various sectors which hampers any comprehensive efforts to tackle this issue. Places like low- and middle-income countries (LMICs) are most vulnerable to the severe outcomes of neonatal sepsis as early diagnosis and immediate treatment are hardly accessible especially in terms of prenatal care of mothers during delivery [21-23]. The two main determinants of neonatal sepsis are preterm delivery and maternal infections [12-16]. Thus, managing any factors related to mothers during their pregnancy and delivery is essential. However, restrictive issues like lack of funds, transportation, cultural differences, restricted access to trained birth attendants, and hygienic delivery techniques still restrain pregnant women from getting adequate prenatal care in many parts of the world [24-25]. Late-Onset Neonatal Sepsis (LONS) is directly related to inadequate handwashing facilities and waste management practices, among other examples of poor hygiene and sanitation infrastructure thus efforts should be made to increase hygiene practices and policies [12]. However, the influence of cultural norms, attitudes, and socioeconomic variables on hygiene behaviors makes the task more difficult. Globally, antibiotic resistance capability of microorganisms is a common threat to treat infections at present. In addition to resource-constrained environments, limited access to microbiological tests, illiteracy,

unawareness, overuse, and misuse of antibiotics contribute to the spread of resistant strains [25-27].

Maternal ignorance and knowledge about health issues like maternal infections, including GBS colonization, urinary tract infections, and sexually transmitted infections greatly [enhance the problem of neonatal sepsis [26-27]. Thus, to address maternal health issues, prenatal screening, treating maternal infections, and providing health education are all essential components. Further, social, and economic factors such as poverty, malnutrition, and cramped living arrangements are major influencing factors of neonatal sepsis [28]. Thus, expected mothers from lower socioeconomic backgrounds could not manage access to wholesome food, sanitary conditions, and secure housing which could be harmful to the health of expectant mothers and newborns [28-29]. To implement a comprehensive newborn sepsis prevention policy, strategies including medical therapies with social, economic, and environmental tactics need to be combined. Further, prevention strategies should focus on measures to decrease antibiotic resistance, provide access to high-quality healthcare, address socioeconomic determinants of health, and encourage cleanliness and hygiene.

1.4 Study Objectives:

a) To identify the maternal risk factors for neonatal sepsis
b) To identify the neonatal risk factors of neonatal sepsis
c) To identify the organisms responsible for neonatal sepsis
d) To create an antibiogram profile of the causative agents of neonatal sepsis

Chapter-2

Literature Review

2.1 Neonatal Sepsis Global Prevalence:

World Health Organization (WHO) has quite prominently announced neonatal sepsis as an imminent global problem around the world. The mortality and morbidity rate of neonatal sepsis is growing uncontrollably amidst the significant medical and healthcare sectors [26]. At present, the mortality rate of neonatal sepsis is 11% globally [27-28]. This indicates the necessity of prompt and adequate preventative policies and treatment methods. Studies regarding the epidemiology of neonatal sepsis are mostly conducted in developed nations thus the real scenario of the illness in lower-middle-income countries (LMIC) is still not fully uncovered. The LMIC nations should be put into priority in terms of neonatal sepsis as the prevalence rate is profoundly higher in those settings. For example, the frequency rate of Early Onset Neonatal Sepsis (EONS) in North America falls between 0.2 and 1 case per 1,000 live births [28]. Late-onset neonatal sepsis (LONS) emerges when the baby is between 3-5 days and up to 3 months of age. The most common pathogens of LONS are *Klebsiella spp.* and *Staphylococcus aureus* and fungi such as Candida spp. which are mostly infected through the hospital setting [29]. In developed nations, the main determinant factor for LONS is claimed to be low birth weight. Studies have recorded that LONS infections are 15% in higher-income nations [80]. Further, meta-analyses have found that the prevalence rate of LONS in higher-income nations is between 1 in 1,800 to 1 in 3,000 live births [29-30]. The rates are way higher in low- and middle-income countries (LMIC) due to insufficient availability and access to primary healthcare facilities. In addition to that, the antibiotic resistance rates of the main causative organisms are between 81-84% in LMIC nations in comparison to the higher-income nations. Thus, many studies have been devoted to understating the antibiogram profile of the key pathogens in both developed and developing nations [31].

The burden of neonatal sepsis cases varies from region to region. In 2002, 4.4 cases per 100,000 were treated in the neonatal intensive care units (NICU) in Australia and New Zealand while the number increased by 2010 recording 181 cases per 100,000 births in China (Table-1) with a 1.3% to 5.4% mortality rate. The following year, 48 cases of sepsis per 100,000 were recorded treated in the NICUs of China, recorded by a meta-analysis [32]. Studies have marked respiratory infections as the main consequence of neonatal sepsis in 40-60% of cases. The rate of culture-positive sepsis cases greatly varied between nations marked by 7% of cases in China and 55% in Spain with no organ failure (Table 1). Studies that provided this information indicated that respiratory infections were the predominant source of infection, accounting for 61% and 41% of cases. The rates of blood culture positivity were found to vary, with 7% of cases in China without organ dysfunction and 55% of all sepsis patients in Spain generating positive results (Table 1). The occurrence of significant underlying comorbidities among children with sepsis varied from 7% in China involving cardiovascular and malnutrition complications to 50% in Australia and New Zealand with congenital heart disease and

immunosuppression (Table 1). America had the highest burden of neonatal sepsis in 2005 marking 89 cases per 100,000 live births whereas Germany had the lowest prevalence in 2007, with only 10 cases per 100,000 live births. However, the United States had the lowest burden of neonatal sepsis, with a rate of 450 cases per 100,000 live births in 1995 whereas India had the prevalence with 17,000 cases per 100,000 live births (Table 1).

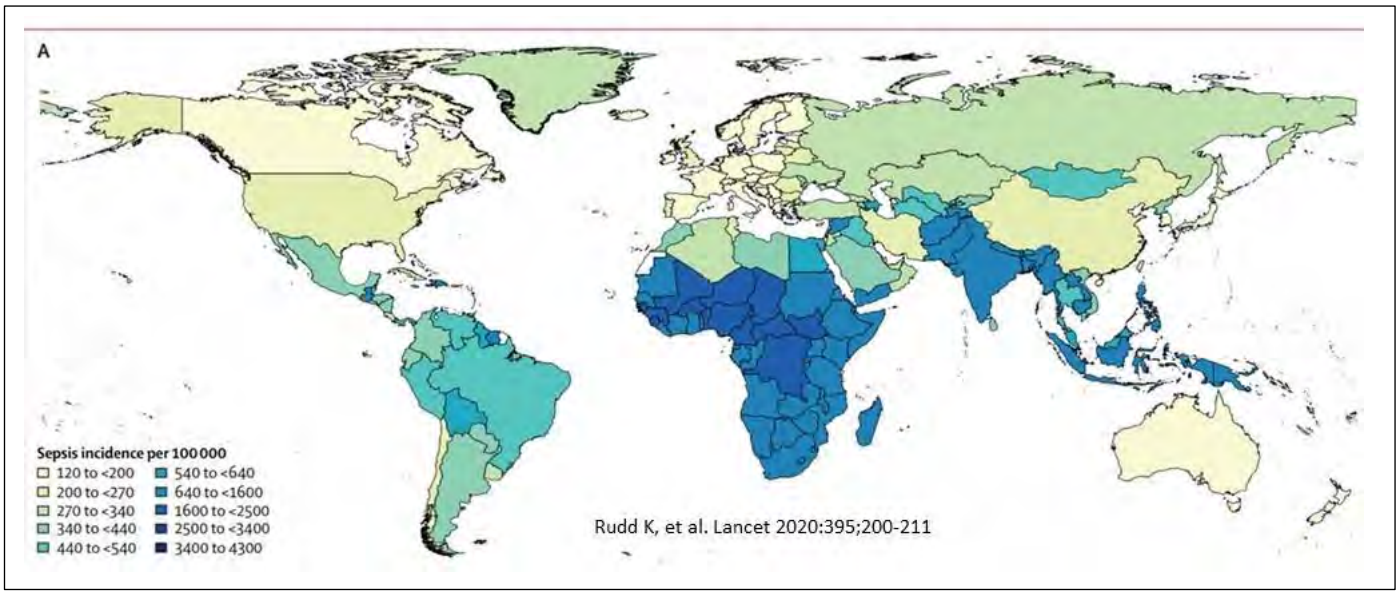


Figure 2: Neonatal Sepsis Global Prevalence -2020 [33]

Table 1: Population-level incidence rates for neonatal sepsis per 100000 live births

Country	Year	Number of Neonatal Sepsis Cases	Incidence per 100000 population	Source
China	2010	153	18·1	[33]
USA	2005	17 542	89	[34]
Canada	2008	851	13·4	[35]
Germany	2013	2071	14·1	[36]
South Korea	1999	206	2570	[37]
Thailand and Myanmar	2009– 2012	187	4480	[38]
India	2006	5	560	[39]
India	2008– 2010	72	2015	[40]
Spain	1999– 2004	153	60·9	[41]
Australia and New Zealand	2013	329	6·5	[42]

2.2 Neonatal Sepsis Prevalence in Developing Countries:

Worldwide, the mortality rates of neonatal sepsis exceed four million cases each year with LMIC nations contributing the most cases. The death rate is 20 per 1000 live births in LMIC nations while the high-income nations have three per 1000 live births. Studies have claimed that these mortality rates are mostly preventable and treatable [43].

Over four million neonatal deaths occur globally each year, with low- and middle-income countries (LMICs) accounting for most of these deaths. Neonatal mortality is predicted to be 20 per 1000 live births in low- and middle-income countries (LMICs) and 3 per 1000 in high-income countries [44]. Preterm birth, respiratory tract infections, diarrheal illnesses, meningitis, newborn sepsis, and neonatal tetanus are among the major preventable or treatable conditions that are the main causes of these deaths. Neonatal sepsis has been repeatedly identified as the main cause of newborn mortality in previous studies [45]. Bangladesh is a fair picture of the situation, with South Asia and sub-Saharan Africa accounting for most instances of newborn sepsis. 2010 saw 1.7 million cases reported globally; in 2013, 39% of newborn sepsis deaths occurred in South Asia. Furthermore, in LMIC countries, the prevalence rate by 2018 was 3930 instances per 100,000 live births. These numbers correspond to an economic cost in LMICs of between 10 and 469 billion US dollars, and in South Asian countries, between 5.29 and 8.73 million US dollars [46]. Within the first seven days of a newborn's life, 42% of deaths occur [47]. Lower-income moms were linked to 2.76% of cases in Southern Ethiopia, but over a million cases were linked to them in Pakistan.

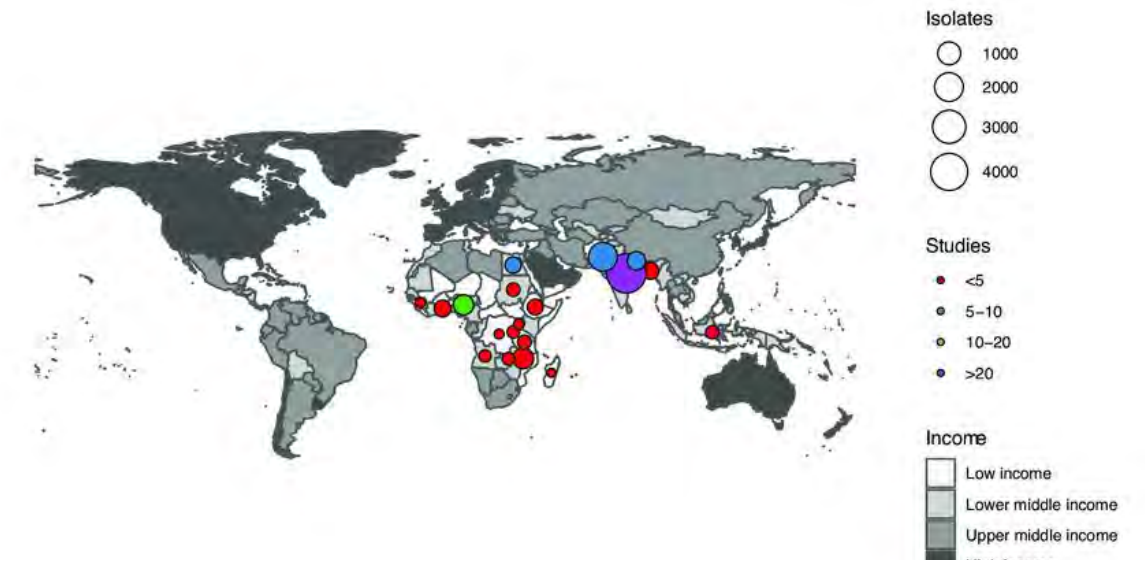


Figure 3: Distribution of included studies in low- and lower-middle-income countries [47]

2.3 Major Outcomes of Neonatal Sepsis:

The kind of pathogenic organism, the timing of the infection, and the effectiveness of treatment all affect how neonatal sepsis turns out. Neonatal sepsis is one of the main causes of newborn death worldwide. Patients with newborn sepsis are at risk of dying if they do not receive prompt medical attention and an early diagnosis. Neonatal sepsis fatality rates remained high despite access to expensive medical facilities and antibiotic treatments; the situation is worse in areas with limited resources. Neurodevelopmental dysfunction, which includes learning difficulties, motor impairment, and cognitive abnormalities, is significant in terms of long-term effects [47].

Moreover, brain damage and neuronal death might result from newborn sepsis. Heart failure, liver dysfunction, acute kidney injury (AKI), and acute respiratory distress syndrome (ARDS) aggravate many cases. Neonatal sepsis-related septic shock must be treated right away to reduce the risk of inadequate tissue perfusion and systemic hypoperfusion [47–48]. There will be an increase in hypotension, insufficient perfusion, and impaired mental status. Moreover, potential long-term effects of neonatal sepsis include bacterial pneumoniae, lung damage caused by sepsis, respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), and chronic lung disease [49]. In terms of growth and development, some of the effects of infection-related metabolic demands and catabolic stress in newborns include growth disruption, failure to thrive, and nutritional inadequacy. Lastly, the psychological influence placed on the. To conclude, the potential consequences of neonatal sepsis include organ failure, neurodevelopmental damage, respiratory troubles, septic shock, and long-term growth and developmental problems.

Chapter-3

Risk Factors of Neonatal Sepsis

3.1 Neonatal Risk Factors:

Plethora of studies in neonatal sepsis has marked low birth weight and premature as the main risk factor for neonatal sepsis than full-term babies [51]. The risk of premature babies is much higher if transmission of maternal interrupts. On the other hand, low APGAR scores, resuscitation, and multiparous mothers are associated with Early onset neonatal sepsis (EONS) indued mortality whereas operations involving invading methods, poor breastfeeding, maternal nutrition, stomach acid getting low, and surgical interventions are linked with late-onset sepsis (LONS) [52].

Figure 4: Most Common Neonatal Risk Factors Associated with Neonatal Sepsis

Neonatal sepsis is associated with several noteworthy risk factors, including non-biological factors such resuscitation techniques, clinical and nursing practices, and neonatal features such as low birth weight, preterm birth, and poor Apgar scores [53]. Moreover, several histochemical variables impacting neonatal sepsis include platelet count, white blood cell count, and c-reactive protein measurements. Neutral cord care in a pool and any form of maternal infection that goes undiagnosed during pregnancy and delivery may also contribute to neonatal sepsis [54]. Because newborns' immune systems are so delicate, a meta-analysis found that decreased gestational age, extremely low birth weight, and maternal risk factors are strongly linked to EONS [55].

3.2 Maternal Risk Factors:

The emergence of sepsis in newborns has frequently been linked to maternal obstetric and clinical features. Numerous investigations conducted worldwide have established a connection between the emergence of newborn sepsis and problems faced by the mother during pregnancy [55-57]. Mother-related risk factors have been linked to Early Onset Neonatal Sepsis (EONS). Particularly in lower-middle-income countries where women's lifestyles are severely impacted and education is only at the elementary school level [59]. Pregnant women are therefore at risk for infections and other problems that might be prevented or treated right away with the right information and lifestyle modifications. Premature rupture of membranes (PROM), prolonged labor, fever during delivery, preterm delivery, urinary tract infection, preeclampsia, diabetes, vaginal infections, intrauterine infections, infections with B streptococcus (GBS), and pregnancy history involving GBS infection have been the focus of numerous studies on the etiological factors for neonatal sepsis to date [60]. Furthermore, with a 1-3% prevalence rate, the likelihood of EONS increases tenfold in mothers who also have both PROM and chorioamnionitis [61]. Vaginal infections and repeated births are other possible risk factors for newborn sepsis. Early treatment with antibiotic prophylactic techniques appeared to reduce the mortality risk in GBS-infected EONS [62].

Maternal age below 20 years and over 30 years is thought to be a risk factor for newborn sepsis with an early onset. According to a 2019 Ethiopian study, infants born to moms older than thirty had a higher risk of developing early-onset neonatal sepsis [63]. Because there is little data on sepsis's course from pregnancy to postpartum or postabortion, it is difficult to determine with accuracy how frequently maternal and neonatal risk factors influence the disease in these nations [2, 10, 11]. Numerous maternal factors have been linked in the literature to neonatal sepsis, such as prolonged labor, missed antenatal care, prolonged membrane rupture, mother infection history, repeated vaginal checkups, intrapartum fever, gestational age, parity, delivery style, preterm, chorioamnionitis, and meconium-stained amniotic fluid [64-67].

Many studies have found that mothers who are younger than 20 years and older than 30 years are likely to pass sepsis infection to their babies. In 2019, an Ethiopian study claimed that EONS is associated with babies born to mothers older than 30 years old [68]. However, there is still a scarcity of accurate data on the association between maternal factors and neonatal sepsis making the prevalence rate of neonatal sepsis difficult to estimate. Especially in lower-middle-income countries where postpartum and postabortion information is crucial to understanding the predisposing factors of neonatal sepsis [69].

Maternal history of hypoxia or intrauterine discomfort and meconium-stained amniotic fluid are the leading causes of EONS according to a meta-analysis. Also, maternal asphyxia results

in immunological damage and interruption of immunological function [70], leading to the dysfunction of the mucosal barrier in neonates which reduces their immunity and making vulnerable to infections [71]. Further, meconium from meconium can enter the amniotic fluid when the baby is hypoxic. As, inside the womb the baby is protected from external bacterial infections through amniotic fluid, thus meconium entering the fluid can lower its ability to fight bacterial development and lower the immunity of the unborn baby. Though premature rupture of membrane in mothers is independently related to chorioamnionitis development, however, in combination they can increase the chances of EONS by 10-fold [72-74]. Therefore, early diagnosis using disease biomarkers should be used promptly to quickly assess and diagnose the disease especially starting from examining for related complications in mothers.

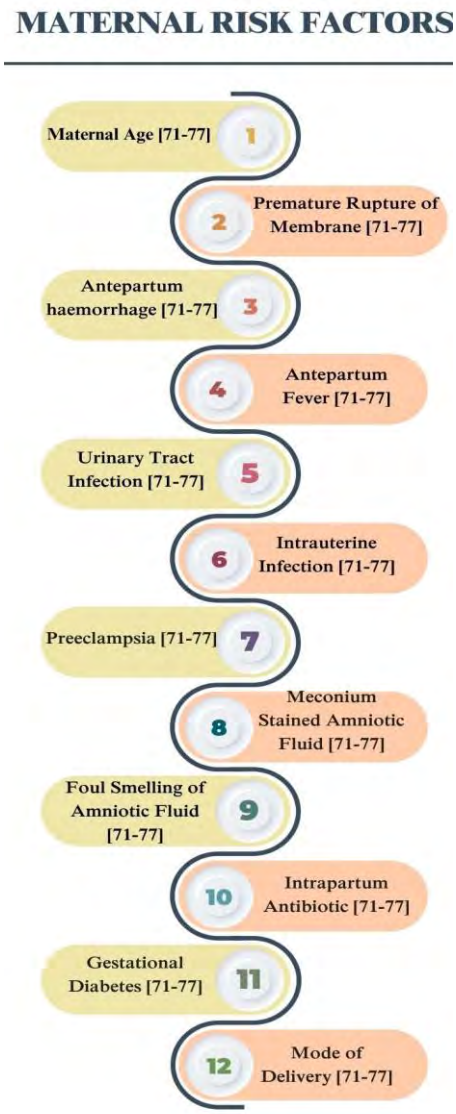


Figure 5: Maternal Risk Factors Associated with Neonatal Sepsis

For early-onset neonatal sepsis (EONS), the transmission of infection through mothers during pregnancy is common. Therefore, mothers developing urinary tract infections, sexually

transmitted infections, vaginal infections, and intrauterine infections will greatly uplift the mortality risk of EONS in babies [75]. Many studies have found that mothers having vaginal inspection history may increase their risk of Gram-negative bacilli infection thus inclining the occurrence of EONS by 3 times [75].

In a nutshell, factors like intrauterine infection, meconium contamination in amniotic fluid, chorioamnionitis, premature membrane rupture, lower gestational age, maternal urinary or reproductive tract infection, perinatal fever, very low birth weight, and vaginal examination ≥three times [75–77] is strongly related with EONS. To reduce that risk, improvement in the care given to pregnant mothers, and recognizing prenatal risk factors to administer antibiotics at the right time to avoid the risk is necessary.

3.3 Major Causative Agents:

In terms of causative organisms, many studies have marked various bacteria as the responsible pathogen for neonatal sepsis including both gram-positive and gram-negative bacteria. So far, quite a few meta-analyses have listed *Escherichia coli (E coli),* Listeria, and a few specific types of streptococci as some of the key causative pathogens of neonatal sepsis. In addition to that, many Group B streptococcus (GBS) has been associated with neonatal sepsis cases [78-80]. Because of the prevalence of maternal colonization of Group B streptococcus (GBS), pregnant women are frequently tested for the infection to identify the risk of neonatal sepsis. Further, studies say that herpes simplex virus (HSV) infects the genitourinary system contaminating the placenta, cervix, amniotic fluid, or vaginal canal thus increasing the risk of mortal illness from HSV [81]. The bacteria may infect the baby during labour by coming out at the time of rupture of the amniotic membranes [82].

Late-Onset Neonatal Sepsis (LONS) is primarily caused by Staphylococci species and *E. coli,* while *Streptococcus, L. monocytogenes*, *E. faecalis,* group D streptococci, α-haemolytic streptococci, and Staphylococcus pneumoniae influenzae type B are the main causative organisms of early-onset neonatal sepsis (EONS) [83]. These microorganisms were also commonly detected in low-birth-weight neonates with sepsis, and in newborns undergoing intravascular catheterization, endotracheal intubation, assisted breathing, surgery, and contact with contaminated tools and staff [84]. Furthermore, *Pseudomonas aeruginosa* was primarily acquired in hospitals through contaminated breathing equipment. [85].

Furthermore, studies have claimed that gram-negative organisms were more prevalent in neonatal sepsis cases than gram-positive organisms. Amongst the gram-negative organisms, *Escherichia coli and Klebsiella pneumoniae* were most isolated whereas *Staphylococcus aureus* was the most common gram-positive bacteria. A Similar study in Bangladesh has recovered these organisms from blood samples of neonatal sepsis suspected infants [86] whereas another Bangladeshi study mostly isolated *Klebsiella pneumoniae* [87]. These findings were supported by studies conducted around the world where *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, and coagulase-negative Staphylococcus* [9, 10, 12, 32] have been the most identified causative pathogen. In most cases, the organisms were transmitted from the mothers to the babies during delivery. Escherichia coli and Klebsiella pneumoniae are abundantly found in gastrointestinal flora and are directly linked with pneumonia, septicemia, and urinary tract infections in mothers [88].

Chapter-4

Diagnosis and Treatment

4.1 Diagnosis and Antibiotic Treatment:

Early diagnosis of neonatal sepsis infection is the most important way to improve the treatment and reduce the mortality rates. Immediate antibiotic treatment and antenatal care is the most effective ways of reducing neonatal sepsis prevalence [88]. Blood culture is the main diagnosis method of neonatal sepsis around the world. However, positive blood cultures are not the only confirmation of neonatal sepsis as negative blood cultures are still considered clinical sepsis as many of the cases still show heightened risk factors and symptoms of neonatal sepsis. Because of this confusion, antibiotic treatment, and intensive care are still given to babies with negative blood cultures. In terms of antibiotic treatment, cefotaxime and vancomycin are administered to babies even if their blood culture comes negative whereas ampicillin and gentamicin [89] are given to patients in neonatal intensive care units (NICUs). To fight and reduce antibiotic resistance issues, accurate diagnosis and proper antibiotic treatment are necessary. In addition to blood culture, C-reactive protein (CRP) and Procalcitonin are also used to detect neonatal sepsis infection. As inclined levels of both the biomarkers in the blood streams indicate bacterial contamination in the blood. However, these biomarkers cannot accurately detect neonatal sepsis cases as they also indicate inflammatory diseases [90]. Further, complete blood count (CBC) is another way of detecting infection in the blood by understanding the yield of Leucocytosis, leukopenia, and immature neutrophils. Also, bacterial sepsis is indicated by a higher than 0.2 Immature to Total neutrophil ratio. Finally, Radiological Imaging like chest X-rays or abdominal ultrasounds can be used to identify the complications of sepsis, such as pneumonia or intra-abdominal abscesses [91].

4.2 Common Diagnosis Method:

The diagnosis processes can sometimes be misguided with false negative blood culture results. False-negative results often happen when the blood volume used for blood culture is not sufficient which often happens when the baby in question has a very low birth weight or is premature [90]. Another reason for the false negative result is the low volume of pathogen present in the blood due to mothers having intrapartum antibiotics to treat maternal diseases like chorioamnionitis Therefore, at least 1 ml of blood should be taken from neonates to have high sensitivity (98%) even when pathogen concentrations are low [90].

So far, blood cultures have proven to be extremely sensitive even though it takes up to seven days to have the result. Which delays the treatment processes and the start of intensive care services for the baby in need. Apart from the blood culture method, other blood biomarkers like CRP, CBC, and Procalcitonin do not accurately indicate infections specifically related to sepsis [91]. Thus, the implementation of genetic markers and molecular identification should be put into priority to promote early diagnosis. Automated multiplex PCR should be done immediately to have fast results. In addition to that, genetic sequencing and identifying biomarkers like bacterial surface antigens should be used frequently as a diagnosis method for neonatal sepsis. An automated multiplex PCR only takes an hour to detect *S. aureus* and *E. coli* infections, however, the results can still be misinterpreted in terms of blood samples [92].
To avoid misinterpretation, comparing results performed in different techniques is an ideal way. Also taking blood samples more than once for multiple cultures can be ideal to avoid false

results. Some species like Coagulase-negative *Staphylococci* (CoNS) and *Streptococcus viridans* are commonly found organisms whereas *Escherichia coli, Staphylococcus aureus,* and *Streptococcus pneumoniae* are typically symptomatic of a real infection [93-94]. Often blood culture is not always used to start the treatment of neonatal sepsis. Sometimes, the treatment gets already started based on the clinical symptoms and characteristics of the baby. In most of cases, babies undergo antibiotic treatment right after admission and blood culture happens resulting in a false negative outcome [95].

4.3 Antibiotic Treatment of Neonatal Sepsis:

In the United States, ampicillin and gentamicin are the most used antibiotics, especially for neonates admitted to NICU [56]. According to Oliver et al in 93% of cases, ampicillin and gentamicin are administered to babies in their first three days of life. On the other, cefotaxime is the most used antibiotic at the Utah hospital in terms of early onset cases and gram-negative infections. In a 2002 survey, 60% of doctors from 35 hospitals with NICU suggested vancomycin as the first line of treatment for suspected late-onset sepsis (LONS) in combination with an aminoglycoside [96]. Due to the emergence of vancomycin-resistant pathogens, cautious use of vancomycin has been recommended for many years (The Hospital Infection Control Practices Advisory Committee, 1995). Research suggests that using empiric vancomycin may not improve short-term outcomes or hospital stays in neonatal Coagulase-negative *Staphylococci* (CoNS) infections [97-98].

According to studies, the increasing use of vancomycin has been increasing antibiotic resistance potential among Coagulase-negative *Staphylococci* (CoNS) resulting in shorter outcomes and increasing hospital stay [99]. Therefore, the use of vancomycin has been instructed to be controlled in neonatal sepsis cases (The Hospital Infection Control Practices Advisory Committee, 1995). Moreover, the practice of antibiotic administration to neonates with neonatal sepsis even before blood culture results come and continue even if the tests are negative, is widespread (100). This practice is directly or indirectly influencing the increasing trend of antibiotic resistance among common pathogens. Many studies have found that antibiotics are often given to neonates even after just analyzing the C-reactive protein levels which is not even a clear indication of sepsis. Research has claimed that antibiotic doses can be discontinued even after 5 days if the blood cultures are negative which is not a common practice around the world [101]. This practice leads to negative complications such as necrotizing enterocolitis, longer hospital admissions, and mortality [102-103]. Further research has also claimed that antibiotic treatment can be stopped after 48 hours. A study by Cantey et al. has claimed that interrupting the administration of antibiotics after 5 days when the culture results are negative leads to better outcomes in comparison to situations where the treatment continues [104].

In lower middle-income countries, access to microbiologists, microbiology testing, and pediatricians is limited therefore diagnosis of neonatal sepsis is mostly based on clinical symptoms. World Health Organization (WHO) has recommended gentamicin and ampicillin penicillin, or cloxacillin for suspected cases. However, gram bacteria have shown multi-drug resistance in these developing and underdeveloped nations [105]. Due to the scarcity of information on antibiogram patterns of the pathogen, taking necessary measures has become difficult. Cephalosporins are the most used first-line drug to treat neonatal sepsis in lower-middle-income countries [106]. Continuously longer antibiotic administration is responsible for adverse outcomes. Many studies have suggested that short courses of antibiotics and stopping the treatment when results come as negative greatly bring positive outcomes. To

avoid the increase of antibiotic resistance among pathogens, antibiotic susceptibility testing before starting the treatment should be prioritized among nations [107]. Finally, comprehensive strategy-oriented quality assurance, local surveillance data, and standardized antibiotic susceptibility testing procedures should be improved.

Across nations, our knowledge of local antimicrobial susceptibilities is crucial in treating newborn sepsis in hospital and community settings. However, very little evidence has been found for decreased susceptibility of Klebsiella species towards first lines of defense drugs [69]. Treatment protocols must be supervised and grounded by clearly stating the risk and benefit analyses. To understand the infrastructures of antimicrobial susceptibility patterns, treatment outcomes, and infection causes guidelines should be maintained both regionally and globally. However, antimicrobials like amikacin, carbapenems, fluoroquinolones, and extended-spectrum penicillin (such as ticarcillin-clavulanate) are not budget-friendly and are hardly available in many developing nations thus stringent stewardship practices needs to be initiated to stop the emergence of resistance [108].

4.4 Antibiotic Resistance in Neonatal Sepsis Treatment:

Clinical symptoms of neonatal sepsis are not well defined and blood culture results often come quite late. In lower-middle-income nations, around 40–50% of neonates are diagnosed with positive blood cultures [70]. To reduce neonatal sepsis-related morbidity and mortality, understanding the common bacterial isolates and their antibiotic susceptibility is mandatory to use the correct antibiotic treatment. Bacterial resistance towards antibiotic drugs is mainly caused by the exhaustive use of antibiotics which has led us to the "post-antibiotic era. National Bacterial Resistance Surveillance Network programs can evaluate resistance both locally and nationally to direct empirical antibiotic usage, monitoring bacterial resistance has become routine practice in public health [71].

World Health Organization suggests the administration of Ampicillin-Gentamicin drugs as the first line of treatment for newborn sepsis [109]. In Iran, Ampicillin, and aminoglycosides such as amikacin or gentamicin drugs are exhaustibly administered to neonatal sepsis patients admitted in neonatal intensive care units (NICUs) [110]. Around 54.0% of isolates of Gram-negative (GN) bacteria are not sensitive to the first-line medicines recommended by the WHO. The percentage of lowered sensitivity in Iran is their constant use and misuse of antibiotics [111]. Like these results, previous research [55–56] found a similar resistance pattern to first-line antibiotics. Around, 50–80% of infants exhibited inclined levels of resistance to ampicillin, cephalosporins, and aminoglycosides in South America, Asia, and Africa [112-114].
In the United States, aminoglycosides were less effective on 17% of *E. Coli* isolates where 67% showed resistance to ampicillin [72]. However, about 10% of these isolates were less sensitive to both gentamicin and ampicillin. According to a report of USA from 2015– 2017, *E. coli* is the responsible agent in around 7.8% of neonatal sepsis cases in NICUs and is resistant to both ampicillin and gentamicin [73].
According to research, *E. coli* and *K. pneumoniae* have gained resistance through plasmid-mediated production of extended-spectrum beta-lactamase (ESBL) which is an alarming public health concern over two decades [112-116]. As a result of the overuse of broad-spectrum antibiotics in neonatal intensive care units (NICUs) multidrug-resistant Gram-negative (GN) bacterial infections are a serious problem in several developing countries. In comparison developed nations. Preventing Gram-negative (GN) bacteria from transmitting between people is the main concern [117]. Thus, infection control practices such as strict hand washing,

appropriate glove use, and efficient sterilization and disinfection techniques should be mandatory to reduce the risk.

Chapter-5

Neonatal Sepsis in Bangladeshi Perspective

5.1 Diagnostic and Treatment Method of Neonatal Sepsis:

The diagnostic and treatment methods in Bangladesh are modified to tailor the standard according to the local healthcare environment in Bangladesh. The most practiced diagnostic method in Bangladesh is for medical professionals to assess symptoms and indicators such as fever or hypothermia, breathing difficulty, eating issues, sluggishness, agitation, and irregular vital signs for neonatal sepsis [116-118]. In Bangladesh, the most frequently used diagnostic method for neonatal sepsis is blood culture which also aids in identifying antimicrobial susceptibility and the causative organism. In addition to that, certain histochemical biomarkers are also used to recognize any infections and inflammation in the blood [117]. To find systemic inflammation or infection, measurement of the concentration of leucocytosis, leukopenia, or a left shift on a complete blood count (CBC) is frequently used. Further, procalcitonin (PCT) and interleukin-6 (IL-6) are used as additional biomarkers for sepsis diagnosis and prognosis. Few imaging tests like ultrasounds or chest X-rays are sometimes conducted to identify severe consequences of sepsis such as pneumonia or the development of an abscess [118]. Apart from, blood culture and biomarker confirmation, Bangladeshi clinicians also consider many clinical characteristics of neonates and babies such as low birth weight, maternal illnesses, and extended labor.

Moreover, in Bangladesh, empirical antibiotic therapy starts even before the conclusive microbiological confirmation. Even with a mere suspicion of sepsis newborns are mostly administered with ampicillin and gentamicin antibiotics in Bangladesh as they protect against both Gram-positive and Gram-negative bacteria to some extent. With the growing prevalence of antibiotic resistance around the world, Bangladesh is providing more concentration on taking initiatives to reduce antibiotic stewardship [118-119]. As a result, Bangladeshi healthcare providers are instructed to provide antibiotics by following prescribing standards to avoid needless usage of antibiotic drugs and utilize the right dosage and length of treatment. Further, supportive care like temperature control, nutritional support, hydration and electrolyte management, and breathing support are vastly required in severe sepsis cases among babies [119]. To improve treatment methods in Bangladesh, frequent monitoring of vital signs, laboratory data, and clinical state is heavily required to evaluate response to treatment and thus tailor the therapy as necessary. Neonatal sepsis complications associated with each patient's situation include respiratory distress syndrome, meningitis, shock, and disseminated intravascular coagulation (DIC) [119]. Family members especially parents of neonatal sepsis should be provided with information and assistance to help them understand their child's treatment plan and get help quickly if necessary. Further, follow-up care to identify any long-term adverse impacts of the infection, monitor for recurrence, and evaluate growth and development are required to treat neonatal sepsis. To sum up, Bangladesh uses a combination of clinical evaluations like laboratory testing, empirical antibiotic medication, supportive care, and close observation to treat neonatal sepsis. To expect improved treatment and diagnostic methods for neonatal sepsis, Bangladesh needs a multidisciplinary approach, adherence to evidence-based guidelines, and antibiotic stewardship principles.

5.2 Cost of Diagnosis and Treatment of Neonatal Sepsis in Bangladesh:

The cost of treatment and diagnosis of neonatal sepsis in Bangladesh varies depending on the severity of the condition, the degree of care provided by the healthcare facility, the availability of resources, and the diagnostic and therapeutic methods. The cost of diagnosis includes blood cultures (2000-2500 BDT) complete blood counts (CBCs) (400 BDT), C-reactive protein (CRP) (600 BDT), and other biomarkers where the price structure of the laboratory, and the volume of tests conducted, may affect the cost of these tests. Further, imaging tests like ultrasounds or chest X-rays (1200-2500 BDT) are also included in the cost as they are required for a diagnosis or to evaluate any severe consequences. In Bangladesh, the overall cost of antibiotic treatment varies based on the type of antibiotic, dose, length of therapy, and mode of administration (oral vs. intravenous) as generic antibiotics (3-5 BDT/Tablet) are less expensive whereas imported or branded ones (25-30 BDT/Tablet) are more costly. The costs are further enhanced by adding expenses of temperature control, nutritional support, fluid and electrolyte management, and breathing support. Also, in severe cases, problems like shock, meningitis, or respiratory distress syndrome require drugs, treatments, and longer hospital stays which increases the cost. The financial outcomes are hugely influenced by hospital stays (4200-12000 BDT/ night NICU cost), doctor visits, nursing care, and other healthcare services received during therapy. Also, medical equipment like incubators, ventilators, and infusion pumps for monitoring, diagnosis (800-2000 BDT per night), and therapy inclines to the overall cost of treating neonatal sepsis in Bangladesh (Table 2, 3).

Table 2: Cost Distribution of Neonatal Sepsis Diagnosis Tests

Test	Cost (BDT)		Source
	Urban	Rural	
Blood Culture	2000-2500	300	Popular Diagnostic Centre, icddr,b
Complete Blood Count	400	160	Popular Diagnostic Centre, icddrb
C-Reactive Protein	600	150	Popular Diagnostic Centre, icddrb
X-Ray	1200-2500	200	Popular Diagnostic Centre, icddrb
Ultrasound	1200-2500	220	Popular Diagnostic Centre, icddrb
Procalcitonin	3000	650	Popular Diagnostic Centre, icddrb
Polymerase Chain Reaction (PCR)	3000	500	Popular Diagnostic Centre, icddrb

Table 3: Cost Distribution of Neonatal Sepsis Treatment Facilities

Facility	Cost (BDT)		Source
	Urban	Rural	
NICU	4200-12000	4000	BIRDEM Hospital, Mymensingh Medical College Hospital
Incubator	9000	2000	BIRDEM Hospital, Mymensingh Medical College Hospital
Ventilator	2790	1500	BIRDEM Hospital, Mymensingh Medical College Hospital
Infusion Pump	8000	1500	BIRDEM Hospital, Mymensingh Medical College Hospital
Doctor Visit	3000	500	BIRDEM Hospital, Mymensingh Medical College Hospital
Nursing Care	3000	500	BIRDEM Hospital, Mymensingh Medical College Hospital

5.3 Knowledge and Awareness of Neonatal Sepsis among Bangladeshi Mothers:

Several factors like educational background, social standing, access to healthcare, and involvement in health education programs influence the knowledge of neonatal sepsis among Bangladeshi mothers. In Bangladesh, a mother's education has been frequently associated with the knowledge of neonatal sepsis-related risk factors as highly educated mothers are probably more knowledgeable and able to avail better health information sources [120]. In Bangladesh,

most mothers do not give frequent visits to prenatal care and other healthcare services thus providing healthcare practitioners fewer chances to inform expectant women about the warning signs of neonatal sepsis, the value of good hygiene, and when to take their babies to the hospital [120]. Frequent home visits, health education workshops, and group discussions to increase public knowledge of mother and child health issues, including neonatal sepsis are required by Bangladeshi community health workers. In Bangladesh, most child health complications are raised by varying levels of customs around labor, postpartum care, and infant care [121]. Further, the level of knowledge on neonatal sepsis among mothers is greatly influenced by cultural customs around labor, postpartum care, and infant care in Bangladesh. Factors like low literacy rates, language barriers, limited access to healthcare services in rural areas, and socioeconomic inequalities may limit mothers' access to information about neonatal sepsis [121-123]. Sufficient funding is required for health education programs, community outreach initiatives, and healthcare infrastructure to guarantee that mothers are informed about infection prevention and neonatal health on time. By attaining sufficient funding, it will be feasible to raise awareness levels and provide moms the confidence to take preventative action to safeguard the health and welfare of their infants [122].

In general, neonatal sepsis is one of the most concerning public health issues in Bangladesh which impacts socioeconomic development, healthcare resources, and maternal and child health outcomes. Neonatal sepsis is one of the primary causes of neonatal mortality and illness in Bangladesh [123-125]. The heavy population, limited access to high-quality healthcare in remote areas, and socioeconomic inequalities can heighten the burden of neonatal sepsis in Bangladesh. The exact prevalence rate of neonatal sepsis in Bangladesh is not clear but research suggests that neonatal sepsis continues to be a major concern, especially in environments with limited resources [126]. Prevalence rates are influenced by multiple risk factors including diseases that mothers acquire during pregnancy or childbirth due to poor prenatal care, unclean delivery methods, restricted access to sanitary facilities and clean water which leads to early membrane rupture, low birth weight, and malnourishment. Further factors like socioeconomic variables including poverty, low levels of education, and ignorance about newborn health issues can influence neonatal sepsis prevalence [127]. In a resource-limited environment like Bangladesh, access to diagnostic facilities and trained healthcare professionals are limited. Diagnostic processes like blood cultures and biomarkers can create complications if clinical signs and symptoms of sepsis can overlap with other neonatal conditions, leading to delays in diagnosis [128]. Issues like antibiotic resistance, a lack of effective antibiotics, insufficient healthcare infrastructure, and gaps in the healthcare delivery system can interrupt Timely and appropriate treatment. Supportive care like feeding, hydration management, and respiratory assistance can improve the morbidity and mortality rates of neonatal sepsis. In underprivileged and rural areas access to healthcare including NICUs (neonatal intensive care units), labs, and qualified medical personnel requires improvement [127-129].
To sum up, a comprehensive strategy including research and innovation, policy interventions tailored to increase the health of mothers and children, health education and awareness-raising, and the building of the healthcare infrastructure is needed to reduce the prevalence and mortality rate of neonatal sepsis in Bangladesh.

Chapter-6

Materials and Methods

6.1 Study Design and Setting:

This was a cross-sectional study conducted in the Neonatal Intensive Care Unit (NICU) of Ad-Din Medical College Hospital, BIRDEM Women and Children Hospital, and Kurmitola General Hospital. All the hospitals are situated in Dhaka, Bangladesh. The samples were collected from September 2023 to March 2024.

6.2 Study Population and Sampling:

The study population was neonates admitted to the NICU of Ad-Din Medical College Hospital, BIRDEM Women and Children Hospital, and Kurmitola General Hospital with suspicion of neonatal sepsis. The sample included blood samples of neonates, sociodemographic data, and medical history of both neonates and their mothers. The total sample size is 60 where 20 samples only have sociodemographic and medical history-related data of neonates and their mothers.

6.3 Inclusion and Exclusion Criteria:

Neonates suspected or diagnosed for neonatal sepsis according to established clinical and haematological criteria of IMNCI (Integrated Management of Neonatal and Childhood Illness) and evidence of positive blood culture results were included as cases in this study. Neonates admitted to the NICU of Ad-Din Medical College Hospital (30 samples and questionnaire), BIRDEM Women and Children Hospital (10 samples and questionnaire), and Kurmitola General Hospital (20 questionnaire data) with a suspicion or diagnosis of neonatal sepsis were included. Neonates admitted to the NICU of these hospitals who were not suspected or diagnosed with neonatal sepsis were excluded from the study.

6.4 Data and Sample Collection:

Following the institution's ethical clearance, mothers and guardians of patients were contacted to obtain their written or verbal agreement for the collection of data and blood samples. Trained paediatric nurses took 0.5 ml blood samples from the clinically suspicious neonates after obtaining written or verbal consent from the mothers of the patients. The BRAC University Life Sciences Laboratory processed the blood samples, which were transported in a 2 ml blood collection tube containing lithium-heparin. The newborns' medical histories, including their Apgar score, birth weight, gestational age, and antibiotic use, were gathered from their medical records in addition to their blood samples. Additionally, prenatal data were gathered from the mothers of the neonates via a phone interview, in-person interview, and review of their medical records using a structured questionnaire. Premature rupture of membrane (PROM), meconium-stained amniotic fluid, foul-smelling amniotic fluid, prolonged labour, urinary tract infection (UTI), preeclampsia, sexually transmitted infection, intrauterine infection, ante-partem haemorrhage, ante-partem fever, and intrapartum antibiotic given four hours before delivery,

and age was among the antenatal factors. These potential risk factors were selected using information from previous studies [31–40].

6.5 Independent Variable:

Age, parity, delivery mode, delivery location, length of rupture of membrane, prolonged labor, UTI, preeclampsia, STD, intrauterine infection, ante-partem haemorrhage, ante-partem fever, intrapartum antibiotic given 4 hours before delivery, premature rupture of membrane (PROM), meconium-stained amniotic fluid, foul-smelling amniotic fluid, and neonatal factors (age, sex of neonate, prematurity (gestational age), weight at birth, and APGAR score) were the variables that were considered independent.

6.6 Blood culture:

500 microliters of the blood sample were enhanced in 5 millilitres of Nutrient Broth/Peptone buffer and incubated at 37 degrees Celsius for 24 hours after the blood was brought to the laboratory. In parallel, 100 microliter blood samples were immediately added to nutritional agar media and allowed to incubate for a full day. This was carried out to confirm and monitor the presence of a blood infection caused by bacteria. The samples were diluted up to 10-2 times in 0.9% saline water after being enriched in the buffer. Following dilution, 100 microliter samples were divided among five distinct selective media: Mannitol Salt Agar with 7.5% NACL, High chrome KPN, Cetrimide Agar, Mannitol Salt Agar, and EMB-Levine media. Every plate was incubated at 37 degrees Celsius for a whole day. The target bacterial pathogens for this investigation were Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Following a 24-hour incubation period, the kind of pathogen was inferred by looking at the colony's form and colour on the specific media.

6.7 Stock Preparation:

After the initial assumption of the pathogenic organism based on morphological observation, a stock sample was prepared using T1N1 (1% tryptone, 1% NACL, and 2% agar) media. A 24-hour fresh bacterial culture on nutrient agar media was used for stock preparation. The 24-hour bacterial cultures were inoculated into T1N1 media by strapping with a needle the incubated for 24 hours. After that paraffin oil was added then the vial/Eppendorf tube was covered with parafilm and kept at room temperature.

6.8 DNA Extraction and Polymerase Chain Reaction (PCR):

DNA was isolated using a boiling technique from a 24-hour culture in Nutrient Agar (NA) media. One loopful of 24-hour NA culture was inoculated in 150 microliters of TE buffer and then vortexed. After that, the mixture is heated up to 95 degrees Celsius for 20 minutes using a heat block machine. Then the mixture is centrifuged at 10,000 rpm for 10 minutes at 4 degrees Celsius. Finally, the supernatant was collected in a different Eppendorf tube and stored at -20 degrees Celsius. After initial identification by observing the colony morphology of the organisms, a species-specific PCR has been done to confirm the molecular-level identification of the isolates.

6.8.1 PCR Conditions for *Klebsiella pneumoniae*:

For Kle*bsiella pneumonia* KPN forward and KPN reverse primers were used with 133bd product size. The total volume of the reaction mixture was 13 microlitres where Target DNA was 2 microliters and PCR mixture was 11 microliters. The PCR mixture included 0.5 microliters each of the 10x forward and reverse primers, 6.5 microliter master mix, and 3.5 microliter nuclease-free water. The reaction mixture was run for 30 cycles with initial temperature and denaturation temperature at 94 degrees Celsius for 10 minutes and 30 sec respectively, followed by an annealing step at 60 degrees Celsius for 45 sec, then an elongation step, and final elongation step at 72 degrees Celsius for 45 sec and 10 minutes respectively [102].

6.8.2 PCR Conditions for *Pseudomonas aeruginosa*:

For *Pseudomonas aeruginosa* PA-SS forward and PA-SS reverse primers were used with 956 bd product size. The total volume of the reaction mixture was 15 microliters where Target DNA was 3 microliters and PCR mixture was 12 microliters. The PCR mixture included 1.5 microliters of each of the 10x forward and reverse primers, 7.5 microliter master mix, and 1.5 microliters nuclease-free water. The reaction mixture was run for 30 cycles with an initial temperature of 95-degree Celsius and denaturation temperature of 94 degrees Celsius for 2 minutes and 20 sec respectively, followed by an annealing step at 58 degrees Celsius for 20 sec, then an elongation step and final elongation step at 72 degrees Celsius for 40 sec and 1 min respectively [103].

6.8.3 PCR Conditions for *E. coli*:

For *E. coli* ECO forward and ECO reverse primers were used with 585 bd product size. The total volume of the reaction mixture was 13 microliters where Target DNA was 2 microliters and PCR mixture was 11 microliters. The PCR mixture included 0.5 microliters each of the 10x forward and reverse primers, 6.5 microliter master mix, and 3.5 microliter nuclease-free water. The reaction mixture was run for 30 cycles with the initial temperature at 95-degree Celsius and denaturation temperature at 94 degrees Celsius for 3 minutes and 45 sec respectively, followed by an annealing step at 58 degrees Celsius for 45 sec, then an elongation step and final elongation step at 72 degrees Celsius for 60 sec and 3 min respectively [104].

6.8.4 Data Visualization Using Gel Electrophoresis:

PCR products were run in gel electrophoresis using 2% agarose gel at 110 volts for 60 minutes visualized under UV light.

6.9 Antibiotic Susceptibility Test:

The Kirby-Bauer disc diffusion method [103] was used three times for the Antimicrobial Susceptibility Test (AST) to establish the susceptibility patterns following CLSI recommendations [104]. Cefepime, Meropenem, Levofloxacin (5 micrograms), Amikacin (30 micrograms), Ciprofloxacin (5 micrograms), Azithromycin (30 micrograms), Ceftazidime (30 micrograms), and Amoxillin (30 micrograms) were among the antimicrobials whose susceptibility was assessed. Multidrug resistance (MDR) was defined as the inability to respond to at least one agent in three or more antimicrobial groups [105].

6.9.1 PCR Amplification of Antibiotic Resistance Genes *blaNDM, blaIMP, blaVIM, blaSPM, blaKPC, and blaOXA_48*:

For the amplification of resistant genes, both forward and reverse primers were used 0.5 µ where PCR was performed where total volume was 20 µl with 5 µl of extracted DNA, 7.5 µl of nuclease-free water, 6.5 µl of PCR master mix. In terms of blaKPC and blaOXA_48, 8.5 µl of nuclease-free water and 5.5 µl of PCR master mix were used. The PCR conditions were set up for 35 cycles, with five minutes of denaturation at 95°C, 45 seconds of annealing and denaturation at 94°C and 60°C, respectively, and one minute of elongation at 72°C. Except for the annealing temperature, which was changed to 55°C for 45 seconds for blaKPC and blaOXA_48. All primer sets also received a final extension of 72°C for 7 minutes. [106].

6.9.2 PCR Amplification of Antibiotic Resistance Genes blaTEM:

A total of 13 µl of reaction volume was used for the PCR, which included 0.5 µ of the forward and 0.5 µ of the reverse primer for each gene. 5 µl of PCR master mix (Maximum PCR Premix Kit i-Taq, iNtRON, Biotechnology, Korea) was mixed with 2 µl of extracted DNA and 6 µl of distilled water. The PCR apparatus was set up for 35 cycles, comprising an initial denaturation phase that lasted for 3 minutes at 94°C, denaturation and annealing that lasted for 30 seconds at 94°C and 50°C, respectively, and elongation that lasted for 2 minutes at 72°C. Finally, all primer sets had a final extension that lasted for 7 minutes at 72°C [106].

6.9.3 PCR Amplification of Antibiotic Resistance Genes blaSHV and blaCTX:

The volume was 13 µl with 0.5 µ of the forward and 0.5 µ of the reverse primer for each gene. 5 µl of PCR master mix was mixed with 2 µl of extracted DNA and 6 µl of distilled water. The PCR conditions were set up for thirty cycles: five minutes of initial denaturation at 94°C, thirty seconds of denaturation and annealing at 94°C and 50°C, respectively, thirty minutes of elongation at 72°C, and seven minutes of final extension at 72°C [106].

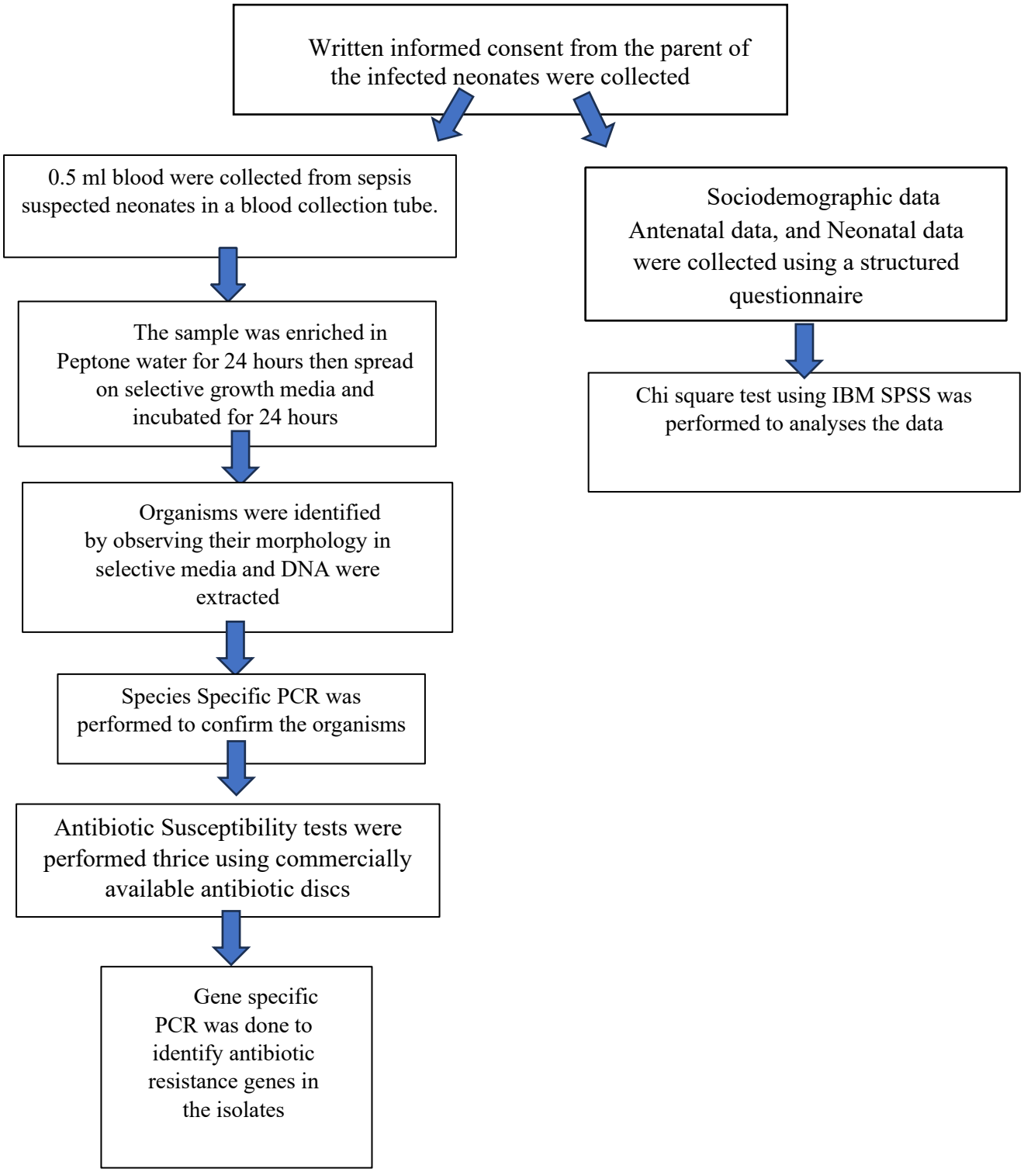
6.9.4 PCR Product Visualization:

The PCR results were seen under a UV lamp after being processed through gel electrophoresis on a 2% agarose gel at 110 volts for 60 minutes.

6.10 Statistical Analysis:

Microsoft Excel and SPSS (IBM version 24.0) were used for all statistical analyses. To identify the risk components for newborn sepsis in all instances, statistical analysis was performed. The chi-square test was used to compare distinctions between groups concerning the outcome variables.

6.10.1 Methodology Outline:



Chapter-7

Results and Analysis

7.1 Sociodemographic Characteristics of the Participants:

A total of 60 neonates (30 from Ad-Din Medical College Hospital, 10 from BIRDEM Women and Children Hospital, and 20 from Kurmitola General Hospital) were included, providing a 100% response rate. Regarding maternal education, 46.7% (28) respondents were Graduates, and 31.7% (19) had high school education. Further, 56.7% (34) were housewives by occupation whereas only 33.3% (20) were doing jobs. The majority (83.3%, 50) of the cases lived in cities. In terms of neonate's age, 48.3% (29) were found under the age of 3 days and 23.3% (14) aged between 3-4 days only. Moreover, most of the neonates were male (68.3%, 41). (see Table 4).

Table 4: Socio-Demographic Characteristics of Neonates and their mothers

Variable	Parameter	Frequency	Percentage	Total Frequency
Neonate's Gender	**Male**	41	68.3	60
	Female	18	30	
Neonatal Age	**<3**	29	48.3	60
	3-7	14	23.3	
	8-11	8	13.3	
	12-16	4	6.7	
	>16	4	6.7	
Maternal Age	**15-20**	10	16.7	60
	21-25	16	26.7	
	26-30	20	33.3	
	31-35	13	21.7	
Maternal Education	**Primary**	0	0	60
	Secondary	6	10	
	Higher Secondary	19	31.7	
	Graduate	28	46.7	
	Post Graduate	6	10	
Mother's Occupation	**Housewife**	34	56.7	60
	Service Holder	20	33.3	
	Business	5	8.3	
	Others	0	0	
Habitation	**Urban**	50	83.3	60
	Rural	10	15	

7.2 Clinical Characteristics of Neonates:

More than half (55%, 33) of the neonates had an early preterm birth with a gestation age of fewer than 34 weeks. Late Pre-Term and Term delivery have been recorded in 15% (9) and 28.3 (17) cases respectively. Regarding the birth weight, the majority of the neonates had a very low birth weight between 1-2 kilograms (53.3%, 32), and the rest were delivered with a weight between 2-3 kilograms (45%,27). The mean gestational age and birth weight were 37.4 ± 2.24 weeks and 1.9 ± .5 kilograms, respectively. In comparison to those who scored ≥7 (30%, 18) in the first minute of life, a greater percentage of newborns (68.3%, 41) had an APGAR score of less than 7. In regard to delivery method and place, almost all the participants were born via C-section (85.3%,51) at the hospital (97%, 58). The majority proportion of the neonates had the onset of illness within the first 7 days of their birth (60%,36). Most of the participants had already started their antibiotic treatment at the time of data collection (85%, 50). (See Table 5).

Table 5: Clinical Characteristics of Neonates

Variable	Parameter	Frequency	Percentage	Total
Neonate Age	3-7	29	48.3	60
	8-11	14	23.3	
	12-16	8	13.3	
	>16	4	6.7	
Gestational Age	Early Preterm (<34 weeks)	33	55	60
	Late preterm (≥34- <37 weeks)	9	15	
	Term (≤37 - <42 weeks)	17	28.3	
Birth Weight (kg)	1-2	32	53.3	60
	2-3	27	45	
Apgar Score (1 min)	<7	41	68.3	60
	≥7	18	30	
Antibiotic Treatment Given	YES	50	85	60
	NO	10	15	
Mode of Delivery	C-Section	51	85.3	60
	Vaginal Delivery	9	14.7	
Place of Delivery	Hospital	58	97	60
	Home	2	3	
Day of Onset of Illness	0-3	36	60	60
	4-28	24	40	

7.3 Pregnancy related complications of Neonatal Mothers:

Fifty-five percent (33) of the mothers were multiparous which means they were giving birth more than once. Further none of the mothers had prolonged labour (more than 24 hours). In this study, 30% (18) of the mothers have suffered from genital bleeding during the third trimester of their pregnancy (Ante-partum haemorrhage). It was also realized 36.7% (22) of mothers had meconium-stained amniotic fluid during birth indicating the chances of infection in infants. Further, only 15% (10) of the mothers had foul smelling of amniotic fluid during pregnancy. Moreover, 30% (18) of mothers had fever during their delivery (Table 6).

Table 6: Pregnancy and Obstetric History of Neonatal Mothers

Variable	Response	Frequency	Percentage (%)	Total Number
Parity	Multiparous	34	55	60
	Primiparous	26	45	
Prolonged Labor (24 hours)	YES	0	0	60
	NO	60	100	
Ante-partum Haemorrhage	YES	18	30	60
	NO	42	70	
Meconium-Stained Amniotic Fluid	YES	22	36.6	60
	NO	38	63.3	
Foul smelling of amniotic fluid	YES	10	15	60
	NO	50	85	
Intrapartum Fever	YES	18	30	60
	NO	42	70	
Premature Rupture of Membrane (PROM)	YES	20	35	60
	NO	40	65	
Urinary Tract Infection	YES	34	55	60
	NO	26	45	
Sexually Transmitted Infection	YES	0	0	60
	NO	60	100	
Preeclampsia	YES	27	45	60
	NO	33	55	
Gestational Diabetes	YES	18	30	60
	NO	42	70	
Administration of Antibiotic before delivery	YES	42	70	60
	NO	18	30	

In terms of premature rupture of membrane (PROM), 35% (20) of the mothers had it. Regarding infections, urinary tract infection (UTI) was recorded in more than half (55%, 33) of the mothers during the third trimester of their pregnancy whereas none of the women bore any sexually transmitted infections. Following that, 45% (27) and 30% (18) of the mothers suffered from preeclampsia and gestational diabetes respectively during their pregnancy. Finally, almost three-fourths of the mothers were given antibiotic drugs 4 hours before their delivery (Table 6).

7.4 Maternal Risk Factors for the Onset of Neonatal Sepsis:

The independent variables include maternal age, parity, prolonged labour, Antepartum Haemorrhage, Meconium-Stained Amniotic Fluid, Foul smelling of amniotic fluid, Intrapartum Fever, Premature Rupture of Membrane (PROM), Urinary Tract Infection (UTI), Sexually Transmitted Infection (STI), Preeclampsia, Gestational Diabetes, Administration of Antibiotic before delivery whereas dependent variable is the occurrence of neonatal sepsis amongst the newborns. The proposed null hypothesis is ‘There is no association between the independent variables and dependent variable’ and the alternative hypothesis is ‘There is an

association between independent variable and dependent variable.' To test the hypothesis chi-square value has been calculated along with the p-value. Following that, a phi measure has been done to calculate the effect of the association between the two variables. The predetermined p-value is 0.05. If the calculated p-value is recorded at less than 0.05 then our observed value will be statistically significant thus the proposed null hypothesis will be rejected. Therefore, an association will be proposed between the two variables. Further, if the observed chi-square value exceeds the critical chi-square value with a degree of freedom (df) 1 and 5% level of significance then an association between the variables is proposed. The effect size of the association can be measured by phi score which ranges between 0-1. The higher the phi measure, the stronger the association is.

Table 7: Maternal Risk Factor of Neonatal Sepsis

Risk Factors	Chi-square Test Observed Value	Chi-Square Critical Value	df	p-value	Phi Measure (Effect)
Maternal Age	1.92	3.84	1	0.092	0.61
Parity	4.33	3.84	1	0.012	0.98
Antepartum haemorrhage	4.22	3.84	1	0.035	0.5
Meconium-Stained Amniotic fluid	5.33	3.84	1	0.036	0.71
Foul Smelling of Amniotic Fluid	1.98	3.84	1	0.61	0.21
Administration of Intrapartum Antibiotic	1.33	3.84	1	0.063	0.23
Intrapartum Fever	1.98	3.84	1	0.072	0.29
Premature Rupture of Membrane (PROM)	3.21	3.84	1	0.063	0.12
Urinary Tract Infection	5.81	3.84	1	0.022	0.98
Preeclampsia	4.1	3.84	1	0.033	0.68
Gestational Diabetes	5.71	3.84	1	0.045	0.56

df: degree of freedom

Six of the eleven variables had a significant overall impact on the risk of newborn sepsis at the 5% level of significance, according to the chi-square association test. The risk of newborn sepsis was significantly correlated with maternal parity (p=0.012, Phi=0.98), with a Chi-square value of 4.33. Additionally, the study discovered a moderate statistical relationship between antepartum haemorrhage and neonatal sepsis, with the phi measure reported at 0.5 (p=0.035; Chi-square value = 4.22). The study also discovered that most cases—33, or 55%—were urinary tract infections in the third trimester of pregnancy in women. These infections had a significant impact on the development of newborn sepsis, with an effect size of 0.98 (p-value=0.022; chi-square value=5.81). Preeclampsia, or high blood pressure during pregnancy, increases the risk of sepsis in newborns more than moderate risk does (p=0.033; chi-square value= 4.1; phi=0.68). Subsequent investigation showed a moderate relationship between the incidence of sepsis and neonates born to women with diabetes during the index pregnancy (p-

value=0.45; chi square=5.71; phi=0.56). Finally, there was a significant correlation between the risk of newborn sepsis and amniotic fluid stained with meconium (p=0.036; chi square= 5.33; phi= 0.71). (Table 7).

7.5 Neonatal Risk Factor for the Onset of Neonatal Sepsis:

Applying the chi-square association test, the low birth weight, Apgar score, and gestational, mode of delivery showed significant effect on the risk of neonatal sepsis. APGAR scores in the first (*p*=0.022; chi square= 4.56; phi= 0.855) are strongly associated with the development of sepsis in newborns. Apgar score lower than 7 provides a greater chance of sepsis development amongst neonates. The probability of developing neonatal sepsis increased with lower birth weight of neonates with a whooping effect size of 0.996 (p-value=0.036; chi square= 4.1). There was no evident pattern in the probability of developing neonatal sepsis based on delivery place.

Table 8: Neonatal Risk Factors for the Onset of Neonatal Sepsis

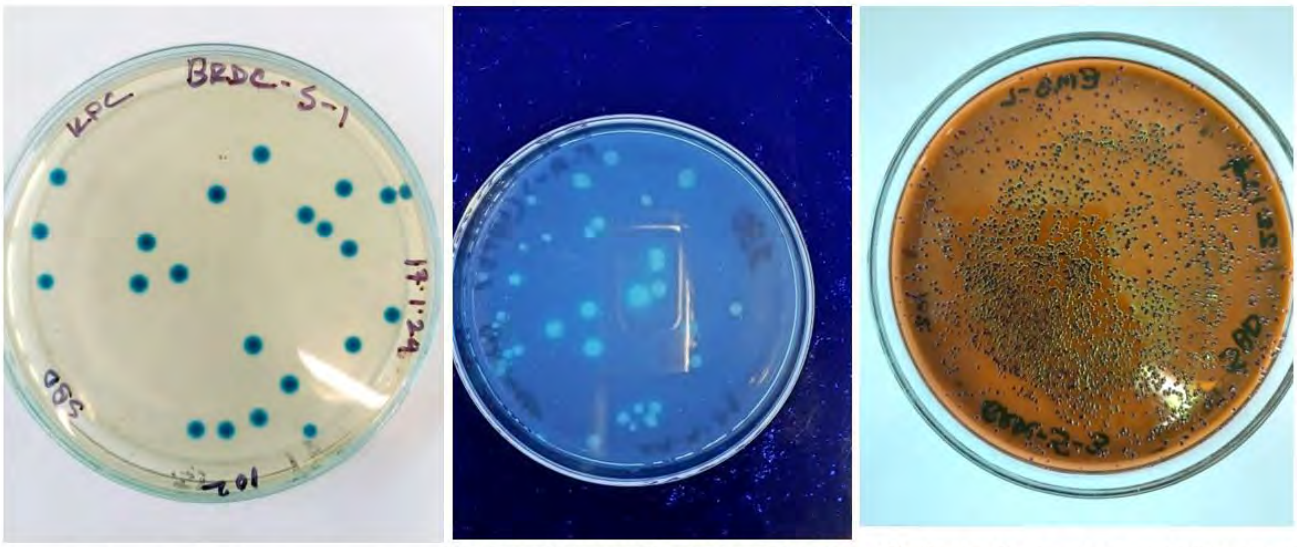
Gestational Age	Chi-square Test Observed Value	Chi-Square Critical Value	df	p-value	Phi Measure (Effect)
Birth Weight	4.1	3.84	1	0.036	0.996
Apgar Score	4.56	3.84	1	0.022	0.855
Mode of Delivery	4.4	3.84	1	0.023	0.569
Place of Delivery	1.37	3.84	1	0.044	0.115
Gestational Age	3.94	3.84	1	0.001	0.864
Neonate Gender	5.23	3.84	1	0.003	0.63

df: Degree of Freedom

In this study, most of the participating neonates were male (68.3%) (Table 3) providing a strong association between gender and sepsis occurrence with an effect size of 0.63 (p-value=0.003; chi square= 5.23). Finally, the Caesarean delivery method was recorded in almost all the cases (85.3%) (table 3) with a moderate influence on sepsis development (p-value= 0.023; chi square= 4.4; phi=0.569) (Table 8).

7.6 Causative Organism of Neonatal Sepsis:

From blood samples of probable sepsis-affected newborns, Escherichia coli was the most isolated gram-negative bacteria (19, 47.5%), followed by Klebsiella pneumoniae (16, 40%) and Pseudomonas aeruginosa (16, 40%) (Table 7). Pseudomonas aeruginosa was most frequently isolated from late-onset (8–16 days) newborn sepsis patients, while Klebsiella pneumoniae and Escherichia coli were the most frequent causes of early-onset neonatal sepsis (0–7 days) (Table 9).



A) Blue colonies indicating the presence of *Klebsiella pneumoniae* in High chrome KPC media

B) Green fluorescent colonies indicating the presence of *Pseudomonas aeruginosa* in Cetrimide Base Agar media

C) Green Sheen colonies indicating the presence of *E. coli* in EMB- Levine media

Figure 6: Culture Positive Isolates in Selective Media

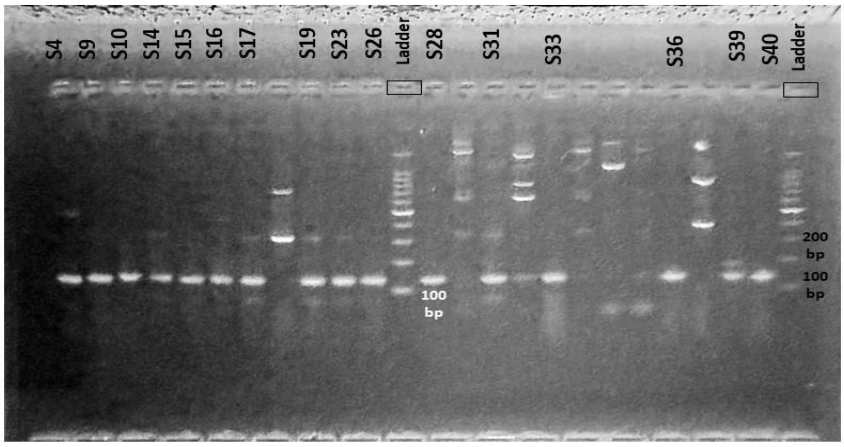


Figure 7: On 2% agarose gel electrophoresis, the KPN gene was amplified using polymerase chain reaction (PCR). Lanes 12 and 24 contain a 100 bp DNA ladder. Samples 4, 9, 10, 14, 15, 16, 17, 19, 23, 26, 28, 31, 33, 36, 39, and 40 are Klebsiella pneumoniae positive samples with a typical band size of (133 bp).

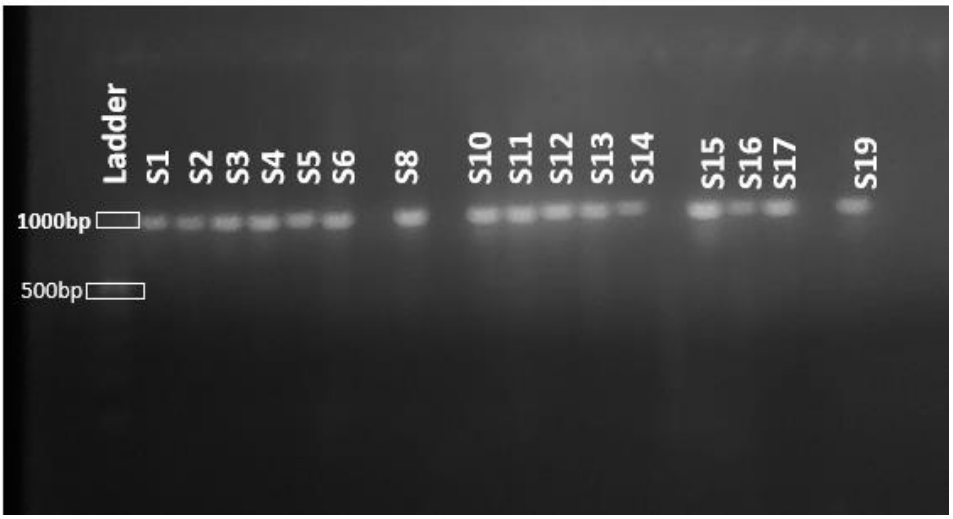


Figure 8: PA-SS gene amplification using Polymerase Chain Reaction (PCR) on 2% agarose gel electrophoresis: Samples 1, 2, 3, 4, 6, 8, 10, 11, 13, 14, 15, 16, 17, and 19 are Pseudomonas aeruginosa positive samples with a typical band size of (956 bp), as shown in lane 1 of the DNA ladder.

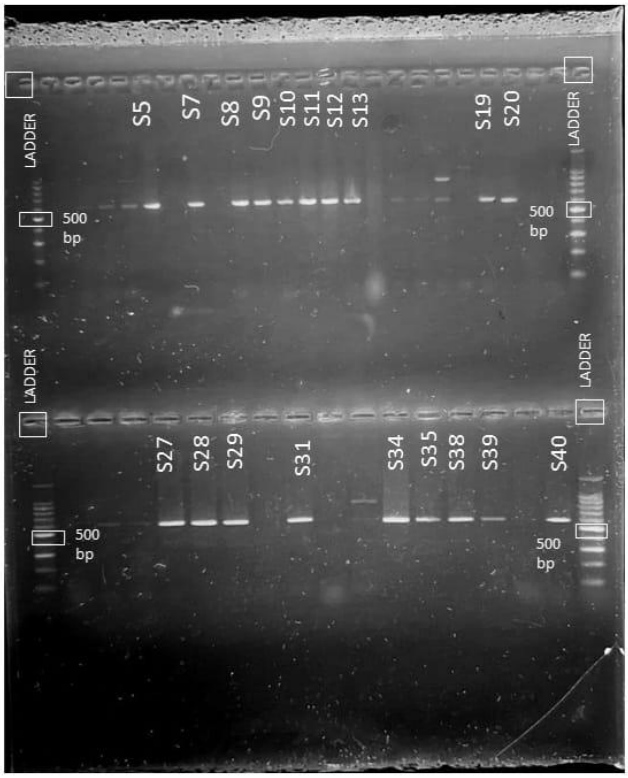


Figure 9: The ECO1 gene was amplified using polymerase chain reaction (PCR) on 2% agarose gel electrophoresis. Samples 5, 7, 8, 9, 10, 11, 12, 13, 19, 20, 27, 28, 29, 31, 34, 35, 38, 39, and 40 are E. coli-positive samples with a typical band size of (585 bp).

Table 9: Causative Organism of Neonatal Sepsis

Pathogenic Organism	Presence	Frequency	Percentage	Total Number
Klebsiella pneumoniae	Yes	16	40	40
	NO	24	60	
Pseudomonas aeruginosa	YES	16	40	40
	NO	24	60	
E. coli	YES	19	47.5	40
	NO	21	52.5	

Further, the highest percentage of *E. coli followed* (73.7%) *by Klebsiella pneumoniae* (50%) then *Pseudomonas aeruginosa* were isolated from cases with early preterm delivery. However, in terms of birth weight, most of the *Pseudomonas aeruginosa* isolates (56.25%) were found in the blood samples of patients with a birth weight between 1-2 kg whereas *Klebsiella pneumoniae* (68.75%) was prominently found in neonates with 2-3 kg birth weight.

Table 10: Causative Organism Found in Different Neonatal Characteristics

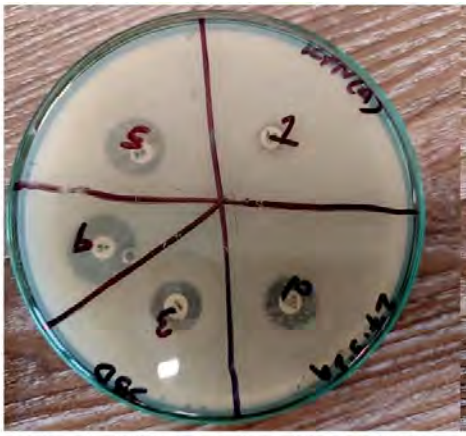
Organism	**Day of Onset Total N (%)**		**Gestational Age Total N (%)**			**Birth weight (kg) Total N (%)**		**Apgar Score Total N (%)**	
	0-3	**4-28**	**Early Preterm**	**Late Preterm**	**Term**	**1-2**	**2-3**	**>7**	**<7**
Klebsiella pneumoniae	12(75)	4(25)	8 (50)	5 (31.25)	3(18.75)	5 (31.25)	11(68.75)	5(31.25)	11(68.75)
Pseudomonas aeruginosa	9(56.25)	7(43.75)	6(37.5)	5 (31.25)	5(31.25)	9 (56.25)	7(43.75)	13(81.25)	3(18.75)
E. coli	14(87.5)	5(26.31)	14(73.7)	3 (15.8)	2 (10.5)	7(36.84)	12(63.16)	6(31.58)	13(68.42)

Moreover, the highest proportions of *Klebsiella pneumoniae* (68.75%) and *E. coli* (68.42%) were the causative agents of neonatal sepsis in cases with Apgar score lower than 7, conversely, *Pseudomonas aeruginosa* (81.25%) was dominant in cases with greater than 7 scores (Table 10).

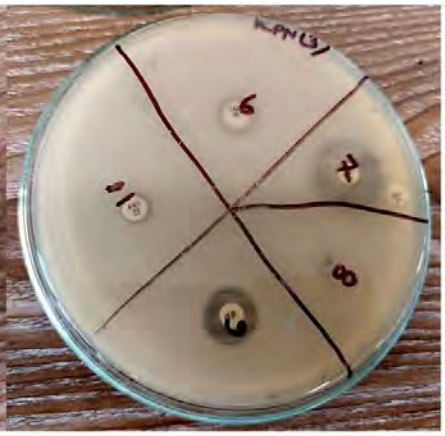
7.7 Antibiotic Resistance Pattern:

Klebsiella pneumoniae was found to be highly resistant to Ciprofloxacin (5 µg) (100, 16), Colistin (10 µg) (93.75%, 15), Cefepime (30 µg) (87.5%, 14), Ceftazidime (30 µg) (87.5&, 14), Meropenem (10 µg) (75%, 12), Amoxillin (30 µg) (75%, 12) (Table 9). More than 50% *Klebsiella pneumoniae* isolates showed resistance towards 8 out of 10 antibiotic drugs (Figure 11). Following that, *Pseudomonas aeruginosa* was extremely resistant to Ceftazidime (30 µg) (81.25%, 13) and Amoxillin (30 µg) (68.75%, 11) whereas half of the isolates were no susceptible to Azithromycin (30 µg) (50%, 8) (Figure 12 (B)). Similarly, almost three-fourth quarter of *E. coli* showed resistance to Ceftazidime (30 µg) (73.68%, 14) and trimethoprim-sulfamethoxazole 8 (100%). E. coli was resistant to Colistin (10 µg) and Amoxillin (30 µg) at the rate of 1 (5.26%) and 2 (10.52%) respectively (Figure 12 (A)). On the other hand, almost all identified bacteria showed least resistance rate to Levofloxacin (5 µg) (Table 9) whereas all the *Pseudomonas aeruginosa and E. coli* isolates showed sensitivity *towards* Meropenem (10

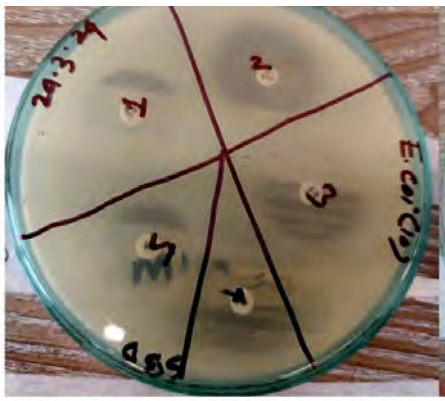
µg), Piperacillin/Tazobactam (100/10 µg), Amikacin (30 µg), and Ciprofloxacin (5 µg) (Table 10)



A) *Klebsiella pneumoniae*: Antibiotic Susceptibility Disc Diffusion Test on Muller-Hinton Agar media; Numbers 1, 2, 3, 4, 5 are representing antibiotic drugs Cefepime (30 µg), Meropenem (10 µg), Piperacillin/Tazobactam (100/10 µg), Levofloxacin (5 µg), Amikacin (30 µg)

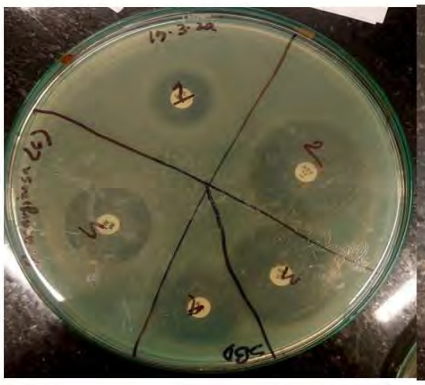


B) *Klebsiella pneumoniae*: Antibiotic Susceptibility Disc Diffusion Test on Muller-Hinton Agar media; Numbers 6, 7, 8, 9, 10 are representing antibiotic drugs Colistin (10 µg), Ciprofloxacin (5 µg), Azithromycin (30 µg), Ceftazidime (30 µg), Amoxillin (30 µg) respectively

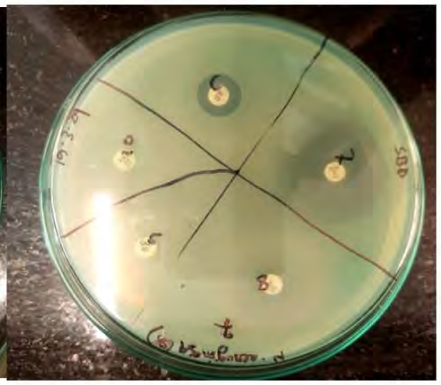


C) *E. coli* : Antibiotic Susceptibility Disc Diffusion Test on Muller-Hinton Agar media; Numbers 1, 2, 3, 4, 5 are representing antibiotic drugs Cefepime (30 µg), Meropenem (10 µg), Piperacillin/Tazobactam (100/10 µg), Levofloxacin (5 µg), Amikacin (30 µg) respectively

D) *E. coli*: Antibiotic Susceptibility Disc Diffusion Test on Muller-Hinton Agar media; Numbers 6, 7, 8, 9, 10 are representing antibiotic drugs Colistin (10 µg), Ciprofloxacin (5 µg), Azithromycin (30 µg), Ceftazidime (30 µg), Amoxillin (30 µg) respectively



E) *Pseudomonas aeruginosa*: Antibiotic Susceptibility Disc Diffusion Test on Muller-Hinton Agar media; Numbers 1, 2, 3, 4, 5 are representing antibiotic drugs Cefepime (30 µg), Meropenem (10 µg), Piperacillin/Tazobactam (100/10 µg), Levofloxacin (5 µg), Amikacin (30 µg) respectively



F) *Pseudomonas aeruginosa*: Antibiotic Susceptibility Disc Diffusion Test on Muller-Hinton Agar media; Numbers 6, 7, 8, 9, 10 are representing antibiotic drugs Colistin (10 µg), Ciprofloxacin (5 µg), Azithromycin (30 µg), Ceftazidime (30 µg), Amoxillin (30 µg) respectively

Figure 10: Antibiotic Susceptibility Disc Diffusion Test of Isolated Organisms on Muller-Hinton Agar Media

Table 11: Antibiotic Resistance Pattern of Neonatal Sepsis Causative Agents:

Sl	Antibiotic	Organism								
		Klebsiella pneumonia (n=16)			*Pseudomonas aeruginosa (n=16)*			*E. coli (n=19)*		
		R n (%)	**I n (%)**	**S n (%)**	**R n (%)**	**I n (%)**	**S n (%)**	**R n (%)**	**I n (%)**	**S n (%)**
1	**Cefepime (30 µg)**	14 (87.5)	0	2 (12.5)	3 (18.75)	9 (56.25)	4 (25)	1 (5.26)	2 (10.52)	16 (84.21)
2	**Meropenem (10 µg)**	12 (75)	3 (18.75)	1 (6.25)	0	0	16 (100)	0	1 (5.26)	18 (94.73)
3	**Piperacillin/Tazobactam (100/10 µg)**	10 (62.5)	2 (12.5)	4 (25)	0	1 (6.25)	15 (93.75)	0	0	19 (100)
4	**Levofloxacin (5 µg)**	6 (37.5)	2 (12.5)	8 (50)	0	2 (12.5)	14 (87.5)	0	0	19 (100)
5	**Amikacin (30 µg)**	9 (56.25)	2 (12.5)	5 (31.25)	0	0	16 (100)	0	0	19 (100)
6	**Colistin (10 µg)**	15 (93.75)	1 (6.25)	0	2 (12.5)	2 (12.5)	12 (75)	1 (5.26)	2 (10.52)	16 (84.21)
7	**Ciprofloxacin (5 µg)**	16 (100)	0	0	0	0	16 (100)	0	3 (15.79)	16 (84.21)
8	**Azithromycin (30 µg)**	13 (81.25)	2 (12.5)	1 (6.25)	8 (50)	0	8 (50)	3 (15.79)	4 (21.05)	12 (63.15)
9	**Ceftazidime (30 µg)**	14 (87.5)	2 (12.5)	0	13 (81.25)	3 (18.75)	0	14 (73.68)	2 (10.52)	3 (15.79)
10	**Amoxillin (30 µg)**	12 (75)	3 (18.75)	1 (6.25)	11 (68.75)	1 (6.25)	4 (25)	2 (10.52)	2 (10.52)	15 (78.95)

R= Resistant; S= Sensitive; I= Intermediate

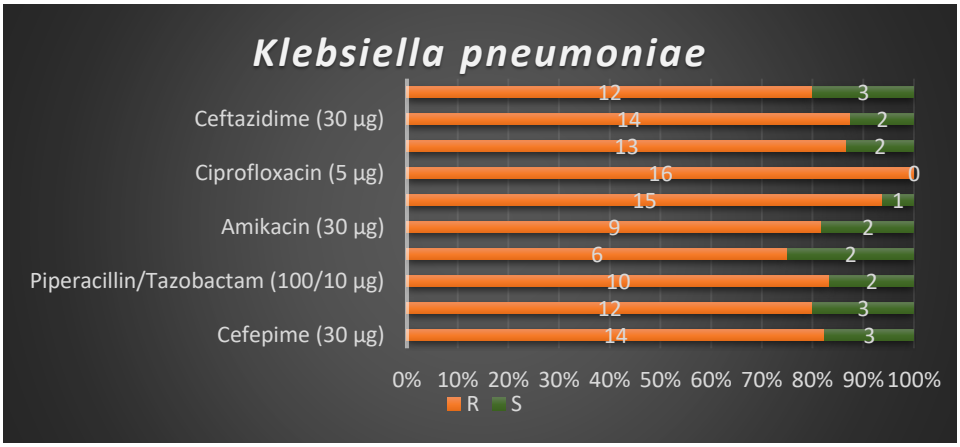
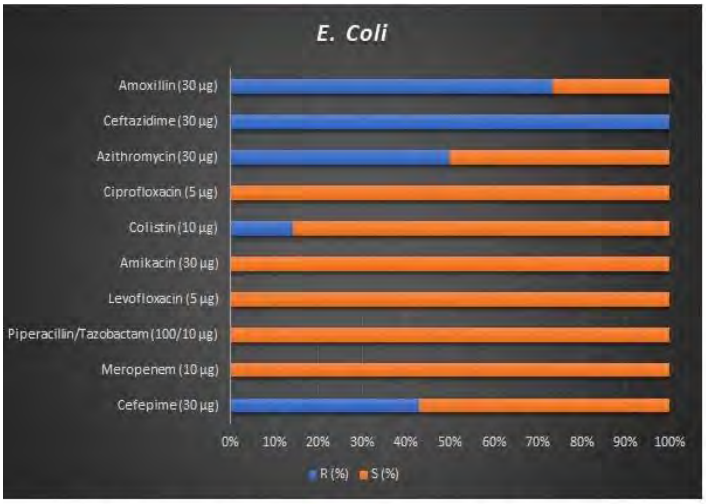


Figure 11: Antibiotic Resistance Pattern of *Klebsiella pneumoniae*



A) Antibiotic Resistance Pattern of *E. coli*

B) Antibiotic Resistance Pattern of *Pseudomonas aeruginosa*

Figure 12: Antibiotic Resistance Patterns of A) *E. coli* and B) *Pseudomonas aeruginosa*

7.8 Multidrug Resistance Pattern of Neonatal Sepsis Causative Organism:

The proportion of *Klebsiella pneumoniae* isolates that were resistant to three or more antimicrobial agent classes was around 67%. Merely 13% of *E. coli* isolates were resistant to three or four different classes of antimicrobial drugs, compared to 20% *of Pseudomonas aeruginosa* isolates that were resistant to three different categories (Figure 13).

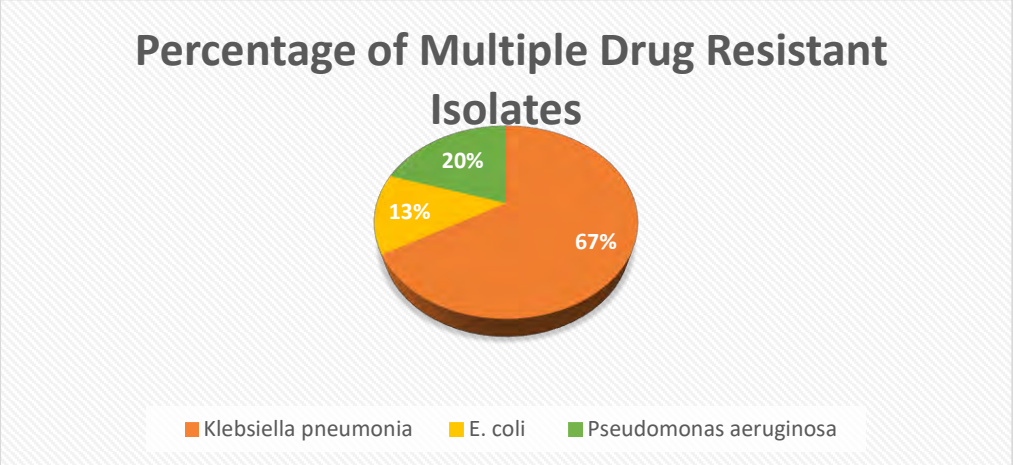


Figure 13: Distribution of Multidrug Resistance Patterns of Isolated Organisms

7.9 Detection of Carbapenem and Beta-Lactamase Resistant Genes:

Sixty-six percent (9/16) of the *Klebsiella pneumoniae* isolates exhibited a single carbapenem resistance gene (blaNDM) according to the PCR assay, while the remaining 62.5% of isolates carried two beta-lactamase resistance genes (blaSPM and blaSVH). Nine isolates (56.25%) were found to be positive for the blaNMD and blaSPM genes, with blaSVH (6.25%) following closely behind. The remaining three genes, blaOXA-48, blaVIM, and blaIPM, blaCTX, blaKPC, and blaTEM, were absent from all isolates (Table 12). Gene product PCR amplification is displayed in Figures 14, 15, and 17. Nevertheless, *E. Coli* carried three genes that were resistant to beta-lactamases: blaSPM, blaTEM, and blaCTX. Five (26.31) *E. coli* isolates possessed the blaSPM gene (Figure 19), while only three (15.78%) had the blaTEM and blaCTX genes (Figures 16, 17). However, only three (18.75%) of the isolates of *Pseudomonas aeruginosa* possessed the single beta-lactamase gene blaVIM and were devoid of the other genes listed in Table 11 (Figure 18).

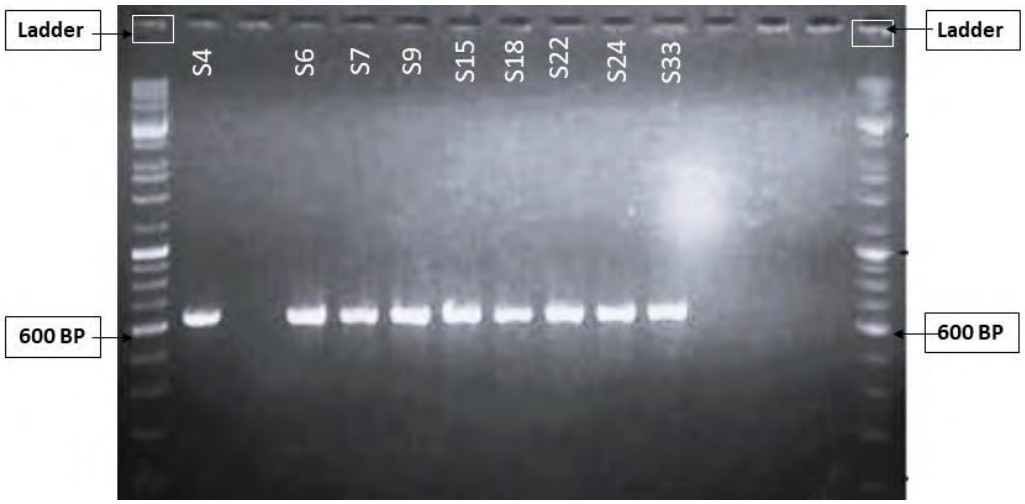


Figure 14: 2% agarose gel electrophoresis using Polymerase Chain Reaction (PCR) to amplify the blaNDM gene: lanes 1 and 14: DNA ladder 1 kb; samples 4, 6, 7, 9, 15, 18, 22, 24, 33 are blaNDM-containing *Klebsiella pneumoniae* isolates with a typical band size of (624bp).

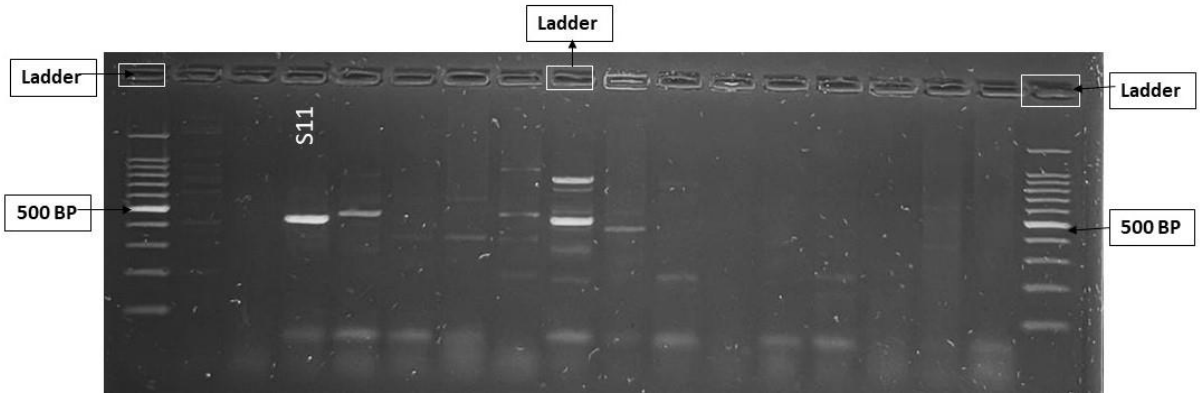


Figure 15: Using a 2% agarose gel electrophoresis, the blaSVH gene was amplified using Polymerase Chain Reaction (PCR). Lanes 1 and 18 had a 100 bp DNA ladder, and samples 11 had blaSVH-containing *Klebsiella pneumoniae* isolates with a typical band size of 450 bp.

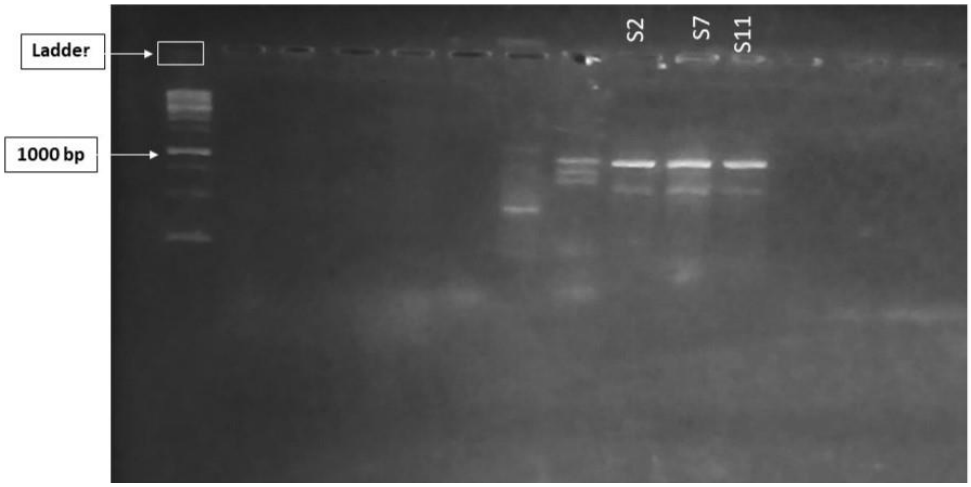


Figure 16: blaTEM gene amplification using Polymerase Chain Reaction (PCR) on 2% agarose gel electrophoresis: Samples 2, 7, and 11 are blaTEM isolates of *E. coli* with a typical band size of 980 bp. Lane 1: DNA ladder 1 kb.

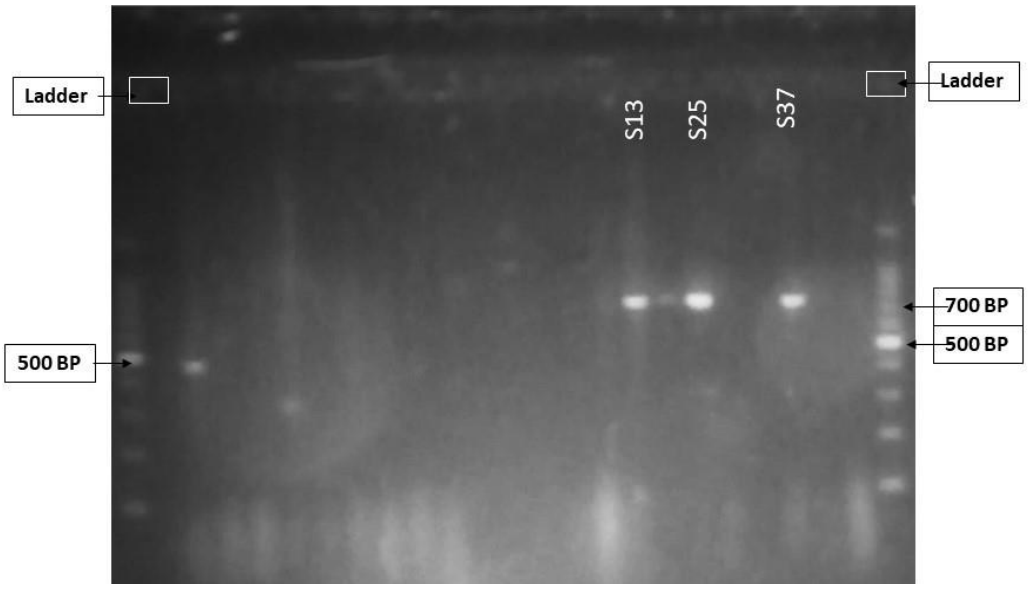


Figure 17: Using 2% agarose gel electrophoresis and Polymerase Chain Reaction (PCR), the blaCTX gene was amplified. Samples 13, 25, and 37 were blaCTX-containing *E. coli* isolates with a mean band size of 759 bp. Lanes 1 and 25 were DNA ladders of 100 bp.

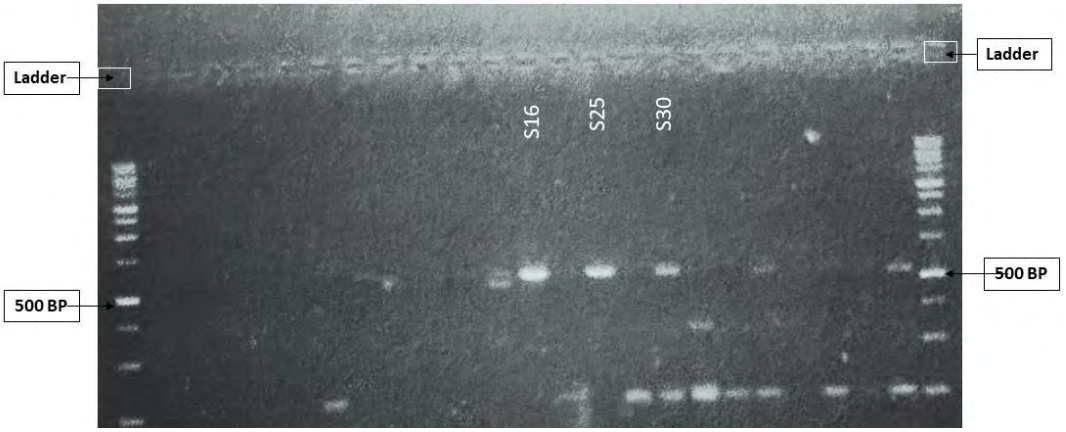


Figure 18: Using 2% agarose gel electrophoresis and Polymerase Chain Reaction (PCR) to amplify the blaVIM gene, samples 16, 25, and 30 are blaVIM-containing isolates of *Pseudomonas aeruginosa* with a typical band size of 502 bp. Lanes 1 and 25 represent DNA ladder 100.

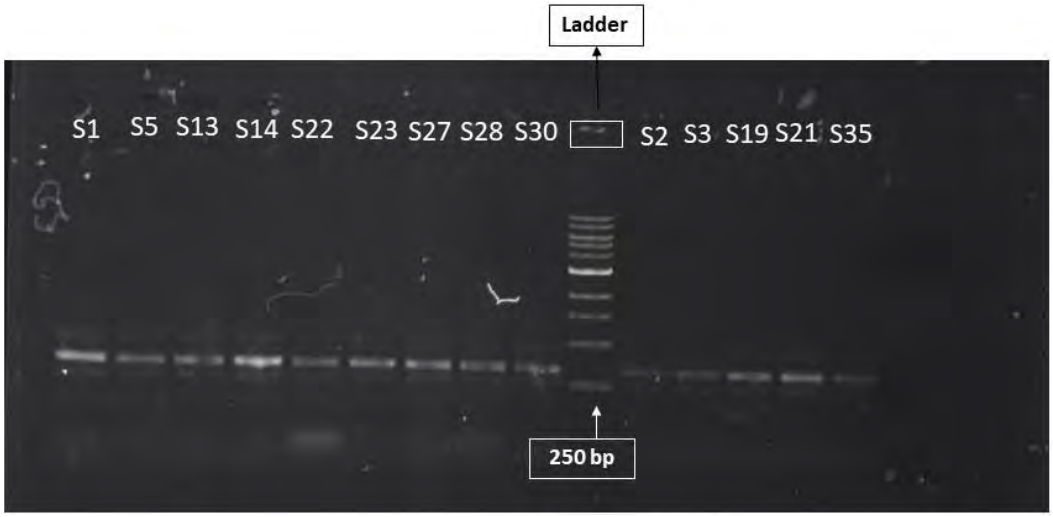


Figure 19: blaSPM gene amplification using Polymerase Chain Reaction (PCR) on 2% agarose gel electrophoresis: lane 10: 1 kb DNA ladder; *Klebsiella pneumoniae* isolates with blaSPM samples 1, 5, 13, 14, 22, 23, 27, 28, and 30 have a typical band size of 271 bp; *E. coli* isolates with blaSPM samples 2, 3, 13, 19, 21, and 35 have a typical band size of 271 bp.

Table 12: The Number of Bacterial Strains Positive for Carbapenem and Beta Lactamase-Resistant Genes.

Antibiotic-Resistant Gene	Organisms		
	Klebsiella pneumoniae ***N=16*** ***n (%)***	***Pseudomonas aeruginosa*** ***N=16*** ***n (%)***	***E. coli*** ***N=19*** ***n (%)***
bla_{SPM}	**9 (56.25%)**	**0**	**5 (26.31)**
bla_{VIM}	**0**	**3 (18.75)**	**0**
bla_{NDM}	**9 (56.25)**	**0**	**0**
bla_{IPM}	**0**	**0**	**0**
bla_{KPC}	**0**	**0**	**0**
bla_{oxa-48}	**0**	**0**	**0**
bla_{CTX}	**0**	**0**	**3 (15.78)**
bla_{SVH}	**1 (6.25)**	**0**	**0**
bla_{TEM}	**0**	**0**	**3 (15.78)**

Chapter-8

Discussion

This study aimed to assess the association between neonatal and maternal risk factors and the emergence of sepsis in newborns admitted to Kurmitola General Hospital, Addin Medical College Hospital, and BIRDEM Women and Child Hospital in Dhaka, Bangladesh, encompassing both early- and late-onset cases. Sixty samples all were collected, of which twenty contained only data pertinent to the sociodemographic and health histories of the mothers and infants. Among the diagnosed neonatal sepsis cases, 60% were found as Early-Onset Neonatal Sepsis (EONS), and 40% were found as Late-Onset Neonatal Sepsis (LONS). However, a prevalence study in Dhaka showed a lower proposition EONS case (35%) [107] On the other hand, similar studies conducted in other lower-middle income countries (LMIC) like India (67%) [108], Nepal [78%] [109], Ethiopia (77%) [110], and Ghana (82%) [111] showed higher EONS prevalence like this study. The study population of this study were neonates mostly from urban settings, especially male participants who were dominant, with a major proportion having early preterm birth and very low birth weight. This demographic profile aligns with findings from similar studies in LMICs, indicating a consistent trend of neonatal vulnerability in resource-constrained environments [108-111].

In terms of maternal risk factors, this study found that neonates whose mothers had urinary tract infections (UTI) during their third trimester had a potential influence on the development of sepsis. In this study, more than half of the patients' mothers had UTIs during their pregnancy. Several meta-analysis studies from around the globe have zeroed in on maternal infection or UTI as one of the potential determinants of neonatal sepsis development. Studies have revealed the possibility of any kind of maternal infection transferring to the newborn through the birth canal or the utero resulting in neonatal sepsis [112]. Further, another key finding of this study is the association of multiparous mothers with neonatal sepsis. This study found that most of the participating mothers were multiparous which means they had given birth once or twice before. Through the chi-square association test, parity stood out as one of the influencing variables on the development of sepsis in infants. Although our findings were consistent with other LMIC countries like India and Ghana [108, 111], a similar etiological factor evaluation study conducted in a tertiary care hospital in Rajshahi, Bangladesh did not find any such association [113].
Furthermore, this study showed that mothers suffering from preeclampsia and gestational diabetes during pregnancy were statistically associated with neonatal sepsis development which aligns with the study findings by Siakwa et al and Shah et al in Ghana and Nepal [114-115]. Studies have found that both preeclampsia and diabetes during pregnancy may weaken the mother's immune system thus interrupting the transfer of antibodies to the baby which makes the baby more immunosuppressant and more prone to any kind of infection after or during birth [114-115]. This study also revealed that maternal history of antepartum hemorrhage and meconium-stained amniotic fluid during pregnancy and delivery are statistically associated with neonatal sepsis. These findings are supported by meta-analyses studies around the world as haemorrhage leads to early pre-term birth, lower birth weight, prematurity, and infections in babies [116]. In terms of meconium-stained amniotic fluid (MSAF), the present study correlated with studies conducted by Unnisa et al. and Becker et al. as meconium influences the bacterial growth in the amniotic fluid lowering the strength of the

mother's immune system hence making the baby more prone to infections [117-119]. The current study, however, did not find any connection between neonatal sepsis and maternal risk factors, such as advanced maternal age, prolonged labor, premature rupture of the membrane, intrapartum fever, administration of antibiotics before delivery, and foul-smelling amniotic fluid, which increases the risk of an infection ascending from the birth canal into the amniotic fluid [120–121]. Even though many other studies have found an association, this study did not address these issues.

This study discovered a gender-specific vulnerability linked to newborn sepsis, with a significant statistical influence from the male gender. Saudi Arabian research conducted in a hospital reported similar results [122]. Furthermore, there was a high correlation seen between the development of newborn sepsis and early preterm birth, low birth weight, and low Apgar scores. Numerous other studies have also associated low birth weight, low Apgar scores, and preterm birth with an elevated risk of infant sepsis [123]. Conversely, a small number of studies claimed that low birth weight or preterm delivery had little to do with infant infection [19, 26].

Regarding the causative organisms of this study, gram-negative bacteria such *as Klebsiella pneumonia, Pseudomonas aeruginosa, and E. coli* showed predominance. In comparison to that, studies from other lower-middle-income countries (LMIC) reported similar patterns of bacterial isolates in terms of neonatal sepsis cases. In the present study, *Escherichia coli* was the predominant gram-negative bacteria followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* Another similar study in Mymensingh, Bangladesh by Quddus et al mostly isolated these three organisms in the highest proportion [124]. Further, Begum et al reported *Klebsiella pneumoniae* as the most isolated organism of their study in Bangladesh [125]. However, most of the blood samples used in this investigation came from patients who had Early Onset Neonatal Sepsis (EONS), and the primary cause of EONS in our study samples was E. coli. In terms of worldwide frequency, the most often documented causal microorganisms of newborn sepsis are coagulase-negative *Staphylococcus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Escherichia coli* [9, 10, 12, 32]. Babies who are colonized by Escherichia coli and *Klebsiella pneumoniae* represent a serious risk of developing newborn sepsis since these microorganisms are part of the gut flora and can cause septicaemia, pneumonia, and UTIs [28]. In LMIC countries, particularly in sub-Saharan Africa and South Asia, the probability of identifying gram-negative bacteria as the primary causal pathogen in cases of newborn sepsis is remarkably greater, with *Klebsiella pneumoniae* being the predominant organism. [126].
This study found a whooping prevalence of multidrug-resistant *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Escherichia coli* which represents the imminent risk of antimicrobial resistance in neonatal sepsis. The detection of carbapenem and beta-lactamase-resistant genes further indicated the genetic basis of resistance among these pathogens, posing significant challenges for antibiotic therapy. One of the biggest obstacles to the clinical management of newborn sepsis is antibiotic resistance. Most of the identified microorganisms in this investigation were resistant to multiple drugs. Most of the organisms exhibited a high level of resistance to widely used antibiotics such as amoxicillin, cefepime, azithromycin, and ceftazidime. Similar trends in antibiotic resistance have been documented in Ethiopia [108–111], Bangladesh [125], India [108], and Nepal [109]. Almost all the isolated *Klebsiella pneumoniae* of this study showed resistance towards all the 10 antibiotic drugs of six different classes. Further, the resistance towards carbapenem and beta-lactamase classes of drugs was 100%. These findings were like studies previous in Bangladesh [125], India [108], and Nepal [109]. Further, both *E. coli* and *Pseudomonas aeruginosa* showed the highest resistance towards ceftazidime and amoxillin. To sum up, the antibiogram profile of our isolated organisms, and lesser sensitivity towards carbapenem and beta-lactamase classes of antibiotics

were more prominent. This observation was further verified by assessing the genetic determinants of carbapenem and beta-lactamase resistance genes in our isolates.
In the present study, more than half of the *Klebsiella pneumoniae* isolates showed three beta lactamase-resistant genes including blaSPM, blaNDM and blaSHV. Although many previous studies have reported *E. coli* to be showing most prevalence of blaSPM gene, this study reported only 5 isolates to be having blaSPM gene [122]. Further, very few isolates of *E. coli* had blaCTX and blaTEM genes whereas many literatures have claimed E. coli to be having the most blaTEM gene. In terms of blaCTX, *Pseudomonas aeruginosa* is the most referred organism, however, the present study did not find the blaCTX gene in any of *Pseudomonas aeruginosa* isolates. On the other hand, three of the *Pseudomonas aeruginosa* isolates had blaVIM gene in this study which aligns with other study findings where the prevalence of blaVIM gene was higher in *Pseudomonas aeruginosa* [126-130].

To sum up, the study highlights the complex influence of socio-demographic factors, clinical characteristics of neonates, obstetrics features of mothers, and microbial determinants in neonatal sepsis within a resource-limited context. Identifying key risk factors and antibiotic resistance profiles underscores the urgent need to strengthen health systems, enhance antimicrobial stewardship, and implement infection control measures to mitigate the burden of neonatal sepsis and safeguard the health of vulnerable newborns in Bangladesh.

Chapter-9

Prospects, Limitations and Conclusion

9.1 Future Prospects:

The prospects of neonatal sepsis-related research in Bangladesh have promising future potential which can greatly enhance our knowledge of the condition and enhance infant health outcomes. To yield extensive epidemiological data on neonatal sepsis risk factors, setting up national registries to collect data on neonatal sepsis can direct future studies and public health campaigns. For real-time detection of neonatal sepsis, mobile health technology, telemedicine platforms, and electronic medical records must be implemented. In Bangladesh's resource-constraint environment, developments in molecular diagnostics, biomarker discovery, and point-of-care testing need to be implemented to attain quick, affordable, and reliable diagnostic methods with precision and promptness. Further, to control maternal, neonatal, and environmental-related etiological factors for neonatal sepsis, longitudinal cohort studies and case-control analyses need to be done countrywide. To identify the countrywide influence of these risk factors in neonatal sepsis incidence, severity, and outcome, specially designed preventative strategies and personalized management approaches need to be taken. Most of all, maternal knowledge and awareness regarding their pregnancy-related health need to be heightened. Mothers needs to test themselves for infections and other related complications during their pregnancy to take appropriate treatment and take preventative measures to avoid the risk of neonatal sepsis development in their babies. Also, to encourage mothers to take their pregnancy period health seriously, awareness programs need to be implemented. Further, to control maternal risk factors studies cantered on healthcare delivery paradigms, capacity building, and quality improvement initiatives need to be implemented. To control antibiotic-resistant organisms, nationwide antimicrobial resistance surveillance systems should be started. Further, antibiotic susceptibility testing needs to be done frequently before starting the antibiotic treatment. Therefore, a database on antibiotic-resistance patterns of neonatal sepsis-responsible organisms with their genetic profiles detailing in the resistant genes they possess needs to be built to lower the treatment time. Further, sensible usage of antibiotic drugs, research on antimicrobial resistance trends, and antibiotic prescribing practice needs to be done

Bangladeshi mothers' healthcare-seeking behavior is mostly influenced by cultural norms and beliefs. Studies of awareness can clarify these cultural determinants and aid in the creation of culturally competent treatments that motivate parents to get their newborns the proper medical attention. Awareness of the warning signs and symptoms of neonatal sepsis could lead to early detection and effective treatment from healthcare professionals and caregivers. Hence, mortality and morbidity could be greatly reduced by raising awareness. By creating an better understanding of the risk factors for neonatal sepsis, such as unhygienic birth and postnatal care, improved preventative techniques can be achieved. To prevent infections, awareness campaigns to encourage early vaccination, sterilization of medical equipment, and good handwashing. Teaching parents—especially mothers—the association between their antenatal complications and neonatal sepsis development is a necessary step to raise awareness. To achieve this, community health initiatives, public relations campaigns, and instructional materials provided in medical facilities are required.

Warning symptoms of neonatal sepsis can be identified early by taking initiatives for health education and community-based participatory research which can help caregivers in Bangladesh become more aware of newborn sepsis and seek medical attention when necessary. In conclusion, raising knowledge of neonatal sepsis in Bangladesh through research is crucial to lowering the infant mortality rate and enhancing the general health of neonates. Important information on prevalence, risk factors, and optimal treatment and preventative strategies is provided by this research. Bangladesh can improve early detection, encourage preventive actions, and eventually save the lives of many newborns by raising awareness.

9.2 Limitations of the Study:

This study attempted to evaluate the risk factors and causative organisms of neonatal sepsis in Dhaka, Bangladesh. The attempt to include patients from three different hospitals in Dhaka, Bangladesh marks the novelty of this study as most of the previous studies conducted in Bangladesh only studied one hospital. Despite all the efforts, there are some notable limitations of the study. Firstly, the sample size was a lot less than a statistically appropriate sample size, despite being multi-center study. Secondly, this study was a cross-sectional study rather than a case-controlled study. Due to the limited access to samples and negative controls the comparisons could not be made between cases and a control group. Thirdly, many of the obstetrics and pregnancy histories were collected by interviewing the mothers instead of accurate medical records as mothers did not provide consent on acquiring them. Finally, molecular-based characterization of all the multidrug resistance genes in the isolates could not be done due to the lack of specific primers in the laboratory. As a result, it is advisable to take the suggested results cautiously as they represent the actual scenario. It is also advised to carry out a multicentre study with a larger sample size and an equivalent number of healthy controls from Dhaka, Bangladesh, to confirm the risk factors discovered in our research. Further, to completely understand the general epidemiology and resistance mechanism of the organisms responsible for neonatal sepsis, it is advised to comprehensively characterize the multidrug-resistant bacteria by molecular techniques. Accurate data availability and accessibility in Bangladesh is one of the major limitations in doing any type of research on Neonatal Sepsis. In this study, a major portion of the participants were not born in the hospitals they were admitted for neonatal sepsis. For example, participants from BIRDEM Women and Child Hospital and Kurmitola General Hospital were out-born in the hospitals. This situation led to inaccurate medical history-related documentation as many of the participants were born in rural healthcare facilities where standardized reporting procedures or electronic medical record systems are scarce. This incomplete data was passed on to BIRDEM and Kurmitola Hospital which resulted in irregularities in the documentation hence the data on cases of neonatal sepsis become fragmented or incomplete as a result. Moreover, the infrastructure of healthcare facilities is quite complex and non-reachable, especially for a young student seeking data for research. Bangladeshi healthcare facilities are especially skeptical regarding collaborating with other institutional students on research related to infants and women. The most difficult task for this study was attaining ethical clearance and permission to collect neonatal sepsis samples and data. Thus, this study could not acquire the standard volume (2 ml) of blood samples from neonatal sepsis patients which led to a lower concentration of bacterial load in the samples. Another crucial obstacle for this study was attaining data from the mothers of the neonates. In many instances, mothers were hesitant to talk about the complications related to their private parts, also, many of them never diagnosed themselves with any kind of infection during their

pregnancy. The ignorance and poor knowledge of mothers regarding their pregnancy-related history were one of the main restraints during sample collection. The limitation was heightened by the skepticism and conservative attitude of the fathers. In many instances, fathers showed skepticism in letting their wives answer the questions. Thus, many fathers provided the pregnancy-related information themselves instead of letting their wives do it. Lastly, this study included three big hospitals situated in Dhaka city with only 60 data, thus, this cannot accurately represent the overall context of Bangladesh's population.

The risk factors for neonatal sepsis are influenced by a variety of factors like socioeconomic status, healthcare infrastructure and ethnic diversity thus multicentre studies with similar methodology and a range of participant demographics are required to guide for national public health actions.

9.3 Conclusion:

The findings of this study conclude that the chances of developing neonatal sepsis were prominent in male babies in the 3 days of their lives. Further, neonates born with low and very low weights with Apgar scores lower than 7 in the first minute stood out as a determinant in sepsis development in neonates. Further, maternal pregnancy histories like antepartum haemorrhage, meconium-stained amniotic fluid, urinary tract infection, preeclampsia, gestational diabetes, and parity were reported as the key influencing factors for potential sepsis occurrence in infants. Therefore, to reduce the prevalence and fatality rates of neonatal sepsis, proper prenatal screening is required for the early detection and treatment of maternal infections and other problems during pregnancy, as well as for the identification of high-risk pregnancies for appropriate perinatal management of neonates. The most isolated organisms identified by this investigation were *E. Coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa*. Additionally, this study revealed a significant frequency of multidrug-resistant *Klebsiella pneumoniae.* According to the above-mentioned findings, fluoroquinolone and aminoglycoside classes of drugs should be used as empirical antibiotic treatment in the case of *Klebsiella pneumoniae* infection and avoid carbapenem and beta-lactamase classes of antibiotics. On the other hand, carbapenem and beta-lactamase types of antibiotics should be preferred to treat sepsis in terms of *E. coli* and *Pseudomonas aeruginosa* infections. Finally, the optimal course of antibiotic treatment should be determined as soon as possible based on the culture and sensitivity assessment to reduce the likelihood of developing resistance to these drugs. The identified risk factors related to maternal infections, neonatal clinical characteristics and intrapartum risk factors in this study could lead to enhanced intrapartum care practices, prenatal screening, and maternal immunization in the future. The findings indicate the immediate need for preventive measures to reduce the mortality rates in Bangladesh. The findings also emphasize the necessity of safe delivery procedures, establishing screening and treatment protocols for maternal infections, and administering intrapartum antibiotic prophylaxis when needed. The findings most importantly indicate the necessity of improving prenatal care in Bangladesh. Ultimately, enhancing maternal health will lower the risk of infection transfer from mother to child. Further, proactive steps need to be taken to make the mothers and other caregivers better educated to enhance the value of prompt medical attention, good hygiene habits, and early illness detection. In addition to that, this study initiates a basis for evidence-based treatment guidelines about the use of proper antibiotics and provides suggestions for managing problems and supportive care. Finally, this study suggests uplifting the safeguarding techniques for the health and well-being of infants in the nation by identifying the root causes, adopting targeted interventions, boosting education, and upgrading healthcare infrastructure.

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Appendix A

Finding Etiological Factors for the Onset of Neonatal Sepsis Diagnosed in Tertiary Care Hospitals in Dhaka, Bangladesh

Questionnaire form

Reg. No **Date of enrolment:**

Name of the patient (Neonate): **DOB:**

Neonatal Gender:

- **Male**
- **Female**

Name of the mother:

Mother's Age: _____

- 15- 20
- 21-25
- 26-30
- 31-35
- 36-40
- Above 40

Address:

Phone no:

Economic Status:
Income Per Month (Taka): _____

Mothers Education:

1. Primary
2. Secondary
3. Higher Secondary
4.Graduate
5. Postgraduate

Mothers' Occupation:

1.Housewife
2.Service Holder
3. Business
3. Others_____

Antenatal Risk Factors

- Maternal Parity _____

o Primiparous
o Multiparous

- Mode of Delivery

o Vaginal Delivery
o C-section

- Place of Delivery

o Home
o Hospital
o Within Transportation

- Time of Water Breakage (Membrane Rupture)

---------- hours Before Delivery

Table 1. Antenatal Risk Factors

Risk Factor	Yes	No
Prolonged Labor (24 hours)		
Ante-Partem haemorrhage (Bleeding from genital tract during pregnancy)		
Meconium-stained amniotic fluid		
Foul smelling of amniotic fluid		
Intrapartum antibiotic given >4 hours before delivery		
Intrapartum Fever (Fever during Labor/ C-section)		
Premature rupture of membrane (Water Breakage)		
UTI during third trimester		
Presence of Sexually Transmitted Infection		
Preeclampsia (High Blood Pressure		
Diabetes		

Neonatal Factors

- Gestational age of delivery: _____
  - Early Preterm (<34 weeks)
  - Late Preterm (≥34- <37 weeks)
  - Term (≥37 - <42 weeks)

- Birth Weight: _____
  - < 1500 gm
  - 1500 gm - < 2500 gm
  - 2500 gm – 4000 gm

- Day of the onset of illness: _____
  - 0-<7
  - 7-28
- Has antibiotic treatment started?
  - Yes
  - No
- Starting date of antibiotic treatment

  _____
- Name of the antibiotic given:

  _____

- Platelet Count (mcL):

  _____
  - >150,000
  - <150,000

- **Apgar Score:**
- 1 min
  - <7
  - ≥7
- 5 min
  - <7
  - ≥7
- 10 min
  - <7
  - ≥7

I understand that this data would be used for research purposes. My and my child's names/ identities would be kept confidential. I declare that the information provided above is true to my knowledge and I give permission to use my and my child's history for research.

_____ _____ _____

Name Signature Date

Appendix B

Finding Etiological Factors for the Onset of Neonatal Sepsis Diagnosed in Tertiary Care Hospitals in Dhaka, Bangladesh

লিখিত সম্মতি পত্র

প্রিয় অংশগ্রহণকারী,

আমি ব্র্যাক ইউনিভার্সিটি এর শিক্ষার্থী হিসেবে, '**Finding Etiological Factors for the Onset of Neonatal Sepsis Diagnosed in Tertiary Care Hospitals in Dhaka, Bangladesh'** নামক একটি গবেষণামূলক কাজে একজন তথ্য সংগ্রহনকারী হিসেবে কাজ করছি। এই গবেষণা পরিচালনা করার জন্য আপনার কাছ থেকে কিছু প্রাসঙ্গিক তথ্য এবং আপনার নবজাতক এর থেকে ২ মিলিলিটার রক্ত সংগ্রহ করা এবং পরীক্ষা করার অনুমতি প্রয়োজন। পরবর্তীতে ব্র্যাক ইউনিভার্সিটি এর লাইফ সাইন্স ল্যাব এ ব্লাড কালচার, এন্টিবায়োটিক ডিস্ক ডিফিউশন পদ্ধতি, এবং পিসিআর পদ্ধতি এর মাদ্ধমে রক্তে অবস্থিত নিওনেটাল সেপসিস রোগ এর কার্যকরী ব্যাকটেরিয়া এবং এর ওপর কোন কোন এন্টিবায়োটিক ঔষধ কাজ করে না তা বের করা হবে। এই গবেষণায় আপনার অংশগ্রহণ সম্পূর্ণরূপে ঐচ্ছিক এবং কেবল আপনার লিখিত অবহিত সম্মতি পত্রে স্বাক্ষর পরবর্তীতে তথ্য এবং রক্ত সংগ্রহ করা হবে। উক্ত গবেষণায় তথ্য সংগ্রহের জন্য ১০-১৫ মিন সময় ব্যাপী আপনার সাক্ষাৎকার নেয়া হবে। এই সাক্ষাৎকারে প্রদানকৃত তথ্যের দ্বারা আপনার এবং আপনার নবজাতক এর গোপনীয়তা কিছুতেই ক্ষুন্ন করা হবে না। এই গবেষণায় অংশগ্রহণ করে আপনার এবং আপনার নবজাতক এর অন্য কোনো ধরণের অসুবিধা যেমন - মানসিক/ সামাজিক/ আর্থিক/ শারীরিক সমস্যার সম্মুখীন হবেন না। আপনার এই গবেষণায় অংশগ্রহণের বিপরীতে কোন পারিশ্রমিক বা সম্মানী প্রদান করা হবে না। এই গবেষণার জন্য সংগৃহিত তথ্য কেবল মাত্র এই গবেষণার কাজেই ব্যবহার করা হবে। এই গবেষণায় তথ্য প্রদান এর যে কোন সময় আপনি আপনার অংশগ্রহণ প্রত্যাহার করার সম্পূর্ণ অধিকার রাখেন। এই গবেষণা নিওনেটাল সেপসিস রোগ প্রতিরোধ গবেষণা সম্পর্কে জ্ঞান বৃদ্ধি করে স্বাস্থসেবা উদ্যোগের উন্নতি করতে পারে।

যোগাযোগ এর তথ্য:

গবেষণা সম্পর্কে আপনার যদি কোনো প্রশ্ন বা উদ্বেগ থাকে তবে দয়া করে নির্দ্বিধায় গবেষকের সঙ্গে যোগাযোগ করুন।

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সম্মতি

আমি এই সম্মতি পত্রে প্রদত্ত তথ্য পড়েছি এবং বুঝতে পেরেছি। আমি স্বেচ্ছায় "**Finding Etiological Factors for the Onset of Neonatal Sepsis Diagnosed in Tertiary Care Hospitals in Dhaka, Bangladesh'** শীর্ষক গবেষণায় অংশ নিতে এবং আমার নবজাতক এর অংশহগ্রহন এর সম্মতি প্রদান করছি। এই গবেষণায় সংগৃহিত তথ্য গবেষণা কাজে ব্যবহারের অনুমতি প্রদান করছি।

অংশহনকারীর নাম:

অংশহনকারীর স্বাক্ষর:

তারিখ:

তথ্য সংগ্রহণকারীর নাম:

তথ্য সংগ্রহণকারীর স্বাক্ষর: