Identification of Convenient Diagnosis Methods of Premature Membrane Rupture (PROM) for Rural Areas of Developing Countries: A Multicenter Case-Control Study

A dissertation submitted to the Shahjalal University of Science and Technology in partial Fulfillment of the requirements for the MS degree in Biochemistry and Molecular Biology

Submitted by

Registration No: 2020423004

MS Session: 2020-21

September 2023

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Dedicated to

My Beloved Parents

and

My Younger Sister

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Contents

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- Acknowledgements (except examiner's copy).
- Guidelines to writing MS dissertation.
- Abbreviations.
- List of tables, graphs, and figures.
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List of Abbreviations

PROM - Premature Rupture of Membrane

ANC - Antenatal Care

AMR - Antimicrobial Resistance

MR - Methyl Red

VP - Voges-Proskauer

MRSA - Methicillin-Resistant Staphylococcus aureus

DNA - Deoxyribonucleic Acid

RNA - Ribonucleic Acid

PCR - Polymerase Chain Reaction

ICU - Intensive Care Unit

BMI - Body Mass Index

EDTA - Ethylenediaminetetraacetic Acid

ELISA - Enzyme-Linked Immunosorbent Assay

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Abstract

Premature Rupture of Membranes (PROM) during pregnancy is a multifaceted obstetric complication with intricate associations. This study investigates the complex interplay of demographic, clinical, and microbial factors in PROM, focusing on microbial relationships. Age emerged as a critical factor, with higher prevalence rates in the 31 to 40 age group (53%) compared to 16 to 20-year-olds (29%). Economic status played a role, notably, the upper-income group exhibited a higher incidence (60%). Educational status revealed intriguing patterns, with individuals lacking formal education at greater risk (39%), while those with 'Higher Education' displayed a lower likelihood (11%). Consanguineous marriages significantly correlated with higher PROM risk (78%) compared to non-consanguineous unions (22%). Multivariate logistic regression analysis underscored heightened risk in the 31 to 40 age group, individuals with no formal education, and consanguineous marriages. Clinical factors unveiled a web of risk elements. 'Below-average nutrition' was associated with a higher risk (76%), whereas 'good' nutrition reduced risk (8%). The absence of itching during pregnancy decreased the risk (AOR: 0.606), while a previous PROM history didn't significantly influence recurrence risk (AOR: 0.25). Factors like recent injury within 48 hours (AOR: 1.713), fewer than 5 ANC visits (AOR: 3.406), high blood pressure (AOR: 1.55), and the presence of discharge (AOR: 1.562) all correlated with increased PROM risk. Microbial analysis of samples from diverse hospitals revealed microbial communities in 116 samples, with dominant aerobic microbial presence in 9 samples and 7 showcasing dominant anaerobic microbial communities. Microbial species identification exposed Staphylococcus spp. (35 samples), Bacillus spp. (33 samples), Streptococcus spp. (11 samples), Escherichia coli (11 samples), and Enterococcus spp. (9 samples). Furthermore, varying antibiotic resistance patterns emphasize the need for tailored treatments based on specific microbial isolates. This study offers a comprehensive view of PROM's multifactorial nature, focusing on microbial communities and antibiotic resistance. The findings stress the importance of considering microbial relationships in PROM analysis, providing insights for precise interventions.

Keywords: Premature Rupture of Membranes (PROM), Obstetric Complication, Microbial Communities, Antibiotic Resistance Patterns, Demographic Factors, Clinical Factors, Multivariate Logistic Regression, Consanguineous Marriages, Educational Status, Economical Status

Chapter 1:
Introduction

1. Introduction

1.1 Premature Rupture of Membrane (PROM):

The premature rupture of the membrane (PROM) stands as a pivotal obstetric occurrence, precipitating when the amniotic sac fractures before labor's inception **(Figure 1)**. This phenomenon, affecting approximately 10% of pregnancies (Enjamo *et al.*, 2022a), yields consequential ramifications for both maternal and fetal well-being, compelling a profound understanding of its significance in the realm of comprehensive prenatal care and therapeutic management.

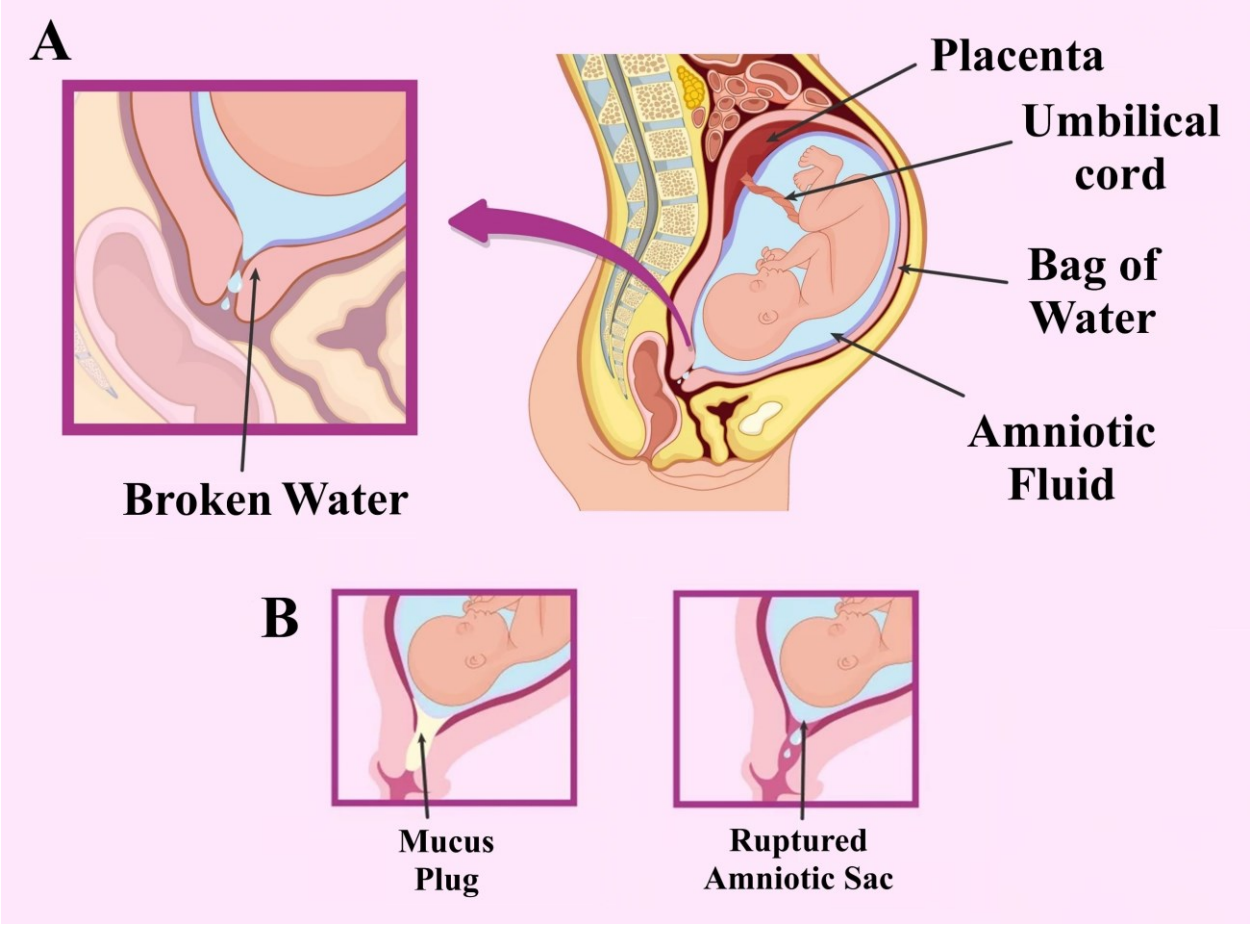

Figure 1. A graphical representation of Premature Rupture of Membrane (PROM); **A.** Water break and the onset of PROM, **B.** The comparative view of mucus plug and ruptured amniotic sac. (Adapted and modified from (https://www.invitra.com/en/amniotic-fluid-leakage))

A quintessential dimension of PROM's exploration lies in its capacity to unveil potential complications that might ensue during pregnancy. Evidently, the susceptibility to infections such as chorioamnionitis, a precursor to premature labor and newborn sepsis, is notably amplified

among women afflicted by PROM (Ocviyanti and Wahono, 2018). Swift identification of these potential challenges equips healthcare practitioners with the acumen to diligently oversee the patient and forestall adversities that could imperil both maternal and neonatal health.

The pivotal role of PROM as a risk catalyst for preterm birth further underscores its gravity. This precipitous rupture of the amniotic sac sets forth a chain of events culminating in the elevation of preterm birth risk. The pivotal role of amniotic fluid in fetal development intensifies this risk manifold. The depletion of this essential fluid could precipitate umbilical cord compression, retarding fetal growth and orchestrating the onset of preterm labor (Ocviyanti and Wahono, 2018). Indeed, the spectrum of healthcare encompasses closely monitoring PROM's occurrences, frequent prenatal visits, and meticulous vigilance during the labor and delivery phases, although these pursuits usher a commensurate escalation in healthcare expenditures. Furthermore, the exigency for specialized neonatal care for preterm infants born post-PROM augments the financial encumbrance borne by healthcare systems.

The intricate enigma surrounding Premature Rupture of Membranes (PROM) extends its influence into the domain of maternal and neonatal health, demonstrating a discernibly pronounced inclination towards unfavorable consequences. This intricate interplay begets an augmented predisposition to untoward maternal and neonatal outcomes, encompassing the emergence of intrauterine infections, preterm labor, and neonatal sepsis. Despite its relatively modest occurrence, approximately constituting 3% of all pregnancies, PROM plays a pivotal role as a determinative factor contributing to nearly one-third of preterm births. It is noteworthy that the diagnostic spectrum of PROM primarily relies on a foundation anchored in historical data, complemented by physical examinations. However, the inherent intricacy within the realm of PROM diagnosis necessitates the pursuit of confirmatory assessments in instances of uncertainty. The pinnacle of diagnostic precision resides in the collection of amniotic fluid, a process facilitated by the introduction of a sterile speculum into the vaginal canal, allowing for cervix visualization. Unfortunately, alternative diagnostic modalities such as atrazine and fern tests pale in comparison due to their inferior sensitivity and specificity (Ladfors *et al.*, 1997).

The management of PROM pivots on the gestational age at diagnosis and the presence of maternal or fetal complexities. Swift delivery is advocated for pregnancies surpassing 34 weeks, as a preventative measure against infection and stillbirth risks (Mercer *et al.*, 1999). In the realm of pregnancies spanning 24 to 34 weeks, a strategy of expectant management prevails, characterized by vigilant maternal and fetal surveillance to detect indications of infection and preterm labor (Berghella *et al.*, 2020). Notably, antibiotic therapy emerges as a recommended course of action

for all women diagnosed with PROM, effectively mitigating the peril of intrauterine infection (Ghafoor, 2021)

While the fatality rate associated with PROM remains relatively subdued, it is essential to recognize the significant morbidity it engenders for both the maternal and neonatal spheres. Among the maternal complexities, chorioamnionitis, postpartum hemorrhage, and sepsis emerge as prevailing concerns (Ghafoor, 2021). In tandem, the neonatal landscape is fraught with challenges, encompassing preterm birth, respiratory distress syndrome, and sepsis, underscoring the multifaceted nature of the impact that PROM bestows (Turrentine *et al.*, 2019). It is imperative to recognize that substantial physiological effects manifest in expectant mothers as PROM advances. Women encountering PROM might grapple with uncertainty or anxiety concerning their pregnancy's outcome, particularly those predisposed to preterm delivery or complications during labor or delivery. Nonetheless, adeptly administering appropriate counseling and support to these expectant mothers holds the potential to alleviate stress and enhance their mental well-being in a majority of instances.

PROM stands as a profound pregnancy complication necessitating meticulous vigilance and adept handling. Although the prevalence of PROM remains relatively modest, the consequential maternal and neonatal morbidity remains substantial. The diagnosis of PROM hinges largely on comprehensive historical evaluation and physical assessment, with supplementary confirmatory tests at one's disposal. Effective management of PROM is contingent upon discerning factors such as gestational age and the existence of maternal or fetal complications. Further exploration is indispensable to enhance our comprehension of the intricate pathophysiology and optimal management strategies concerning PROM.

In summary, the significance of premature rupture of the membrane extends across multiple dimensions. Ensuring a comprehensive understanding of the condition's repercussions for both maternal and fetal health is of paramount importance in delivering appropriate prenatal care and preemptively addressing potential complications. By meticulously identifying the interrelated risks associated with PROM, researchers, and medical practitioners can proactively oversee patients, mitigating the risk of preterm labor and neonatal sepsis. This comprehensive approach stands to exert a significant positive influence on both healthcare systems and the well-being of the families affected.

1.2 Global Prevalence and Impact of PROM

The global prevalence and impact of PROM are matters of substantial concern within the realm of maternal and child health, commanding attention due to their potential to cause a cascade of health, economic, and social implications. This section explores the profound worldwide prevalence and far-reaching impact of PROM, shedding light on its significance within the context of both developed and developing countries **(Figure 2).**

The occurrence of PROM is by no means a rarity, traversing geographical and socio-economic boundaries. The prevalence of PROM, often regarded as the rupture of the amniotic sac before the onset of labor, is a noteworthy concern. It is estimated that approximately 10% of all pregnancies globally are affected by PROM, translating to a significant proportion of maternal and neonatal populations (Ghafoor, 2021). The implications of this statistic are far-reaching, underlining the urgent need for effective preventive strategies and improved diagnostic methodologies. The prevalence of PROM persists across diverse populations, ranging from developed nations with advanced healthcare systems to resource-constrained regions where access to adequate care is limited.

While the impact of PROM is not confined to fetal health alone; it casts a substantial shadow on maternal well-being. Women experiencing PROM are at an augmented risk of developing infections, chorioamnionitis, and postpartum hemorrhage. The ripple effect of these complications can translate into prolonged hospital stays, invasive interventions, and escalated healthcare expenditures (Endale *et al.*, 2016). The potential progression of PROM-associated infections to systemic sepsis poses grave threats to maternal mortality, particularly in areas where access to prompt and appropriate medical attention is restricted.

The consequences of PROM extend far beyond the confines of maternal health, as neonatal outcomes are significantly impacted. Premature birth is a common consequence of PROM, culminating in a heightened risk of neonatal morbidity and mortality. Neonates born as a result of PROM-induced preterm birth are vulnerable to respiratory distress syndrome, intraventricular hemorrhage, and sepsis (Turrentine *et al.*, 2019).

A lens through which to view the prevalence and impact of PROM is one of global disparities. Developing countries, characterized by inadequate healthcare infrastructure and limited access to quality care, bear a disproportionate burden of PROM-related complications (Misau, Al-Sadat and Bakari Gerei, 2010).

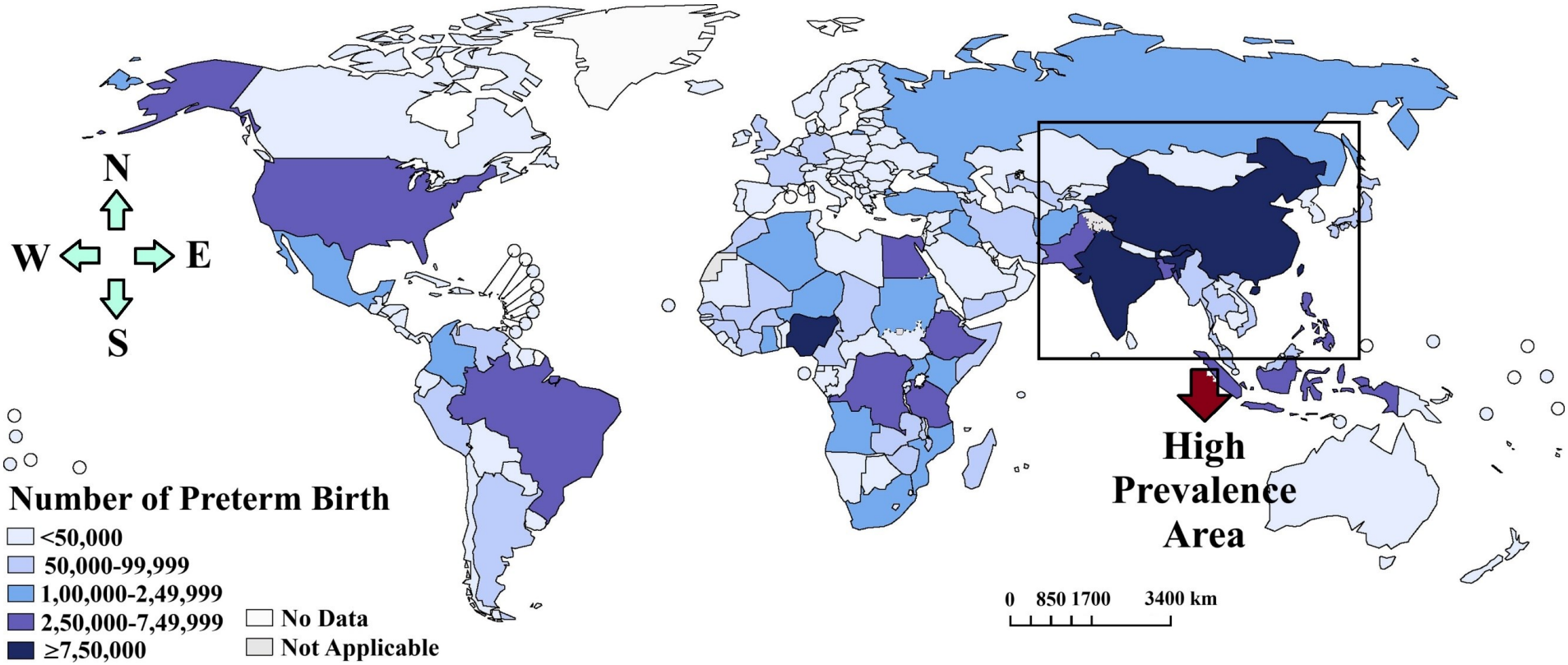

Figure 2. An illuminating global prevalence visualization delineating the worldwide distribution of PROM and the incidence of preterm births across diverse regions. (Adapted and modified from (Chawanpaiboon *et al.*, 2019)).

In these settings, the challenges of geographical remoteness, cultural factors, and economic constraints conspire to hinder timely diagnosis and intervention. The disparities in PROM prevalence and outcomes underscore the urgent need for equitable healthcare interventions that address the unique challenges faced by vulnerable populations.

In conclusion, the global prevalence and impact of PROM resonate across regions and socio-economic strata, posing multifaceted challenges to maternal and neonatal health, healthcare systems, and socio-economic stability. As the nexus of healthcare, science, and policy converge to confront these challenges, comprehensive strategies are warranted to mitigate the impact of PROM on maternal and neonatal health. The subsequent sections of this discourse will unravel the diverse causes of PROM, explore preventive strategies, delve into multifaceted treatment approaches, and navigate the uncharted waters of future directions and research gaps, all in pursuit of enhancing maternal and neonatal outcomes worldwide.

1.3 Diverse Causes of PROM

The underlying causes of PROM are multifaceted and involve intricate interactions between biological, environmental, and maternal factors. This section unveils the diverse causes that contribute to the occurrence of PROM, shedding light on the complex tapestry of influences that intertwine to precipitate this critical obstetric event **(Figure 3)**.

One pivotal realm within the landscape of PROM causality involves microbial influences. Ascending infections from the vaginal flora, characterized by pathogens such as Group B *Streptococcus (GBS)*, *Escherichia coli*, and *Ureaplasma urealyticum*, have been implicated in the weakening of fetal membranes and the subsequent rupture (Rzanek-Głowacka *et al.*, 2003). The delicate balance between commensal and pathogenic microorganisms within the vaginal environment underscores the potential for microbial dysbiosis to tip the scales toward an inflammatory cascade that weakens the membranes.

Rationally, the intricate interplay between the maternal immune response and fetal membranes plays a defining role in PROM causation. Immunological alterations, including both hyperresponsiveness and inadequate immune regulation, can contribute to the degradation of collagen and other structural components that maintain the integrity of the amniotic sac (Thorsen *et al.*, 1998; Rzanek-Głowacka *et al.*, 2003). Inflammatory mediators such as cytokines and prostaglandins are key players in this complex interplay, orchestrating a cascade of events that can culminate in PROM.

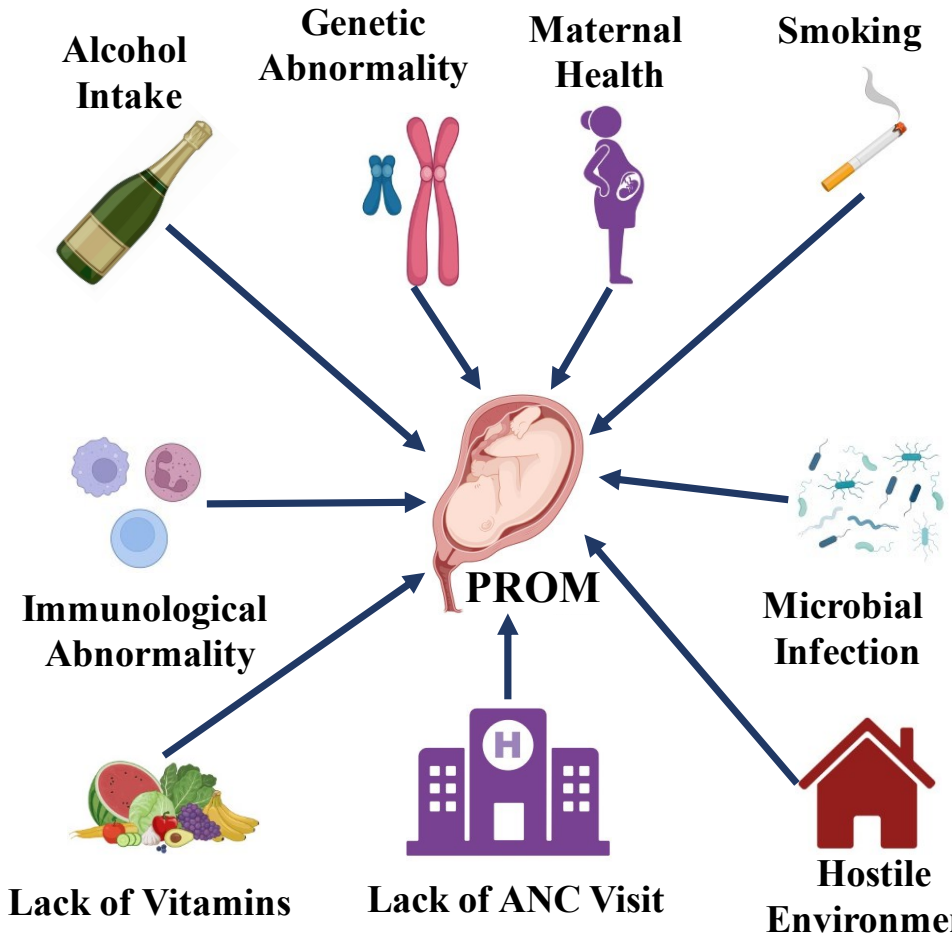

Figure 3. The predominant risk factors, primarily responsible for significantly contributing to the incidence of PROM.

In addition, exposure to environmental factors, both within and outside the maternal environment, has emerged as a potential contributor to PROM. Ambient pollution, exposure to toxins, and lifestyle choices such as smoking are implicated in the weakening of fetal membranes and the initiation of the rupture process (Dadvand and Nieuwenhuijsen, 2019). The intricate web of environmental exposures adds yet another layer of complexity to the multifactorial etiology of PROM.

Moreover, genetic predisposition to PROM represents an area of burgeoning interest within the realm of causality. Variations in genes related to collagen metabolism, inflammation, and immunity have been linked to altered risk profiles for PROM (Tulina *et al.*, 2019). Understanding the genetic underpinnings of PROM holds promise in delineating subgroups of pregnant individuals at higher risk, allowing for targeted interventions and personalized approaches to care.

Furthermore, endocrine factors and hormonal shifts during pregnancy exert significant influences on the integrity of fetal membranes. Variations in hormone levels, particularly those related to

progesterone and estrogen, can impact the tensile strength of the amniotic sac (Robinson and Klein, 2012). Hormonal fluctuations, influenced by factors such as gestational age and maternal health status, contribute to the dynamic equilibrium that determines membrane integrity. Infections during pregnancy, particularly those involving the genitourinary tract, can trigger an inflammatory response that propagates to the fetal membranes. Inflammation-induced changes compromise the structural integrity of the membranes, rendering them susceptible to rupture (Ocviyanti and Wahono, 2018). The cascading impact of infection-induced inflammation underscores the intricate web of causality that underlies PROM.

Hence, the diverse causes of PROM paint a complex and multifaceted portrait of a condition rooted in intricate interactions. Microbiological, immunological, genetic, hormonal, and environmental factors converge to precipitate the rupture of fetal membranes, often leading to preterm birth. This nuanced understanding underscores the need for a comprehensive approach to prevention, diagnosis, and management, which must consider the interplay of these diverse influences. As we navigate the dynamic landscape of PROM etiology, innovative research, and targeted interventions offer the promise of unraveling this intricate tapestry to ultimately enhance maternal and neonatal outcomes.

1.4 Microbiological and Immunological Perspectives on PROM

The complex interplay of microbiological and immunological factors stands as a pivotal nexus within the intricate realm of Premature Rupture of Membranes (PROM). This critical obstetric event, characterized by the untimely rupture of the amniotic sac, is often underpinned by a delicate equilibrium between microbial colonization and maternal immune responses. This section delves into the intricate microbiological and immunological perspectives that shape the dynamics of PROM, unraveling the multifaceted interactions that contribute to its occurrence.

Consequently, the vaginal microbiota, an intricate consortium of microorganisms, wields a significant influence on the integrity of fetal membranes (Baud *et al.*, 2023). A delicate equilibrium between beneficial commensals and potentially pathogenic species governs the vaginal environment. Disruptions in this equilibrium, often exacerbated by factors like sexual activity, hormonal fluctuations, and infections, can tip the scales toward a dysbiotic state. Such dysbiosis, characterized by shifts in microbial composition, can promote inflammatory responses and compromise the structural integrity of fetal membranes.

To a broad spectrum, the interplay between microbial colonization and maternal immune responses represents a pivotal facet in the dynamics of PROM. The maternal immune system,

poised to maintain a fine balance between tolerance and defense, interacts with vaginal microorganisms. This interaction is mediated by pattern recognition receptors (PRRs) that recognize microbial molecules, initiating immune responses (Romano-Keeler and Weitkamp, 2015). Dysregulated immune responses can contribute to inflammation-induced membrane weakening, offering insight into the underlying mechanisms of PROM. Immunological processes within the decidua, the maternal-fetal interface, play a defining role in PROM pathogenesis. Complex interactions between immune cells, cytokines, and growth factors are orchestrated to ensure successful implantation and gestation. Dysregulation of these processes, influenced by factors like infections and inflammation, can disrupt tissue remodeling and contribute to the rupture of fetal membranes (PrabhuDas *et al.*, 2015). This delicate balance between immunomodulation and tissue homeostasis underscores the vulnerability of the amniotic sac.

Additionally, the inflammatory responses lie at the heart of the link between microbiological colonization and PROM. Inflammatory cytokines, including interleukins and tumor necrosis factor-alpha (TNF-α), orchestrate the degradation of collagen, a key structural component of fetal membranes (Romano-Keeler and Weitkamp, 2015). Collagen degradation weakens the membranes, rendering them susceptible to mechanical stress and rupture.

In a nutshell, the microbiological and immunological perspectives on PROM unveil a landscape of intricate interactions that dictate maternal immune responses, microbial colonization, and inflammation-induced tissue dynamics. The delicate equilibrium between commensal and pathogenic microorganisms within the vaginal environment, governed by immune responses, shapes the vulnerability of fetal membranes. As we navigate this complexity, elucidating the nuances of microbial-host interactions and immunological pathways offers a nuanced understanding of PROM's pathogenesis. This comprehension, in turn, informs innovative interventions that target both microbiological dysbiosis and inflammatory cascades to ultimately enhance maternal and neonatal outcomes.

1.5 Prevention Strategies for PROM

Premature Rupture of Membranes (PROM), a critical obstetric event with far-reaching implications, underscores the imperative for effective prevention strategies to enhance maternal and neonatal health outcomes. The untimely rupture of the amniotic sac necessitates a proactive approach that addresses the multifaceted factors contributing to PROM. This section delves into the realm of prevention strategies, illuminating a spectrum of interventions aimed at reducing the incidence of PROM and its associated complications **(Figure 4)**.

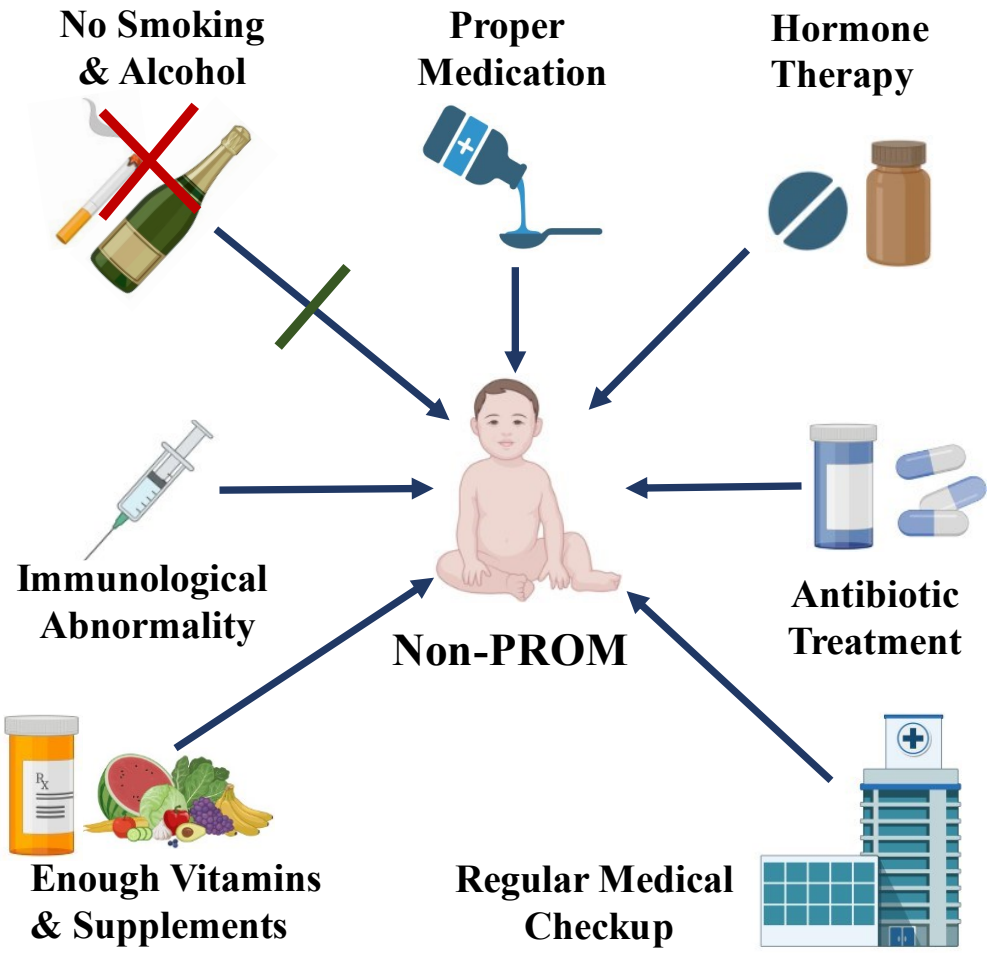

Figure 4. Prospective preventive approaches for mitigating the adverse medical outcomes associated with PROM.

Initially, antenatal care emerged as a cornerstone for PROM prevention, emphasizing the importance of early and regular prenatal visits. Comprehensive antenatal care enables healthcare providers to monitor pregnancy progression, detect potential risk factors, and provide tailored education to expectant mothers. Education on proper hygiene, nutrition, and lifestyle modifications equips women with the knowledge to minimize risk factors that can contribute to PROM (Ahmed and Manzoor, 2019).

Furthermore, a critical avenue for PROM prevention revolves around the mitigation of infection risks. Strategies encompass screening and treating genitourinary infections, such as bacterial vaginosis and sexually transmitted infections, during prenatal visits. The administration of antibiotics to women at high risk of infection can significantly reduce the likelihood of PROM (McDonald, Brocklehurst and Gordon, 2007). By tackling infections, healthcare providers can address a key contributory factor to PROM. Nutritional interventions assume significance in the prevention of PROM. Adequate maternal nutrition, fortified with essential vitamins and minerals, supports fetal development and strengthens the integrity of fetal membranes. Strategies promoting

folic acid supplementation, iron-rich diets, and optimal weight gain contribute to maternal health and potentially mitigate the risk of PROM (Keats *et al.*, 2021).

In addition, the assessment of cervical length, often through ultrasound examinations, offers a window of opportunity for early PROM prediction and prevention. A short cervix has been linked to an increased risk of PROM and preterm birth. Timely interventions, such as cervical cerclage, can be considered for women with a short cervix, effectively reducing the risk of PROM-associated complications (Reicher, Fouks and Yogev, 2021). The avoidance of tobacco smoke, both active and passive, is paramount in reducing the risk of PROM (Golechha, 2016). By addressing modifiable risk factors, women can contribute to a healthier pregnancy and reduce their susceptibility to PROM.

Moreover, for women with a family history of PROM or genetic predispositions, genetic counseling and screening assume significance. Early identification of genetic markers associated with collagen metabolism, inflammation, and hormonal regulation can empower healthcare providers to tailor preventive interventions (Phadke, 2004). Genetic insights offer the potential for personalized approaches to PROM prevention.

In summary, the landscape of PROM prevention strategies encompasses a multidimensional spectrum of interventions that address risk factors, empower expectant mothers, and leverage medical advancements. By investing in antenatal care, infection prevention, nutritional supplementation, and cervical assessment, healthcare providers can navigate a landscape that holds the promise of minimizing PROM incidence and its far-reaching consequences. These prevention strategies, grounded in evidence-based practices, illuminate a path toward enhancing maternal and neonatal health outcomes, ushering in a future where the occurrence of PROM is mitigated, and the trajectories of pregnancy are optimized.

1.6 Multifaceted Treatment Approaches for PROM

The intricate tapestry of Premature Rupture of Membranes (PROM) necessitates a nuanced and multifaceted approach to treatment. This critical obstetric event, marked by the untimely rupture of the amniotic sac, demands interventions that address the immediate clinical complexities while considering the broader implications for maternal and neonatal health. This section delves into the realm of treatment approaches, unveiling a spectrum of interventions that navigate the intricacies of PROM management.

Firstly, in case of PROM occurring before 37 weeks of gestation, expectant management is often considered. This approach involves careful monitoring of maternal and fetal well-being, with interventions implemented if complications arise. Close surveillance aims to prolong pregnancy while minimizing the risk of maternal and neonatal infection (Shazly *et al.*, 2020). Expectant management recognizes the delicate balance between extending gestation and averting potential harm. Consequently, corticosteroids emerge as a cornerstone in the treatment of PROM-associated preterm birth. Administration of antenatal corticosteroids, such as betamethasone or dexamethasone, accelerates fetal lung maturation and reduces the risk of neonatal respiratory distress syndrome (Roberts *et al.*, 2017). This intervention, when administered within the appropriate window, contributes to enhancing neonatal outcomes and mitigating the impact of preterm birth.

Secondly, the role of antibiotics in PROM treatment is twofold: addressing maternal infections and reducing neonatal complications. Antibiotic therapy, guided by the specific pathogens identified, can help manage maternal infections, minimize the risk of chorioamnionitis, and prevent neonatal sepsis (Lin *et al.*, 2023). Customized antibiotic regimens align with the microbiological landscape and contribute to comprehensive PROM management. Additionally, the timing of delivery stands as a pivotal decision point in PROM management. For PROM cases beyond 34 weeks of gestation, immediate delivery is often recommended to mitigate the risk of infection and ensure optimal neonatal outcomes. In cases of PROM between 24 and 34 weeks, the timing of delivery is meticulously weighed against potential complications, fostering a delicate balance between maternal and fetal well-being (Shazly *et al.*, 2020).

Thirdly, for the selected cases of PROM, where the volume of amniotic fluid is compromised, amnioinfusion may be considered. This intervention involves the infusion of sterile fluid into the amniotic cavity to replenish lost fluid and enhance fetal well-being. Additionally, continuous fetal monitoring plays a crucial role in PROM management, allowing healthcare providers to assess fetal heart rate patterns and respond to potential distress (Hofmeyr and Kiiza, 2016). Moreover, surgical interventions, such as cerclage and cervical occlusion, assume significance in specific cases of PROM-associated preterm birth. Cerclage, the suturing of the cervix to prevent its premature dilation, can be considered for women with cervical insufficiency. Cervical occlusion, achieved through the placement of a stitch to close the cervix, can also be utilized to prolong pregnancy in certain scenarios (Alfirevic, Stampalija and Medley, 2017).

In conclusion, the realm of multifaceted treatment approaches for PROM navigates a complex landscape, where clinical decision-making is guided by a synthesis of evidence-based practices,

maternal and fetal well-being, and individualized considerations. From expectant management to antibiotic therapy, from corticosteroid administration to surgical interventions, the tapestry of PROM treatment embodies a commitment to optimizing maternal and neonatal outcomes. As healthcare providers navigate this intricate landscape, their actions are poised to shape the trajectories of pregnancy, birth, and the early postpartum period, nurturing a future where the impact of PROM is mitigated, and the potential for improved maternal and neonatal health is realized.

1.7 Future Directions and Research Gaps in PROM

The dynamic landscape of Premature Rupture of Membranes (PROM) beckons us toward a future marked by innovation, discovery, and the bridging of knowledge gaps. This critical obstetric event, characterized by the untimely rupture of the amniotic sac, calls for continued research endeavors that unravel the complexities of PROM, inform evidence-based practices, and shape the course of maternal and neonatal healthcare. This section casts a forward gaze, illuminating the future directions and research gaps that hold promise in the domain of PROM.

It's a matter of fact that a pivotal future direction lies in the refinement of risk stratification strategies for PROM. Robust predictive models that integrate genetic, environmental, and clinical factors can offer a personalized approach to risk assessment. Identifying high-risk populations early in pregnancy empowers healthcare providers to implement tailored preventive interventions, ultimately minimizing PROM incidence and its subsequent impact (Evans *et al.*, 2023). In addition, the exploration of genetic and molecular underpinnings of PROM remains a fertile field for research. Investigating genetic variants associated with collagen metabolism, inflammation, and hormonal regulation offers insights into susceptibility and pathogenesis. Molecular studies dissecting the mechanistic aspects of fetal membrane integrity hold potential for novel therapeutic targets and preventive strategies (Cunningham *et al.*, 2020).

In addition, the forthcoming research endeavors can revolutionize PROM detection through the development of non-invasive and point-of-care diagnostic tools. Biosensors, biomarkers, and imaging techniques can facilitate rapid and accurate identification of PROM, enabling timely interventions. Innovative approaches that balance sensitivity, specificity, and ease of use have the potential to transform the diagnostic landscape (Sin *et al.*, 2014). Moreover, the identification of reliable biomarkers for early PROM prediction represents an avenue of high promise. The exploration of biochemical, immunological, and genetic markers in maternal serum and amniotic fluid holds potential for early detection and risk assessment. These markers can be harnessed to stratify pregnant women, enabling timely interventions and personalized care (Hornaday, Wood

and Slater, 2022). The future of PROM research envisions collaborative endeavors that span borders and unite expertise. International collaborations can facilitate comparative studies, enabling insights into geographical variations, genetic predispositions, and cultural influences on PROM incidence. These initiatives foster a rich tapestry of data, enabling researchers to decipher multifaceted factors contributing to PROM (Enjamo *et al.*, 2022a).

Moreover, the ongoing research can delve deeper into the role of lifestyle and environmental factors in PROM risk. Exploring the impact of nutrition, socioeconomic status, stress, and exposure to environmental toxins can uncover modifiable factors that influence the occurrence of PROM. These insights inform preventive strategies that encompass holistic approaches to maternal health (Serio, De Donno and Valacchi, 2023). Consequently, the upcoming landscape of PROM research embraces interventions tailored to the ethnic and cultural diversity of populations. Acknowledging disparities in PROM incidence and outcomes across ethnic and racial groups, research efforts can focus on understanding cultural perceptions, genetic variations, and social determinants that shape vulnerability. Culturally sensitive interventions can mitigate disparities and improve outcomes (Enjamo *et al.*, 2022a).

Furthermore, the trajectory of PROM extends beyond the immediate event, necessitating longitudinal studies that illuminate the long-term outcomes for both mothers and neonates. Investigating the impact of PROM on neurodevelopment, cognitive functioning, and chronic health conditions provides insights into the enduring consequences. These studies guide comprehensive care strategies and inform policies that address lifelong outcomes (Turrentine *et al.*, 2019). Additionally, collaborative efforts between maternal-fetal medicine specialists, neonatologists, and developmental experts can optimize outcomes for PROM-associated preterm birth cases. These models prioritize continuity of care, early interventions, and holistic support (Sullivan *et al.*, 2023).

In conclusion, the future of PROM research and practice is brimming with opportunities to deepen our understanding, refine interventions, and enhance maternal and neonatal health outcomes. The pursuit of enhanced risk stratification, molecular insights, diagnostic innovations, and culturally sensitive approaches paves a promising horizon. As researchers, clinicians, and advocates embark on this journey, the collective efforts are poised to reshape the landscape of PROM, ushering in a future where prevention, early detection, and optimized care converge to nurture healthier beginnings.

1.8 Aims and Objectives

Prior investigations into the identification of risk factors associated with premature rupture of the membrane (PROM) have encompassed diverse geographic contexts, predominantly within African settings. In the context of Bangladesh, a series of studies have addressed PROM and its implications among expectant women. However, it is noteworthy that the majority of these studies have primarily focused on the outcomes of PROM and preterm premature rupture of the membrane (PPROM), lacking the comprehensive insights necessary for crafting effective prevention strategies. The research endeavors dedicated to uncovering the risk factors underlying PROM have often adopted a cross-sectional observational approach, which has limitations in probing the deeper causative roots of this condition.

Moreover, it is important to highlight that the critical examination of vaginal and urinary tract pathogens, recognized as significant modifiable risk elements for PROM, has not been extensively explored within these prior investigations. Furthermore, these studies have predominantly centered within the confines of urban tertiary care hospitals, neglecting the unique dynamics of rural locales such as Kishoreganj in Bangladesh. Consequently, an evident knowledge gap persists, motivating the present study to address these shortcomings comprehensively.

The upcoming study seeks to comprehensively investigate diverse PROM risk factors, categorizing them into personal, behavioral, social, and pathophysiological dimensions, while also exploring infections from common pathogens. Moreover, it will evaluate biochemical and immunological markers, unexplored in our country's context, as potential predictors of PROM.

This research endeavors to significantly contribute to PROM prediction and prevention, ultimately reducing maternal and neonatal mortality and morbidity. It aspires to be a beacon of progress, advancing maternal and fetal health outcomes. The specific objectives encompassed the following key facets:

I. Exploration and detection of interrelated risk factors contributing to the incidence of PROM.

II. Identification and characterization of anaerobic, aerobic bacteria, and pathogens directly associated with PROM.

III. Comprehensive analysis to ascertain patterns of antimicrobial resistance within the context of PROM.

IV. Development of a specialized framework for the early detection of PROM utilizing targeted markers.

Chapter 2:
Material and Methods

2. Materials and Methods

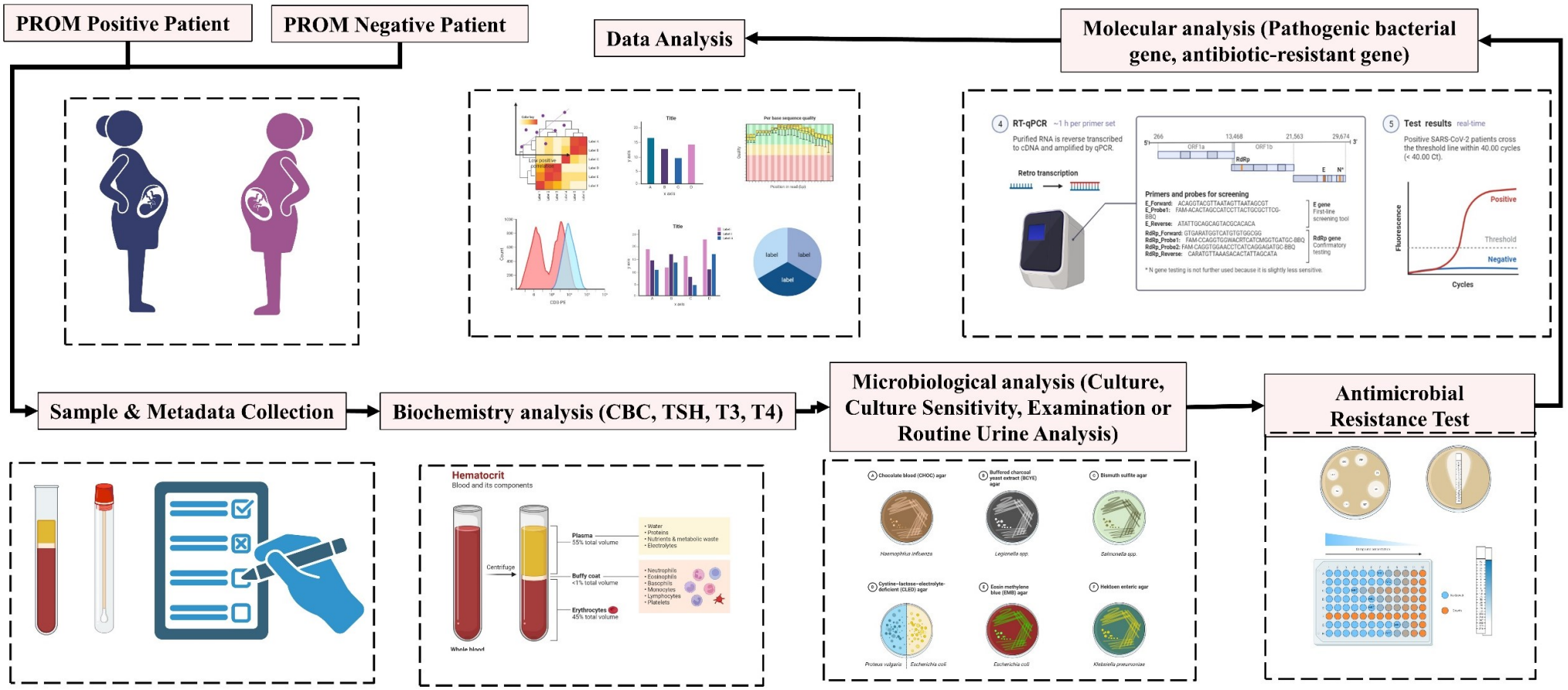

Figure 5. A brief overview of the detail process throughout the study.

2.1 Study Design

This investigation employed a case-control study design, wherein the cases were individuals affected by PROM, while the controls were individuals unaffected by PROM. The study encompassed a total of 390 participants (pregnant women), with 290 individuals serving as the control group (PROM negative) and 100 individuals constituting the case group (PROM positive).

Each participant willingly contributed by completing a comprehensive questionnaire and providing both blood and vaginal samples. These invaluable contributions were pivotal in facilitating the thorough investigation conducted in this study. This experimental study was carried out in the Advanced Molecular lab and Microbiology lab under the Department of Microbiology at President Abdul Hamid Medical College and Hospital, Kishoreganj. The main purpose of this study was to identify pathogens and other risk factors associated with PROM disease following Microbiology, Biochemistry, Immunology, and Molecular aspects.

2.2 Study Area

All of the samples in this study were collected from the Kishoreganj area under the Kishoreganj district of Bangladesh from January to July 2023 **(Figure 6A-B).** The study participants in this case-control study selected from women of gestation age from 28 weeks and above, to be managed in three hospitals of Kishoregonj Districts namely, President Abdul Hamid Medical College and Hospital, Shahid Sayed Nazrul Islam Medical College and Kishoregonj Sadar Hospital after having written consent from them.

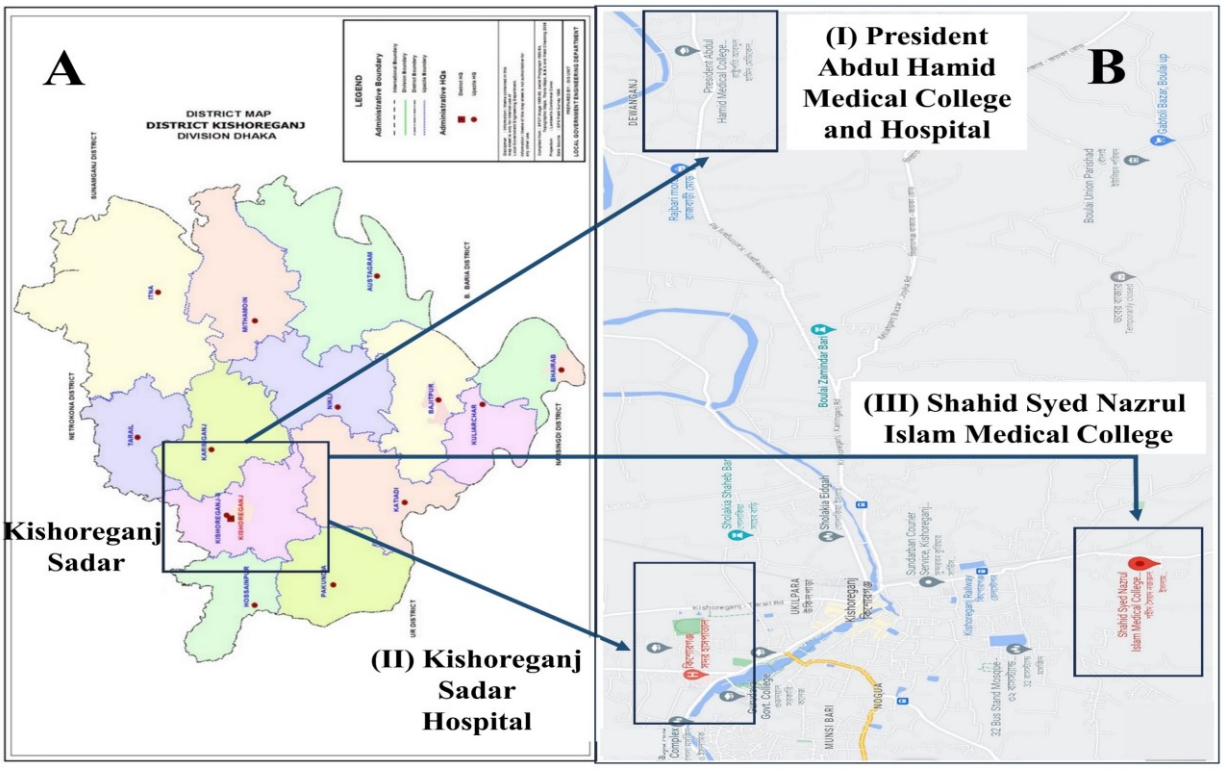

Figure 6. The sample collection areas, the strategic locations for obtaining blood, urine, and vaginal samples from participants, **A.** The geographic location of Kishoreganj within the broader context of the Kishoreganj district. **B.** Specific sampling points, a more detailed overview of the collection areas.

2.3 Investigating Diverse Samples

Employing a methodical approach, sterile tools such as cotton swab sticks, spoons, syringes, urine, and blood collection tubes were utilized to collect an assortment of samples. These samples were procured from three distinct hospitals located in the Kishoreganj district of Bangladesh, as depicted in **Figure 2**. This sampling encompassed three types of specimens: blood, urine, and high vaginal swabs. To ensure effective preservation and transit, five different transport media were selected: Anaerobic Transport Media (ATM), Stuarts Media, Normal Saline Media, Amies Medium with Charcoal, and Viral transport medium (VTM), outlined in **Figure 8**.

Throughout the process, meticulous records were kept, associating each sample with relevant metadata. Samples were refrigerated at 4°C during transportation to the laboratory, ensuring their integrity for subsequent analysis. Different types of sterile tubes (Blood Collection Tubes, Urine Collection Tubes, High Vaginal Swab Collection Tubes, Anaerobic Transport Media (ATM) Tubes, Stuart's Media Tubes, Normal Saline Media Tubes, Amies Medium with Charcoal Tubes and Viral Transport Medium (VTM) Tubes) were employed for sample collection, with accompanying blanks to monitor potential contamination.

Figure 7. Unlocking insights from PROM samples: A visual journey, **A.** Diverse samples safeguarded in specialized transport media, **B.** Sample symphony - Individual specimens nestled in sterile plastic pouches, **C.** Liquid clues encased - Urine samples embracing urine collection tubes, **D.** Precision tagging - Patient IDs merged with corresponding samples, **E.** A glimpse of diversity - Array of unpacked individual samples, **F.** Guardian of quality - The ice box ensuring sample integrity in transit.

2.4 Data Collection

An approved consent form **(Figure 31)** reviewed by the Ethical Review Committee had been issued to the individuals and hence a detailed questionnaire form **(Figure 32-33)** was filled up by them. Most of the questions included several risk factors as well as recognized risk factors for PROM cases in first-world countries.

2.4.1 variables

The dependent variable: Premature Rupture of Membrane confirmed by clinical features (painless gush of fluid that leaks out of the vagina and a change in color of nitrazine paper) and sterile speculum examination. The independent variables included but not limited to socio-demographic data, gravidity status, gestational age, body mass index (BMI), history of cesarean section, miscarriage, any known chronic illness of the mother, coital history immediately before rupture membrane, high fever of the mother, any known infection, anemia, presence of offending bacteria in high vaginal swab culture, urine positive for the culture of bacteria, etc. **(Figure 32-33)**.

2.4.2 Data Validation and Quality Control

To guarantee the precision and reliability of our data, rigorous steps were taken. The validation process encompassed meticulous cross-checking against source documents and employing double data entry. Advanced statistical tools were harnessed for thorough data cleaning and screening, further reinforcing the data's integrity. Any inconsistencies encountered were meticulously addressed through consultation and rigorous verification procedures. This multi-layered approach significantly fortified the credibility and robustness of our research outcomes.

2.5 Data Analysis

2.5.1 Training and Standardization

Before data collection, research personnel underwent comprehensive training to ensure consistency and accuracy. Training encompassed protocol understanding, sample collection techniques, questionnaire administration, and data entry procedures.

2.5.2 Data Management

Data were securely stored and managed using a centralized electronic database. Stringent access controls and encryption protocols ensured data confidentiality and integrity. Regular backups were conducted to prevent data loss. Participant information was de-identified using unique identifiers for privacy protection. Data quality was maintained through routine checks and validations. Audit trails tracked data interactions, and retention policies aligned with ethical and regulatory standards

were established. This approach ensured data security, accuracy, and compliance throughout the study.

2.5.3 Software and Tools

For efficient data management, the software program REDCap (Research Electronic Data Capture) (https://www.project-redcap.org/) was employed for data entry and storage. This secure and user-friendly platform facilitated data collection, minimized errors, and ensured confidentiality. Subsequently, Microsoft Excel 365 was utilized for data cleaning and organization, ensuring data accuracy and consistency. Statistical analyses were conducted using IBM SPSS Statistics (version 26) (https://www.ibm.com/us-en), a robust software tool widely recognized for its capabilities in data analysis and interpretation.

2.5.4 Statistical Analysis

The relationships between variables, differences, and associations were assessed through a range of statistical tests. To explore the associations between categorical variables, Chi-square tests were applied. Logistic regression was employed to identify predictors of outcomes. The statistical significance level was set at $p < 0.05$ for all analyses. These rigorous statistical approaches allowed for the comprehensive examination of the data and meaningful interpretation of findings.

2.6 Microbiological Assay

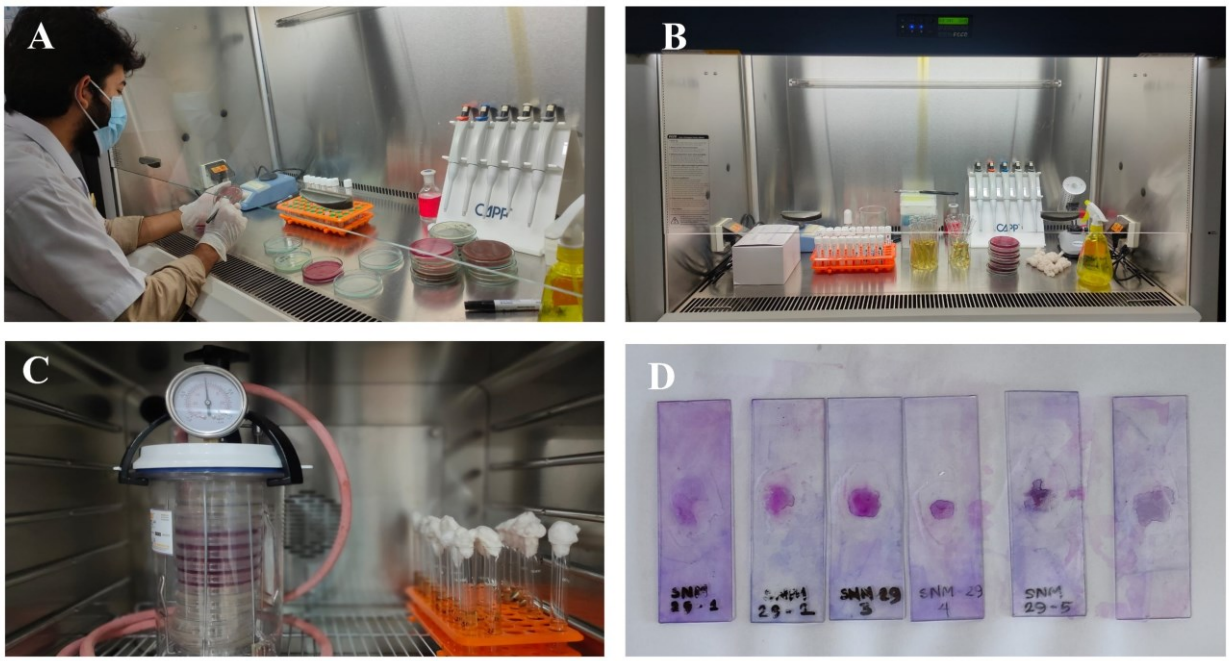

Figure 8. Microbiological assay under processing; **A.** Bacterial culture in progress; **B.** Laminar airflow workstation with essential components. **C.** Anaerobic gas jar containing a culture plate; **D.** Microscope slides prepared for Gram staining and subsequent microscopic examination.

2.6.1 Isolation of Bacteria

The method adopted for microbiological culture adhered to a standardized protocol, ensuring consistent and reliable results for the cultivation of both aerobic and anaerobic bacteria from the collected samples. To discern and isolate these microbial entities, an array of nutrient-rich media was judiciously employed. For the cultivation of both aerobic and anaerobic bacteria, the following media were meticulously selected: Nutrient Agar, MacConkey Agar, UTI Media, and MRSA Media. For the specialized cultivation of anaerobic bacteria, the use of an anaerobic gas jar with a meticulously calibrated gas pack and an indicator was a critical step. A single loopful of each sample was meticulously inoculated onto dedicated petri plates, serving as the breeding ground for bacterial growth. Ensuring optimal conditions, these plates underwent an incubation process at a temperature of 37°C, spanning overnight.

2.6.2 Characterization of Isolates

The potential soil isolates selected from the primary and secondary screening were characterized by standard morphological, microscopical, biochemical, and molecular identification methods.

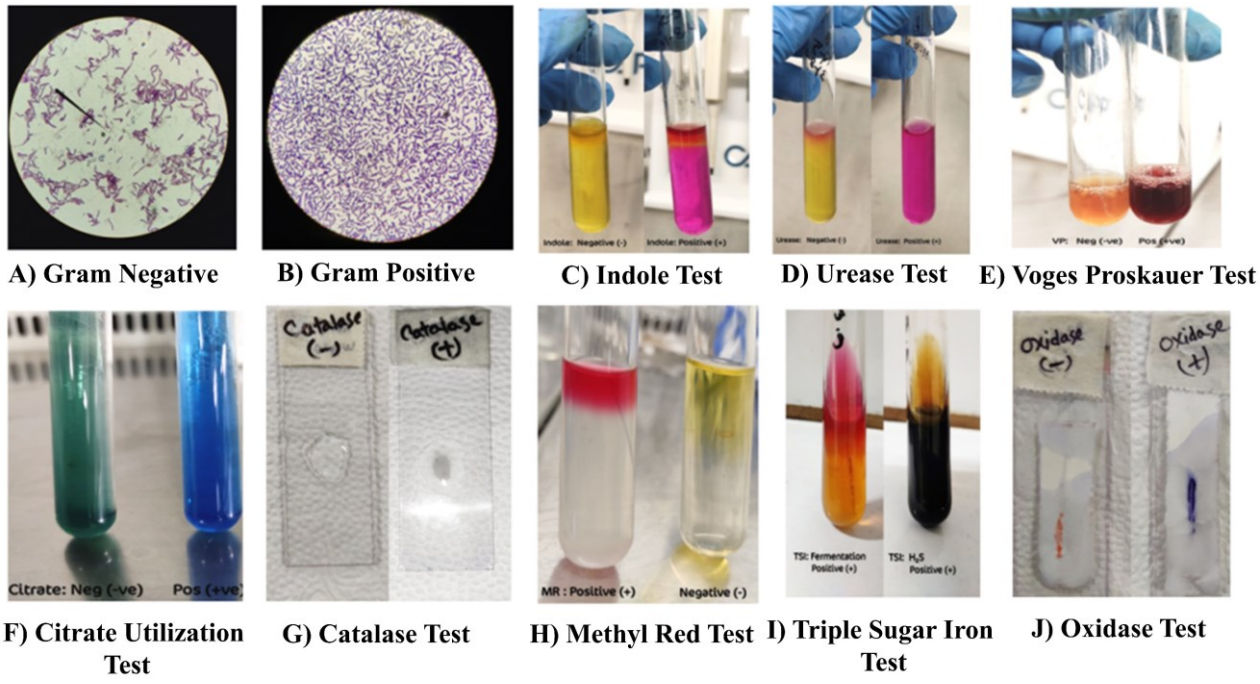

Figure 9. Microscopic observation and several biochemical tests

2.6.2.1 Microscopical Characterization

In the pursuit of accurate bacterial classification, Gram's staining method was meticulously employed. This method involved placing a drop of distilled water on clean glass slides, followed by smearing a loop-full suspension of each bacterial strain. After air-drying and heat-fixing, crystal violet was applied, followed by mordant iodine and 95% ethyl alcohol for decolorization. Counterstaining with safranin was performed, and the stained slides were air-dried before

examination under an oil immersion light microscope at 100X magnification. This comprehensive technique enabled the classification of bacterial strains into Gram-positive and Gram-negative categories, contributing to precise bacterial identification and characterization.

2.6.2.2 Biochemical Characterization

Following preliminary investigations, isolates underwent thorough identification through a battery of biochemical tests. These tests encompassed oxidase, catalase, methyl red, Voges-Proskauer, citrate utilization, motility indole urease, and triple sugar iron assays. To ensure accurate outcomes, cultures were meticulously incubated at 37°C for 1-2 days.

I. Catalase test:

- A sterile wooden stick facilitated the transfer of a small colony onto a clean, dry glass slide.
- A droplet of 3% H2O2 was meticulously placed on the glass slide.
- The emergence of oxygen bubbles was meticulously observed, indicative of the catalase reaction.

II. Oxidase test:

- Filter paper strips of Whatman's No. 1 were immersed in a freshly prepared 1% solution of tetramethyl-phenylene-diamine dihydrochloride before being placed in a petri dish.
- The colonies slated for testing were individually picked using sterile wooden sticks and gently smeared over the damp area.
- Rapid assessment of a positive or negative reaction was accomplished by noting the presence or absence of a vibrant deep-purple hue within 5-10 seconds.

III. MIU test:

- A sterile loop enabled the isolation of a single colony, which was then vertically stabbed into the MIU medium, leaving a 1/3 segment from the base of a test tube.
- Each test tube underwent a 24-hour incubation period at 37°C.
- Following incubation, 2-3 drops of Kovac's reagent were introduced into each tube, with the emergence of a pink-red color ring indicating an indole-positive reaction.

IV. TSI test:

- A sterile straight loop was used to vertically stab the prepared TSI agar initially through the medium's center to the tube's base.
- Subsequently, the surface of the agar slant was streaked.

- Tubes were loosely capped during a 24-hour incubation period at 37°C.

V. Citrate Utilization test:

- The slant of Simmon's Citrate Agar was meticulously streaked back and forth using a light inoculum derived from the center of a well-isolated colony.
- Incubation was carried out at 37°C for a span of 4-7 days.
- The observation of a color shift from green to blue along the slant indicated citrate utilization.

VI. MR-VP test:

- For a well-isolated microbe, MRVP broth was prepared in two distinct test tubes, one labeled as MR and the other as VP.
- The isolated single colony of the microorganism was individually inoculated into the test tubes using a sterile loop.
- After 48 hours of incubation, the introduction of methyl red into the MR broth and alpha-naphthol along with potassium hydroxide into the Voges-Proskauer broth enabled the observation of color changes, indicating respective reactions.

2.6.3 Stock Preservation of the Isolates

Each isolate was streaked aseptically on EMB agar plates for stock culture and incubated at 37°C overnight for single colony formation. After incubation, single colonies for each isolate were inoculated into a sterile microcentrifuge tube containing 750 µl nutrient broth and 250 µl glycerol. Then performed vortex and the stock culture for each isolate was kept at -20°C. Duplicate sets of microcentrifuge tubes were prepared for each isolate and maintained at -80°C.

2.6.4 Antimicrobial Sensitivity Testing

In this pivotal segment, the antimicrobial sensitivity of the test isolates was meticulously evaluated using the well-established and standardized agar-disc-diffusion method, commonly known as the Kirby-Bauer method (Jones *et al.*, 1985), derived from the pioneering work of Bauer (Bauer *et al.*, 1966). To ensure robustness and precision, the study harnessed commercially available discs in tandem with Mueller-Hinton agar, an industry-standard medium known for its reliability (HIMEDIA Limited, India) (refer to Appendix-I for details).

I. Isolate Cultivation and Inoculum Preparation: A judicious protocol was adopted for cultivating each isolate. Thirty-nine isolates were reinvigorated on EMB agar plates from the stock culture. After isolating 1-2 pure colonies, the aseptic technique was employed to transfer them into test tubes, each containing 5mL of nutrient broth, utilizing a sterile inoculating loop.

II. Turbidity Standardization and Inoculum Adjustment: The inoculated broth was meticulously incubated at 37°C until it attained or exceeded the turbidity of the 0.5 McFarland standards. McFarland 0.5 turbidity standard was meticulously prepared by skillfully blending 0.05 mL of 1.175% barium chloride dehydrate (BaCl2.2H2O) with 9.95 mL of 1% sulfuric acid (H2SO4), adhering to the formulation described by McFarland (McFARLAND, 1907).

III. Achieving Optical Equivalence: The turbidity of the actively growing broth culture underwent precision adjustment with sterile broth, meticulously aligned to achieve optical comparability with the benchmark set by the 0.5 McFarland standards.

2.6.4.1 Inoculation of Test Plates

MHA plates were inoculated with the working culture according to the following processes (Gunasegaran *et al.*, 2011). a) After adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension and rotated several times pressing firmly on the inside wall of the respective culture tube above the fluid level. This removed excess inoculum from the swab. b) The dried surface of the MHA plate was inoculated by streaking the swab over the entire sterile agar surface. The streaking was repeated twice more rotating the plate approximately 60° each time to ensure an even distribution of inoculum. At last, the rim of the agar was swabbed. The process was carried out by maintaining standard biosafety procedures inside a class II Biosafety Cabinet (ESCO, Singapore). c) The lid was left ajar for 3-5 minutes but not more than 15 minutes, so that any excess surface moisture could be absorbed before applying the antibiotic discs.

2.6.4.2 Impregnation of Antibiotics Discs on Inoculated Agar Plates

Five-six sterile antibiotic discs (HiMedia Laboratories Limited., India) were placed onto each inoculated agar plate. The discs were pressed down individually to ensure complete contact with the agar surface. The discs on the agar surface were not closer than 24 mm from center to center. The plates were placed inverted in the incubator (Memmert, Germany) set at 37°C within 15 minutes of the impregnation of antibiotics discs.

2.6.4.3 Reading plates and Interpretation

After 16-18 hours of incubation, each plate was examined for the zone of inhibition, uniformly circular with a confluent lawn of growth. The diameters of zones of complete inhibition (judged by the unaided eye) were measured, including the diameter of the disc. The zone was measured to the nearest whole millimeter. The faint growth of tiny colonies could be detected only with a magnifying lens at the edge of growth inhibition, and the sizes of zones of inhibition were interpreted according to the performance standards for antimicrobial susceptibility testing. Finally,

organisms were reported as susceptible, intermediate, or resistant to various antibiotics based on CLSI & EUCAST, 2013 **(Table 1)**.

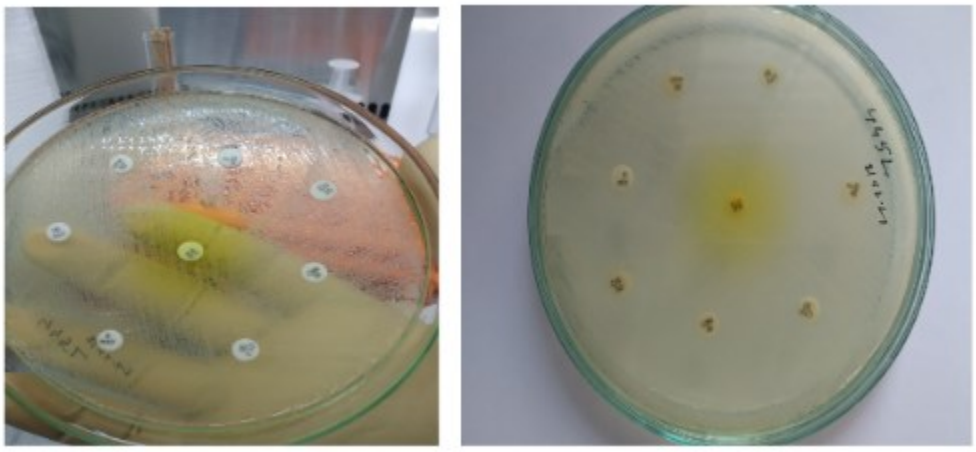

Figure 10. Antibiogram test of PROM bacterial isolates

Table 1. Antibiotic susceptibility patterns of various bacterial groups: A comparative scenario

Category of antibiotic		Disc concentration	Abbreviation	Zone Diameter Interpretive Criteria (nearest whole mm)			Reference
				S (≥)	**I**	**R (≤)**	
Group-I cell wall synthesis inhibitors							
Beta-lactams	Penicillin G	10 µg	P	29	-	28	CLSI 2018
	Cefoxitin	30 µg	FOX	22	-	21	CLSI 2018
	Ampicillin	10 µg	AMP	29	-	28	CLSI 2018
	Ceftazidime	30 µg	CAZ	18	15-17	14	CLSI 2018
	Cefepime	30 µg	CPM	25	19-24	18	CLSI 2018
	Cefotaxime	30 µg	CTX	23	15-22	14	CLSI 2018
Group-II Protein synthesis inhibitors							
Macrolides	Azithromycin	15 µg	AZM	18	14-17	13	CLSI 2018
Aminoglycoside	Gentamicin	10 µg	GEN	15	13-14	12	CLSI 2018
	Amikacin	30 µg	AK	17	15-16	14	CLSI 2018
Phenicols	Chloramphenicol	30 µg	C	18	13-17	12	CLSI 2018
Group-III Nucleic acid synthesis inhibitors							
Fluoroquinolones	Ciprofloxacin	5 µg	CIP	50	23-49	23	EUCAST 2018
	Levofloxacin	5 µg	LEV	50	23-49	23	EUCAST 2018
Group-IV Cell membrane inhibitors							
Lipopeptides	Colistin sulphate	10 µg	C	11	9-10	8	(Charteris *et al.*, 1998)
Group-V Folic acid synthesis inhibitors							
Sulfonamides	Co-Trimoxazole	25 µg	COT	16	11-15	10	CLSI 2018

2.7 Molecular Assay

2.7.1 DNA Extraction (Boil DNA Extraction)

Bacterial DNA was extracted following the boil DNA and commercial kit method.

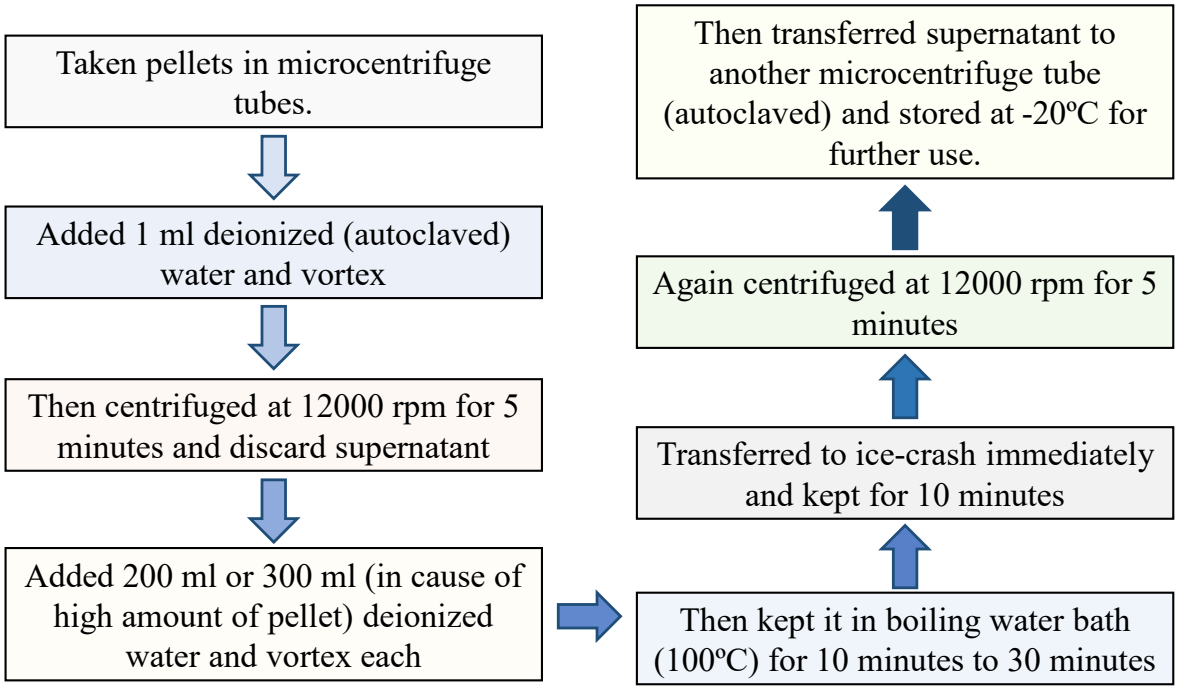

Figure 11. DNA Extraction Protocol Utilizing Boil DNA Method

2.7.2 Polymerase Chain Reaction (PCR)

Multiplex polymerase chain reaction (Multiplex PCR) refers to the use of polymerase chain reaction to amplify several different DNA sequences simultaneously (as if performing many separate PCR reactions all together in one reaction). This process amplifies DNA in samples using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler. The primer design for all primer pairs has to be optimized so that all primer pairs can work at the same annealing temperature during PCR. Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple sequences at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis. Alternatively, if amplicon sizes overlap, the different amplicons may be differentiated and visualized using primers that have been dyed with different color fluorescent dyes.

I. 16S rRNA Amplification

16S rRNA of the selected isolates (*MRSA*, ESBL positive *E. Coli, Bacillus spp., Streptococcus spp.*) was amplified by the 16S rRNA PCR method. 16S rRNA universal primers included for this methodwere:

- 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′),
- 1492 R (5′- CCGTCAATTCMTTTRAGTTT-3′) (Sambo *et al.*, 2018)

PCR was carried out in a 25 µl volume reaction mixture containing:

- 1 µl of each primer,
- 3 µl of crude template DNA and
- 10 µl One taq 'Quick load' 2x master mix.
- 10 µl nuclease-free water

The PCR reaction was performed with below conditions:

- Initial denaturation at 95°C for 5 minute, 35 cycles of final denaturation at 95°C for 1min,
- Annealing at 50°C for 30s and
- Initial extension at 72°C for 1 min with a final extension at 72°C for 5 min.

DNA fragments amplified were 1400 bp in size. In this PCR, a positive control (ATCC's microorganism) and a negative control were used. The amplified PCR products were electrophoresed on 1.5 % agarose gel at 80V for 1 hour. The gel was stained with 10% Ethidium Bromide and then visualized.

II. Pathogen Specific Assay

Table 2. Primers and PCR conditions for bacterial pathogens detection

Bacteria	**Primer**	**Annealing Tem.**	**Product Size**
mecA *(Staphylococcus aureus)*	F: 5′-AAA ATC GAT GGT AAA GGT TGGC-3′ R: 5′-AGT TCT GCA GTA CCG CAT TTGC-3′ (McClure *et al.*, 2020)	56 ^{0}C	533 bp
bla $_{SHV}$ *(Escherichia coli)*	F: 5′-CAC TCA AGG ATG TAT TGTG-3′ R: 5′-TTA GCG TTG CCAGTG CTCG-3′ (Dallenne *et al.*, 2010)	62 ^{0}C	713 bp
bla $_{CTX-M-15}$ *(Escherichia coli)*	F: 5′-CAC ACG TGG AAT TTA GGG ACT 3′ R: 5′-GCC GTC TAA GGC GAT AAA CA-3′. (Pagani *et al.*, 2003)	53 ^{0}C	996 bp
fenD	F: 5′-TTT GGC AGC AGG AGA AGT TT-3′	60 ^{0}C	964 bp

(Bacillus Subtilis)	R: 5′ GCT GTC CGT TCT GCT TTT TC -3′ (Ramarathnam *et al.*, 2007)		

2.7.3 Preparation of 1.5% Agarose gel

1.05g agarose powder mixed well into 70 ml deionized water and boiled for 5 minutes. Then cooled at room temperature for gel formation.

I. Preparation of TAE buffer

20.0 ml 50X TBE was dissolved in 980 ml distilled water for the preparation of 1x×1000 ml TAE buffer, this solution was stored at room temperature.

II. Preparation of staining solution

200.0 ml TAE buffer was mixed with 4 drops of Ethidium Bromide (Et-Br) for preparation of staining solution, this solution was stored at room temperature.

III. Gel documentation

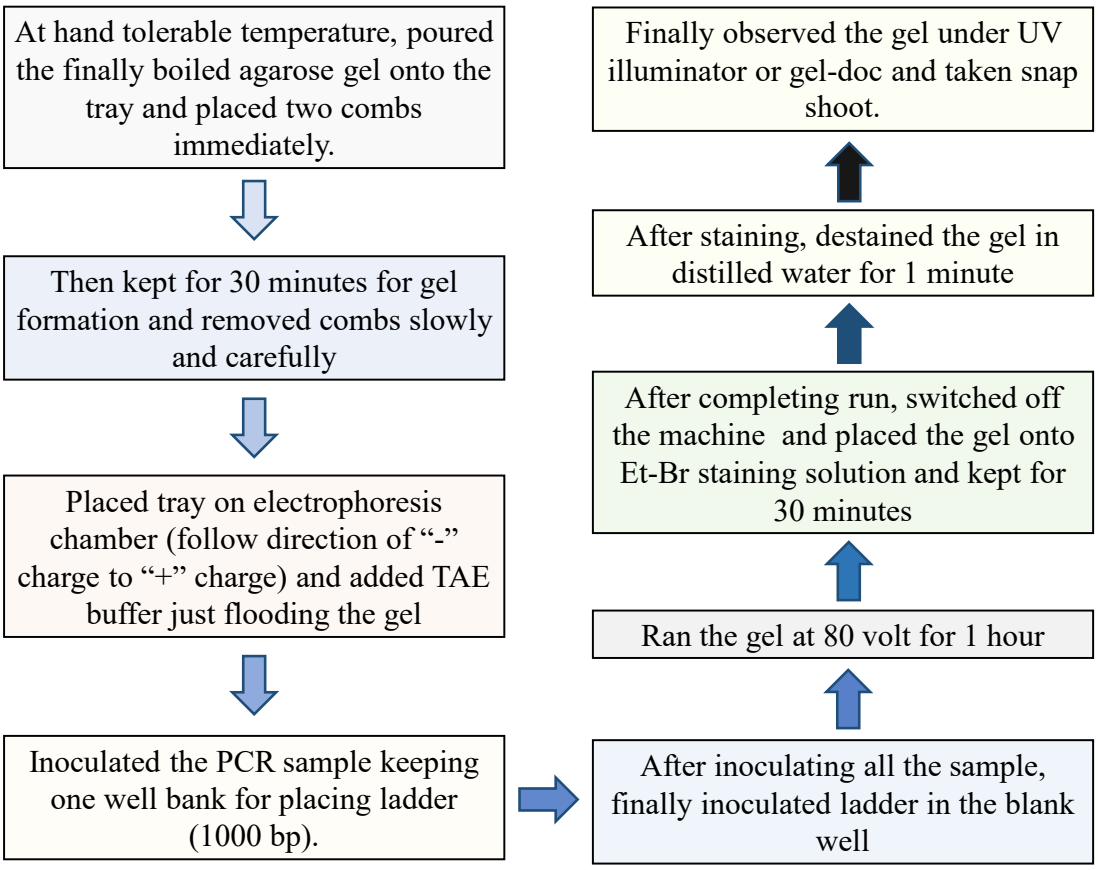

Figure 12. Gel Documentation: Protocol and Procedure

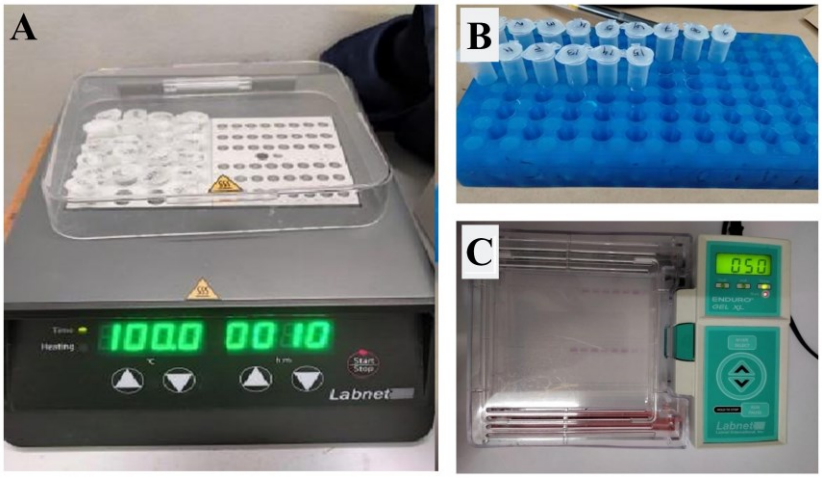

Figure 13. DNA Extraction and Agarose Gel Electrophoresis, **A.** Extraction by boiling technique; **B.** Extracted DNA is prepared for further molecular analysis; **C**. Apparatus for PCR product analysis.

2.8 Hematological and Immunological Assay

In the realm of diagnostic medicine, serological tests hold paramount significance as they encompass an array of pivotal blood analyses. The intricacies of these procedures are expounded below:

I. CBC: The skin overlaying a prominent arm vein was carefully cleansed, and a sterile needle was used to procure a blood specimen into a designated tube. Subsequently, the array of blood components, encompassing red blood cells, white blood cells, and platelets are quantified.

II. Thyroid Hormone Evaluation: Analogously, the designated arm vein site was cleansed, and a blood sample was collected using a sterile needle. Consequently, the levels of thyroid-stimulating hormone (TSH) and thyroid hormones, specifically T3 and T4, within the sample were measured.

III. Creatinine, Ferritin, CRP, and AFP Assessment: Likewise, the skin surrounding the chosen arm vein was meticulously disinfected, and a blood sample was extracted using a sterile needle. The obtained specimen then made its way to the laboratory for thorough examination. Following this, the levels of these markers were ascertained.

2.9 Ethical considerations

The study was reviewed and approved by the ethics committee of the Directorate General of Health Sciences (DGHS), Bangladesh, and by the National Research Ethics Committee (NREC) of the Bangladesh Medical Research Council (BMRC). President Abdul Hamid Medical College and Hospital ERC Review Board for Human Subjects Protection looked over the work based on some criteria.

Chapter 3:

Results

3. Results

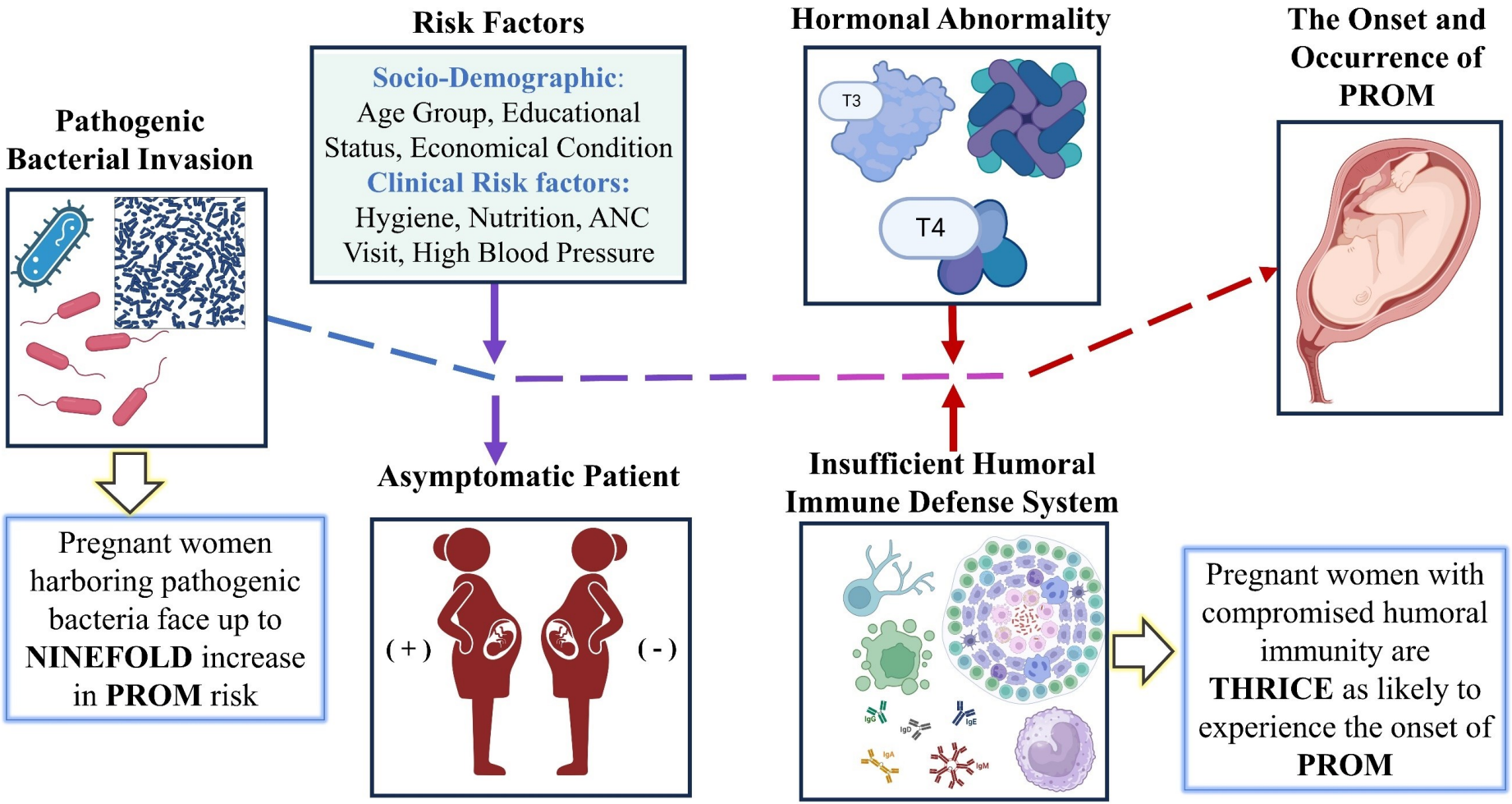

Figure 14. A concise recapitulation of the key study insights and observations

3.1 Statistical Analysis

The study unveiled several significant associations between various demographic and medical factors and the occurrence of PROM **(Table 3, Figure 15-18).** Notably, age emerged as a critical factor, with higher prevalence rates in specific age groups. Among women aged 16 to 20, PROM was observed in 29 cases, constituting 29% of this age group, while in the 31 to 40 age range, PROM was found in 53 cases, accounting for 53%. These figures contrast with the 21 to 30 age group, where PROM was noted in 18 cases, representing 18% of that group.

Economic status also played a pivotal role. Within the lower-income group, 12 cases of PROM were recorded, making up 12% of this category. In contrast, the middle-income group had 28 cases (28%), while the upper-income group showed the highest prevalence, with 60 cases (60%). Educational status demonstrated intriguing patterns. PROM was more prevalent among individuals with no education, constituting 39% of this group. In contrast, the highest level of education appeared to correlate with a lower risk of PROM, with the "Higher Education" category accounting for only 11% of cases.

Consanguinity in marriages exhibited a significant influence. In cases where consanguineous marriages were present, PROM occurred in 78 cases, representing 78% of that category. Conversely, in non-consanguineous marriages, PROM was observed in 22 cases, making up 22%. Nutrition status also played a role, with 76 cases (76%) of PROM found in individuals with below-average nutrition, while the "Average" group accounted for 16 cases (16%) and the "Good" group for 8 cases (8%).

Additional factors such as itching during pregnancy, a history of previous PROM, a history of injury in the last 48 hours, the number of ANC visits, high blood pressure, discharge, pus cell and epithelial cell abnormalities, abnormal TSH, T3, T4, ferritin, and AFP levels, and the presence of anemia all showed statistically significant associations with PROM.

These findings underscore the complex interplay of demographic and medical factors in the occurrence of PROM, highlighting the importance of considering a broad spectrum of risk factors in its assessment and management.

Table 3. A Chi-Square Analysis Revealing Significant Risk Factors Associated with PROM Incidence

Risk Factors	Category	PROM	N (%)	Non-PROM	N (%)	Total	N (%)	p-value
Age Group	16 to 20	29	29.00%	98	33.79%	127	32.56%	<0.01
	21 to 30	18	18.00%	134	46.21%	152	38.97%	
	31 to 40	53	53.00%	58	20.00%	111	28.46%	
Economical Condition	Lowe Income Group	12	12.00%	144	49.66%	156	40.00%	<0.01
	Middle Income Group	28	28.00%	72	24.83%	100	25.64%	
	Upper-Income Group	60	60.00%	74	25.52%	134	34.36%	
Educational Status	No Education	39	39.00%	31	10.69%	70	17.95%	<0.04
	Primary Education	29	29.00%	54	18.62%	83	21.28%	
	Secondary Education	21	21.00%	79	27.24%	100	25.64%	
	Higher Education	11	11.00%	126	43.45%	137	35.13%	
Consanguinity	Yes	78	78.00%	44	15.17%	122	31.28%	<0.0001
	No	22	22.00%	246	84.83%	268	68.72%	
Nutrition	Below Average	76	76.00%	56	19.31%	132	33.85%	<0.001
	Average	16	16.00%	78	26.90%	94	24.10%	
	Good	8	8.00%	156	53.79%	164	42.05%	
Itching	Yes	67	67.00%	112	38.62%	179	45.90%	<0.001
	No	33	33.00%	178	61.38%	211	54.10%	
Previous History of PROM	Yes	78	78.00%	78	26.90%	156	40.00%	<0.001
	No	22	22.00%	212	73.10%	234	60.00%	
Relation to Coitus	Yes	73	73.00%	101	34.83%	174	44.62%	<0.001
	No	27	27.00%	189	65.17%	216	55.38%	
Any history of injury in the last 48 hours	Yes	69	69.00%	112	38.62%	181	46.41%	<0.001
	No	31	31.00%	178	61.38%	209	53.59%	
ANC Visit	Less Than 5 Times	71	71.00%	77	26.55%	148	37.95%	<0.001
	More than 5 Times	29	29.00%	213	73.45%	242	62.05%	
High Blood Pressure	Yes	64	64.00%	192	66.21%	256	65.64%	<0.001
	No	36	36.00%	98	33.79%	134	34.36%	
Discharge	Present	61	61.00%	113	38.97%	174	44.62%	0.0029
	Absent	39	39.00%	177	61.03%	216	55.38%	
Pus Cell	Normal	41	41.00%	174	60.00%	215	55.13%	0.0107
	Abnormal	59	59.00%	116	40.00%	175	44.87%	
Epithelial Cell	Normal	43	43.00%	171	58.97%	214	54.87%	0.0336

	Abnormal	57	57.00%	119	41.03%	176	45.13%	
TSH	Normal	45	45.00%	165	56.90%	210	53.85%	<0.001
	Abnormal	55	55.00%	125	43.10%	210	53.85%	
T3	Normal	32	32.00%	173	59.65%	205	52.56%	0.01
	Abnormal	68	68.00%	117	40.35%	185	47.44%	
T4	Normal	43	43.00%	169	58.27%	212	54.36%	0.036
	Abnormal	57	57.00%	121	41.73%	178	45.64%	
Ferritin	Normal	46	46.00%	166	57.24%	212	54.36%	<0.001
	Abnormal	54	54.00%	124	42.76%	178	45.64%	
AFP	Normal	41	41.00%	177	61.03%	218	55.90%	0.0107
	Abnormal	59	58.00%	113	38.97%	172	44.10%	
Anemia	Mild	7	7.00%	144	49.66%	151	38.72%	<0.0001
	Moderate	13	13.00%	96	33.10%	109	27.95%	
	Severe	32	32.00%	39	13.45%	71	18.21%	
	Very Severe	48	48.00%	11	3.79%	59	15.13%	

3.1.1 Sociodemographic Spectrum

I. Chi-square Portrait

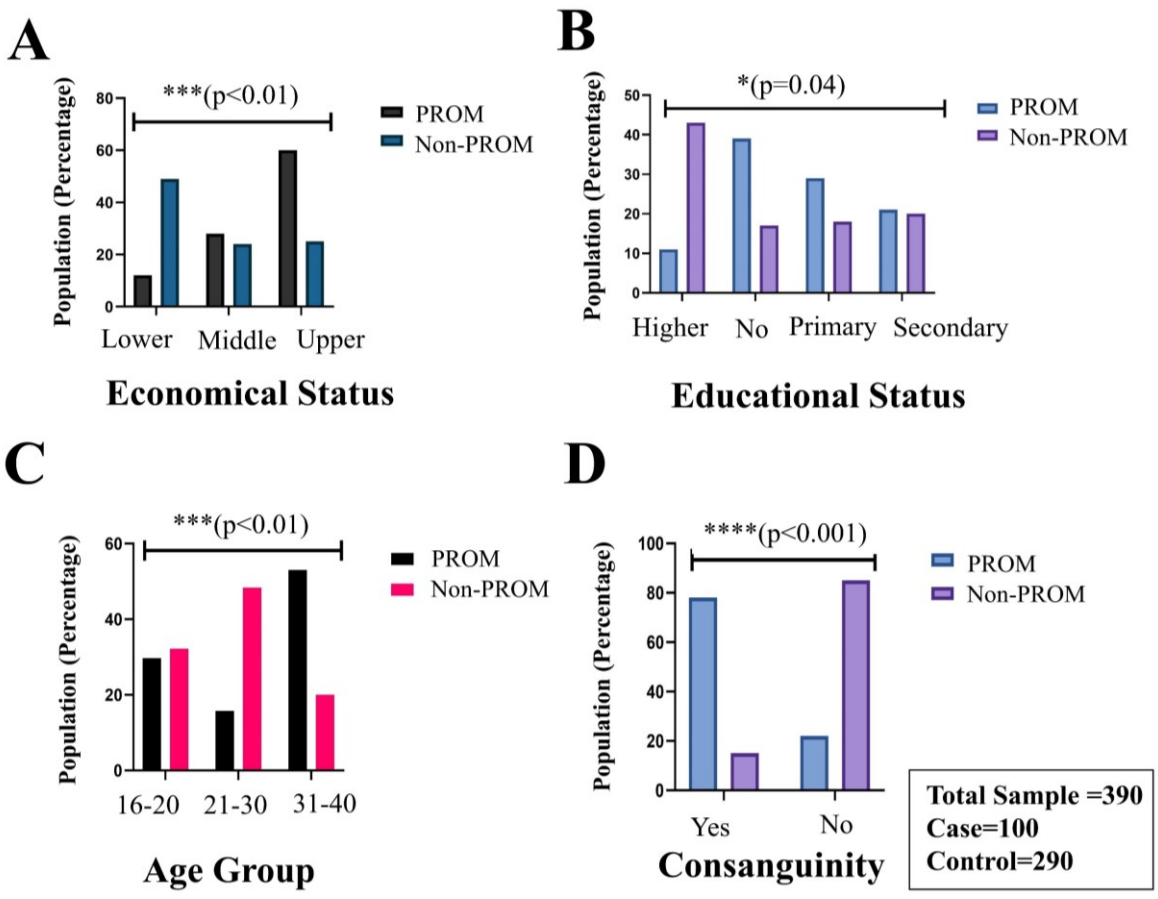

Figure 15. Significant sociodemographic factors having a crucial role in the onset of PROM; **A** economic status, **B.** Educational Status, **C.** Age Group, and **D.** Consanguinity

II. Multivariate Logistic Regression model

In this regression analysis, we have investigated the risk factors associated with PROM, utilizing adjusted odds ratios (AORs) and their corresponding 95% confidence intervals (CIs) to provide a more comprehensive understanding **(Table 4).**

Table 4. A multivariate regression model showing the association of sociodemographic risk factors on the onset of PROM.

Risk Factors	Category	SE	AOR	95% CI		p-value
				Lower	Upper	
Age Group	16 to 20	-	1	-	-	-
	21 to 30	0.258	0.865	0.527	1.231	0.001
	31 to 40	0.347	3.001	1.52	5.925	0.002
Economical Condition	Lowe Income Group	-	1	-	-	-
	Middle Income Group	0.271	1.7	1	2.891	0.05
	Upper-Income Group	0.52	1.627	0.587	4.512	0.35
Educational Status	Higher Education	-	1	-	-	-
	No Education	0.351	2.734	1.374	5.441	0.004
	Primary Education	0.523	3.19	1.145	8.885	0.026
	Secondary Education	0.274	2.198	1.284	3.763	0.004
Consanguinity	Yes	0.572	3.361	1.095	10.321	0.034
	No	-	1	-	-	-

A. Age Group: The age group variable demonstrates notable disparities in the risk of PROM. Within the 21 to 30 age group, the adjusted odds ratio (AOR) is 0.865, with a 95% CI ranging from 0.527 to 1.231 and a statistically significant p-value of 0.001. This suggests a reduced likelihood of PROM in this age category compared to the reference group (16 to 20). Conversely, the 31 to 40 age group displays a substantial increase in risk, with an AOR of 3.001, a 95% CI spanning from 1.52 to 5.925, and a significant p-value of 0.002.

B. Economical Condition: The middle-income group reveals a slightly elevated risk of PROM, as indicated by an AOR of 1.7 and a 95% CI of 1.0 to 2.891, with a p-value of 0.05. The upper-income group, while exhibiting a higher AOR of 1.627, lacks statistical significance ($p = 0.35$) compared to the reference group, the lower-income category.

C. Educational Status: For individuals with no education, the AOR is 2.734, with a 95% CI ranging from 1.374 to 5.441 and a significant p-value of 0.004. Similarly, the primary education group has an AOR of 3.19 (95% CI: 1.145 - 8.885, p = 0.026), and the secondary education group presents an AOR of 2.198 (95% CI: 1.284 - 3.763, p = 0.004). These findings indicate a notable increase in PROM risk for those with lower levels of education.

D. Consanguinity: Those in consanguineous marriages exhibit an AOR of 3.361 (95% CI: 1.095 - 10.321, p = 0.034), signifying a significantly higher risk of PROM compared to individuals in non-consanguineous marriages.

3.1.2 Clinical Spectrums

I. Chi-square Portrait

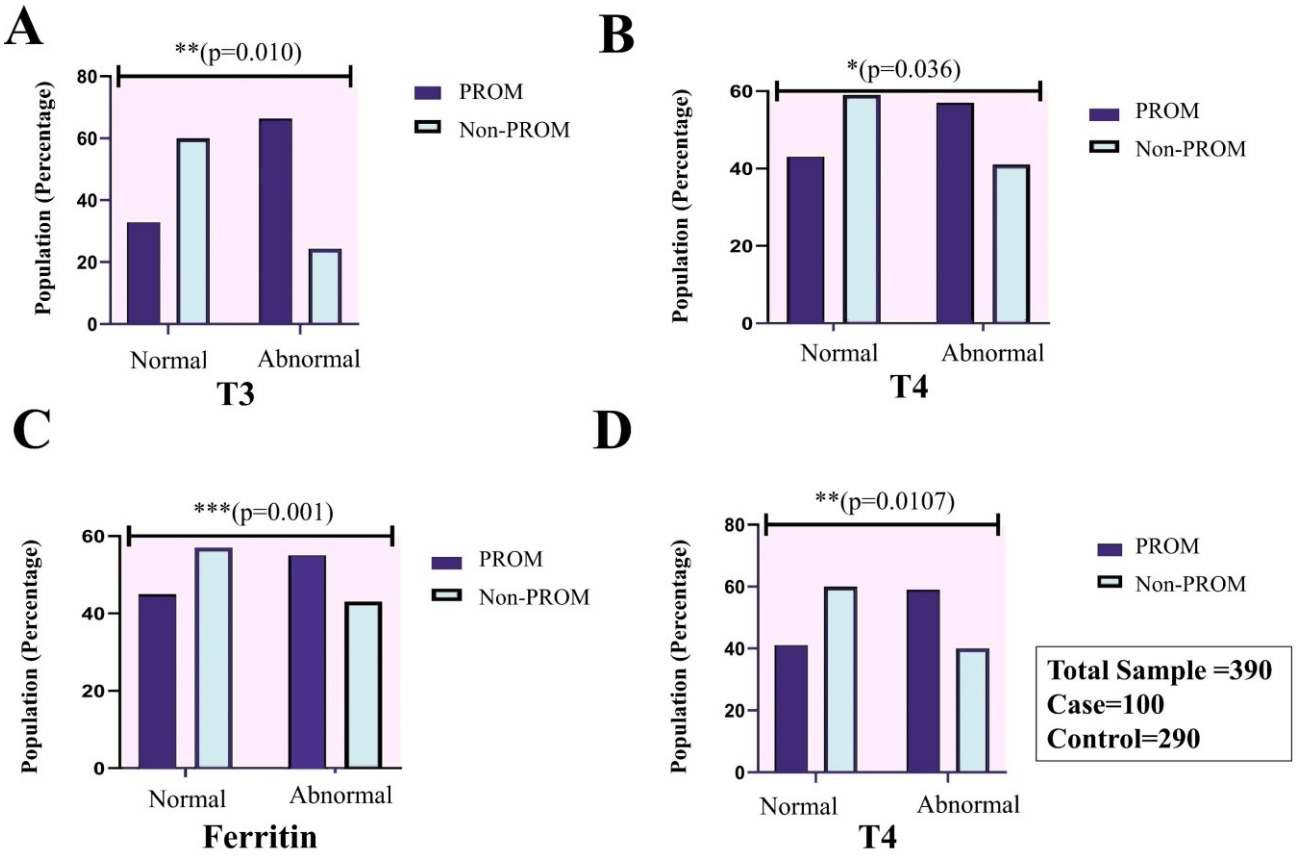

Figure 16. Significant hormonal factors influencing PROM incidence: Chi-Square Test Analysis; **A** T3, **B.** T4, **C.** Ferritin and **D.** AFP.

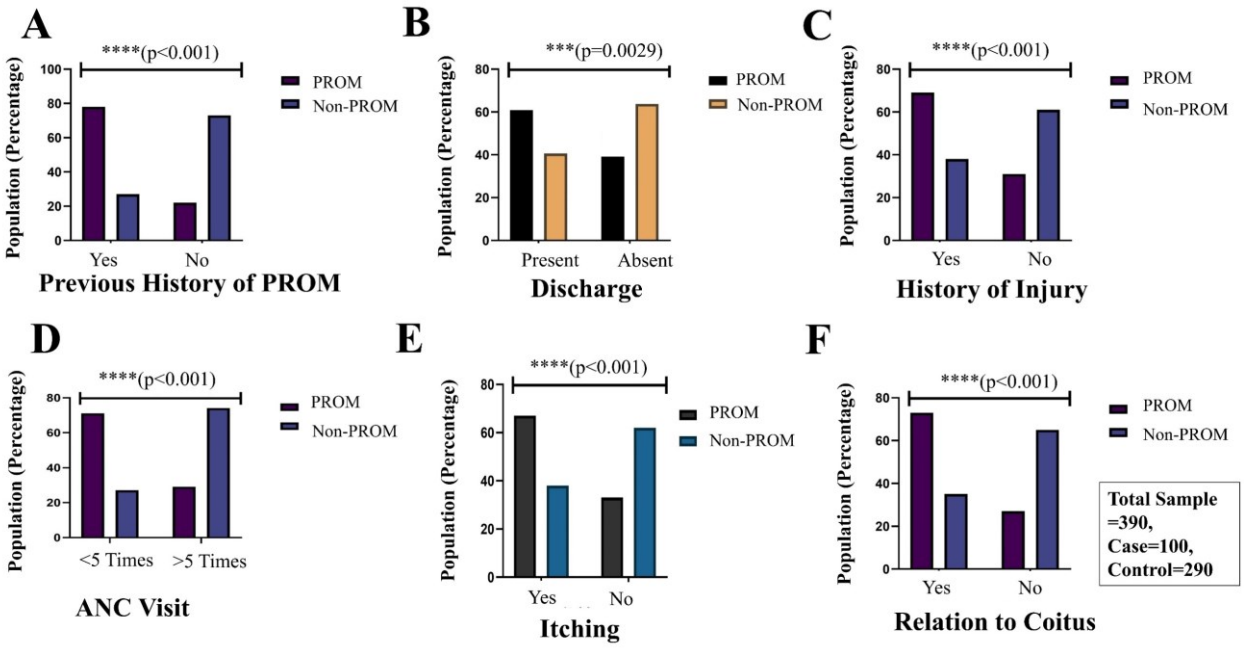

Figure 17. Significant clinical factors have a crucial role in the onset of PROM based on the chi-square test. **A** Previous history of PROM, **B.** Vaginal Discharge, **C.** History of injury in the last 48 hours, **D.** ANC visit, **E.** Itching, and **F.** Relation to coitus

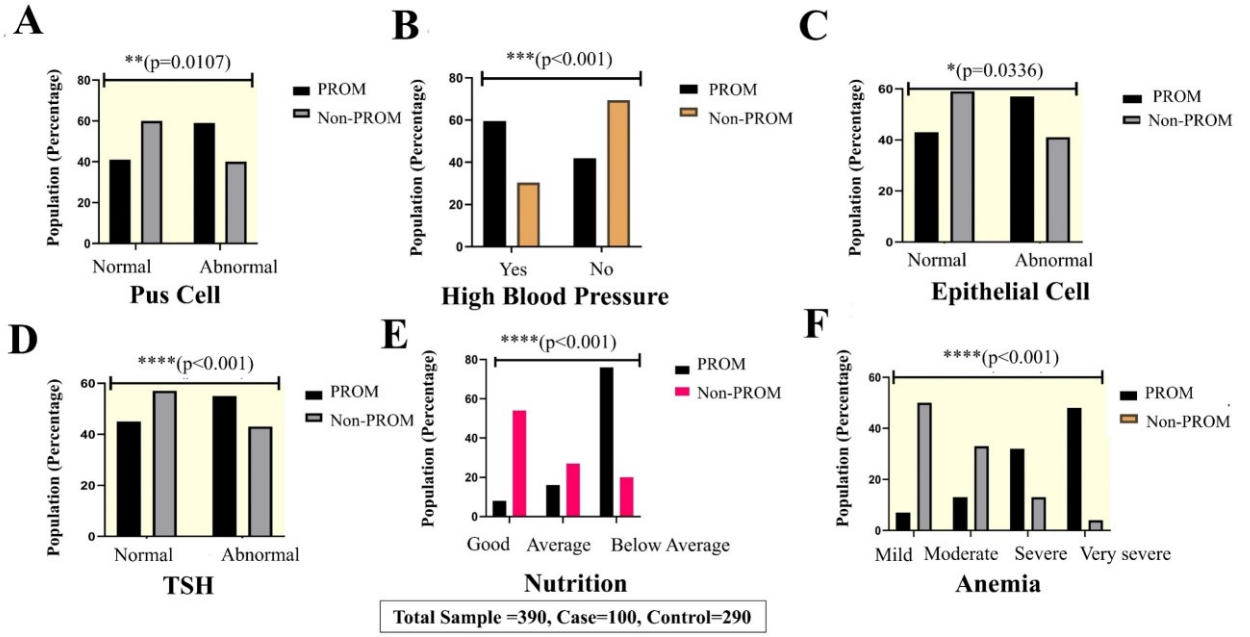

Figure 18. Significant immunological factors influencing PROM incidence: Chi-Square Test Analysis; **A** Pus Cell, **B.** High Blood, **C.** History of injury in the last 48 hours, **D.** ANC visit, **E.** Itching, and **F.** Relation to coitus.

II. Multivariate Logistic Regression Model

In this regression analysis, the impact of clinical factors on the risk of PROM was explored **(Table 5)**.

A. Nutrition: Individuals with below-average nutrition have a significantly increased risk of PROM, as indicated by an AOR of 1.592 (95% CI: 0.975 - 2.598, p = 0.043). Conversely, those with a good nutritional status exhibit a notably reduced risk, with an AOR of 0.504 (95% CI: 0.278 - 0.837, p = 0.036) compared to the reference group, individuals with average nutrition.

B. Itching: The absence of itching is associated with a significantly lower risk of PROM, with an AOR of 0.606 (95% CI: 0.388 - 0.948, p = 0.028).

C. Previous History of PROM: Having a previous history of PROM does not appear to significantly influence the risk of recurrent PROM (AOR: 0.25, 95% CI: 1.374 - 5.441, p = 0.24).

D. Relation to Coitus: The frequency of coitus does not have a substantial impact on the risk of PROM (AOR: 3.361, 95% CI: 1.095 - 10.321, p = 0.534 for individuals with coitus, and AOR: 1 for those without).

E. Any History of Injury in the Last 48 Hours: Individuals with a history of recent injury within the last 48 hours face an increased risk of PROM, with an AOR of 1.713 (95% CI: 1.058 - 2.773, p = 0.029).

F. ANC Visit: Those who have had less than 5 ANC visits have a substantially higher risk of PROM, with an AOR of 3.406 (95% CI: 0.388 - 0.948, p = 0.028) compared to those with more than 5 visits.

G. High Blood Pressure: High blood pressure is associated with an increased risk of PROM, with an AOR of 1.55 (95% CI: 1.033 - 2.326, p = 0.034).

H. Discharge: The presence of discharge is linked to a higher risk of PROM, with an AOR of 1.562 (95% CI: 1.043 - 2.34, p = 0.03).

Table 5. Multivariate logistic regression analysis of clinical risk factors associated with PROM.

Risk Factors	Category	SE	AOR	95% CI		p-value
				Lower	Upper	
Nutrition	Average	-	1	-	-	-
	Below Average	0.25	1.592	0.975	2.598	**0.043**
	Good	0.21	0.504	0.278	0.837	0.036
Itching	Yes	-	1	-	-	-
	No	0.228	0.606	0.388	0.948	0.028
Previous History of PROM	Yes	-	1	-	-	-
	No	0.351	0.25	1.374	5.441	0.24

Relation to Coitus	Yes	0.572	3.361	1.095	10.321	0.534
	No	-	1	-	-	-
Any history of injury in the last 48 hours	Yes	0.246	1.713	1.058	2.773	0.029
	No	-	1	-	-	-
ANC Visit	Less than 5 Times	0.345	3.406	0.388	0.948	0.028
	More than 5 Times	-	1	-	-	-
High Blood Pressure	Yes	0.207	1.55	1.033	2.326	0.034
	No	-	1	-	-	-
Discharge	Present	0.206	1.562	1.043	2.34	0.03
	Absent	-	1	-	-	-

In this regression analysis, the influence of immunological and hormonal factors on the risk of PROM was investigated **(Table 6).**

A. Pus Cell: Abnormal pus cell counts are significantly associated with an increased risk of PROM, as indicated by an AOR of 1.611 (95% CI: 1.071 - 2.421, p = 0.02).

B. Epithelial Cell: Similarly, abnormal epithelial cell counts are linked to a higher risk of PROM, with an AOR of 1.562 (95% CI: 1.042 - 2.341, p = 0.03).

C. Thyroid-Stimulating Hormone (TSH): Abnormal TSH levels are associated with a moderately increased risk of PROM, with an AOR of 1.352 (95% CI: 0.741 - 2.441, p = 0.02).

D. Triiodothyronine (T3): Abnormal T3 levels are linked to an elevated risk of PROM, with an AOR of 1.527 (95% CI: 0.842 - 2.591, p = 0.01).

E. Thyroxine (T4): Abnormal T4 levels are also associated with a higher risk of PROM, with an AOR of 1.599 (95% CI: 0.963 - 2.691, p = 0.02).

F. Ferritin: Abnormal ferritin levels significantly increase the risk of PROM, with an AOR of 1.672 (95% CI: 1.128 - 2.732, p = 0.02).

G. Alpha-Fetoprotein (AFP): Abnormal AFP levels are associated with an elevated risk of PROM, with an AOR of 1.765 (95% CI: 1.383 - 2.911, p = 0.02).

H. Anemia: The risk of PROM increases with the severity of anemia, ranging from moderate (AOR: 1.344, 95% CI: 0.893 - 1.78, p = 0.02) to severe (AOR: 2.311, 95% CI: 1.678 - 2.899, p = 0.05), and very severe (AOR: 3.361, 95% CI: 1.095 - 10.321, p = 0.03).

Table 6. Multivariate logistic regression analysis of immunological and hormonal risk factors associated with PROM.

Risk Factors	Category	SE	AOR	95% CI		p-value
				Lower	Upper	
Pus Cell	Abnormal	0.208	1.611	1.071	2.421	0.02
	Normal	-	1	-	-	-
Epithelial Cell	Abnormal	0.206	1.562	1.042	2.341	0.03
	Normal	-	1	-	-	-
TSH	Normal	-	1	-	-	-
	Abnormal	0.351	1.352	0.741	2.441	0.02
T3	Normal	-	1	-	-	-
	Abnormal	0.276	1.527	0.842	2.591	0.01
T4	Normal	-	1	-	-	-
	Abnormal	0.187	1.599	0.963	2.691	0.02
Ferritin	Normal	-	1	-	-	-
	Abnormal	0.236	1.672	1.128	2.732	0.02
AFP	Normal	-	1	-	-	-
	Abnormal	0.168	1.765	1.383	2.911	0.02
Anaemia	Mild	-	1	-	-	-
	Moderate	0.345	1.344	0.893	1.78	0.02
	Severe	0.455	2.311	1.678	2.899	0.05
	Very Severe	0.572	3.361	1.095	10.321	0.03

3.2 Microbial Isolation and Systematic Observation

The present study aimed to investigate the Premature Rupture of the Membrane of Pregnant Women (PROM) by collecting samples from three hospitals: Sayed Nazrul Hospital, Sadar Hospital, and President Abdul Hamid Medical College Hospital in Kishoreganj. The total number of samples collected was 390, with the majority from President Abdul Hamid Medical College Hospital (n=150), followed by Sadar Hospital (n=120), and Sayed Nazrul Hospital (n=120). The collection of samples from different hospitals was essential to ensure the representation of a diverse population and to minimize sampling bias. Among the 390 samples, 290 were marked as control,

While 352 were favorable for microbial growth (38 samples had lost their quality due to sample transportation and other limitations), the microbial analysis revealed the presence of microbial communities in 116 samples (92 of them were bacterial samples and 24 of them were fungal growth), while the rest showed no significant microbial growth on culture and sensitivity testing. Among the positive samples, 9 had dominant aerobic microbial presence, while 7 had dominant anaerobic microbial communities.

Further differentiation of the dominant microbial species among the positive samples revealed 35 samples identified as *Staphylococcus spp.*, 33 as *Bacillus spp.*, 11 as *Streptococcus spp.*, 11 as *Escherichia coli,* and 9 as *Enterococcus spp.* **Table 7** presents a selection of representative bacterial isolates along with their respective strain names. This complements the information provided in **Table 8**, which details the morphological characteristics of these isolates.

Figure 19. Microbiological Insight: A comprehensive examination of bacterial growth in TSB broth

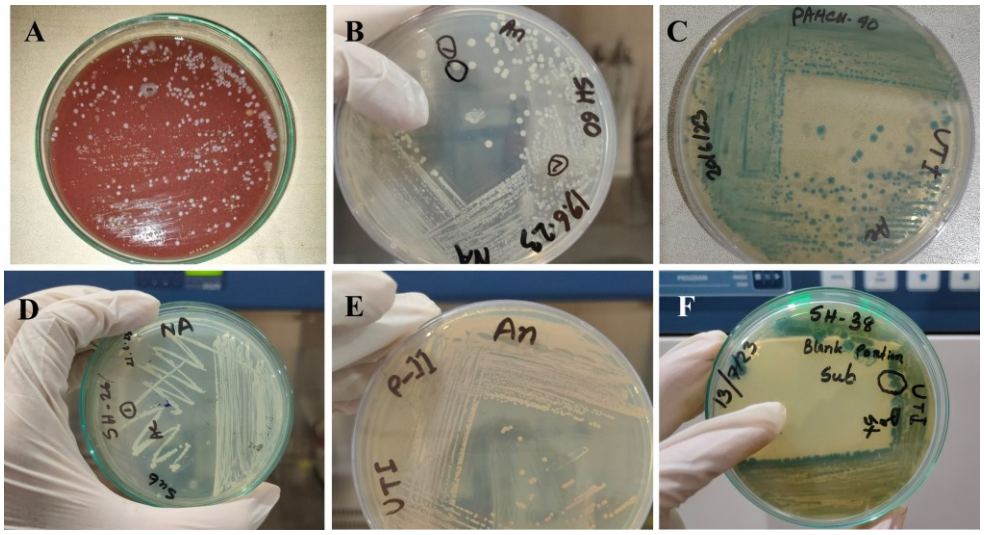

Figure 20. Microbial Proliferation Across Diverse Growth Media and Conditions; **A**. Flourishing Growth on Chocolate Agar Medium, **B.** Robust Anaerobic Growth on Nutrient Agar Plate, **C.** Vigorous Aerobic Growth on UTI Agar Plate, **D.** Successful Subculture on Nutrient Agar in an

Oxygen-Rich Environment, **E-F.** Efficient Subculture on UTI Agar Plate Under Anaerobic Conditions.

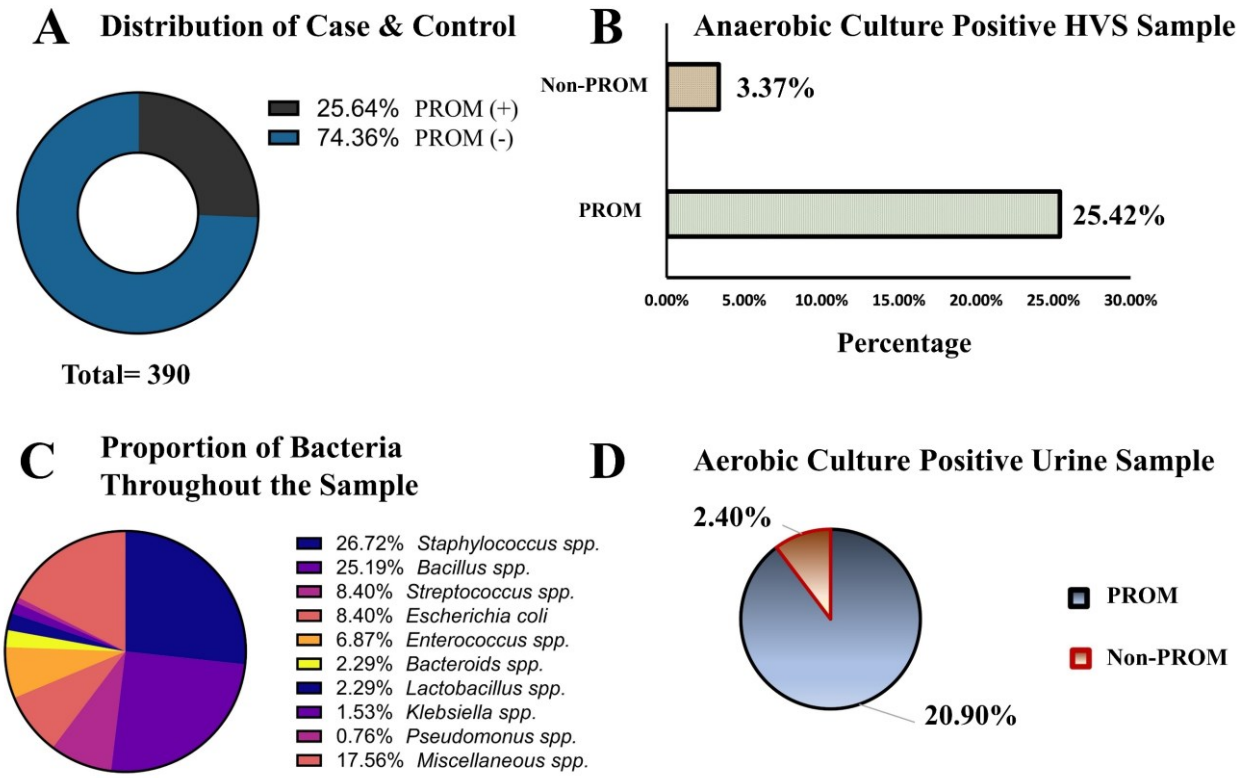

Figure 21. An in-depth exploration of the bacterial spectrum: **A.** Distribution of cases and controls, **B.** Positive anaerobic cultures in HVS samples, **C.** Proportions of bacterial strains across the sample set, **D.** Positive aerobic cultures in urine samples.

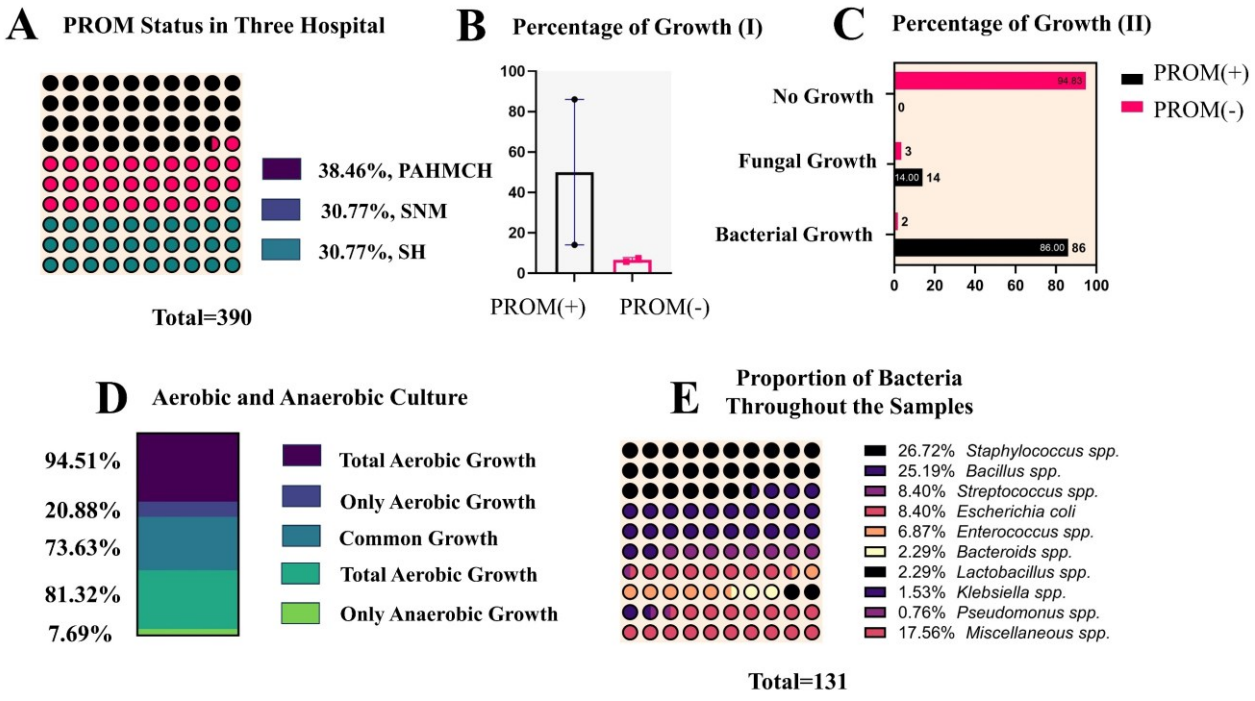

Figure 22. A comparative overview of bacterial growth in this study. **A.** Prom status in three hospitals, **B-C.** Percentage of growth, **D.** Aerobic and anaerobic growth spectrum, **E.** Proportion of Bacteria throughout the sample.

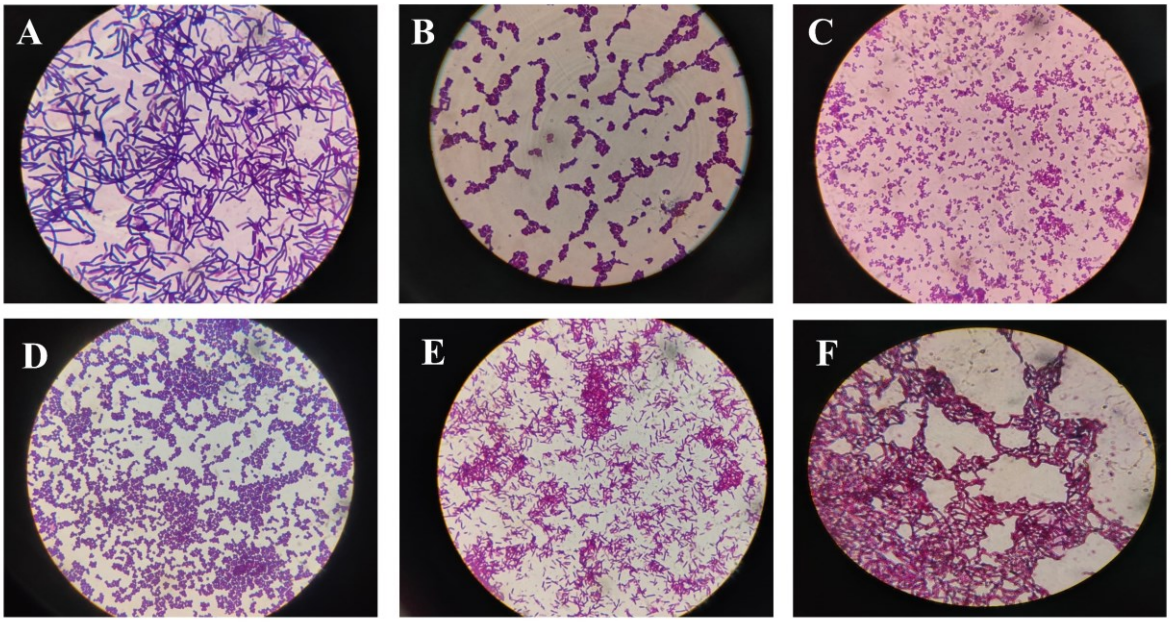

Figure 23. Microscopic examination of gram-stained bacterial isolates: **A and F.** *Bacillus spp.*, **B and D.** *Staphylococcus spp.*, **C.** *Enterococcus aerogenes*, **E.** Gram-Negative *Bacillus spp*.

3.3 Species Identification: Precision in Microbial Revelation

Within this crucial section, a meticulous journey unfolds as the precise identities of the microbial inhabitants are unveiled. Employing cutting-edge techniques and exhaustive analyses, these unique species are methodically classified and cataloged. This comprehensive profiling establishes the foundation for profound insights and further scientific exploration.

Figure 24. Demonstrating key biochemical tests with representative test tubes for bacterial identification.

Table 7. A representative group of prominently identified bacteria within isolates from PROM-positive patients: a comprehensive insight

PROM Status	Sample ID	Nutrient Agar	MacConkey agar	HiCrome UTI Agar	Blood Agar	Chocolate Agar	Organism Name
Positive	PAHMCH 24	Growth	No Growth	Growth	Growth	Growth	*Bacillus cereus*
Positive	SNM 30	Growth	No Growth	No Growth	Growth	Growth	*Bacillus cereus*
Positive	PAHMCH 13	Growth	No Growth	Growth	Growth	Growth	*Bacillus coagulans*
Positive	SH 14	Growth	No Growth	No Growth	Growth	Growth	*Bacillus coagulans*
Positive	SH 61	Growth	No Growth	No Growth	Growth	Growth	*Bacillus subtilis, Bacillus cereus*
Positive	SH 62	Growth	Growth	No Growth	Growth	Growth	*Enterobacter Aerogens*
Positive	PAHMCH 61	Growth	Growth	Growth	Growth	Growth	*Enterococcus spp.*
Positive	PAHMCH 62	Growth	Growth	Growth	Growth	Growth	*Enterococcus spp.*
Positive	SNM 55	Growth	Growth	Growth	Growth	Growth	*Escherichia coli*
Positive	PAHMCH 03	Growth	No Growth	Growth	Growth	Growth	*Escherichia coli*
Positive	PAHMCH 19	Growth	No Growth	Growth	Growth	Growth	*Klebsiella pneumoniae*
Positive	SH 54	Growth	No Growth	Growth	Growth	Growth	*Klebsiella pneumoniae*
Positive	PAHMCH 95	Growth	Growth	Growth	Growth	Growth	*Lactobacillus spp.*
Positive	PAHMCH 70	Growth	Growth	Growth	Growth	Growth	*Lactobacillus spp.*
Positive	SNM 83	Growth	Growth	Growth	Growth	Growth	*Lactobacillus spp.*
Positive	SNM 14	Growth	No Growth	Growth	Growth	Growth	*Micrococcus lotus, Bacillus spp.*
Positive	SNM 10	Growth	No Growth	Growth	Growth	Growth	*Micrococcus spp., Escherichia coli*
Positive	SH 17	Growth	No Growth	Growth	Growth	Growth	*Pseudomonus flurosence*
Positive	SNM 61	Growth	No Growth	Growth	Growth	Growth	*Staphylococcus areus, Enterococcus faecalis*
Positive	SH 06	Growth	No Growth	Growth	Growth	Growth	*Staphylococcus areus, Staphylococcus epidermis*
Positive	PAHMCH 27	Growth	No Growth	Growth	Growth	Growth	*Staphylococcus aureus*

Positive	SH 59	Growth	No Growth	Growth	Growth	Growth	*Staphylococcus aureus, Enterococcus spp.*
Positive	PAHMCH 23	Growth	Growth	Growth	Growth	Growth	*Staphylococcus aureus, Escherichia coli, Micrococcus lotus, Acinetobacter spp.*
Positive	PAHMCH 58	Growth	Growth	Growth	Growth	Growth	*Staphylococcus aureus, Escherichia coli, Micrococcus lotus, Acinetobacter spp.*
Positive	SNM 29	Growth	Growth	No Growth	Growth	Growth	*Staphylococcus aureus, Escherichia coli, Micrococcus lotus, Acinetobacter spp.*
Positive	SH 69	Growth	No Growth	No Growth	Growth	Growth	*Staphylococcus aureus, Micrococcus spp.*
Positive	SNM 18	Growth	No Growth	No Growth	Growth	Growth	*Staphylococcus aureus, Staphylococcus epidermis*
Positive	PAHMCH 28	Growth	No Growth	Growth	Growth	Growth	*Staphylococcus aureus,Bacillus spp.*
Positive	SNM 32	Growth	No Growth	No Growth	Growth	Growth	*Streptococcus spp.*
Positive	SNM 38	Growth	No Growth	No Growth	Growth	Growth	*Streptococcus spp.*
Positive	PAHMCH 21	Growth	No Growth	Growth	Growth	Growth	*Streptococcus spp., Bacillus spp.*

Table 8. Comprehensive Analysis of Morphological Characteristics of Identified Bacteria

Isolates	**Isolation Media**	**Colony Characteristics**		
		Color	**Shape and Size**	**Margin**
E. Coli	MacConkey Agar	Pink	Gram-negative rod-shaped,medium, flat, and smooth colonies, non-mucoid	Entire and Circular
Klebsiella spp.	MacConkey Agar	Pink to red	Gram-negative rod-shaped, large, mucoid colonies	Entire and Circular
Enterobacter spp.	MacConkey Agar	Pink	Round, medium, smooth colonies	Entire
Acinetobacter spp.	MacConkey Agar	Pale/colorless	Round, small, pale colonies	Circular
Pseudomonas spp.	MacConkey Agar	Pale/colorless	Round, small, flat and smooth colonies	Swell and Regular
Staphylococcus aureus	Nutrient Agar	Yellow/White	Round, convex, large, sphered shape	Irregular
Bacillus spp.	Nutrient Agar	Slightly Yellow/White	Gram-positive rod-shaped, large, rough, opaque, fuzzy colonies with jagged edges	Irregular
Streptococcus spp.	Blood & Chocolate Agar	Greenish or brownish coloration	Spherical and small, smooth, and round or irregular in shape.	Irregular

3.4 Antibiogram: Identification of Bacterial Isolates

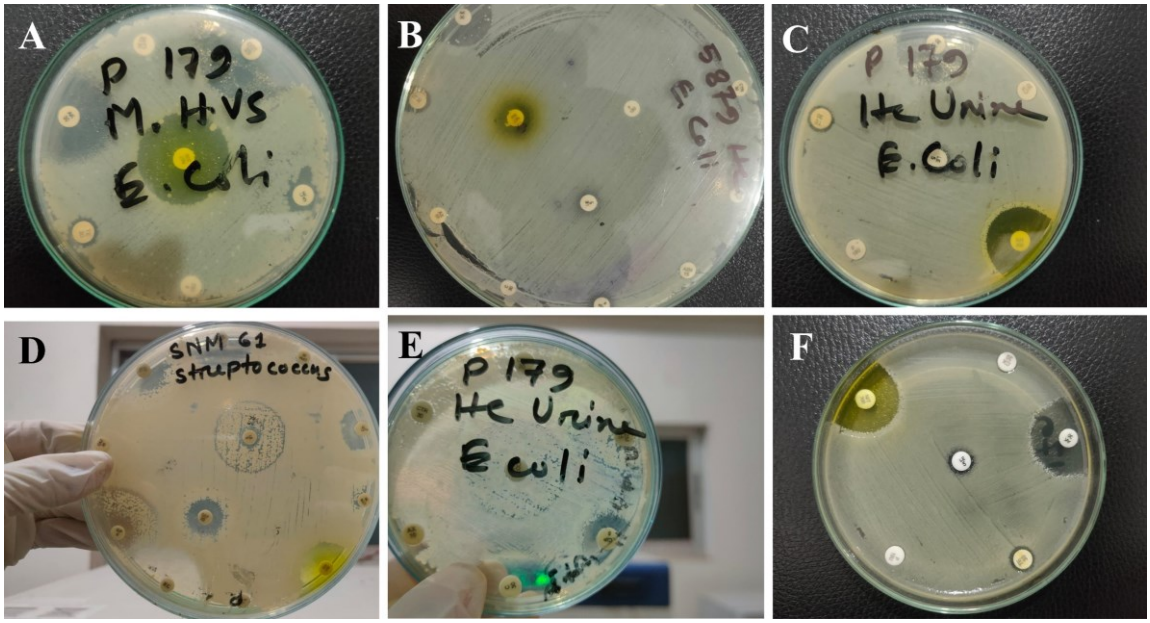

Figure 25. Antibiotic Resistance Profiles Across Isolates: *Escherichia coli* (A, B, C, and E), and *Streptococcus species* (D, F) Exhibit Distinctive Resistance Patterns. Notably, Isolates B and D Demonstrate Pronounced Resistance.

In our pursuit of a comprehensive understanding of the bacterial isolates, we delved into the intricate realm of Antimicrobial Resistance (AMR). Consequently, we elucidate the resistance of various microorganisms, including but not limited to *Enterococci, E. coli, Lactobacilli, Staphylococcus spp., Streptococcus spp., Bacillus spp., Candida, Bacteroids,* etc. to an array of antibiotics **(Table 9-11)**. The tested antibiotics include AK (Amikacin), AX (Amoxicillin), AZM (Azithromycin), CPM (Cefepime), CTX (Cefotaxime), CX (Cefoxitin), CAZ (Ceftazidime), CTR (Ceftriaxone), C (Chloramphenicol), CIP (Ciprofloxacin), CD (Clindamycin), CL (Colistin), COT (Cotrimoxazole), GEN (Gentamicin), LE (Levofloxacin), LZ (Linezolid), NIT (Nitrofurantoin), P (Penicillin), TE (Tetracycline), IMP (Imipenem), CFM (Cefixime) and AMX (Amoxicillin).

Table 9. Antibiotic resistance patterns (%) across bacterial isolates: *E. coli* & *Enterococcus spp*., *Staphylococcus spp., Streptococcus spp., Bacillus spp.*

Anti-biotic Pannel	***Bacillus spp.***			***E coli & Enterococcus spp.***			***Staphylococcus spp.***			***Streptococcus spp.***		
Pattern	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
AK	28%	6%	66%	36%	17%	47%	16%	7%	77%	12%	1%	88%
AX	49%	9%	43%	53%	15%	32%	44%	12%	44%	30%	5%	66%
AZM	42%	8%	50%	46%	16%	38%	44%	12%	44%	73%	16%	12%
CPM	84%	11%	5%	93%	6%	1%	87%	9%	4%	93%	4%	3%

CTX	52%	16%	33%	79%	21%	0%	77%	14%	10%	28%	69%	3%
CX	88%	9%	3%	89%	8%	3%	88%	9%	3%	73%	25%	1%
CAZ	73%	27%	0%	70%	20%	10%	76%	24%	0%	99%	1%	0%
CTR	21%	7%	72%	57%	13%	30%	50%	26%	23%	25%	67%	8%
C	58%	16%	27%	59%	12%	29%	58%	2%	39%	50%	1%	49%
CIP	15%	27%	58%	13%	4%	83%	14%	5%	81%	31%	4%	65%
CD	65%	14%	21%	77%	19%	4%	71%	5%	24%	74%	4%	23%
CL	67%	19%	14%	55%	21%	23%	61%	20%	19%	42%	42%	16%
COT	33%	5%	63%	20%	9%	71%	26%	7%	67%	34%	62%	5%
GEN	27%	6%	67%	13%	8%	79%	14%	7%	79%	9%	19%	72%
LE	27%	12%	61%	22%	13%	65%	20%	13%	67%	13%	34%	53%
LZ	53%	0%	47%	56%	0%	45%	54%	0%	46%	83%	0%	17%
NIT	60%	13%	27%	67%	17%	16%	63%	15%	22%	57%	26%	17%
P	89%	9%	2%	77%	20%	2%	83%	15%	2%	79%	17%	4%
TE	48%	16%	36%	56%	27%	17%	52%	22%	26%	7%	17%	75%
IMP	87%	14%	0%	92%	8%	0%	86%	15%	0%	85%	15%	0%
CFM	57%	15%	29%	62%	18%	20%	59%	16%	24%	68%	2%	30%
AMX	75%	13%	12%	82%	13%	5%	79%	13%	8%	41%	23%	36%

The antibiotic resistance patterns among various bacterial species were examined, revealing distinct trends **(Table 9)**. Notably, Bacillus spp. demonstrated lower resistance percentages across most antibiotics, with the highest susceptibility to CPM (93%) and AZM (73%). In contrast, E. coli & Enterococcus spp. exhibited mixed resistance profiles, with substantial resistance to CPM (84%) and CX (88%), but relatively high susceptibility to AK (66%) and LE (61%). Staphylococcus spp. displayed moderate resistance, particularly to CPM (87%) and CIP (83%), yet showed sensitivity to a few antibiotics like COT (71%). Streptococcus spp. revealed diverse resistance patterns, with some strains highly resistant to certain antibiotics, such as CTR (72%), while remaining susceptible to others.

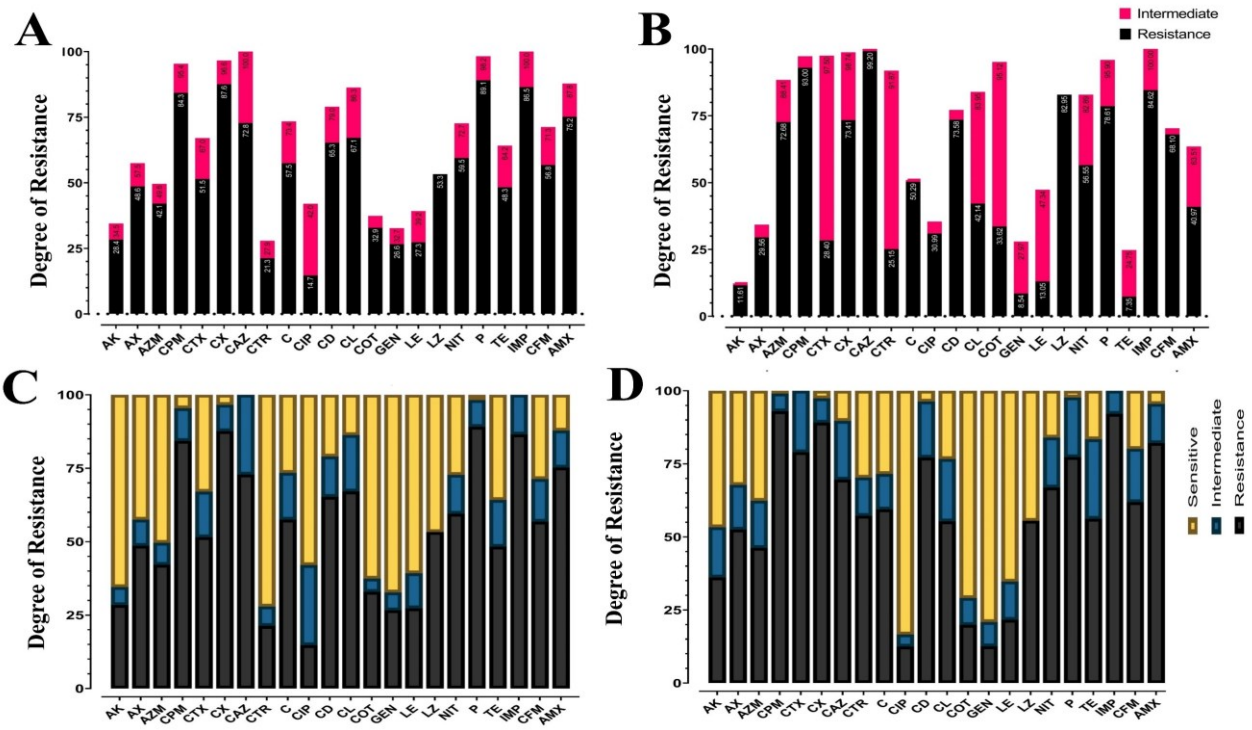

Figure 26. A comprehensive comparative analysis of antibiotic resistance patterns between **A, C.** *Bacillus spp.* and **B, D.** *Escherichia. coli,* highlighting significantly divergent susceptibility profiles.

A captivating glimpse into antibiotic resistance patterns among various bacterial species is provided **(Figure 26,27)** with a comprehensive antibiotic resistance pattern **(Figure 28)**. The contrast between *Bacillus spp.* and *E. coli* is highlighted **(Figure 26)**, with *E. coli* showing higher resistance. This emphasizes the importance of tailored antibiotic treatments and ongoing resistance monitoring. A comparative unique susceptibility profile between Streptococcus spp. with *Staphylococcus spp.* is observed **(Figure 27)**. These findings underscore the importance of tailored antibiotic selection in clinical settings, considering the variations in resistance among different bacterial species.

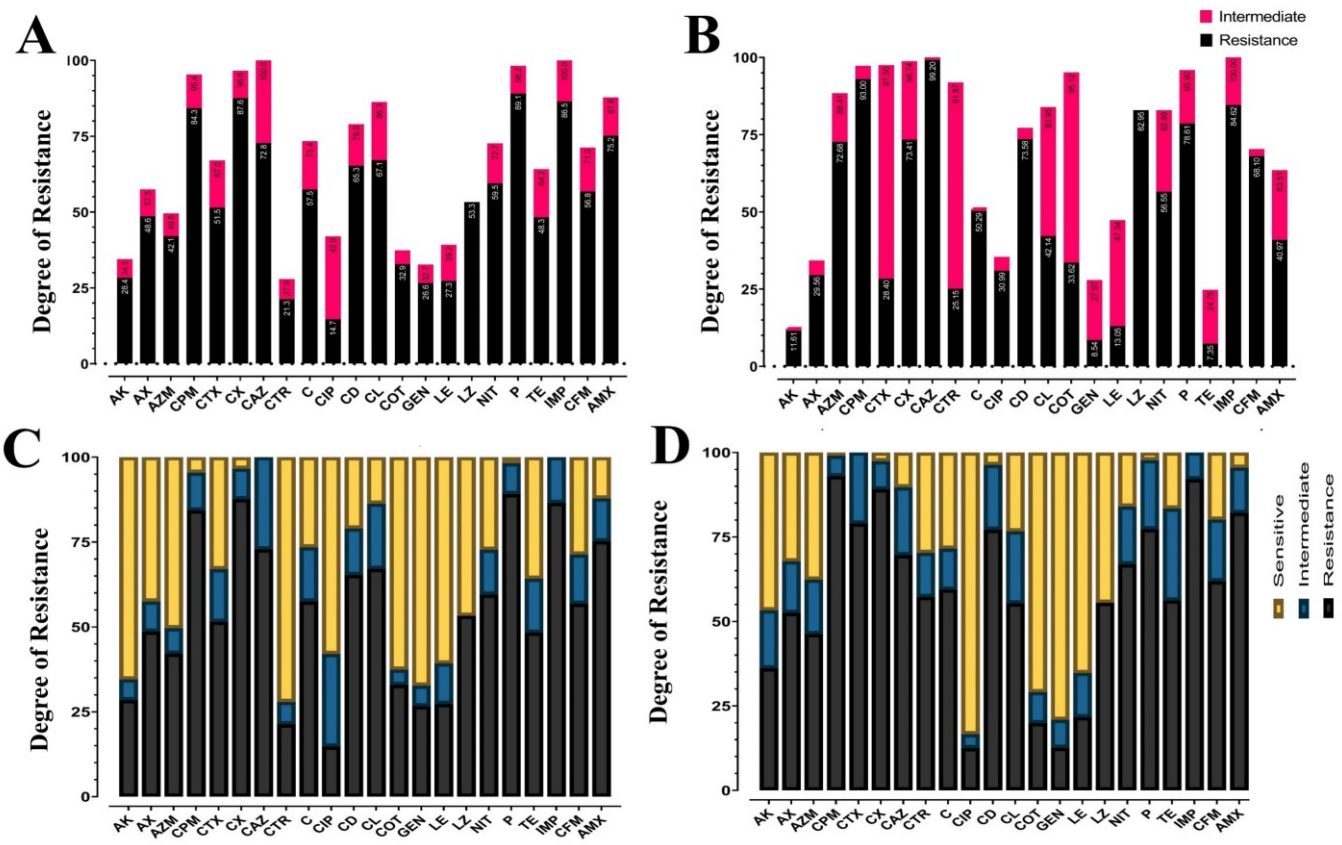

Figure 27. A comprehensive comparative analysis of antibiotic resistance patterns between **A, C.** *Streptococcus spp.* and **B, D.** *Staphylococcus spp.*, highlighting significantly divergent susceptibility profiles.

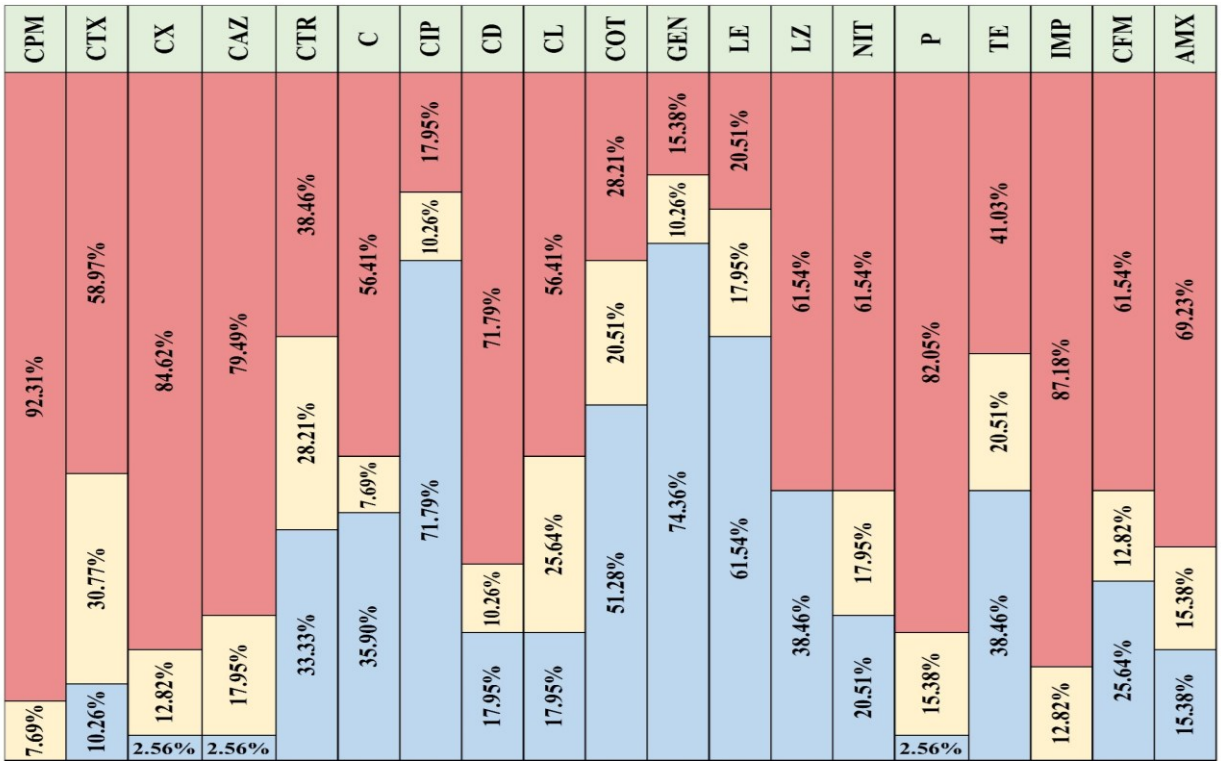

Figure 28. A comprehensive antibiotic resistance pattern across the bacterial spectrum

Table 10. A comprehensive insight into bacterial characteristics and a summarized antibiotic resistance patterns of clinical isolates

P. ID	AGE	HVS For C/S	URINE C/S	R/E, M/E	Organism	Sensitive	Resistant	TSI				MIU			Citrate	MR	VP	Catalase	Oxidase	Gram St.	Aerobic
								Slant	But	H_2S	Gas	Motility	Indole	Urease							
PAHMCH-03	30	*Enterococci*			*Enterococci*	AK, CIP, GEN, NIT	CTX, CTR, LE, CN, CPM, CFM, COT	A	A	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH-08	35	*E.coli*	*N/G*		*E.coli*	AK, IPM, NIT, GEN,	CN, CXM, CTR, CTX, CIP, CPM	A	A	-	+	-	+	-	-	+	-	+	-	-	-
PAHMCH-10	25	*E.coli*			*E.coli*	IMI, AK,GEN, CTR, CTX, CPM	NIT, CN, CXM	A	A	-	+	-	+	-	-	+	-	+	-	-	-
PAHMCH-26	22	*Lactobacilli, Candida*	*N/G*		*Lactobacilli, Candida*			A	A	-	-	-	-	-	-	-	-	-	-	+	-
PAHMCH-39	20	*Peptostaptococcus*	*Enterococci*		*Enterococci*	AK, GEN, NIT, IMI, CIP	CTX, CTR, CXM, CPM, CN	A	A	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH-40	20	*N/G*	*E.coli*	*F(+) CL(+)*	*E.coli*	AK, GEN, IMI, CIP	CTX, NIT, CN, CTR, CPM	A	A	-	+	-	+	-	-	+	-	+	-	-	-
PAHMCH-43	20	*N/G*	*E.coli*	*F(-) CL(+)*	*E.coli*	NIT, GEN, AK, IPM	CXM, CTX, CIP, CPM, CTR, CN	A	A	-	+	-	+	-	-	+	-	+	-	-	-
PAHMCH-45	19	*Bacteroides*	*N/G*	*F(-) CL(+)*	*Bacteroids*	AK, GEN, IMI, CIP, NIT,	NIT,CTX, CTR, CFM, CXM, CPM	A	A	-	+	-	+	-	-	+	-	+	-	-	-
PAHMCH-46	25	*N/G*	*Enterococci*		*Enterococci*	AK, CIP, CTR, CPM, CTX, GEN,IPM	NIT, CXM,	A	A	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH-47	20	*E.coli*	*Enterococci*		*Enterococci*	AK, CTR, CPM, CXM, CIP, IPM, CTX	NIT	A	A	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH-48	20	*N/G*	*E.coli*		*E.coli*	AK, IMI, GEN, NIT, CIP, CTR	CPM, CXM, CTX	A	A	-	+	-	+	-	-	+	-	+	-	-	-
PAHMCH-53	22	*Lactobacilli*	*N/G*	*F(-) CL(+)*	*Lactobacilli*			A	A	-	-	-	-	-	-	-	-	-	-	+	-
PAHMCH-55	35	*E.coli*	*N/G*	*F(-) CL(+)*	*E.coli*	NIT, GEN, AK, IPM	CXM, CTX, CIP, CPM, CTR, CN	A	A	-	+	-	+	-	-	+	-	+	-	-	-

PAHMCH -61	**20**	***N/G***	***Enterococci***		***Enterococci***	**AK, CIP, GEN, IMI**	**CPM, CXM, NIT, CTX, CTR**	**A**	**A**	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH -62	**20**	***Stephylococcus***	***Enterococci***		***Enterococci***	**AK, GEN,CIP,NIT,CTR**	**CPM,CXM,IMI,CTX**	**A**	**A**	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH -63	**19**	***N/G***	***Enterococci***		***Enterococci***	**AK,GEN,IMI,CIP, NIT**	**CXM,CTX,CTR,CPM**	**A**	**A**	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH -68	**39**	***N/G***	***Enterococci***		***Enterococci***			**A**	**A**	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH -70	**20**	***Lactobacilli***	***Enterococci***		***Enterococci***	**AK, IMI, GEN,**	**CIP, CXM, CTX, CTR, NIT, CPM**	**A**	**A**	-	-	-	-	+	+	-	-	-	-	+	-
PAHMCH -95	**23**	***N/G***	***Enterococci***		***Enterococci***	**AK, CPM, CTR, IPM, CIP, GEN, CTX**	**NIT, CXM**	**A**	**A**	-	-	-	-	+	+	-	-	-	-	+	-
SH-01	**30**	***H VS***			***E. coli***	**AK, CTX, IPM, CIP, NIT, CTR, GEN**	**LOM, CN, CXM, CFM**	**A**	**A**	-	+	-	+	-	-	+	-	+	-	-	-
SH-20	**26**	***E.coli***	***N/G***		***E.coli***	**AK, GEN, NIT, CIP, CTR, CPM, CTX, IMI**	**CN, CXM**	**A**	**A**	-	+	-	+	-	-	+	-	+	-	-	-
SH-32	**27**	***Lactobacillus***	***N/G***		***Lactobacillus***			**A**	**A**	-	-	-	-	-	-	-	-	-	-	+	-
SH-35	**29**	***Lactobacillus***	***N/G***		***Lactobacillus***			**A**	**A**	-	-	-	-	-	-	-	-	-	-	+	-
SH-87	**19**	***Peptosteptococcus***	***N/G***		***E.coli***	**GEN, CTR, AK, IPM, CIP**	**NIT, CXM, CPM, CTX**	**A**	**A**	-	+	-	+	-	-	+	-	+	-	-	-
SNM -65	**19**	***N/G***	***E.coli***		***E.coli***	**AK,GEN,CIP,IMI**	**CPM,CXM,CTR,NIT,CTX**	**A**	**A**	-	+	-	+	-	-	+	-	+	-	-	-
SNM-04	**28**	***Lactobacilli***	***N/G***		***Lactobacilli***			**A**	**A**	-	-	-	-	-	-	-	-	-	-	+	-
SNM-28	**35**	***Lactobacilli***	***N/G***		***Lactobacilli***			**A**	**A**	-	-	-	-	-	-	-	-	-	-	+	-
SNM-30	**22**	***Lactobacillus***	***N/G***		***Lactobacillus***			**A**	**A**	-	-	-	-	-	-	-	-	-	-	+	-
SNM-54	**25**	***Bacteroides***	***E.coli***		***Bacteroids (E.coli)***	**(HVS)- AK,CTR, CXM, CIP, CTX, CN, IPM**	**(HVS)- NIT, GEN**	**A**	**A**	-	+	-	+	-	-	+	-	+	-	-	-
SNM-55	**25**	***N/G***	***E.coli***		***E.coli***	**CPM, CIP,IPM,NIT, AK, GEN, CTR, CTX**	**CXM, CN**	**A**	**A**	-	+	-	+	-	-	+	-	+	-	-	-
SNM-56	**22**	***Lactobasili***	***N/G***		***Lactobacilli***			**A**	**A**	-	-	-	-	-	-	-	-	-	-	+	-
SNM-58	**35**	***Bacteroides***	***N/G***	***F(-) CL(-)***	***Bacteroids***	**AK, GEN, IMI, CIP, NIT**	**CTR, CTX, CFM, CN, CPM**	**A**	**A**	-	+	-	+	-	-	+	-	+	-	-	-
SNM-68	**25**	***Lactobacilli***	***N/G***		***Lactobacilli***			**A**	**A**	-	-	-	-	-	-	-	-	-	-	+	-
SNM-83	**28**	***N/G***	***Enterococci***		***Enterococci***	**AK, CIP, IMI, GEN, NIT, CPM**	**CXM, CTX, CTR**	**A**	**A**	-	-	-	-	+	+	-	-	-	-	+	-

Table 11. Clinical bacterial isolates: Unveiling the tapestry of antibiotic resistance.

Patients ID	Isolates	Sensetivity	Resistance	Category of antibiotic Resistance	Comment
SH-38 Y	***Staphylococcus Aureus***	AK, AX, AZM, CTR, CIP, CL, GEN, LE	CPM, CTX, CX, CAZ, C, CD, LZ, NIT, P, TE	Macrolides, Beta-lactams, Phenicols, Nitrofurans, Oxazolidinones, Tetracyclines	MDR
SH 38 Cream	***Staphylococcus Aureus***	AK, AX, AZM, CTR, CIP, GEN, LE	CPM, CTX, CX, CAZ, C, CD, LZ, NIT, P	Macrolides, Beta-lactams, Phenicols, Nitrofurans, Oxazolidinones	MDR
SH 38	***Staphylococcus Aureus***	AK, AX, AZM, CIP, CL GEN, LE	CPM, CTX, CX, CAZ, C, CD, COT, LZ, NIT, P	Macrolides, Beta-lactams, Phenicols, Nitrofurans, Oxazolidinones, Sulfonamides	MDR
SH-25	***Staphylococcus Aureus***	AK, AX, CTR, C, COT, GEN, LE, LZ, TE	AZM, CPM, CTX, CX, CAZ, CIP, CD, CL, NIT, P	Macrolides, Beta-lactams, Nitrofurans, Fluoroquinolones, Nitrofurans, Lipopeptides	MDR
SH-38 Cream2	***Staphylococcus Aureus***	AK, CIP, GEN, LE	AX, AZM, CPM, CTX, CX, CAZ, C, CD, CL, COT, LZ, NIT, P, TE	Macrolides, Beta-lactams, Phenicols, Nitrofurans, Sulfonamides, Lipopeptides, Tetracyclines, Oxazolidinones	MDR
SH-61	***Staphylococcus Aureus***	AK, CTX, C, CIP, CL, GEN, LE, LZ, TE	AX, AZM, CPM, CX, CAZ, CTR, CD, COT, NIT, P	Macrolides, Beta-lactams, Sulfonamides, Nitrofurans,	MDR
SH 56 NA	***Staphylococcus Aureus***	CIP, COT, GEN	AK, AX, AZM, CPM, CX, CAZ, C, LZ, NIT, P, TE	Aminoglycosides, Macrolides, Beta-lactams, Phenicols, Tetracyclines, Nitrofurans, Oxazolidinones	MDR
SNM 86 (W+Y)	***Staphylococcus Epidermis***	AK, COT, LE, NIT	AX, AZM, CPM, CX, CAZ, C, CIP, CL, GEN, LZ, P, TE	Macrolides, Beta-lactams, Phenicols, Tetracyclines, Oxazo-lidinones, Aminoglycosides, Lipopeptides, Fluoroquinolones	MDR
SNM-61	***Streptococcus spp.***	AK, GEN, LE, LZ, NIT,	AX, AZM, CPM, CTX, CX, CAZ, CTR, CIP, CD, CL, COT, P, TE	Macrolides, Beta-lactams, Fluoroquinolones, Lipopeptides, Sulfonamides, Tetracyclines	MDR
SH-38 Cream	***Streptococcus spp.***	AK, AX, C, CIP,CL, GEN, LE, TE	AZM, CPM, CTX, CX, CAZ, CD, LZ, NIT, P	Macrolides, Beta-lactams, Nitrofurans, Oxazolidinones	MDR
5879 - Hc	***E. Coli***	CD, CL	AK, AX, AZM, CPM, CTX, CX, CAZ, CTR, C, CIP, COT, GEN, LE, LZ, NIT, P, TE, IMP, CFM, AMX	Macrolides, Beta-lactams, Phenicols, Tetracyclines, Oxazo-lidinones, Aminoglycosides, Sulfonamides, Fluoroquinolones, Nitrofurans, Carbapenems, Cephalosporins	**PDR**
P-179 Hc	***E. Coli***	CD	AK, AX, AZM, CPM, CTX, CX, CAZ, CTR, C, CL, CIP, COT, GEN, LE, LZ, P, TE, IMP, CFM, AMX	Macrolides, Beta-lactams, Phenicols, Tetracyclines, Oxazo-lidinones, Aminoglycosides, Sulfonamides, Fluoroquinolones, Carbapenems, Cephalosporins	**PDR**
P-179 M HVS	***E. Coli***	AK, CD, CL, COT, GEN, NIT	AX, AZM, CPM, CTX, CX, CAZ, CTR, C, CIP, LE, LZ, P, TE, IMP, CFM, AMX	Macrolides, Beta-lactams, Phenicols, Fluoroquinolones, Oxazolidinone, Tetracyclines, Carbapenem, Cephalosporin	MDR

SNH-10	***E. Coli***	AK, AX, AZM, CTX, CTR, CIP, CD, COT, GEN, LE, LZ, TE, CFM	CPM, CX, CAZ, C, CL, NIT, P, IMP, AMX	Beta-lactams, Phenicols, Lipopeptides, Nitrofurans, Carbapenems, Cephalosporins	MDR
P-41 UTI	***E. Coli***	AK, AZM, CTX, CAZ, CIP, GEN, LE, CFM	AX, CPM, CX, CTR, C, CD, CL, COT, LZ, NIT, P, TE, IMP	Beta-lactams, Phenicols, Macrolides, Lipopeptides, Sulfonamides, Oxazolidinones, Nitrofurans, Tetracyclines, Carbapenems	MDR
SH-62 NA.Ae	***Bacillus spp.***	AK	AX, AZM, CPM, CTX, CAZ, CTR, C, CL, CD, CIP, COT, GEN, LZ, P, TE	Beta-lactams, Phenicols, Macrolides, Lipopeptides, Fluoro-quinolones, Sulfonamides, Aminoglycosides, Oxazolidinones, Tetracyclines	**PDR**
P 39	***Bacillus spp.***	CTX, CIP, COT, LE	AX, AZM, CPM, CAZ, CTR, C, CD, CL, LZ, P, TE	Beta-lactams, Phenicols, Macrolides, Lipopeptides, Oxazolidinones, Tetracyclines	MDR
SH-62	***Bacillus spp.***	AK, AZM, CIP, COT, GEN	AX, CPM, CTX, CAZ, CTR, C, CD, CL, LE, LZ, P, TE	Beta-lactams, Phenicols, Macrolides, Lipopeptides, Fluoroquinolones, Oxazolidinones, Tetracyclines	MDR
SH-67	***Bacillus spp.***	-	AX, AZM, CPM, CTX, CAZ, CTR, C, CL, CD, COT, GEN, LZ, P	Beta-lactams, Phenicols, Macrolides, Lipopeptides, Sulfonamides, Aminoglycosides, Oxazolidinones	**XDR**
P-40 uti	***Bacillus Subtilis***	AK, AX, CIP, COT, GEN, LE	AZM, CPM, CTX, CAZ, CTR, C, CD, CL, LZ, P, TE	Beta-lactams, Phenicols, Macrolides, Lipopeptides, Oxazolidinones, Tetracyclines	MDR
SH-02 NA	***Bacillus spp.***	AZM, C, COT, GEN, LE	AK, AX, CPM, CTX, CAZ, CTR, CL, LZ, P	Aminoglycosides, Beta-lactams,Lipopeptides, Oxazolidinones	MDR
SNM-40 Cream	***Bacillus spp.***	CIP	AK, AX, AZM, CPM, CTX, CAZ, CTR, C, CD, CL, COT, LE, LZ, P, TE	Aminoglycosides, Beta-lactams, Macrolides, Phenicols, Lipopeptides, Sulfonamides, Fluoroquinolones, Oxazolidinones, Tetracyclines	**PDR**
SH-38 Blank	***Baillus Coagulans***	AX, CTX, C, CIP, CL, COT, GEN	AK, CPM, CAZ, CD, LE, LZ, P	Aminoglycosides, Beta-lactams, Macrolides, Fluoroquinolones, Oxazolidinones	MDR
SH-38 (B+C) Blank	***Bacillus Subtilis***	AX, CTR, CIP, COT, GEN, LE	AK, AZM, CPM, CTX, CAZ, C, CD, CL, LZ, P	Aminoglycosides, Beta-lactams, Macrolides, Phenicols, Lipopeptides, Oxazolidinones	MDR
SH-38 Purple	***Bacillus Subtilis***	AX, CTR, C, CIP, LE, LZ, TE	AK, AZM, CPM, CAZ, CD, CL, GEN, P	Aminoglycosides, Beta-lactams, Macrolides, Lipopeptides	MDR
SH-38 (B+P) Purple	***Bacillus Coagulans***	AK, AZM, C, GEN, LZ, TE	AX, CPM, CTX, CAZ, CTR, CD, COT, LE, P	Beta-lactams, Macrolides, Sulfonamides, Fluoroquinolones	MDR
SH-38 Inhibit	***Bacillus Amyloliquifaciens***	AK, AX, AZM, CIP, GEN, LE	CPM, CTX, CAZ, C, CD, LZ, P, TE	Beta-lactams, Phenicols, Macrolides, Oxazolidinones, Tetracyclines	MDR

A demographic perspective by encompassing patients spanning a wide age range, from 19 to 39 years, underscoring the prevalence of these clinical isolates across distinct age cohorts **(Table 10)**. Notably, certain specimens like *Lactobacilli* and *Candida* manifest mixed infections involving diverse organisms, signifying the presence of polymicrobial infections within clinical scenarios. The bacterial entities featured in this table are predominantly Gram-negative, and for precise characterization and classification, an array of biochemical assessments including citrate utilization (TSI), MR, VP, catalase, and oxidase tests were diligently conducted. Additionally, an intriguing observation arises as a majority of these isolates exhibit limited or absent aerobic motility, offering valuable insights into their metabolic attributes and motility dynamics under the specific experimental conditions employed.

A meticulous analysis of the antibiotic resistance profiles for clinical isolates reveals noteworthy findings **(Table 11)**. Several Staphylococcus Aureus strains (SH-38 Y, SH 38 Cream, SH 38, SH-25, SH-38 Cream2, SH-61) showcase a concerning trend of multidrug resistance (MDR), demonstrating resistance to various antibiotic categories, including macrolides, beta-lactams, phenicols, nitrofurans, oxazolidinones, and tetracyclines. Moreover, *Staphylococcus epidermis* (SNM-86 W+Y) exhibits resistance primarily to macrolides, beta-lactams, phenicols, tetracyclines, oxazolidinones, aminoglycosides, lipopeptides, and fluoroquinolones, signifying the importance of tailored therapeutic approaches.

The *Streptococcus spp.* strains (SNM-61, SH-38 Cream) display resistance to multiple antibiotic classes, including macrolides, beta-lactams, fluoroquinolones, nitrofurans, and oxazolidinones, emphasizing the necessity of personalized treatment strategies. *E. Coli* strains (5879 - Hc, P-179 Hc, P-179 M HVS, SNH-10, P-41 UTI) exhibit varying degrees of resistance, with some demonstrating pan-drug resistance (PDR) to multiple antibiotic categories, such as macrolides, beta-lactams, phenicols, tetracyclines, oxazolidinones, aminoglycosides, sulfonamides, fluoroquinolones, nitrofurans, carbapenems, and cephalosporins, underscoring the critical importance of judicious antibiotic selection.

In the case of *Bacillus spp.* strains (SH-62 NA-Ae, P 39, SH-62, SH-67, P-40 uti, SH-02 NA, SNM-40 Cream, SH-38 Blank, SH-38 (B+C) Blank, SH-38 Purple, SH-38 (B+P) Purple, SH-38 Inhibit), resistance patterns are observed against beta-lactams, macrolides, lipopeptides, fluoroquinolones, sulfonamides, aminoglycosides, oxazolidinones, and tetracyclines, reinforcing the need for tailored treatment regimens.

These findings underscore the critical importance of precision medicine in combating antibiotic resistance, necessitating individualized treatment strategies based on the resistance patterns of

specific bacterial isolates to optimize clinical outcomes and mitigate the spread of antibiotic resistance.

3.5 Identification of Pathogenic Bacteria

3.5.1 16S rRNA amplification

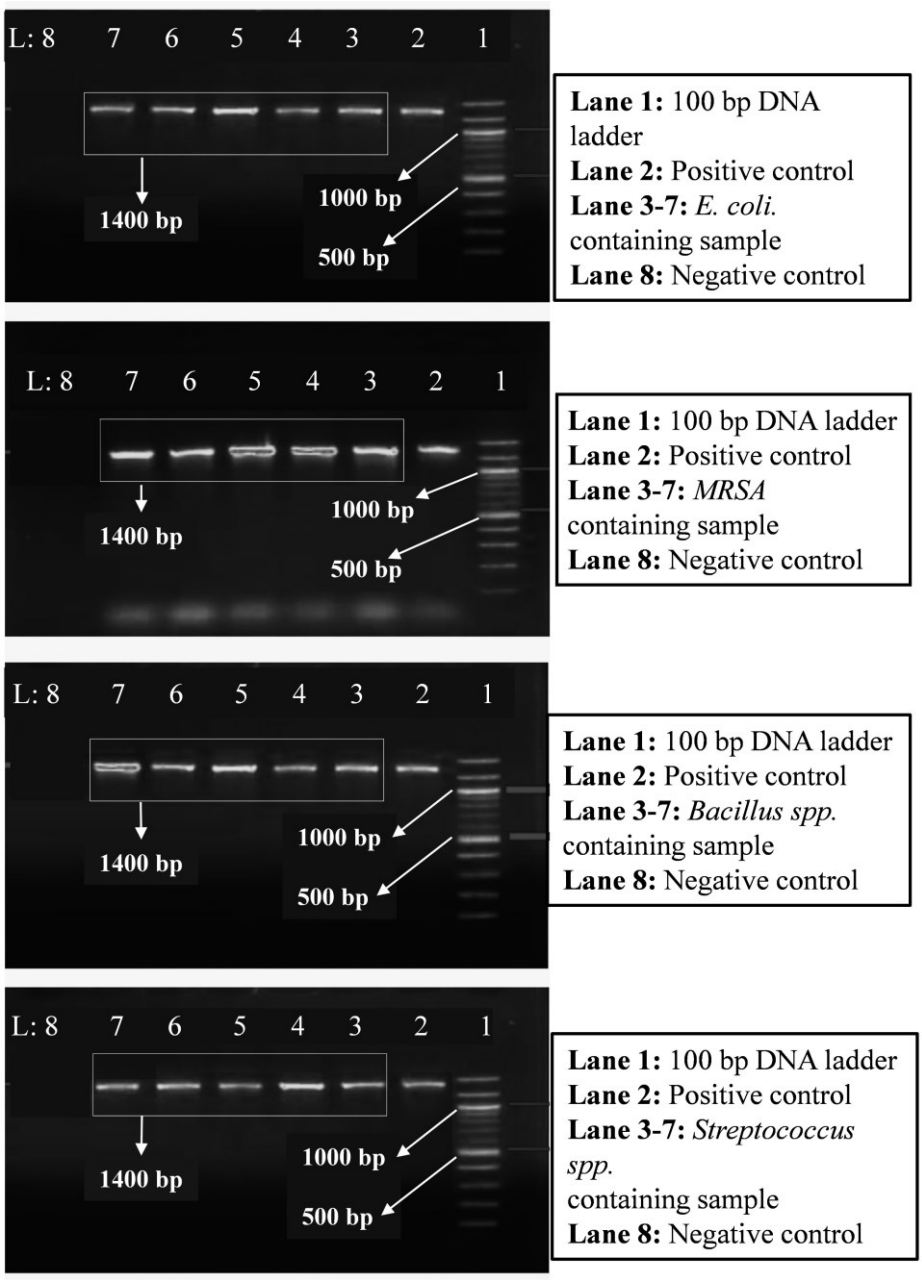

Figure 29. Visualization of 16S rRNA PCR products by gel electrophoresis

The 16S rRNA gene was amplified from boil DNA samples, (ESBL positive *E. Coli, MRSA, Bacillus spp., Streptococcus spp.*) by 16S rRNA PCR. The annealing temperature for 16S rRNA PCR was 50º C. The amplified product of 16S rRNA PCR was verified on 1.5% agarose gel at

80V for 1 hour. The selected isolates and the positive control (ATCC's microorganism) created a band in the same line. All of them were 1.4 kb in size **(Figure 29).**

3.5.2 PCR Method for Identification of Specific Bacteria

I. PCR method for ESBL producing *E. Coli.:* Using a 25 µl volume reaction mixtures, PCR method was performed for ESBL producing *E. Coli.*. The annealing temperature for bla-$_{SHV}$ gene was 62ºC. For detection of ESBL producing gene (bla-$_{SHV}$), PCR reactions were done and the following results were obtained by gel electrophoresis. We found 8 ESBL producing *E. Coli* for bla-$_{SHV}$ primer (80%) among the 10 isolates of ESBL producing *E. Coli.* PCR results of bla-$_{SHV}$ gene expression was observed at 713 bp **(Figure 30A)**

II. Methicillin resistance *Staphylococcus aureus*: Using a 25 µl volume reaction mixtures, PCR method was performed for Methicillin resistance *Staphylococcus aureus*. The Annealing temperature for mecA gene was 56ºC. For the detection of oxacillin resistance gene of *Staphylococcus aureus* (*mecA*) PCR reactions were done and the following results were obtained by gel electrophoresis. We found 9 amplicons for *mecA* gene (81.81%) among the 11 isolates of *Staphylococcus aureus*. PCR results of *mecA* gene expression was identified at 533 bp position **(Figure 30B)**.

III. Fengycin producing *Bacillus subtilis*: Using a 25 µl volume reaction mixtures, PCR method was performed for fengycin producing *Bacillus subtilis.* Fengycin is an antifungal lipopeptide complex produced by Bacillus subtilis. It inhibits filamentous fungi but is ineffective against yeast and bacteria. The Annealing temperature for gene was 60ºC. For the detection of fengycin producing gene of bacillus (fenD) PCR reactions were done and the following results were obtained by gel electrophoresis. We found 9 amplicons for fenD gene (90%) among the 10 isolates of *Bacillus subtilis.* PCR results of *mecA* gene expression was identified at 964 bp position **(Figure 30C)**.

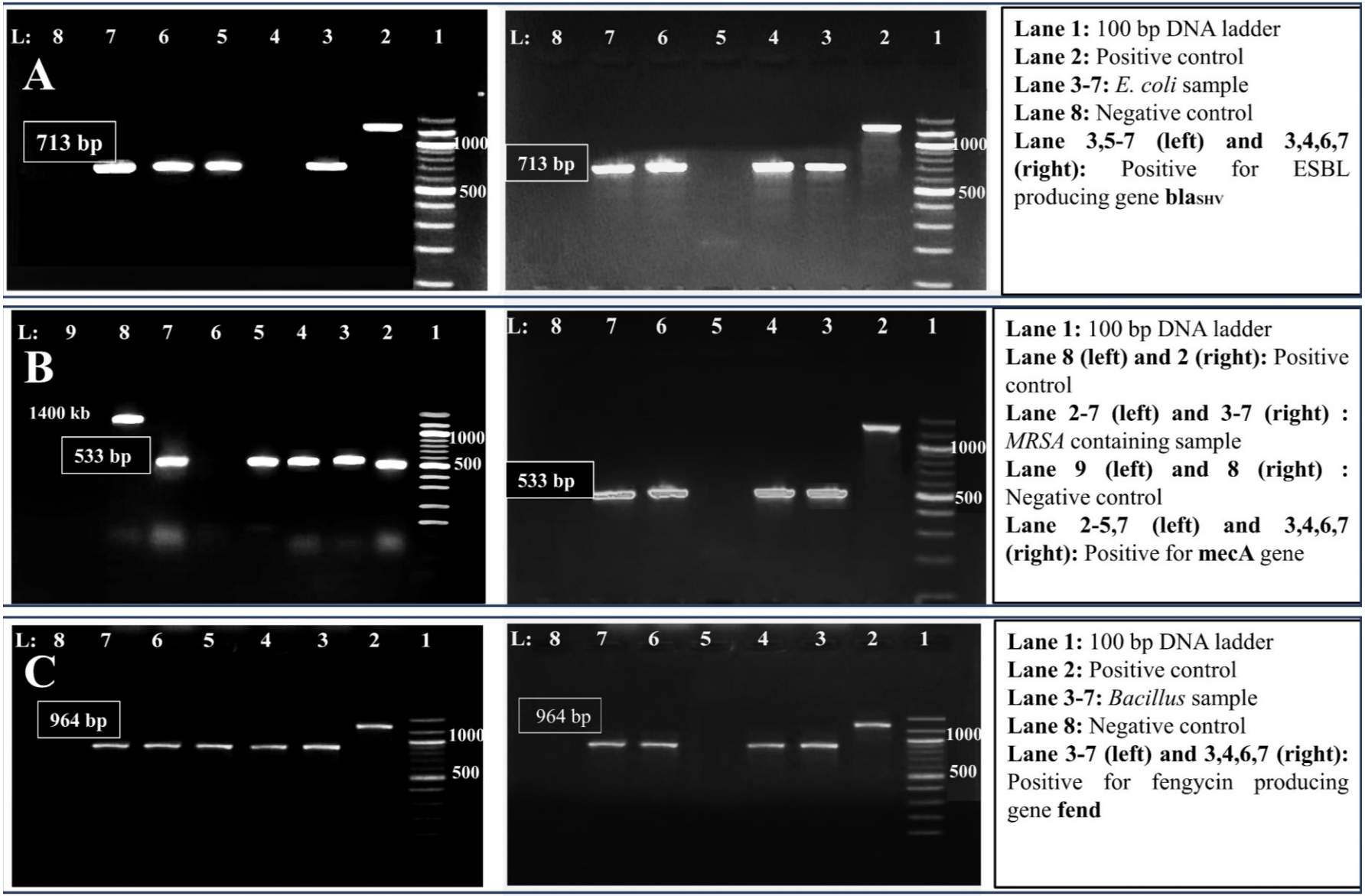

Figure 30. Visualization of PCR products by gel electrophoresis. **A.** ESBL producing (bla $_{SHV}$) *Escherichia coli*; **B.** Methicillin resistance *Staphylococcus aureus*; **C.** Fengycin producing *Bacillus subtilis*.

Chapter 4:
Discussion and Conclusion

4. Discussion and Conclusion

4.1 Discussion

The occurrence of Premature Rupture of Membrane (PROM) in pregnancy presents a multifaceted challenge, impacting both maternal and fetal health. With its prevalence affecting approximately 10% of pregnancies, PROM underscores the need for an in-depth exploration of its consequences and management strategies (Enjamo *et al.*, 2022b). This discussion investigates the intricate web of factors associated with PROM, from its role as a risk factor for preterm birth to the diagnostic and therapeutic approaches employed in its management. By unraveling the complexities of PROM, an enhanced view on the pathophysiology and more effective strategies for prevention and treatment have been established.

In this section, we investigate the multifaceted findings of our study, exploring significant associations, revealing microbial secrets, and deciphering the intricacies of antimicrobial resistance. Our investigation, which spans demographic and medical factors, microbial communities, and antibiotic susceptibility, unveils a nuanced picture of Premature Rupture of Membrane (PROM) among pregnant women. Through this comprehensive analysis, we seek to contribute to the body of knowledge that informs clinical practice and improves outcomes for expectant mothers and their infants.

Initially, our study uncovers a web of connections between various demographic and medical factors and the occurrence of PROM. Accordingly, age emerges as a pivotal determinant, with a substantial prevalence shift across age cohorts. Notably, PROM incidence spikes among women aged 31 to 40, suggesting that older maternal age is a significant risk factor. This finding aligns with prior research highlighting the complex relationship between maternal age and PROM (Enjamo *et al.*, 2022b). At the same time prompts questions about the biological mechanisms at play. Investigating age-related changes in the fetal membranes or hormonal influences could lead to breakthroughs in understanding the pathophysiology of PROM.

Additionally, economic status plays a crucial role, with a clear trend of increased PROM prevalence in higher-income groups. This may reflect disparities in access to healthcare and lifestyle factors associated with economic well-being. In contrast, educational status reveals a more intricate pattern, with PROM risk rising among individuals with lower education levels. Consanguinity in marriages presents a stark contrast, as consanguineous marriages exhibit a significantly higher risk of PROM. This finding suggests a potential genetic component in PROM susceptibility. Further genetic studies, including whole-genome sequencing, could uncover

specific genetic markers or mutations associated with PROM. Identifying these genetic factors might enable the development of predictive tools to assess an individual's risk of PROM and guide personalized interventions (Menon and Richardson, 2017).

Consequently, nutrition status also emerges as a significant factor, with below-average nutrition linked to a substantially higher risk of PROM. This observation emphasizes the need for nutritional interventions and support for expectant mothers, particularly those in vulnerable populations (Young *et al.*, 2018). Additional factors, including itching during pregnancy, previous PROM history, recent injury, ANC visit frequency, high blood pressure, and various laboratory abnormalities, all exhibit statistically significant associations with PROM. These multifaceted findings underscore the complex interplay of demographic and medical factors in PROM occurrence and highlight the need for comprehensive risk assessment and tailored interventions.

Notably, our investigation takes a crucial turn as we explore microbial communities in the context of PROM. Samples from three hospitals represent a diverse population, minimizing sampling bias and enhancing the generalizability of our findings. Among the samples, microbial analysis reveals significant microbial growth in a substantial proportion. The presence of microbial communities, both aerobic and anaerobic, underscores the role of microorganisms in PROM. While previous research has established a link between infections and PROM (Nakubulwa *et al.*, 2015), our study goes further by identifying specific microbial species. The specific microbial species identified, including *Staphylococcus spp*., *Bacillus spp., Streptococcus spp., Escherichia coli,* and *Enterococcus spp*., shed light on the complex microbial landscape associated with PROM.

Furthermore, the presence of polymicrobial infections involving diverse organisms like *Lactobacilli and Candida* is a notable discovery. Research often focuses on single pathogens (Nakubulwa *et al.*, 2015), but polymicrobial infections may have distinct pathophysiological mechanisms . Investigating the interactions and synergistic effects of multiple microorganisms could provide insights into more effective treatment strategies and the prevention of recurrent infections. The observation that a majority of the microbial isolates exhibit limited or absent aerobic motility is intriguing. This points to potential metabolic adaptations of these microbes under specific conditions. Investigating the metabolic pathways involved and their impact on PROM could reveal novel targets for therapeutic intervention (Liu *et al.*, 2021). Understanding how these microbes thrive in the unique environment of the fetal membranes might yield breakthroughs in infection prevention.

In addition, we embark on a meticulous journey to identify and classify the precise identities of these microbial inhabitants and establish a comprehensive profile of the microbial species present

in our study population. Understanding the role of these microbes in PROM could pave the way for groundbreaking diagnostic and treatment strategies (Zhao, Hu and Ying, 2023). For instance, targeted antibiotics or probiotics tailored to the specific microbial profile might offer novel approaches to prevent or manage PROM. Accordingly, Antimicrobial Resistance (AMR) takes center stage as we scrutinize the susceptibility of various microorganisms to a range of antibiotics. This rigorous analysis reveals distinct resistance patterns among different bacterial species. Notably, *Bacillus spp*. exhibit lower resistance percentages across most antibiotics, whereas *E. coli* and *Enterococcus spp.* display mixed resistance profiles. *Staphylococcus spp.* and *Streptococcus spp.* demonstrate moderate resistance, emphasizing the need for tailored antibiotic selection in clinical practice.

On top of that, a captivating glimpse into antibiotic resistance patterns among various bacterial species showcased the variability in resistance profiles (**Figures 31**,**32**). The variability in antibiotic resistance profiles among different bacterial species highlights the complexity of antibiotic resistance and the need for individualized treatment strategies. Tailored antibiotic selection based on the resistance patterns of specific bacterial isolates could revolutionize the management of infectious complications during pregnancy and beyond, reducing the overuse of broad-spectrum antibiotics and curbing antibiotic resistance (Eleje *et al.*, 2014). The identification of highly resistant strains within specific species presents an opportunity for in-depth genomic analysis. Investigating the genetic mechanisms driving resistance in these strains could lead to innovative therapeutic targets or the development of new antibiotics.

Finally, to extend the picture in a remarkable scale, a robust demographic perspective in association with bacterial infection highlights the prevalence of clinical isolates across distinct age cohorts **(Table 10)**. This nuanced view of patient demographics offers valuable insights into the population at risk for PROM-associated infections. Additionally, our biochemical assessments, including citrate utilization, MR, VP, catalase, and oxidase tests, provide a robust foundation for precise characterization and classification of microbial isolates. Moreover, the antibiotic resistance profiles for clinical isolates revealed a concerning trend of multidrug resistance among certain *Staphylococcus aureus* strains **(Table 11)**. *Staphylococcus epidermis* exhibits resistance primarily to specific antibiotic categories, emphasizing the importance of tailored therapeutic approaches. The *Streptococcus spp.* strains display resistance to multiple antibiotic classes, highlighting the necessity of personalized treatment strategies. *E. Coli* strains exhibit varying degrees of resistance, with some demonstrating pan-drug resistance, underscoring the critical importance of judicious antibiotic selection. *Bacillus spp.* strains also display resistance patterns,

reinforcing the need for tailored treatment regimens. Our findings illuminate the vital importance of precision medicine in combating antibiotic resistance. Tailored treatment strategies based on the resistance patterns of specific bacterial isolates are crucial for optimizing clinical outcomes and mitigating the spread of antibiotic resistance.

In conclusion, our study unravels the intricate tapestry of PROM, revealing the interplay of demographic and medical factors, microbial communities, and antibiotic resistance. These multifaceted findings provide a comprehensive understanding of PROM and underscore the need for personalized interventions and ongoing research to improve maternal and neonatal health. Our research paves the way for more precise diagnostic and therapeutic approaches, offering hope for healthier outcomes for expectant mothers and their infants.

Moreover, our study unveils promising prospects, from pinpointing microbial communities linked to PROM to the notion of tailored antibiotic therapy. These revelations forge new paths in research, promising enhanced maternal and neonatal health. The revelation of PROM-associated microbial communities lays the groundwork for innovative diagnostics and treatments. Simultaneously, the antibiotic resistance variability underscores the importance of personalized approaches in fighting infections during pregnancy and beyond. These pivotal insights drive transformative progress in obstetrics and infectious disease management.

4.2. Conclusion

Globally, pregnancy outcomes dance to a diverse rhythm, with Premature Rupture of Membranes (PROM) as a significant player, affecting 4 to 10% of pregnancies, casting its impact on both mothers and newborns. Despite the concerted efforts of medical intervention, the PROM puzzle remains. Our study unraveled the rich tapestry of PROM, with age, economic status, and education as the maestros orchestrating this intricate composition. Older maternal age, higher income, and lower education levels took center stage, conducting a higher risk of PROM. We also embarked on a fascinating journey into the microbial world within fetal membranes, where Staphylococcus, Bacillus, Streptococcus, Escherichia coli, and Enterococcus played their solo roles, potentially holding the key to PROM's enigma. The grand finale was our discovery of antibiotic resistance, a crescendo that underscores the need for tailored treatment, potentially revolutionizing the battle against infectious complications during pregnancy. With this symphony of findings, we've opened new doors to research and innovative treatments, illuminating the future of obstetrics and infectious disease management for brighter maternal and neonatal health outcomes.

Chapter 5:
References

5. References

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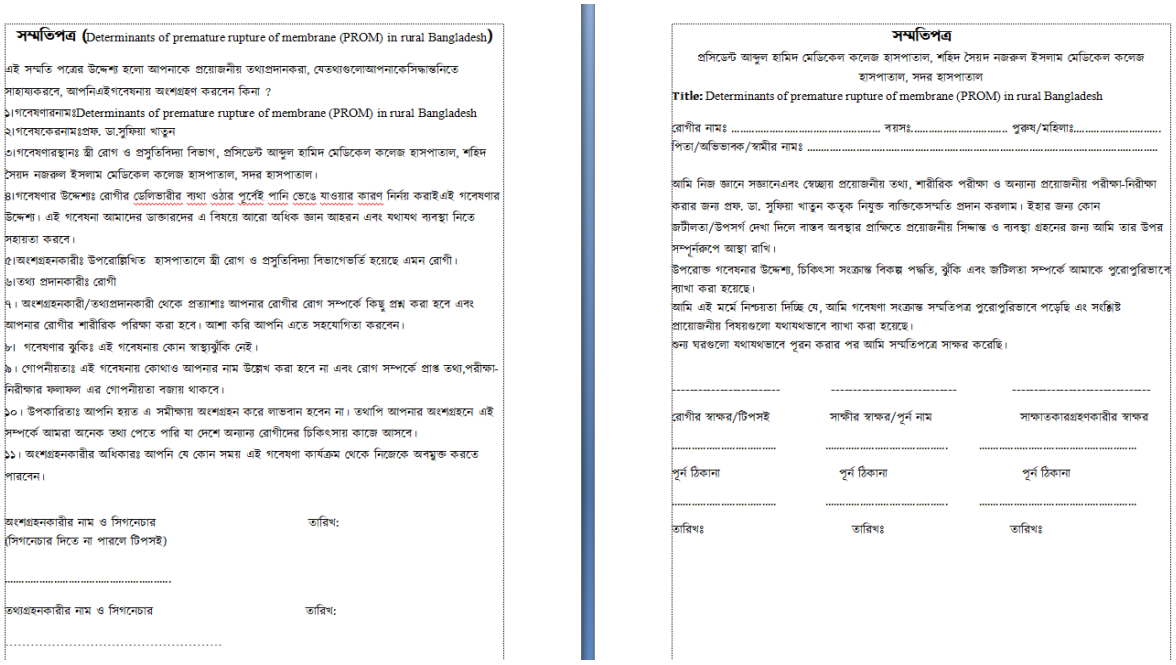
Chapter 6:
Appendix

6. Appendix



সম্মতিপত্র (Determinants of premature rupture of membrane (PROM) in rural Bangladesh)

এই সম্মতি পত্রের উদ্দেশ্য হলো আপনাকে প্রয়োজনীয় তথ্যপ্রদানকরা, যেতথ্যগুলোআপনাকেসিদ্ধান্তনিতে সাহায্যকরবে, আপনিএইগবেষনায় অংশগ্রহণ করবেন কিনা ?

১।গবেষণারনামঃDeterminants of premature rupture of membrane (PROM) in rural Bangladesh

২।গবেষকেরনামঃপ্রফ. ডা.সুফিয়া খাতুন

৩।গবেষণারস্থানঃ স্ত্রী রোগ ও প্রসুতিবিদ্যা বিভাগ, প্রসিডেন্ট আব্দুল হামিদ মেডিকেল কলেজ হাসপাতাল, শহিদ সৈয়দ নজরুল ইসলাম মেডিকেল কলেজ হাসপাতাল, সদর হাসপাতাল।

৪।গবেষণার উদ্দেশ্যঃ রোগীর ডেলিভারীর ব্যথা ওঠার পূর্বেই পানি ভেঙে যাওয়ার কারণ নির্নয় করাইএই গবেষণার উদ্দেশ্য। এই গবেষনা আমাদের ডাক্তারদের এ বিষয়ে আরো অধিক জ্ঞান আহরন এবং যথাযথ ব্যবস্থা নিতে সহায়তা করবে।

৫।অংশগ্রহনকারীঃ উপরোল্লিখিত হাসপাতালে স্ত্রী রোগ ও প্রসুতিবিদ্যা বিভাগেভর্তি হয়েছে এমন রোগী।

৬।তথ্য প্রদানকারীঃ রোগী

৭। অংশগ্রহনকারী/তথ্যপ্রদানকারী থেকে প্রত্যাশাঃ আপনার রোগীর রোগ সম্পর্কে কিছু প্রশ্ন করা হবে এবং আপনার রোগীর শারীরিক পরিক্ষা করা হবে। আশা করি আপনি এতে সহযোগিতা করবেন।

৮। গবেষণার ঝুকিঃ এই গবেষনায় কোন স্বাস্থ্যঝুঁকি নেই।

৯। গোপনীয়তাঃ এই গবেষনায় কোথাও আপনার নাম উল্লেখ করা হবে না এবং রোগ সম্পর্কে প্রাপ্ত তথ্য,পরীক্ষা-নিরীক্ষার ফলাফল এর গোপনীয়তা বজায় থাকবে।

১০। উপকারিতাঃ আপনি হয়ত এ সমীক্ষায় অংশগ্রহন করে লাভবান হবেন না। তথাপি আপনার অংশগ্রহনে এই সম্পর্কে আমরা অনেক তথ্য পেতে পারি যা দেশে অন্যান্য রোগীদের চিকিৎসায় কাজে আসবে।

১১। অংশগ্রহনকারীর অধিকারঃ আপনি যে কোন সময় এই গবেষণা কার্যক্রম থেকে নিজেকে অবমুক্ত করতে পারবেন।

অংশগ্রহনকারীর নাম ও সিগনেচার (সিগনেচার দিতে না পারলে টিপসই) ..... তারিখ:

তথ্যগ্রহনকারীর নাম ও সিগনেচার ..... তারিখ:

সম্মতিপত্র

প্রসিডেন্ট আব্দুল হামিদ মেডিকেল কলেজ হাসপাতাল, শহিদ সৈয়দ নজরুল ইসলাম মেডিকেল কলেজ হাসপাতাল, সদর হাসপাতাল

Title: Determinants of premature rupture of membrane (PROM) in rural Bangladesh

রোগীর নামঃ ..... বয়সঃ..... পুরুষ/মহিলাঃ.....

পিতা/অভিভাবক/স্বামীর নামঃ .....

আমি নিজ জ্ঞানে সজ্ঞানেএবং স্বেচ্ছায় প্রয়োজনীয় তথ্য, শারীরিক পরীক্ষা ও অন্যান্য প্রয়োজনীয় পরীক্ষা-নিরীক্ষা করার জন্য প্রফ. ডা. সুফিয়া খাতুন কতৃক নিযুক্ত ব্যক্তিকেসম্মতি প্রদান করলাম। ইহার জন্য কোন জটীলতা/উপসর্গ দেখা দিলে বাস্তব অবস্থার প্রাক্ষিতে প্রয়োজনীয় সিদ্ধান্ত ও ব্যবস্থা গ্রহনের জন্য আমি তার উপর সম্পূর্নরুপে আস্থা রাখি।

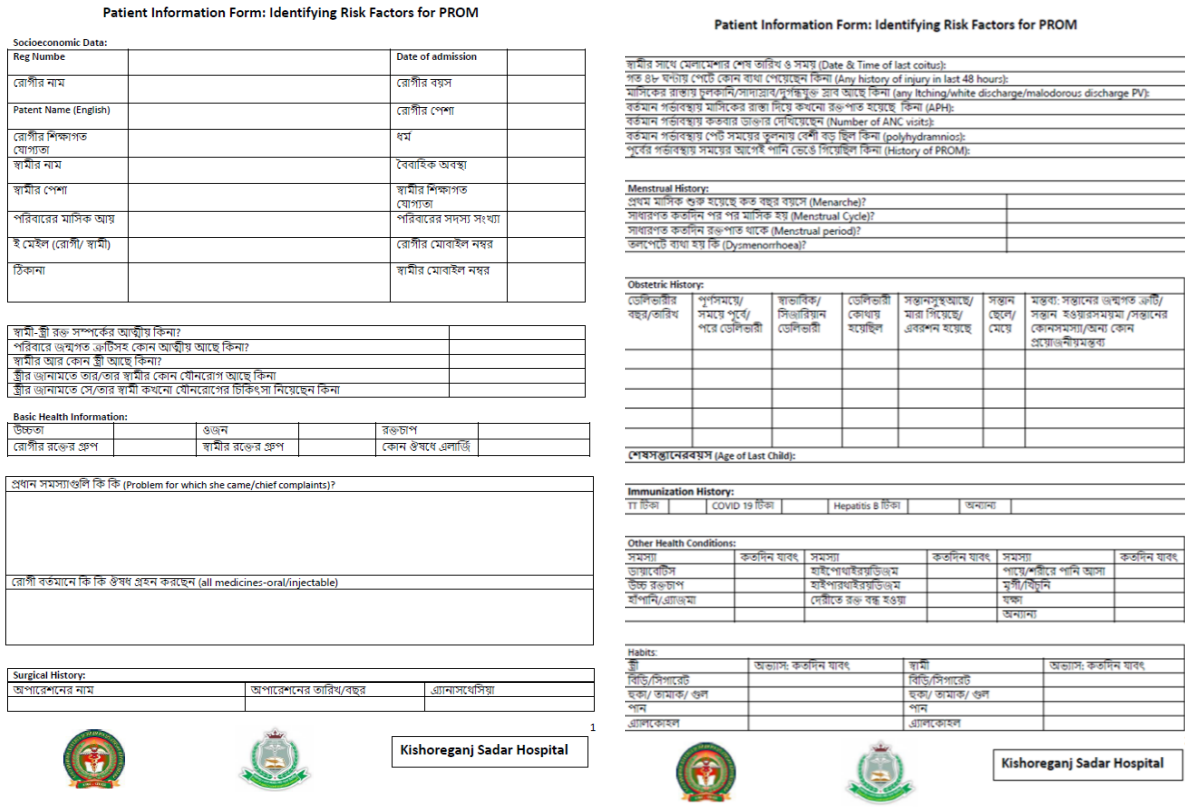
উপরোক্ত গবেষনার উদ্দেশ্য, চিকিৎসা সংক্রান্ত বিকল্প পদ্ধতি, ঝুঁকি এবং জটিলতা সম্পর্কে আমাকে পুরোপুরিভাবে ব্যাখা করা হয়েছে।

আমি এই মর্মে নিশ্চয়তা দিচ্ছি যে, আমি গবেষণা সংক্রান্ত সম্মতিপত্র পুরোপুরিভাবে পড়েছি এং সংশ্লিষ্ট প্রায়োজনীয় বিষয়গুলো যথাযথভাবে ব্যাখা করা হয়েছে।

শুন্য ঘরগুলো যথাযথভাবে পূরন করার পর আমি সম্মতিপত্রে সাক্ষর করেছি।

রোগীর স্বাক্ষর/টিপসই	সাক্ষীর স্বাক্ষর/পূর্ন নাম	সাক্ষাতকারগ্রহণকারীর স্বাক্ষর
পূর্ন ঠিকানা	পূর্ন ঠিকানা	পূর্ন ঠিকানা
তারিখঃ	তারিখঃ	তারিখঃ

Figure 31. Patients consent form of PROM research which is reviewed by President Abdul Hamid Medical College ERC and BMRC



Patient Information Form: Identifying Risk Factors for PROM

Socioeconomic Data:

Reg Numbe		Date of admission	
রোগীর নাম		রোগীর বয়স	
Patent Name (English)		রোগীর পেশা	
রোগীর শিক্ষাগত যোগ্যতা		ধর্ম	
স্বামীর নাম		বৈবাহিক অবস্থা	
স্বামীর পেশা		স্বামীর শিক্ষাগত যোগ্যতা	
পরিবারের মাসিক আয়		পরিবারের সদস্য সংখ্যা	
ই মেইল (রোগী/ স্বামী)		রোগীর মোবাইল নম্বর	
ঠিকানা		স্বামীর মোবাইল নম্বর	

স্বামী-স্ত্রী রক্ত সম্পর্কের আত্মীয় কিনা?	
পরিবারে জন্মগত ত্রুটিসহ কোন আত্মীয় আছে কিনা?	
স্বামীর আর কোন স্ত্রী আছে কিনা?	
স্ত্রীর জানামতে তার/তার স্বামীর কোন যৌনরোগ আছে কিনা	
স্ত্রীর জানামতে সে/তার স্বামী কখনো যৌনরোগের চিকিৎসা নিয়েছেন কিনা	

Basic Health Information:

উচ্চতা		ওজন		রক্তচাপ	
রোগীর রক্তের গ্রুপ		স্বামীর রক্তের গ্রুপ		কোন ঔষধে এলার্জি	

প্রধান সমস্যাগুলি কি কি (Problem for which she came/chief complaints)?
রোগী বর্তমানে কি কি ঔষধ গ্রহন করছেন (all medicines-oral/injectable)

Surgical History:

অপারেশনের নাম	অপারেশনের তারিখ/বছর	এ্যানাসথেসিয়া

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Patient Information Form: Identifying Risk Factors for PROM

স্বামীর সাথে মেলামেশার শেষ তারিখ ও সময় (Date & Time of last coitus):
গত ৪৮ ঘন্টায় পেটে কোন ব্যথা পেয়েছেন কিনা (Any history of injury in last 48 hours):
যৌনিকের রাস্তায় চুলকানি/সাদাস্রাব/দুর্গন্ধযুক্ত স্রাব আছে কিনা (any itching/white discharge/malodorous discharge PV):
বর্তমান গর্ভাবস্থায় যৌনিকের রাস্তা দিয়ে কখনো রক্তপাত হয়েছে কিনা (APH):
বর্তমান গর্ভাবস্থায় কতবার ডাক্তার দেখিয়েছেন (Number of ANC visit):
বর্তমান গর্ভাবস্থায় পেট সময়ের তুলনায় বেশী বড় ছিল কিনা (polyhydramnios):
পুর্বের গর্ভাবস্থায় সময়ের আগেই পানি ভেঙে গিয়েছিল কিনা (History of PROM):

Menstrual History:

প্রথম মাসিক শুরু হয়েছে কত বছর বয়সে (Menarche)?	
সাধারণত কতদিন পর পর মাসিক হয় (Menstrual Cycle)?	
সাধারণত কতদিন রক্তপাত থাকে (Menstrual period)?	
তলপেটে ব্যথা হয় কি (Dysmenorrhoea)?	

Obstetric History:

ডেলিভারীর বছর/তারিখ	পূর্ণসময়ে/ সময়ে পূর্বে/ পরে ডেলিভারী	যাভাবিক/ সিজারিয়ান ডেলিভারী	ডেলিভারী কোথায় হয়েছিল	সন্তানসুস্থআছে/ মারা গিয়েছে/ এবরশন হয়েছে	সন্তান ছেলে/ মেয়ে	মন্তব্য: সন্তানের জন্মগত ত্রুটি/ সন্তান হওয়ারসময়মা /সন্তানের কোনসমস্যা/অন্য কোন প্রয়োজনীয়মন্তব্য

শেষসন্তানেরবয়স (Age of Last Child):

Immunization History:

TT টিকা		COVID 19 টিকা		Hepatitis B টিকা		অন্যান্য	

Other Health Conditions:

সমস্যা	কতদিন যাবৎ	সমস্যা	কতদিন যাবৎ	সমস্যা	কতদিন যাবৎ
ডায়াবেটিস		হাইপোথাইরয়ডিজম		পায়ে/শরীরে পানি আসা	
উচ্চ রক্তচাপ		হাইপারথাইরয়ডিজম		নূর্বী/খিচুনি	
হাঁপানি/এ্যাজমা		দেহাঁতে রক্ত বন্ধ হওয়া		যক্ষা	
				অন্যান্য	

Habits:

স্ত্রী	অভ্যাস: কতদিন যাবৎ	স্বামী	অভ্যাস: কতদিন যাবৎ
বিড়ি/সিগারেট		বিড়ি/সিগারেট	
হুক্কা/ তামাক/ গুল		হুক্কা/ তামাক/ গুল	
পান		পান	
এ্যালকোহল		এ্যালকোহল	

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Figure 32. Data collection form designed to collect the data from the individual pregnant woman.

Patient Information Form: Identifying Risk Factors for PROM

Examinaion:	
Height of Uterus in CM:	Fetal heart sound:
Uterine contraction:	Fetal movement:
Watery discharge PV:	Oligohydramnios/Polyhydramnios:
PV bleeding (APH):	Blood stained discharge PV (Show):
Only Whitish discharge PV:	Meconium stained discharge PV:
Effacement:	Whitish & malodorous discharge PV:
Cervical Os:	Station:

Investigations:	
CBC	**USG**
Hemoglobin:	Date of Last USG (on/after admission):
TC:	Number of fetus:
Lymphocyte:	Fetal age in week:
Neutroplil:	Fetus dead/alive:
Eosiniphil:	AFI:
Monocyte:	EDD:
Platelet:	
Blood Sugar:	**High Vaginal Swab CS:**
RBS/Fasting/	
2HABF/2HAG	
HbS Ag:	
Urine RE: RBC:	
Pus Cell:	
Urine Sugar:	
Urine albumin:	
Urine CS:	

Treatment	
Treatment	Outcome
Conservative Rx:	Discharge on:
Delivered: NVD or CS	Baby weight:
Condition of Mother:	Condition of baby:

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Figure 33. Data collection form (continued)