PERINATAL ANTIBIOTIC PROPHYLAXIS AND NEONATAL SEPSIS IN INDONESIA

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ABSTRACT

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Neonatal sepsis is a major health problem in lower-middle income countries (LMIC). Antibiotic prophylaxis is one of the most common practices to neonatal sepsis. Little is known about how antibiotic prophylaxis affects neonatal sepsis epidemiology in LMICs. Mounting evidence suggests that antibiotics have a substantial effect on the microbiome and influences newborns' susceptibility to infection. This dissertation aims to 1) estimate the prevalence, incidence, and risk factors of neonatal sepsis and perinatal antibiotic prophylaxis use in Palembang, Indonesia; 2) assess the effects of perinatal antibiotic prophylaxis on neonatal sepsis incidence; and 3) explore the effects of perinatal antibiotic prophylaxis on newborns' gut microbiomes and evaluate whether microbiome features mediate the association of antibiotic prophylaxis with neonatal sepsis. To provide preliminary data, a retrospective study was conducted at Mohammad Hoesin Hospital, Indonesia, reviewing 306 neonatal sepsis cases and 3,657 deliveries between 2016 and 2018. Then, a prospective cohort recruited 1,002 mother–viable newborn pairs admitted for delivery at two Indonesian hospitals. Newborns were followed until the age of 28 days or until sepsis was observed. Adjusted relative risk and average treatment effect (ATE) of antibiotic prophylaxis for neonatal sepsis were estimated. Lastly, a nested case-control study matched 53 newborns with sepsis to 102 healthy infants by mode of delivery. Newborns' gut microbiomes from meconium and stool specimens were profiled using 16S ribosomal RNA sequencing. Mediation analysis assessed the relationships among perinatal antibiotic prophylaxis, newborns' microbiome features, and neonatal sepsis. The preliminary study showed

that the neonatal sepsis hospital admission prevalence was 14.1%. The percentages of early-onset sepsis and late-onset sepsis were comparable. The proportion of culture-negative sepsis was 44%. Overall, 62.6% of all isolated organisms were multidrug-resistant bacteria. The prevalence of prophylactic antibiotic use during delivery was 47.1%. Premature rupture of membrane (PROM) and C-section were some factors that were strongly associated with prophylactic antibiotic use. In the cohort study, the cumulative incidence of neonatal sepsis was 10.4 per 100 live births. The proportion of culture-negative sepsis was three times higher than in the preliminary study. The neonatal sepsis risk was increased by PROM, foul-smelling amniotic fluid, high maternal leukocyte count, low birth weight, mixed feeding, and fasting. Of the newborns studied, 72% were exposed to antibiotic prophylaxis. The estimate of the ATE of perinatal antibiotic prophylaxis on neonatal sepsis was 0.10 ($p< 0.0001$). The causal effect was more robust for postnatal prophylaxis alone or with maternal exposure. The meconium and follow-up stool specimens of newborns with sepsis had a significantly lower alpha diversity than non-sepsis newborns. The microbiome analysis found that the meconium of newborns exposed to perinatal antibiotic prophylaxis exhibited a distinct gut microbiome community compared to the unexposed group. Although there were few suppression effects across perinatal antibiotic prophylaxis, microbiome features, and neonatal sepsis, no significant mediation effects were found. This study corroborates that neonatal sepsis incidence remains high despite the high use of perinatal antibiotic prophylaxis. Considering that the post-antibiotic era is nearing, there is an urgent need for non-antibiotic prevention strategies that are feasible in LMICs, which often have crippling resource constraints. Given this need, this dissertation elucidates the microbiome's potential role in the causal pathway of neonatal sepsis and is an advancement toward manipulation of the gut microbiome to prevent neonatal sepsis and injudicious antibiotic use.

Dedicated to my Papi and Papa, in loving memory.

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KEY TO ABBREVIATIONS

ACOG – American College of Obstetricians and Gynecologist

AST – Antimicrobial Susceptibility Testing

aOR – Adjusted Odds Ratio

aRR – Adjusted Relative Risk

ATE – Average Treatment Effect

CDC – Centers for Disease Control and Prevention

CI – Confidence Interval

CoNS- Coagulase-Negative Staphylococcus

$COPSAC_{2010}$ - Copenhagen Prospective Studies on Asthma in Childhood 2010

EOS – Early Onset Sepsis

GBS – Group B Streptococcus

HIC – High Income Country

HIV – Human Immunodeficiency Virus

IAP – Intrapartum Antibiotic Prophylaxis

ICD-10-CM – International Classification Disease-10-Clinical Modification

IPW- Inverse Probability Weighting

IQR – Interquartile Range

KMC – Kangaroo Mother Care

LDA – Linear Discriminant Analysis

LefSe - Linear Discriminant Analysis Effect Size

LMIC – Low- and Middle- Income Country

LOS – Late Onset Sepsis

MDR – Multiple Drug Resistant

NICHD – National Institute of Child and Human Development

Ob/Gyn – Obstetrics and Gynecologist

OR – Odds Ratio

OUT – Operational Taxonomic Unit

PERMANOVA – Permutational Analysis of Variance

PROM – Premature Rupture of Membranes

PPROM – Preterm Premature Rupture of Membranes

QIIME2 - Quantitative Insights into Microbial Ecology 2

SD – Standard Deviation

UniFrac - Unique Fraction Metric

US – United States

VLBW – Very Low Birth Weight

WHO – World Health Organization

CHAPTER 1 - BACKGROUND, AIMS, and APPROACHES

1.0 Background

1.0.1 Overview of neonatal sepsis

Neonatal sepsis is a clinical syndrome involving bloodstream infection in infants aged 28 days or younger (1). To this day, neonatal sepsis remains one of the leading causes of morbidity and mortality among newborns. A comprehensive review of 1,270 studies of sepsis incidence from 1957 to 2016, by Fleischmann-Sturzek, estimated that global population-based sepsis incidence was 22 per 1,000 live births, equivalent to nearly three million cases annually (2). The study shows significantly different disease burden estimates reported from high-income countries than reports from low- and middle-income countries (LMIC): the incidence of neonatal sepsis in LMIC is twofold to fourfold higher than in high-income countries (3,4). In terms of mortality, in 2018 the World Health Organization (WHO) reported that, globally, neonatal sepsis was responsible for over 1,000 deaths per day, accounting for almost 15% of all neonatal deaths (5). Five years prior, in 2013, Oza et al. estimated that, globally, from all cause-specific deaths, the proportion of death caused by neonatal sepsis was also over 15% (4). Other studies also showed that of total sepsis-related neonatal deaths, almost 40% occurred in LMICs (2,6,7). Additionally, newborns in low-income countries have a higher risk of death due to neonatal sepsis—34 times higher, in fact—compared to newborns in high-income countries (3,4). Despite the large amount of available data on neonatal sepsis incidence and mortality from LMIC, these numbers still may not reflect the actual burden of neonatal sepsis due to the paucity of high-quality data. The WHO stated that from all WHO state members, high-quality vital registration data are available only for one-third of them, which are dominated by high-income countries (8). Data on neonatal sepsis in LMIC in Asia mostly reports the prevalence of neonatal sepsis hospital admission from

single-center center, which range from 5 to 45.9% of neonates admissions (9–12). Studies from Indonesia are also limited; some reported that the neonatal admission prevalence in some hospitals in Indonesia is between 5% and 25% (11–13). Hence, there is a critical need for data on population-level epidemiology of neonatal sepsis from LMIC.

With the knowledge of neonatal sepsis' prevalence, it is important to understand that neonatal sepsis is classified in two groups, depending on the infant's age at the presentation of symptoms: early-onset sepsis (EOS), when symptoms occur within the first 72 hours of life, and late-onset sepsis (LOS), when the onset occurs after 72 hours of life (1). EOS is generally caused by the transmission of pathogens from the mother's genitourinary system to the newborn or fetus. These pathogens can ascend the vagina, the cervix, and the uterus, infecting the amniotic fluid. Neonates can also become infected in utero or during delivery as they pass through the vaginal canal. The reported key factors that increase the risk of neonatal sepsis include chorioamnionitis, GBS colonization, prolonged rupture of membranes (more than 18 hours), prematurity, and low birth weight (7,14,15). LOS is usually caused by pathogen transmission from the surrounding environment after delivery, such as contact from healthcare workers, contact from caregivers, or invasive procedures that disrupt the newborn's mucosa, including resuscitation. A small proportion of LOS may also be caused by a late manifestation of vertically transmitted infection (14,15).

Another key understanding to grasp regarding neonatal sepsis is that the etiological agents of neonatal sepsis in high-income countries differ from those seen in LMIC. In high-income countries, the most common bacterial pathogens for EOS include Group B *Streptococcus* (GBS), *Escherichia coli*, coagulase-negative *Staphylococcus (*CoNS), *Haemophilus influenzae*, and *Listeria monocytogenes*. For LOS, the leading etiological agent is CoNS species, especially

Staphylococcus epidermis (7,14). On the other hand, in LMIC, previous studies reported that the pathogen profiles of EOS and LOS are similar, dominated by gram-negative organisms—mainly represented by *Klebsiella spp.* and *E. coli*. Of the gram-positive organisms, *S. aureus* and CoNS are the most commonly isolated, while GBS is extremely rare (7,16). The reason for the differences in the etiological agents of neonatal sepsis between high-income and LMIC remains uncertain. It may reflect an actual difference in the distribution of causative agents between countries or be due to differences in the case definition of sepsis, the capability to perform blood cultures, or published reports from short periods of surveillance. In LMIC, there are also significant diagnoses of neonatal sepsis without a blood culture or cases that never reached healthcare facilities (thus, being classified as unreported), leading to missing information on the etiological cause of neonatal sepsis and misclassified.

Even with all this knowledge, years of clinical experience with the care of neonates with confirmed or suspected sepsis, identifying newborns with sepsis is still challenging, especially in LMIC (7,17). Currently, the gold standard for establishing a diagnosis of neonatal sepsis is through a blood or CSF culture (15). However, there are major challenges in obtaining a positive culture, even in countries with excellent resources: for example, limited blood volume obtained from neonates, the presence of low or intermittent bacteremia, previous antibiotic exposure, and the low specificity of blood culture due to microbial contamination during sampling (15,18,19). Therefore, even though some clinical manifestations in neonates are not a reliable indicator of illness, history and physical examination combined with other laboratory markers, such as leukocyte count and acute-phase reactants, remain the cornerstone of clinical practice (15,20). The signs and symptoms of neonatal sepsis can range from nonspecific signs to hemodynamic collapse. Early symptoms may include temperature instability, irritability, lethargy, jaundice,

tachycardia, mottled skin, or poor feeding. Later symptoms may include respiratory distress, liver or bone marrow dysfunction, disseminated intravascular coagulation, hypothermia, or hypotension with poor perfusion and shock (15,19,20). This condition enables a diagnosis of neonatal sepsis both clinically with or without microbiological proof (20). A condition when a newborn is presume to have symptomatic infection but no bacterial cause identified is referred as culture-negative sepsis (18). A study showed that in high-income countries, the ratio of culture-proven versus culture-negative sepsis ranges from 1:6 to 1:12 (18). Due to limited resources, it is expected that the proportion of culture-negative neonatal sepsis in LMIC is higher compared to high-income countries. A study in Indonesia reported that the proportion of culture-proven sepsis was nearly 2% (21). The high proportion of culture-negative sepsis complicates the management of neonatal sepsis, especially in the era of antibiotic resistance when antibiotics should be use judiciously.

However, studies show that the mortality rate between culture-proven and culture-negative sepsis is similar, indicating that those with culture-negative sepsis were also severely ill (22,23). Therefore, some of these culture-negative sepsis cases are not likely to be truly negative, as they may be caused by unusual organisms that are not screened for in routine practice, including virus, or the clinical syndrome is caused by a noninfectious agent. Conventional microbiological methods also frequently fail to identify pathogens due to technical issues or traits intrinsic to the microorganism that limit detection. Therefore, future research must focus on identifying, developing, and refining a rapid, sensitive, and specific diagnostic tools for neonatal sepsis that can be widely used in LMIC, where the disease is most prevalent. These tools should be able to screen for and identify all relevant pathogens in neonatal sepsis, regardless of prior antimicrobial exposure, with the results not being limited by the obtained

volume of blood. Moreover, most importantly, these tools' usage should be feasible in low-resource settings.

1.0.2 Neonatal sepsis prevention

Multiple preventative strategies have been implemented to prevent neonatal bacterial infection during antenatal care, during labor and delivery, and after birth. Most of these strategies are to prevent early-onset sepsis. In prenatal care, the interventions are mainly aimed to prevent factors linked to sepsis, such as preterm birth and low birth weight, GBS screening in the week of 35-37 of pregnancy, and maternal vaccination (24–27). Clinical trials showed that improving maternal nutritional status with adequate level of calories, multiple micronutrients and supplementation significantly reduces prematurity rates and low birth weight, which are the predominant risk factors associated with neonatal sepsis (28,29). In addition, screening and comprehensive management of illnesses during pregnancy associated with preterm and low birth weight, such as HIV or other diseases that are more common in LMIC (like malaria and tuberculosis), will indirectly prevent neonatal sepsis (30–32). Also, maternal vaccination provides neonates with appropriate antibodies as soon as they are born, preventing further infection. Examples of proven vaccinations that can protect neonates against infection through the transfer of maternal antibodies via the placenta include tetanus, diphtheria, pertussis, and influenza (27).

Another key issue in the understanding of this syndrome is the importance of sanitation. Studies showed strong evidence that practices with clean delivery kits and handwashing protocols can reduce the rates of neonatal sepsis in both home and healthcare settings (33,34). Studies from LMICs also indicate that antisepsis interventions, such as a vaginal and umbilical

cord wipe with chlorhexidine, during labor and delivery may impact neonatal sepsis rates (35,36). A widely used strategy to prevent neonatal sepsis during delivery is intrapartum antibiotic prophylaxis (IAP). IAP has been highly effective in reducing early-onset neonatal bacterial infection in high-income countries (37,38). After introducing IAP, the US has dramatically reduced the incidence of early-onset neonatal bacterial sepsis caused by GBS, from 1.7 per 1,000 live births in 1993 to 0.26 per 1,000 in 2010 (37). In high-income countries, there are two approaches for implementing IAP: screening-based and risk factor approaches. In the screening-based approach, all pregnant women should be screened for anogenital GBS colonization, starting between 36 0/7 to 36 6/7 weeks gestation. Those with positive a GBS culture are encouraged to receive IAP. In the risk factor approach, IAP is given if the mother has one or more intrapartum risk factors. The risk factors include gestational age less than 37 weeks, duration of membrane rupture greater than or equal to 18 hours, and intrapartum temperature greater than or equal to 100.4 °F (38.0 °C). In high-income countries, when IAP is recommended, clear protocols that state the type, dose, and interval of the given antibiotic are in place to manage women with risk factors for GBS neonatal sepsis (37,39). The success of these approaches has resulted in IAP being widely adopted worldwide, with varying degrees of consistency, including in LMIC; however, even studies consistently reported showed that the incidence of GBS in LMIC is extremely low (16,39–43). In most low-income countries, prophylactic antibiotic administration relies heavily on clinical risk factors and physicians' clinical judgment. The goal is not solely limited to prevent GBS infection, but also to limit other pathogenic bacterial infections. The type of antibiotics used varies as well (37,38).

In addition to IAP, after delivery, the WHO recommends giving prophylactic antibiotics to neonates with documented risk factors for infection to prevent early-onset sepsis (44). The risk

factors include membranes rupturing greater than 18 hours before delivery, maternal temperature over 38 °C before delivery or during labor, and the presence of foul-smelling or purulent amniotic fluid. Antibiotic prophylaxis will be given for two days and will only continue if there is a sign of sepsis. There are a few other suggested strategies to prevent neonatal sepsis. After delivery, healthcare providers' handwashing can reduce neonatal sepsis and infection rates, especially in hospitals (33,34). Additionally, kangaroo mother care (KMC) for preterm and low birth weight newborns supports physiological stabilization of the newborn and beneficial early neonatal maternal microbial flora colonization (45,46). KMC in LMIC has been shown to promote earlier breastfeeding, weight gain, and early discharge from the hospital, all of which are significant factors in preventing neonatal sepsis (47,48). Breastfeeding has been repeatedly shown to reduce the risk of neonatal sepsis (49). Breastmilk contains secretory IgA, lysozymes, white blood cells, and lactoferrin. These human milk immunologic components have been shown to encourage the growth of healthy lactobacilli and reduce the growth of *E. coli* and other gram-negative pathogenic bacteria (50). Similarly, the early oropharyngeal application of maternal colostrum to very low birth weight infants may have corresponding immunomodulatory benefits (51). Currently, there is growing evidence that suggests probiotics (live microorganisms which when administered in adequate amounts confer a health benefit to the host) and synbiotics (combination of probiotics and non-viable food component with health benefits on the host associated with microbiota modulation) are beneficial in reducing neonatal sepsis (52,53). In a meta-analysis of 18 clinical trials, the administration of probiotics decreased the risk of LOS by 20% compared with no intervention (54). A large-randomized trial, enrolling more than 4,000 newborns in rural India, showed that using an oral symbiotic preparation of Lactobacillus plantarum, along with fructooligosaccharide, significantly reduced the incidence of sepsis (RR

0.60, 95% CI 0.48–0.74) (52). Up to now, studies continue to try to develop cost-effective and simplistic strategies to prevent neonatal sepsis.

1.0.3 Antibiotic prophylaxis use during delivery and neonatal sepsis

Given that IAP and the administration of antibiotic prophylaxis to high-risk neonates is one of the measures to prevent neonatal sepsis and adopted worldwide, these practices have inevitably led to the increased use of antibiotics during the perinatal period—increasing the proportion of neonates exposed to antibiotics during their early-life. Even in the US, since the introduction of IAP, the use of antimicrobials has more than doubled (55). In addition to being exposed to antibiotic prophylaxis for IAP or directly, as recommended by the WHO, to prevent neonatal sepsis, a newborn can be exposed to antibiotic prophylaxis due to a C-Section delivery (56). Some healthcare centers also recommend giving antibiotic prophylaxis during deliveries with premature membrane rupture (57,58). In high-income countries, it is estimated that more than 45% of neonates are exposed to some type of antibiotic given to their mother immediately before delivery (59,60). In LMIC, the proportion of neonates exposed to antibiotics is expected to be much higher. A study in a tertiary hospital in India reported that more than 90% of the deliveries received antibiotics (61). No Indonesian study was found on the use of maternal antibiotic prophylaxis (62). Although the WHO recommends giving antibiotic prophylaxis to newborns with documented risk factors for infection, there is very limited data on the usage rate of antibiotic prophylaxis in newborns (44). There was a study in 2001 at a tertiary hospital in Indonesia that reported that 35% of all in-hospital deliveries included the newborn receiving prophylactic antibiotics for EOS.

Of course, the high use of antibiotics during the perinatal period is of concern among experts. Previous studies suggested that antibiotic exposure during labor increases the risk of various adverse events to both the mother and newborn, such as non-GBS neonatal sepsis, late-onset sepsis, antibiotic-resistant bacterial infection, maternal and infant microbiome alteration, increased risk of allergic diseases, obesity, and long-term functional impairment in children (63–66). In terms of neonatal infection or sepsis, although the introduction of IAP has substantially reduced EOS from GBS, it did not reduce the rate of LOS. Some studies even showed an increment of LOS incidence after IAP policy implementation, although this trend may likely be due to the improved survival of premature infants (67,68). An early study evaluating neonatal mortality from sepsis, before and after IAP recommendations, in the US found an increasing trend in mortality from LOS (69). Another potential concern is that increased antibiotic use associated with the wide use of IAP might lead to more severe or antimicrobial-resistant etiologies of sepsis. In all age ranges, sepsis due to gram-negative bacterial infection carries a higher risk of severe sepsis, septic shock, and mortality than gram-positive infection (70). The fatality rate of gram-negative neonatal sepsis was reported at 36 to 55%, which is much higher than gram-positive neonatal sepsis at 18 to 27% (71). Neonatal gram-negative infections were also associated with significant neurological consequences, along with an increased length of hospital stays and costs (72,73).

Moreover, gram-negative pathogens have a higher rate of antibiotic resistance, reaching greater than 70% for third-generation cephalosporins and greater than 20% for last-resort antibiotics such as carbapenems (16,74). Concerns that IAP may increase the incidence of gram-negative and antibiotic-resistant neonatal sepsis have been supported by several studies comparing incidence rates before and after IAP guidelines were developed. An increase of gram-

negative neonatal sepsis cases from 0.29 to 1.3 per 1,000 live births was reported during the 1990s (75). Similarly, *E. coli* sepsis at 15 neonatal centers in the Neonatal Research Network of the National Institute of Child Health and Human Development (NICHD) increased from 3.2 to 6.8 per 1,000 live births (76). A study from Taiwan also reported an increase of EOS due to *E. coli*, from 40.9 to 70% (77). On the other hand, others reported that the incidence of *E. coli* associated with EOS remained stable, in the era before and after IAP implementation (78). Regarding antibiotic resistance rates, the studies' results are still conflicting. In an analysis of San Francisco and Atlanta data for the CDC Active Bacterial Core Surveillance, the rate of ampicillin resistance in EOS attributable to *E. coli* in preterm newborns increased from 29% in 1998 to 84% in 2000 (79). In contrast, Schrag et al., in a study of 132 cases of early-onset *E. coli* infection, did not find an association between IAP and EOS attributable to ampicillin-resistant *E. coli* (80). Although there are multiple publications on how IAP impacts neonatal sepsis epidemiology, there is limited literature that discusses the effect of other types of prophylactic antibiotics, including neonatal antibiotic prophylaxis, that are also used in the perinatal period.

Culture-negative neonatal sepsis is also frequently related to antibiotic exposure, particularly maternal antibiotics (18,81). However, since most publications on neonatal sepsis only include culture-proven sepsis, there is a paucity of data on culture-negative sepsis. Therefore, there are limited studies on the possibility of how maternal antibiotic exposure might contribute to the incidence of culture-negative neonatal sepsis. Considering that cases of culture-negative sepsis often outnumber culture-proven sepsis, with some studies reporting the lack of mortality differences between those groups, further studies are required to optimize the management of neonatal sepsis and prevent overuse of antibiotics (18,22,23).

1.0.4 The impact of antibiotic exposure on the role of the gut microbiome and its relationship with neonatal sepsis

It is suggested that the development of the gut microbiome may starts before birth (82). The most important factors that influence the establishment of the microbiome during the neonatal period include mode of delivery, type of infant feeding, gestational age, infant hospitalization, and early-life antibiotic exposure (83). Multiple pieces of evidence indicate that both long or short regimens of antibiotics before, during, and after birth also disrupt the natural microbiome assembly (84). In mice, prenatal antibiotics decrease the diversity and structure of the microbiota (85). In humans, intrapartum antibiotic use has been associated with decreased bacterial diversity of the neonate's first stool (86,87) and lower abundance of *Lactobacilli* and *Bifidobacterium* in the neonatal gut (87–90). IAP administration was also correlated with a reduction in *Actinobacteria* and *Bacteroidetes*, along with increased in *Proteobacteria* (91,92). In addition, another study in Spain suggested that newborns who have been exposed to IAP experience an increase of potentially pathogenic microorganisms, including *Campylobacteriaceae* or *Helicobacteriaceae* (93). Although *Campylobacteriaceae* infection in neonates is rare, there were a few reports that reported *Campylobacter* in neonates(94,95). While *Helicobacter* infection has been linked with the occurrence of colic infantile (96,97). In terms of postnatal antibiotic exposure, a study in Ireland showed that the gut microbiota of newborns who were treated with Ampicillin and Gentamycin within 48 hours after birth had significantly higher proportions of Proteobacteria and significantly lower proportions of Actinobacteria compared to healthy newborns (98). Tanaka in Japan, reported that newborns that was treated with broad spectrum antibiotic during their first four days of life showed less microbiome diversity compared to antibiotic-free newborns (90).

Substantial evidence has shown that early normal bacterial colonization is essential for normal development of the gut (99), strengthening and promoting gut barrier integrity (100), protecting against pathogens (101), and regulating host immunity (102,103). It is also suggested that the microbiome can influence the development of the immune cells, particularly neutrophils, which play a crucial role in the defense against microbial infection (104). Therefore, alteration in the gut microbiome or dysbiosis has been linked to increased neonatal sepsis (105–107). In a mouse model study, preventing dysbiosis of the neonatal mouse's gut microbiome protected it against sepsis (108). However, no human data have confirmed this association.

In line with studies that showed that newborns exposed to antibiotics have a less diverse microbiome compared to those who were unexposed to antibiotics, bacterial-profiling studies also found that newborns with sepsis have lower bacterial diversity compared to healthy newborns. Studies observed that there is an alteration in the gut microbiome community relatively to the community found in the healthy population or dysbiosis in neonatal sepsis cases (109–111). Compared to healthy newborns, the gut microbiome of newborns with sepsis had a relatively higher proportion of *Proteobacteria* and *Firmicutes*, which was similar to the comparison between newborns exposed to IAP and not (109,110). Stewart et al., who studied over 600 stool samples from newborns with and without sepsis, reported that *Bifidobacteria* were found only in stool samples from healthy newborns, which is in line with what was found in the comparison between infants who were exposed and unexposed to antibiotics (88,89,107) . These findings showed a possible indication that antibiotic prophylaxis exposure in neonates influence microbial assembly in the neonates gut and the occurrence of neonatal sepsis.

Yet, connecting the association between antibiotic exposure, microbiome, and neonatal sepsis, it is plausible that prophylactic antibiotics are selectively choosing specific communities

that may be dysfunctional, with a higher abundance of antibiotic resistant opportunistic pathogens. This dysfunctional microbiota may further increase the host's susceptibility to sepsis (109). Although still inconsistent, some studies observed benefits of probiotics in preventing neonatal sepsis, which lends further support to the role of gut microbiota in sepsis. While a Cochrane Review of 19 studies did not find significant benefits of probiotics in reducing LOS risk in preterm infants (RR 0.91, 95% CI 0.80-1.03) (112), a follow-up meta-analysis of which analyzed 18 more randomized controlled studies than the Cochrane review concluded that probiotic supplementation reduced the risk of LOS in preterm infants (RR 0.86, 95% CI 0.78-0.94) (113). Further, when *Bifidobacterium* strains were used as probiotics in two clinical trials, no reduction in neonatal sepsis was found (114,115). By contrast, in a recent trial in India, a 40% reduction in newborn sepsis was observed when administering a combination of *Lactobacillus plantarum* and fructooligosaccharide (52). Based on the current findings, although previous studies have shown that prophylactic antibiotics, particularly IAP, are effective in preventing EOS due to GBS in high-income countries, there is an indication that prophylactic antibiotics are changing the epidemiology of neonatal sepsis – increasing the incidence of non-GBS and gram-negative neonatal sepsis cases, LOS, and antibiotic-resistant sepsis. Further studies in different settings with a higher burden of neonatal sepsis are needed to re-evaluate the impact of prophylactic antibiotics on sepsis incidence, how they could be affected by the newborn's gut microbiome, and whether the microbiome mediates the prophylactic antibiotic impact on the risk of sepsis.

1.1 Current challenge and gaps in knowledge

Despite continued advances in neonatal care, sepsis remains a leading cause of morbidity and mortality among infants. Maternal and neonatal antibiotic prophylactics are a widely used

medical intervention for improving neonatal and maternal outcomes. Although the burden of neonatal sepsis and the use of antibiotics in routine practice is higher in LMIC, epidemiologic literature from these countries is sparse. Past literature has often been limited to studies from high-income countries that have lower incidence of neonatal sepsis, along with a stricter regulation on prescribing antibiotics (75,77,116).

Once again, to this day, there is no internationally agreed upon definition of neonatal sepsis (117). In clinical practice, the diagnosis of neonatal sepsis can be established solely based on the clinical manifestation. The inability to successfully isolate a microbial pathogen from blood cultures and/or cerebrospinal fluid does not exclude the diagnosis of neonatal sepsis (7). Therefore, there are many neonates that are diagnose and treated as "probable or possible sepsis" or often referred as culture-negative neonatal sepsis (118). Studies have shown that currently most neonatal sepsis cases treated with a regiment of antibiotics are cases of culture-negative sepsis (18). However, most publications on neonatal sepsis only include microbiologically proven sepsis. Culture-negative sepsis, which uses up to 10 times more antibiotics for culture-proven sepsis, is largely ignored in epidemiological studies (81). Therefore, more studies are needed that could provide a deeper insight into the distribution of culture-negative sepsis along with studies that investigate the clinical application of non-culture based methods for the diagnosis and management of neonatal sepsis.

In terms of preventing neonatal sepsis, mainly EOS, administering prophylactic antibiotics right before and after delivery is one strategy. This strategy consists of (1) administrating IAP to prevent EOS due to GBS (first introduced in the US) and (2) giving prophylactic antibiotics directly to newborns with documented risk factors for infection, as recommended by the WHO (37,44). Given that the common cause of EOS in high-income

countries is GBS infection, the introduction of IAP has substantially reduced the incidence of EOS, which led to a decrease in overall neonatal incidence (119,120). The success of IAP usage has led to a wide adoption of this strategy by multiple countries, including LMIC whose leading cause of EOS is not GBS, with varied levels of consistency (7,38,77). However, not long after introducing IAP, multiple studies showed that IAP had no effect on LOS, indicating that it might increase the risk of neonatal sepsis cases from other pathogens (55,121). These negative impacts are particularly concerning, considering re-evaluating the efficacy or impact of IAP on neonatal sepsis by conducting a randomized clinical trial may no longer be ethical. The same reason may also hold in assessing the efficacy of newborns' prophylactic antibiotic that the WHO recommended. A high-quality observational study may help to address this gap in knowledge.

Another factor regarding antibiotic use is that it is known to have a strong impact on host-associated microbial communities (122). Alterations in gut microbiota due to antibiotic exposure have increased the risk of neonatal sepsis (105–107). Thus, it is plausible that antibiotic exposure is differentially selecting specific communities that may be dysfunctional with a higher abundance of antibiotic resistance determinants and resistant opportunistic pathogens, and this dysfunctional microbiota may further increase the host's susceptibility to sepsis. Interestingly, an animal study has shown that preventing dysbiosis of gut microbiome by administrating *Lactobacillus murinus* protected against late-onset sepsis (108). However, there are limited human studies that explore the role of microbiome alteration in sepsis occurrence and the relationship of this alteration to prophylactic antibiotic use.

1.2 Study aims

This dissertation investigates neonatal sepsis epidemiology in a location with extensive use of perinatal antibiotic prophylaxis and its impact on the neonate gut microbiome. The overarching goal is to better understand how antibiotic prophylaxis practices during the perinatal period influence the changing epidemiology of neonatal sepsis, determining whether gut microbiomes mediate any effect of antibiotic prophylaxis on sepsis risk. An additional implication of this dissertation is providing further evidence on a potential alternative of a non-antibiotic-based strategy to prevent neonatal sepsis that will be beneficial in the era of antibiotic resistance.

Below are the specific aims of this dissertation:

Specific Aim 1. To estimate the prevalence, incidence, and risk factors of neonatal sepsis and perinatal antibiotic prophylaxis exposure in Palembang, Indonesia.

Specific Aim 2. To assess the impact of perinatal antibiotic prophylaxis exposure on neonatal sepsis incidence.

Specific Aim 3. To explore the effect of perinatal antibiotic prophylaxis exposure on the newborns' gut microbiome and evaluate whether the microbiome feature mediates the association between perinatal antibiotic prophylaxis and neonatal sepsis.

1.3 Approach

To achieve the aims of this dissertation, a series of study designs was conducted. First of all, a retrospective study was completed to provide preliminary data on the prevalence of neonatal sepsis hospital admission and prophylactic antibiotic use during delivery at one of the study sites, reviewing all deliveries and neonatal sepsis cases from January 1st, 2016, to

December 31st, 2016. Then, a prospective hospital-based birth cohort study was conducted to assess the impact of prophylactic antibiotics on neonatal sepsis. The study took place at two referral hospitals in Palembang, Indonesia, from September 2019 to March 2021. Finally, because the estimated incidence of the outcome, i.e., neonatal sepsis, was relatively low and performing fecal microbiota analysis to the entire cohort was too costly, a nested case-control design was used to investigate the effect of perinatal antibiotic prophylaxis exposure on the infant gut microbiome and explore the infant microbiome's role in the occurrence of neonatal sepsis.

CHAPTER 2 MANUSCRIPT 1 – CLINICAL AND BACTERIOLOGICAL PROFILE OF CULTURE-NEGATIVE AND CULTURE-PROVEN NEONATAL SEPSIS IN PALEMBANG, INDONESIA

2.0 Abstract

2.0.1 Background & Aim:

Culture-negative and multidrug-resistant neonatal sepsis frequently occur in lower- and middle-income countries (LMIC) and complicate neonatal sepsis management. These conditions contribute to a high neonatal mortality rate and accelerate the misuse of antibiotics. However, the extent of culture-negative and multidrug-resistant neonatal sepsis in LMICs remains poorly characterized. This study aims to describe culture-negative and culture-proven neonatal sepsis epidemiology and the antimicrobial resistance patterns in Palembang, Indonesia.

2.0.2 Methods:

A retrospective review of the medical records of all neonatal admissions between January 2016 and December 2018 was conducted at a tertiary-level referral hospital in Indonesia. The maternal and neonatal characteristics and microbiological results of the identified sepsis cases were obtained and analyzed.

2.0.3 Results:

Three hundred and fifty-six neonatal sepsis cases were admitted from 2016 to 2018, accounting for 14.1% of neonatal hospital admissions. The proportion of early-onset and late-onset sepsis of all admitted cases were comparable (49.7% vs. 50.3%), with an 18.1% case fatality rate. The proportion of culture-negative sepsis was 44%. The fatality rates between culture-proven and culture-negative sepsis cases did not differ statistically (aOR 1.80 , 95% CI 0.93-3.47). Coagulase-negative staphylococci (30.9%), *Klebsiella pneumoniae* (18.1%), and *Acinetobacter*

spp. (10.7%) were the most frequently isolated pathogens. Overall, 62.6% of all isolated organisms were multidrug-resistant bacteria, with a high prevalence of extended-spectrum cephalosporin-resistant and carbapenem-resistant strains.

2.0.4 Conclusion:

Culture-negative sepsis accounts for a significant proportion of neonatal sepsis cases. Early- and late-onset and culture-negative and culture-proven neonatal sepsis contribute to a comparable proportion of neonatal sepsis morbidity and mortality. There is an alarmingly high prevalence of resistance to extended-spectrum cephalosporin and carbapenem in neonatal sepsis cases.

2.1 Background

Neonatal sepsis is a significant cause of morbidity and mortality among newborns. Worldwide, the estimated neonatal sepsis incidence is 22 per 1,000 live births (2). In 2018, the World Health Organization (WHO) estimated that neonatal sepsis was responsible for over 1,000 deaths per day globally, accounting for 15% of all neonatal deaths (5). The highest burden of neonatal sepsis occurs in LMICs, where the incidence is twofold to fourfold higher than that in high income countries (HICs). The risk of death in newborn resulting from neonatal sepsis is 34 times higher in LMICs than in high income countries (4). However, these numbers may not reflect the actual burden of neonatal sepsis because of data scarcity from LMICs. High-quality surveillance data are available for only one-third of WHO member countries, and most of these are HICs (8).

Culture-negative sepsis and multidrug-resistant (MDR) neonatal sepsis are conditions that often challenge the management of sepsis in newborns. Blood culture remains the most important microbiological tool in sepsis diagnosis and management; however, the inability to isolate a microbial pathogen does not exclude a sepsis diagnosis. Many neonates, especially those in LMICs, have been diagnosed with sepsis solely based on clinical suspicion, referred to as culture-negative sepsis (123). A study showed that blood culture positivity among neonatal sepsis cases could be as low as 0.5% (124). Recent reports suggest that antibiotic use is up to 16 times greater for culture-negative sepsis therapy than for culture-proven sepsis therapy (18). In addition, culture-negative sepsis cases treated with antibiotics are largely ignored in epidemiological studies. Therefore, more studies are needed to obtain a better understanding of culture-negative sepsis, as it also contributes to significant morbidity and mortality.

The high consumption of antibiotics in culture-negative sepsis cases potentially relates to an increased risk of colonization by antibiotic-resistant bacteria in neonates. Over the past decade, neonatal sepsis episodes caused by MDR organisms have become associated with significant increases in mortality rates, particularly in LMICs (3,16). However, the extent of MDR neonatal sepsis in LMICs remains poorly characterized. Consequently, there is a critical need for more data on the population-level epidemiology of MDR neonatal sepsis in these countries to optimize neonatal sepsis management and prevent antibiotic resistance.

This study aimed to review neonatal sepsis epidemiology, including cases of culture-negative and culture-proven neonatal sepsis, and antimicrobial resistance patterns at a tertiary level referral hospital in Indonesia.

2.2 Materials and methods

2.2.1 Study design and variables of interest

This study used data from the medical records of Mohammad Hoesin Hospital from January 2016 to December 2018. The hospital is located in Palembang, Indonesia. It is a government-run teaching hospital that acts as a tertiary-level referral hospital serving patients from five neighboring provinces. The hospital's Neonatal Intensive Care Unit capacity and neonatal ward capacity are 15 and 40 beds, respectively. We identified neonatal sepsis cases based on the International Classification of Disease, 10th Revision, Clinical Modification coded P36 (i.e., bacterial sepsis of newborns) and searched the paper-based medical records of each patient. Sepsis was classified as early-onset sepsis (EOS) when symptoms occurred within the first 72 hours of life and as late-onset sepsis (LOS) when the onset occurred after 72 hours of life (1).

The medical records of the cases were screened for the onset of sepsis, gender, birth weight, gestational age, mode of delivery, place of delivery (in-born or out-born), birth attendant, and mortality. The risk factors for neonatal sepsis included a history of premature rupture of the membrane > 18 hours, maternal antepartum fever, antepartum hemorrhage, and foul-smelling amniotic fluid.

The bacteriological profile was obtained by reviewing the blood culture results database. At the study site, blood cultures were performed following the protocol of the BD BACTEC^{TM} automated blood culture systems. All cultures were incubated aerobically at 37°C for 18-24 hours, and negative cultures were incubated for up to five days for bacteria and nine days for fungi before being reported as negative. Identification and antimicrobial susceptibility testing of all isolates was performed using an automated method from VITEX-2 Compact (Biomérieux) in accordance with the Clinical and Laboratory Standard Institute guideline (125).

The sensitivity of antimicrobial testing results was categorized as susceptible, intermediate, resistant, or not tested. Pathogens were recorded based on their resistance to various antibiotics classes, which included methicillin, vancomycin, extended-spectrum cephalosporin (any one of ceftriaxone, ceftazidime, or cefotaxime), extended-spectrum penicillin (piperacillin), carbapenem (meropenem or imipenem), fluroquinolone (ciprofloxacin or levofloxacin), and aminoglycoside (gentamycin or amikacin). Antimicrobial multidrug resistance was defined as an isolated pathogen classified as intermediate or showing resistance to at least one agent in three or more antimicrobial classes(126).

2.2.2 Statistical analysis

Descriptive statistics and the frequency distribution of all variables of interest were reported as a proportion for categorical variables and as a mean or median for continuous variables. The chi-square or Fisher's exact test was used to analyze categorical variables. Logistic regression was used to determine the significant risk factors for the onset of sepsis, mortality, and antimicrobial resistance. Initially, each factor was tested individually in an univariable regression model. The variables with a p-value < 0.25 were then included in the multivariable regression logistic model to estimate the adjusted odds ratio (aOR). The data processing and analyses were conducted using SAS®, version 9.4 (SAS Institute Inc, Cary, NC).b

2.2.3 Ethics approval

This study was approved by the Michigan State University's Biomedical and Health Institutional Review Board and by the Department of Education and Research Mohammad Hoesin Hospital.

2.3 Results

From 2016 to 2018, the perinatology ward admitted 2517 patients. Among all admissions, 356 (14.1%) newborns were diagnosed with neonatal sepsis from January 2016 until December 2018. We were able to retrieve and review the medical records of 306 (86%) patients: 95 of 118 (80.5%) from 2016, 128 of 141 (90.1%) from 2017, and 83 of 97 (85.6%) from 2018. Across the calendar years, the referral cases, gender, gestational age group, mode of delivery, birth attendant, and cases outcomes showed a similar proportion. Although not statistically significant, in 2018, the proportion of sepsis cases with low birth weight was smaller than that in previous years. The basic characteristics of the neonatal cases are summarized in Table 1.

Table 1. Basic characteristics of neonatal sepsis cases by year (N = 306)

Variable	2016 (n = 95)	2017 (n = 128)	2018 (n = 83)	*p-value*
Place of delivery (%)				
In-born	42 (44.2)	58 (45.3)	36 (43.4)	0.96[a]
Out-born	53 (55.8)	70 (54.7)	47 (56.6)	
Gender (%)				
Male	53 (55.8)	77 (60.2)	59 (71.1)	0.10[a]
Female	42 (44.2)	51 (39.9)	24 (28.9)	
Birthweight (grams)				
Mean (SD)	2,684.2	2,784.1	2,809.3	0.35[b]
Range	(664.5)	(643.2)	(542.2)	
	1,100–4,100	1,200–4,500	1,300–4,000	
Birthweight classification (%)				
1000–1499 grams				0.05[a]
1500–2499 grams	2 (2.1)	4 (4.7)	2 (2.4)	
≥ 2500 grams	30 (31.6)	27 (21.1)	11 (13.3)	
	63 (66.3)	102 (79.7)	70 (84.3)	
Gestational age (weeks)				
Median (IQR)				0.56[c]
Range	38 (36-39)	38 (37-38)	38 (36-38)	
	26–42	28–41	28–42	
Gestational age group (%)				
<28 weeks				
32–<34 weeks	2 (2.1)	0 (0)	0 (0)	0.14[a]
34–<37 weeks	2 (2.1)	6 (4.7)	4 (4.8)	
≥ 37 weeks	25 (26.3)	20 (15.6)	20 (24.1)	
	66 (69.5)	102 (79.7)	59 (71.1)	
Mode of delivery (%)				
Spontaneous	56 (59.0)	71 (55.5)	46 (55.4)	0.43[a]
Vacuum extraction	3 (3.2)	1 (0.8)	4 (4.8)	
Forceps extraction	0 (0)	2 (1.6)	0 (0)	
C-Section	36 (37.9)	54 (42.2)	33 (39.8)	
Birth assistant (%)				
Midwife	37 (39.0)	47 (36.7)	29 (35.0)	0.57[a]
General practitioner	0 (0)	2 (1.6)	1 (1.2)	
Ob/Gyn resident	28 (29.5)	47 (36.7)	34(41.0)	
Ob/Gyn specialist	30 (31.6)	32 (25.0)	19 (22.9)	

Table 1. (cont'd)

Variable	2016 (n = 95)	2017 (n = 128)	2018 (n = 83)	*p-value*
Outcome (%)				
Lived	83 (79.0)	102 (79.7)	70 (84.3)	0.69[a]
Died	19 (20.0)	23 (18.0)	13 (15.7)	
Discharged against medical advice	1 (1.1)	3 (2.3)	0 (0)	

Ob/Gyn:Obstetrics and Gynecology; [a]Chi-square/Fisher's exact [b]Anova [c]Wilcoxon-Mann-Whitney

Of all the cases, 152 (49.7%) were classified as EOS and 154 (50.3%) as LOS. Cases born outside the hospital (out-born) accounted for 48% of all EOS and 63% of all LOS. Out-born cases were more likely to have LOS than cases born in the hospital (in-born) (odds ratio [OR] 1.84, 95% confidence interval [CI] 1.17–2.91). Most of the newborns delivered with EOS were assisted by Obstetrics and Gynecology (Ob/Gyn) residents (37.5%), whereas most LOS cases were assisted by midwives (40.9%). The mean (standard deviation [SD]) birth weight was 2,833.1 ± 588.2 grams for neonates with EOS, whereas for neonates with LOS, the mean (SD) of the birth weight was 2,687.8 ± 652.4 grams. Aside from the place of delivery, we did not find any other association between other neonatal and maternal risk factors for EOS or LOS (Table 2).

Table 2. Neonatal and maternal risk factors for sepsis onset.

Variable (%)	EOS n = 152	LOS n = 154	*p-value**
Out-born	73 (48.0)	97 (63.0)	0.009
Male	93 (61.2)	96 (62.3)	0.84
Birth assistant			0.47
Midwife	50 (32.9)	63 (40.9)	
General practitioner	1 (0.7)	2 (1.3)	
Ob/Gyn resident	57 (37.5)	52 (33.8)	
Ob/Gyn specialist	44 (29.0)	37 (24.0)	
Prematurity	40 (26.3)	39 (25.3)	0.84
Birthweight <1500 grams	33 (21.7)	43 (27.9)	0.21
C-Section	66 (43.4)	57 (37.0)	0.25

Table 2. (cont'd)

Variable (%)	EOS n = 152	LOS n = 154	*p-value**
Premature rupture of membrane (PROM)	53(34.9)	41 (26.6)	0.12
Maternal fever†	4 (2.6)	2 (1.3)	0.45**
Antepartum hemorrhage	5 (3.3)	4 (2.6)	0.72
Foul-smelling amniotic fluid	9 (5.9)	10 (6.5)	0.84

*chi-square **Fisher-exact †1 missing data

However, a stratified analysis based on the delivery site revealed a significant association between sepsis onset and the person who assisted the delivery in the in-born neonatal sepsis cases. LOS was more likely to occur in births delivered by Ob/Gyn residents than by specialist (OR 5.4, 95% CI 1.76–16.78).

2.3.1 Case fatality rates of neonatal sepsis

During the study period, 55 deaths resulting from neonatal sepsis were recorded. Of these, 31 (53.4%) were EOS, and 24 (43.6%) were LOS. Among all death cases, 20 cases (36.4%) were culture-negative sepsis, 27 (49.1%) were culture-proven sepsis, and 8 had missing culture results (14.5%). Among the 27 culture-proven sepsis cases, gram-negative bacteria were the leading cause of death in both EOS and LOS. In culture-proven EOS, 10 deaths were due to gram-negative bacteria, 3 were due to gram-positive bacteria, and 1 was due to fungal infection. In culture-proven LOS, gram-negative bacteria caused 9 deaths, and gram-positive bacteria caused 4 deaths.

Klebsiella pneumoniae was the most common pathogen isolated from all culture-proven EOS-related cases that died, whereas *Acinetobacter* spp. and *Enterobacter* spp. were the two most common pathogens isolated from all culture-proven LOS-related cases that died. Among all

pathogens isolated from all cases of neonatal sepsis-related death, 20 (36.4%) were MDR bacteria; 17 of these were gram-negative bacteria, and 3 were gram-positive bacteria.

In the adjusted model, our study found a significant association between out-born cases and fatality (aOR 2.07, 95% CI 1.09–3.92). Although not statistically significant, we also found that newborns whose birthweight was less than 1,500 grams were twice as likely to die as those whose birthweight > 1,500 grams. (aOR 2.01, 95% CI 0.97–4.15) (Table 3).

Table 3. Predictors of case fatality rates for overall neonatal sepsis cases (N=302)

Variables (%)	Death (+) (n=55)	Death (-) (n=247)	*p-value**	OR (95% CI)	aOR (95% CI)
Late-onset sepsis	24 (43.6)	130 (52.6)	0.23	0.70 (0.39–1.26)	0.56 (0.30–1.05)
Birth assistant	24 (43.6)	87 (35.2)	0.41	--	--
Midwife	0 (0)	3 (1.2)	--	--	--
General Practitioner	15 (27.3)	87 (35.2)	--	--	--
Ob/Gyn resident	16 (29.1)	64 (25.9)	--	--	--
Ob/Gyn specialist (ref)	20 (36.4)	101 (40.9)	--	--	--
C-Section	17 (30.9)	76 (30.8)	0.54	--	--
Premature rupture of membrane	1 (1.8)	5 (2.3)	0.98	--	--
Maternal fever†	37 (67.3)	130 (52.6)	0.99	--	--
Out-born	26 (47.3)	90 (36.4)	0.05	1.85 (1.00–3.42)	2.07 (1.09–3.92)
Female	21 (38.2)	54 (21.9)	0.14	1.54 (0.86–2.79)	1.46 (0.78–2.67)
Very low birth weight (<1,500 gr)	19 (34.6)	60 (24.3)	0.01	2.24 (1.20–4.17)	2.01 (0.97–4.15)
Prematurity	5 (9.1)	14 (5.7)	0.12	1.67 (0.89–3.12)	1.21 (0.59–2.50)

Table 3. (cont'd)

Variables (%)	Death (+) (n=55)	Death (-) (n=247)	*p-value**	OR (95% CI)	aOR (95% CI)
Foul-smelling amniotic fluid	0 (0)	9 (3.6)	0.34	--	--
Antepartum hemorrhage	27 (49.1)	120 (48.6)	0.37	--	--
Culture-proven			0.11	1.07 (0.32–1.13)	1.80 (0.93-3.47)

*Chi-square, Fisher exact †1 missing data

An unadjusted logistic regression model that only includes culture-proven sepsis (n = 147) revealed a probable association between gram-negative pathogens and death than between gram-positive pathogens and death (OR 2.71, 95%CI 1.06–6.94). This significant association persisted after adjusting for birth weight category and prematurity (aOR 2.70, 95%CI 1.05–6.97). An univariable logistic model did not reveal any specific pathogen that significantly increased the risk of fatality (Table 4).

Table 4. Predictors of case fatality rate for culture-proven sepsis only (n=147)

Variables (%)	Death (+) n = 27	Death (-) N=119	*p-value**	OR (95% CI)	aOR (95% CI)
Late-onset sepsis	11 (40.7)	61 (50.8)	0.36	--	--
Birth assistant			0.96	--	--
Midwife	9 (33.3)	40 (33.3)	--	--	--
General Practitioner	0 (0)	2 (1.7)	--	--	--
Ob/Gyn resident	10 (37.0)	48 (40.0)	--	--	--
Ob/Gyn specialist (ref)	8 (29.6)	30 (25.0)	--	--	--
C-Section	9 (33.3)	51 (42.5)	0.41	--	--
Premature rupture of membrane	8 (29.6)	35 (29.2)	0.91	--	--

Table 4. (cont'd)

Variables (%)	Death (+) n = 27	Death (-) N=119	*p-value* *	OR (95% CI)	aOR (95% CI)
Maternal fever†	1 (3.7)	4 (3.4)	0.93	--	--
Out-born	16 (59.3)	50 (50)	0.37	--	--
Female	11 (40.7)	79 (65.8)	0.54	--	--
Very low birth weight (<1,500 gr)	11 (40.7)	29 (24.2)	0.07	2.23 (0.93-5.37)	1.84 (0.64-5.25)
Prematurity	10 (37.0)	30 (25)	0.18	1.83 (0.75-4.42)	1.36 (0.47-3.95)
Foul-smelling amniotic fluid	2 (7.4)	7 (5.8)	0.77	--	--
Antepartum hemorrhage	0 (0)	7 (5.8)	0.97	--	--
Isolates	--	--	0.07	--	--
Gram-positive (ref)	7 (25.9)	60 (50.0)	--	--	--
Gram-negative	19 (70.4)	59 (49.2)	--	2.71 (1.06-6.94)	2.70 (1.05-6.97)
Fungal	1 (3.7)	1 (0.8)	--	8.43 (0.47-150.21)	7.05 (0.37-135.35)

*Chi-square, Fisher exact †1 missing data

Our study site does not require two simultaneous blood cultures to differentiate true coagulase-negative staphylococcal (CoNS) infection from contamination. Therefore, we conducted an additional analysis excluding sepsis cases resulting from CoNS infection, assuming that such cases were not true infection. After excluding CoNS sepsis cases, although the association between gram-negative pathogen and mortality lost its statistical significance, the aOR was similar to the aOR before CoNS exclusion (aOR 2.80, 95% CI 0.59-13.36).

2.3.2 Microbial profile

Overall, 266 (86.9%) cases had blood culture results. Of the 266 blood culture results, 149 (56.0%) were culture proven and 117 (44.0%) were culture negative. Gender, the onset of sepsis, prematurity, and mode of delivery were not associated with the culture results (Table 5). However, culture-proven sepsis was more likely in neonates with birth weights less than 1,500

grams than in those with birthweights equal to or greater than 1,500 grams (OR 1.8, 95%CO 1.01–3.36).

Table 5. Characteristics of culture-proven versus culture-negative sepsis

Variable (%)	Culture proven n = 149	Culture negative n = 117	*p-value**
Out-born delivery	77 (51.7)	72 (61.5)	0.11
Male	96 (64.4)	70 (59.9)	0.44
Late-onset sepsis	72 (48.3)	59(50.4)	0.73
Birthweight <1,500 grams	41 (27.5)	20 (17.1)	**0.04**
Prematurity	40 (26.9)	25 (21.4)	0.30
C-Section	61 (40.9)	49 (41.9)	0.88
PROM/PPROM	44 (29.5)	36 (30.8)	0.83
Foul-smelling amniotic fluid	9 (6.0)	6 (5.1)	0.75
Maternal fever †	5 (3.4)	1 (0.9)	0.23
Antepartum hemorrhage	7 (4.7)	2 (1.7)	0.31

*Chi-square, Fischer exact test †missing 1

Among all culture-proven sepsis cases (n = 149), 78 (52.4%) were caused by gram-negative bacteria, 69 (46.3%) by gram-positive bacteria, and 2 (1.4%) by fungal infection (both *Candida albicans)*. Overall, the most frequently isolated pathogens were CoNS (46, 30.9%), *Klebsiella pneumoniae* (27, 18.1%), and *Acinetobacter* spp. (16, 10.7%). The distribution of the isolated pathogens is shown in Table 6.

Table 6. Isolated pathogens in all culture-proven neonatal sepsis cases

Isolated pathogen	Frequency (%)
Coagulase-negative staphylococci (CoNS)	46 (30.9)
Klebsiella pneumoniae	27 (18.1)
Acinetobacter spp.	16 (10.7)
Pseudomonas aeruginosa	11 (7.4)
Enterobacter spp.	10 (6.7)
Non-beta hemolytic *streptococcus*	10 (6.7)
Staphylococcus aureus	6 (4.0)
Enterococcus spp.	5 (3.4)
Escherichia coli	5 (3.4)
Pantoea spp.	5 (3.4)
Serratia spp.	4 (2.7)
Candida albicans	2 (1.3)

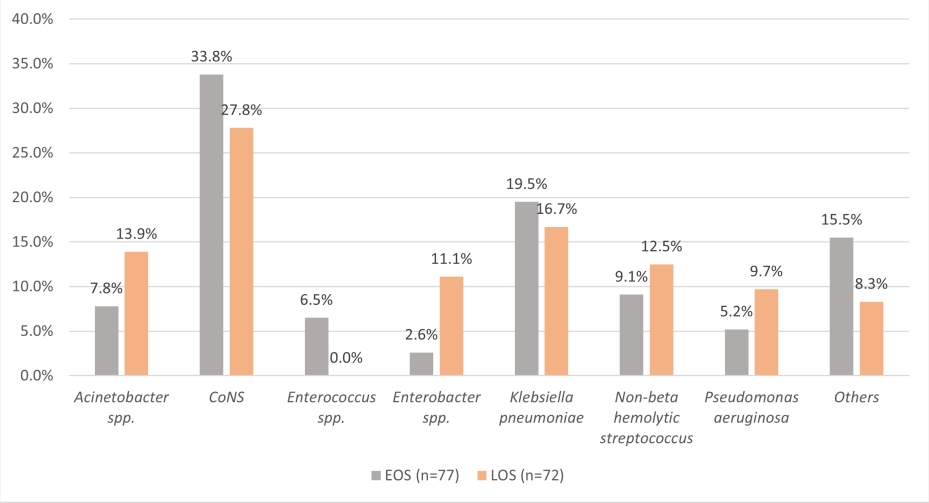
Table 6. (cont'd)

Isolated pathogen	Frequency (%)
Bacillus sp.	1 (0.7)
Kocuria sp.	1 (0.7)

Among the gram-negative bacteria, the predominant bacteria were *Klebsiella pneumoniae* (27, 34.6%), *Acinetobacter baumanii* (11, 14.1%), and *Pseudomonas aeruginosa* (11, 14.1%). The predominant gram-positive bacteria causing neonatal sepsis were CoNS (46, 30.9%) and non-beta hemolytic streptococcus (10, 6.7%).

Among the neonates with culture-proven EOS, CoNS (26, 33.7%) was the most frequently detected pathogen, followed by *Klebsiella pneumoniae* (15, 19.5%) and non-beta hemolytic streptococcus (7, 9.1%). In the LOS cases, the most common causative organisms were also CoNS (20, 27.8%), followed by *Klebsiella pneumoniae* (12, 16.7%), and *Acinetobacter* spp. (10, 13.9%) (Figure 1).

Figure 1. Causative pathogen distribution in EOS and LOS cases

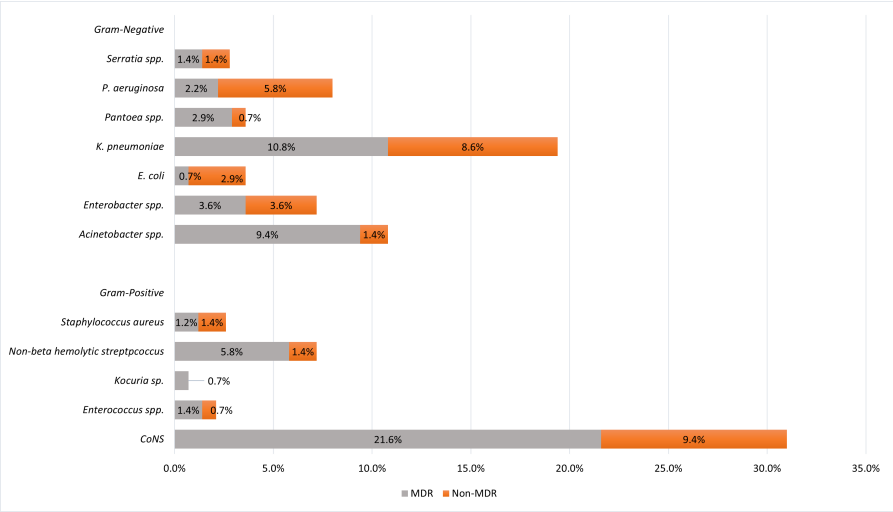


Although further analysis did not reveal an association between gram-negative neonatal sepsis and maternal and neonatal risk factors, the presence of gram-negative bacteria was proportionately more frequent in LOS (55.1% vs. 40.9%, OR 1.8, 95% CI 0.93–3.41). No significant association was noted between neonatal and maternal sepsis risk factors and sepsis caused by specific gram-negative pathogen.

2.3.3 Antimicrobial susceptibility

Among the 149 culture-proven sepsis cases, 10 specimens from these cases did not have antimicrobial susceptibility test (AST) results; two had fungal infections, seven were infected with gram-positive bacteria, and one was infected with gram-negative bacteria. Of the 139 cases with AST results, 87 (62.6%) were MDR bacterial infections. Among the 62 gram-positive bacteria, 44 (71.0%) were MDR, and among the 77 gram-negative bacteria, 43 (55.9%) were MDR. CoNS were the most prevalent MDR bacteria among all gram-positive bacteria, whereas *Klebsiella pneumoniae* were the most prevalent MDR bacteria among the gram-negative pathogens (Figure 2).

Figure 2. Distribution of isolated pathogens in culture-proven neonatal sepsis as determined by antimicrobial susceptibility test (AST) results



This study revealed that 58.3% of the tested *Acinetobacter* spp., 63.6% of the *Pseudomonas aeruginosa*, and 37.2% of the Enterobacteriaceae isolates were carbapenem-resistant strains. In addition, 64.6% of the Enterobacteriaceae isolates were cephalosporin-resistant strains. At the study hospital, cephalosporin is the empiric antibiotic therapy for neonatal sepsis; this study revealed that 78.7% of the tested isolates were resistant to cephalosporins. Table 20 in Appendix A shows the antibiotic resistance patterns of the major isolated organisms.

Univariable analysis did not reveal significant associations between MDR pathogens and various maternal and neonatal sepsis risk factors. This study did not show an association between MDR and mortality (OR 1.08, 95%CI 0.44–2.65). Similar results were found in the analysis that excluded cases of CoNS neonatal sepsis.

2.4 Discussion

Throughout the study, we found that the characteristics of the admitted neonatal sepsis cases were similar. However, over the years, there was a decrease in the proportion of neonatal sepsis cases with low birth weight, from 31.6% in 2016 to 13.3% in 2018. The possible reason for this is that over the years, there has been an improvement in healthcare facilities in Indonesia; an increasing number of smaller hospitals now have the capability to manage babies with low birth weight. Therefore, fewer cases of low birth weight needed to be referred to our study site. The incidence of neonatal admissions for sepsis during the study period was 14.1%. Currently, Indonesia does not have national data on neonatal sepsis incidence. However, several studies have reported that the neonatal admission incidence rates in some tertiary hospitals in Indonesia vary between 5% and 25% (11,12). Studies from other LMICs in South Asia have shown admission incidence rates ranging from 20.5 to 45.9% (9,10). The variation in incidence rates

between referral hospitals in Indonesia may be due to the differences in the healthcare referral systems implemented during each study period and hospital capacity. In 2014, Indonesia's government developed a new national health insurance scheme that changed the referral policy and restricted those cases referred to tertiary-level hospitals. The admission incidence difference with other LMICs may also be explained by differences in patient socio-demographic status, accessibility to the healthcare facilities, and the definition of neonatal sepsis used in the different studies.

The proportions of EOS and LOS in this study were similar (49.7% vs. 50.3%). Previous studies have shown a higher EOS burden in LMICs than in high income countries (127,128). Advances in obstetric care, including prophylactic antibiotics, have significantly reduced the incidence of EOS in HICs; however, in LMICs, the incidence of EOS remains high. The occurrence of EOS is frequently associated with colonization of the newborn by vertical transmission from the maternal genital tract, unhygienic birth practices during labor, and ultra-early horizontal transmission from the delivery room or neonatal care units; these problems are more common in LMICs (7,129).

By contrast, LOS reflects community or nosocomial infection more strongly and is highly associated with infant prematurity. Improvements in premature infant survival as a result of advances in neonatal intensive care in HICs have led to increases in LOS incidence (7,129,130). The comparable proportion of LOS and EOS observed in this study may indicate that basic obstetric practices aimed at preventing vertical infection from mother to newborns are still inadequate, despite improvements in overall neonatal care. In this study, LOS was more likely to occur in out-born cases, reflecting community-acquired infection. Poor hygiene, poor cord care,

unhygienic bottle feeding, and the use of prelacteal feeds are common practices in LMICs, especially among people with a low socioeconomic status (131).

We also found that in-born cases assisted by Ob/Gyn residents were more likely to develop LOS than those cases assisted by Ob/Gyn specialists. This may be related to the two-tier healthcare system in hospitals in Indonesia, which frequently leads to discrimination in the provision and quality of care for patients(132). Residents serve more patients with a lower socioeconomic status who occupy crowded wards. A low socioeconomic status predisposes newborns to neonatal infection, and overcrowded wards compromise the healthcare quality given to both the mother and the newborn, thereby increasing newborns' susceptibility to late infection(132,133).

The fatality rate of neonatal sepsis in the present study was 18%, whereas other studies from Indonesia have reported higher fatality rates, from 20% to 67% (11,12). In other LMICs, the fatality rates range from 16% to 46% (9,134). Our study had a smaller proportion of newborns who have a low-birth-weight and are premature, which could explain the lower fatality rate, as neonate mortality is frequently inversely proportional to birth weight and gestational age(134). Our study also revealed that a low birth weight is a higher risk of fatality. The out-born cases were also associated with a higher risk of fatality, most likely because these were the more severe case and were referred from a lower healthcare facility.

Consistent with previous studies, we found increased risk of death in neonates with gram-negative sepsis compared with gram-positive sepsis (71,127). The most common pathogens isolated from cases with both EOS- and LOS-related deaths were the gram-negative bacteria. Sepsis resulting from gram-negative infection carried a higher risk of severe sepsis and death in all age ranges. Gram-negative bacteria are known to be more virulent because of their capability

to evade hosts' immune responses, produce endotoxins that increase the severity of inflammation, and adapt to changing host and environmental conditions through multiple genetic mechanisms (135).

The proportion of culture-negative sepsis in this study was 44%, which is comparable with the proportions reported by studies in other LMICs (10,136). In HICs, the ratio of culture-proven versus culture-negative sepsis ranges from 1:6 to 1:12 (18). Some concerns have been raised regarding the over-diagnosis of sepsis. This leads to a high consumption of unnecessary antibiotics; however, some of these culture-negative sepsis cases may not be truly negative. Our study supported other previous findings regarding a lack of significant fatality differences between culture-negative and culture-positive cases (22,23). This result reflects the fact that newborns with culture-negative sepsis were also severely ill.

The reasons for the large proportion of culture-negative sepsis remain unclear. The low blood volume obtained from newborns is likely a strong reason. In addition, anaerobic blood culture is not routinely performed. Cultures obtained after antibiotic initiation and maternal antibiotic treatment before and during delivery are also possible explanations, especially considering some of the cases in this study were transferred cases that has been treated. Conventional microbiological methods may frequently fail to identify pathogens because of technical issues or traits intrinsic to microorganism that limit sepsis detection. Although new diagnostic approaches have been developed to replace conventional methods, implementation in LMICs will be challenging because of a lack of resources (42,137).

Our study found the same pattern of the predominant pathogen for both EOS and LOS, which was CoNS, followed by *K. pneumoniae,* and *Acinetobacter* spp. Studies from Turkey and Brazil have also reported that CoNS is the leading cause of both EOS and LOS. The 14-years

study from Turkey reported that 64.4% of neonatal sepsis cases were caused by CoNS, whereas in Brazil, the proportion of CoNS was 36.5% (43,138). Other smaller studies from Peru, Egypt, and India also reported similar findings (10,71,139). On the other hand, a large cohort study in India found the same three predominant pathogen - CoNS, *Acinetobacter* spp., and *Klebsiella* spp. - as the most isolated pathogens in their EOS and LOS cases but in a different order. Instead of CoNS, *Acinetobacter* spp. were ranked first as the predominant pathogen (16). Although the determination of CoNS as true pathogen or contamination in neonatal sepsis is still debatable, the consistent findings from multiple studies of CoNS as the most reported causative pathogen in neonatal sepsis cases should be a strong indication that CoNS have an important role in neonatal sepsis. A careful evaluation is needed before determining that CoNS isolation from a blood culture is a contamination, especially when the case is supported with sepsis clinical signs and symptoms and abnormal laboratory findings and when the specimen collection was performed with an appropriate antisepsis protocol. Previous studies have suggested that CoNS bacteremia is associated with a low birth weight and prematurity;(10,14); however, we found that CoNS infection was also frequently found in full-term neonates with a normal birth weight. This may suggest that even in full-term neonates, the immaturity of the immune system and the ineffectiveness of neonate skin and mucous membranes to act as physical barriers may still be associated with these neonate's vulnerability to low-virulence pathogens. However, the search for approaches to increase the ability to distinguish between true bacteremia and contamination should continue.

Consistent with previous studies from LMICs reporting Group B *Streptococcus* (GBS) infection were rarely found, our studies also did not detect any GBS neonatal sepsis cases (7,16). In HICs, the most common cause of neonatal sepsis for EOS and LOS is a gram-positive

organism or GBS for EOS and CoNS for LOS (15,127). Whether differences in the etiological agents of neonatal sepsis between developed and LMICs reflect an actual difference in the causative agents across the globe or can be attributed to differences in the case definition of sepsis. There may also be differences in the capability to perform blood culture, published reports that come from short periods of surveillance, and the numbers of neonatal sepsis cases diagnosed without blood culture or that never reached healthcare facilities and not reported remains uncertain. Therefore, further epidemiological studies that describe the various pathogens causing neonatal sepsis and their changing antibiotic susceptibility profile remains important.

Similar to previous studies, our work revealed a large number of MDR pathogens with resistance to methicillin, cephalosporin, and carbapenem (9,16). This confirm that antibiotic resistance is a major global health problem and needs urgent attention, particularly in LMICs. In HICs, MDR neonatal sepsis accounts for less than 20% of cases, whereas this proportion can reach 40%–80% in HICs (16,140–142). We observed a high prevalence of resistance to extended-spectrum cephalosporin and carbapenem, which significantly complicates sepsis management, especially considering that the first- and second-line empirical antibiotics used at our study site are third-generation cephalosporin and carbapenem, respectively. These first and second-line antibiotics are used until blood culture, and AST results are available, or they are continued as a complete course of treatment of culture-negative sepsis cases. Antimicrobial resistance to cephalosporin mainly due to extended spectrum beta-lactamase (ESBL) production, which readily found and transferred via plasmid(143). While carbapenem resistance is often caused by the production of carbapenemases (carbapenem-hydrolizing enzyme) and beta-lactamase activity coupled with structural mutation (144). ESBL-producing and carbapenem-

resistant Enterobacteriaceae are a serious antibiotic resistant health threat as classified by the CDC in the US and elsewhere (145).

Previous exposure to third-generation cephalosporin and carbapenem has been identified as an independent risk factor for acquiring resistance to gram-negative bacteria, including the use of prophylactic antibiotics during delivery which is a common practice in LMIC (16,142). We addressed the practice of prophylactic antibiotic delivery from the same database in another study report. Other factors responsible for the surge in MDR in LMICs include the non-existence of antibiotic prescription guidelines, the over-the-counter sale of antibiotics, poor sanitary conditions, a lack of basic facilities and practices, and the lack of surveillance regarding organisms that cause infections (16,146,147).

Our study provides an update on neonatal sepsis burden and the bacteriological profile and antibiotic resistance patterns in Indonesia. This study also emphasizes the prevalence of culture-negative sepsis cases, which are generally underreported. This research has some limitations. The data were obtained by reviewing medical records of neonatal sepsis cases. The documentation may have been incomplete, and this would have limited further analysis aimed at finding associations with other potential risk factors for case fatality or late- and early-onset sepsis within the neonatal sepsis population. Another important limitation of this study is given that our study population were limited in sepsis cases only, we do not have the ability to determine the risk factors for neonatal sepsis occurrence. Our work is a single-center study conducted at a tertiary-level referral hospital, so selection bias may have occurred against less severe neonatal sepsis cases. Our findings may not be representative of other neonatal units in the country. Another limitation is that the protocol for blood culture specimen collection at our study site does not require two simultaneous blood cultures, so we may have overestimated the

incidence of CoNS infection. However, at the study site, sepsis diagnosis is made by fulfilling clinical and laboratory criteria, and it was not solely based on blood culture results.

2.5 Conclusion

Our findings showed that EOS and LOS and culture-negative and culture-proven neonatal sepsis cases shared a comparable proportion of neonatal sepsis morbidity and mortality. Our findings emphasized the surge in multidrug antibiotic resistance occurring in LMICs and the need for significant actions that will improve efforts to prevent infection in neonates while controlling the use of antibiotics. Neonatal sepsis remains a global public health issue, so we recommend more comprehensive, extensive, and large-scale studies to better understand the magnitude of the disease. We also advocate the development of alternative, affordable pathogen identification approaches that can serve as add-ons to traditional microbiological techniques to improve the management of neonatal sepsis and the prevention of antimicrobial resistance.

CHAPTER 3 MANUSCRIPT 2 – THE PREVALENCE AND FACTORS ASSOCIATED WITH PROPHYLACTIC ANTIBIOTIC USE DURING DELIVERY: A HOSPITAL BASED-RETROSPECTIVE STUDY IN PALEMBANG, INDONESIA

3.0 Abstract

3.0.1 Background & Aim:

Prophylactic antibiotic usage during delivery is a common practice worldwide, especially in low- to middle-income countries. Guidelines have been published to reduce antibiotic overuse; however, data describing the use of prophylactic antibiotics and clinician adherence to guidelines in low- to middle-income countries remain limited. This study aimed to describe the prevalence of prophylactic antibiotic use, factors associated with its use, and clinician adherence to guidelines.

3.0.2. Methods:

A retrospective review was conducted for all deliveries from January 1, 2016, to December 31, 2018 at a tertiary level hospital in Indonesia.

3.0.3 Results:

The prevalence of prophylactic antibiotic use during delivery was 47.1%. Maternal education level, Ob/Gyn specialist-led delivery, a history of multiple spontaneous or induced abortions, C-section, premature membrane rupture, and antepartum hemorrhage were independently associated with prophylactic antibiotic use. Clinician adherence to the guidelines was 68.9%. Adherence to guidelines was the lowest in conditions where the patient had only one indication for prophylactic antibiotics (aOR 0.36, 95% CI 0.24–0.54).

3.0.4 Conclusions:

The findings showed that the prevalence of prophylactic antibiotic use during delivery was moderate to high. Adherence to local guidelines was moderate. Updating the local prescribing guidelines may improve clinician adherence.

3.1 Introduction

Bacterial infection during labor and delivery is one of the leading causes of maternal and neonatal mortality worldwide, accounting for about one-tenth of the global burden of maternal and neonatal deaths (148,149). While the number of deaths from these infections has decreased considerably in high-income settings, the situation has not improved in many resource-limited settings (2,150). In Indonesia, serious bacterial infections are responsible for about 600,000 newborn deaths every year. Neonatal sepsis is a major cause of neonatal mortality and accounts for 13 per cent of newborn deaths (151). Infection is also one of the three leading causes of maternal death (152).

Reduction of bacterial infections is typically attempted by the prescription of prophylactic antibiotics during labor and delivery as a routine practice. Studies have shown that the use of antibiotics has reduced maternal infections and has improved neonatal outcomes. The incidence of neonatal sepsis has significantly declined in the US since the introduction of guidelines by the Centers for Disease Control and Prevention (CDC) for the prevention of perinatal Group B *Streptococcus* (GBS) (37,119). The benefit of prophylactic antibiotics in reducing infection incidence in women who have undergone a C-section has also been proven (153). Prophylactic antibiotic use for the premature rupture of membranes (PROM) has also been associated with a reduction in neonatal infection (154).

These significant benefits of antibiotic use during labor and delivery have inevitably led to an increased use of antibiotics worldwide (38,155). Even in the US, at some centers, the use of antimicrobials have more than doubled compared to the era before IAP introduction (155). Studies in LMICs have reported that the proportion of deliveries that received antibiotics could reach up to 90% (61,156). In Indonesia, study on prophylactic antibiotic use during delivery is

very limited (62). This situation immediately raised concern among experts, as previous studies had suggested that antibiotic exposure during labor and delivery may increase the risk of various adverse events to both the mother and newborn, including antibiotic-resistant bacterial infection, maternal and infant microbiome alteration, long-term functional impairment in children, and maternal anaphylaxis reaction (63–66). Therefore, many countries, together with their professional organizations of physicians, have published guidelines that specify the recommended conditions for the administration of prophylactic antibiotics. These recommendations are supported by strong evidence on the impact and prevention of inappropriate use [14,23,24]. However, due to disparities in healthcare facilities, the guidelines for prophylactic antibiotic use in labor and delivery and the extent to which practitioners adopt these guidelines vary across countries, especially between high-income and low- to middle-income countries (38). Despite the evidence that antibiotic use and inappropriate antibiotic prescriptions tend to be higher in low-income countries than in high-income countries, the practices of prophylactic antibiotic administration during delivery in low- to middle-income countries are not well characterized (157,158).

Antibiotic consumption (including inappropriate usage) is the major cause of antimicrobial resistance. Therefore, more data on antibiotic use practices from low-income countries, including prophylactic antibiotic practices during delivery, are essential for guiding and controlling the overuse and misuse of antibiotics to mitigate the development of antimicrobial resistance. This study utilized data on antibiotic use during delivery from three consecutive years at a tertiary-level referral hospital in Indonesia. The aims were to describe the prevalence of prophylactic antibiotic use during delivery, the significant factors that were associated with prophylactic antibiotic use, and clinician adherence to local guidelines.

3.2 Materials and Methods

3.2.1 Study Design and Study Population

The study data comprised medical records from Mohammad Hoesin Hospital from January 1, 2016, to December 31, 2018. The hospital is a government-run teaching hospital and serves as the tertiary-level referral hospital for patients from five neighboring provinces. The hospital provides all primary, secondary, and tertiary care and has a near 1000-bed capacity. We identified all deliveries in this hospital based on the International Classification of Disease, 10th Revision, Clinical Modification (ICD-10-CM) codes (i.e., O60, O80–O84) and consulted the paper-based medical records of each patient. The hospital's electronic medical record database indicated that 3957 deliveries had occurred during this period. We were able to collect complete data from 3657 medical records (91.5%), while 338 (8.5%) of the paper-based medical records were either lost or had missing sheets and were not included in the data analysis.

3.2.2 Variables and Measurement

The variable of interest in this study was prophylactic antibiotic use during deliveries. We determined that a prophylactic antibiotic was given when written and when specified as a prophylactic antibiotic in the medical record by the physician in charge. We recorded the maternal conditions that were recommended by the local guidelines for the administration of prophylactic antibiotics. According to the local guidelines, all C-section deliveries (elective and emergency), PROM, and cases with antepartum hemorrhage due to placenta previa were recommended for prophylactic antibiotics (159,160). In addition, the guideline recommended for giving prophylactic antibiotics to mothers who had risk factors for infection, which included a

maternal intrapartum temperature ≥ 38 °C, preterm deliveries (gestational age < 37 weeks), and a maternal leukocyte count > 15,000/mm^3 (160). The type of antibiotic was also recorded.

We also documented other sociodemographic and obstetric variables, such as the mother's age, place of residence, level of education, birth attendant, multiple births, gravidities, parities, number of abortions, and foul-smelling amniotic fluid.

3.2.3 Statistical Analysis

The prevalence of prophylactic antibiotic use during deliveries from 1 January 2016 to 31 December 2018 was reported on a monthly basis. Poisson regression was used to assess the trend in use over the study period. Descriptive characteristics were summarized as frequencies and proportions. Comparisons between groups were assessed with the chi-square test. Logistic regression was used to assess the association of potential risk factors of antibiotic use and clinician adherence. Initially, each factor was tested individually in a univariate regression model. The variables with a p-value < 0.20 and the conditions that were recommended for antibiotic prophylaxis by the guidelines were then included in the final model to estimate the adjusted odds ratio. We derived the estimates (crude and adjusted odds ratio) with the corresponding 95% confidence intervals. A significance level of 0.05 was used in all of the analyses. The data processing and analyses were conducted using SAS®, version 9.4 (SAS Institute Inc, Cary, NC).

3.3 Results

From a total of 3957 recorded deliveries in the hospital during 2016–2018, the medical records of 3657 (92.4%) patients were retrieved: a total of 1087 of 1202 (90.4%) were from

2016, 1227 of 1338 (91.7%) were from 2017, and 1343 of 1417 (94.8%) were from 2018. Overall, the mean age of women who underwent delivery at this hospital was 29.9 years; and most of them were in the group age 17–35 (78.1%). Among the mothers, most were residents of Palembang city (63.8%) and most were high school graduates (72.9%). The gestational age of most deliveries was ≥37 weeks (77.1%), the parity was <5 (98.1%), previous abortions were ≤1 (97.3%), 97.6% had singleton births, 55.3% had vaginal delivery, and most (85.2%) were assisted by obstetrics and gynecology (Ob/Gyn) residents (Table 7). Across the calendar year, the maternal age group, place of residency, parity, multiple births, maternal leukocyte count, cases with antepartum hemorrhage, and foul-smelling amniotic fluid showed similar proportions. However, a significant difference was noted for the mother's education level, birth attendant, number of previous abortions, gestational age, mode of delivery, maternal fever, and PROM cases.

Table 7. Maternal sociodemographic and obstetric characteristics across study period.

Variables	2016	2017	2018	Total	*p* [a]
	n = 1087	*n* = 1227	*n* = 1343	*n* = 3657	
Maternal age group					
<17 years old	5 (0.5)	6 (0.5)	12(0.9)	23 (0.6)	0.21
17–35 years old	851 (78.3)	979 (79.8)	1027 (76.5)	2857 (78.1)	
>35 years old	231 (21.3)	242 (19.7)	304 (22.6)	777 (21.3)	
Mother Education					
No formal education	3 (0.3)	23 (1.9)	7 (0.5)	33 (0.9)	<0.0001
Less than high school	72 (6.7)	120 (9.8)	193 (14.4)	385 (10.5)	
High school graduate	895 (83.2)	898 (73.2)	871 (64.9)	2664 (72.9)	
College or higher	106 (9.8)	130 (10.6)	192 (14.3)	428 (11.7)	
Missing[b]	11 (1.0)	56 (4.6)	80 (5.9)	147 (4.0)	

Table 7. (cont'd)

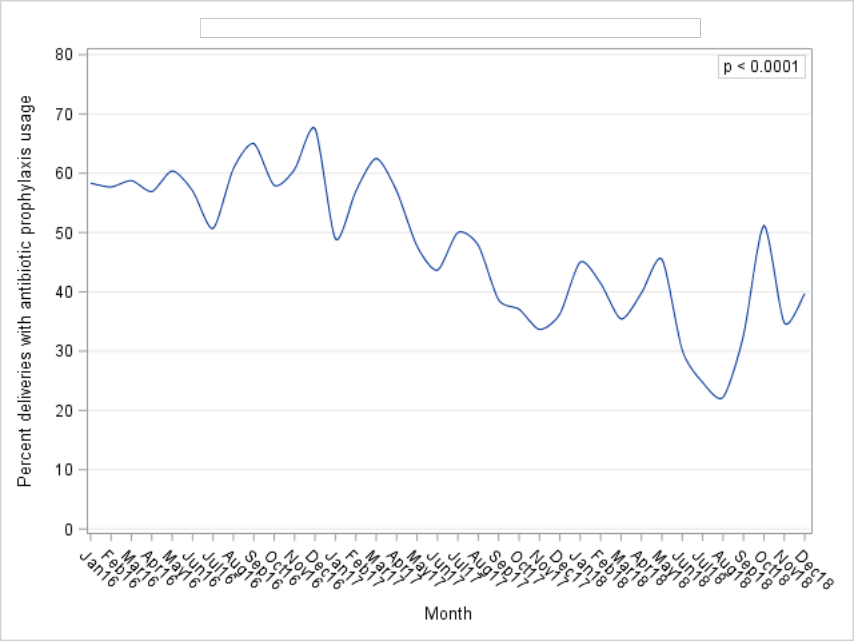
Variables	2016	2017	2018	Total	*p* [a]
Mother's place of residence					
Resident	693 (63.8)	770 (62.6)	870 (64.8)	2333 (63.8)	0.54
Non-resident	393 (36.2)	456 (37.2)	470 (35.0)	1319 (36.1)	
Missing[b]	1 (0.1)	1 (0.1)	3 (0.2)	5 (0.1)	
Birth attendant					
Ob/Gyn	193 (17.8)	177 (14.4)	172 (12.8)	542 (14.8)	0.003
Resident	894 (82.2)	1050 (85.6)	1171 (87.2)	3115 (85.2)	
Parity ≥ 5	15 (1.4)	30 (2.4)	25 (1.9)	70 (1.9)	0.17
Previous abortion >1	34 (3.1)	43 (3.5)	23 (1.7)	100 (2.7)	0.01
Gestational age < 37 weeks	297 (27.3)	239 (19.5)	300 (22.3)	836 (22.9)	<0.0001
Multiple birth	33 (3.0)	21 (1.7)	32 (2.4)	86 (2.4)	0.11
Mode of delivery					
C-section	486 (44.7)	491 (40.02)	658 (49.0)	1635 (44.7)	0.0004
Spontaneous vaginal delivery	575 (52.9)	699 (57.0)	648 (48.3)	1922 (52.6)	
Vacuum extraction	11 (1.0)	14 (1.1)	21 (1.6)	46 (1.3)	
Forceps extraction	15 (1.4)	23 (1.9)	16 (1.2)	54 (1.5)	
Intrapartum temperature ≥ 38 °C	20 (1.8)	17 (1.4)	67 (5.0)	104 (2.8)	< 0.0001
PROM	374 (34.4)	349 (28.4)	312 (23.2)	1035 (28.3)	< 0.0001
Maternal leukocyte count					
>15,000/mm^3	316 (29.1)	333 (27.1)	354 (21.2)	1003 (27.4)	0.25
<15,000/mm^3	674 (62.0)	753 (61.4)	852 (58.3)	2279 (62.3)	
Missing[b]	97 (8.9)	141 (11.5)	137 (20.6)	375 (10.3)	
Antepartum hemorrhage	22 (2.0)	24 (2.0)	16 (1.2)	62 (1.7)	0.2
Foul-smelling amnion fluid	7 (0.6)	17 (1.4)	11 (0.8)	35 (1.0)	0.15

[a] *p*-values from chi-square tests [b] Missing category were not included in the analysis.

3.3.1 Prevalence of Prophylactic Antibiotic Usage

Of the 3657 deliveries, 2730 (74.7%) were given antibiotics during delivery. Of the given antibiotics, sixty-three percent were categorized as prophylaxis, which accounts for 47.1% of all deliveries. The prevalence of prophylactic antibiotic use was 63.6% for C-Section deliveries and 33.7% for vaginal deliveries. A marginal increase occurred at the end of 2018, but the proportion of women receiving prophylactic antibiotics decreased overall during the study, from 59.2% in 2016 to 46.2% in 2017 and to 38.1% in 2018 (Figure 3). The proportion decreased for both vaginal deliveries and C-sections. For vaginal deliveries, the proportion of prophylactic antibiotic use decreased from 42.4% to 33.2% and then to 24.4%. For C-sections, the proportion of antibiotic use also decreased, decreasing from 79.8% to 65.8 and then to 50%. Tests for linear trends over the years, which were based on Poisson regression, detected significant declining rates in prophylactic antibiotic use ($p < 0.0001$).

Figure 3. Percentage of deliveries with prophylactic antibiotic use during 2016–2018.



Among the conditions where prophylactic antibiotics were recommended, the most common condition was C-section, followed by PROM, and then preterm (gestational age < 37 weeks) deliveries (Table 2). During the study period, ampicillin was the most commonly used antibiotic during delivery (64.2%), which was followed by ceftriaxone (34.2%).

The univariate analysis revealed significant associations of maternal educational level, place of residence, birth attendant, frequency of previous abortion, gestational age, mode of delivery, PROM, and antepartum hemorrhage with prophylactic antibiotic use. After adjustment, the associations between prophylactic antibiotic use and place of residence and gestational age lost their statistical significance. However, the associations between prophylactic antibiotic use and maternal education level, birth attendant, frequency of previous abortions, mode of delivery, PROM, and antepartum hemorrhage persisted (Table 8).

Table 8. Association between prophylactic antibiotic use and maternal sociodemographic and obstetric factors.

Variables	Antibiotic Prophylaxis		OR (95% CI)	p [a]	aOR (95%CI)[†]	p [a]
	Yes (n = 1721)	No (n = 1936)				
Maternal age group						
<17 years old	9 (0.5)	14 (0.7)	0.75 (0.32–1.72)	0.13	1.04 (0.32–3.35)	0.34
17–35 years old	1323 (76.9)	1534 (79.2)	Ref		Ref	
>35 years old	389 (22.6)	388 (20.0)	1.16 (1.00–1.36)		1.17 (0.94–1.48)	
Mother Education[b]						
No formal education	13 (0.8)	20 (1.1)	0.84 (0.41–1.75)	0.009	0.83 (0.30–2.36)	0.01

Table 8. (cont'd)

Variables	Antibiotic Prophylaxis		OR (95% CI)	p [a]	aOR (95%CI)[†]	p [a]
	Yes	No				
Mother Education[b]						
Less than high school	159 (9.6)	226 (12.2)	0.92 (0.68–1.22)		0.91 (0.61–1.37)	
High school graduate	1298 (78.5)	1366 (73.6)	1.23 (0.99–1.54)		1.39 (1.04–1.86)	
College or higher	184 (11.1)	244 (13.2)	Ref		Ref	
Resident	1055 (61.3)	654 (33.9)	Ref	0.003	Ref	0.08
Non-resident	666 (38.7)	1278 (66.2)	1.23 (1.08–1.41)		1.19 (0.98–1.45)	
Birth attendant						
Ob/Gyn	298 (17.3)	244 (12.6)	1.45 (1.21–1.75)	<0.0001	1.29 (1.001–1.66)	0.049
Resident	1423 (82.7)	1692 (87.4)	Ref			
Parity						
<5	1691 (98.3)	1896 (98.0)	Ref	0.48	--	--
≥5	30 (1.7)	40 (2.1)	0.84 (0.52–1.36)			
Previous abortion						
≤1	1663 (96.6)	1894 (97.8)	Ref	0.03	Ref	0.02
>1	58 (3.4)	42 (2.2)	1.57 (1.05–2.35)		1.88 (1.11–3.17)	
Gestational age < 37 weeks	450 (26.2)	384 (19.9)	1.42 (1.21–1.66)	<0.0001	1.13 (0.89–1.41)	0.31
Multiple birth	41 (2.4)	45 (2.3)	1.03 (0.69–1.57)	0.91	--	--
Mode of delivery						
C-section	1040 (60.4)	595 (30.7)	3.45 (3.01–3.96)	<0.0001	7.96 (6.40-9.91)	<0.0001
Spontaneous vaginal delivery	646 (37.5)	1276 (65.9)	Ref		Ref	
Vacuum extraction	16 (0.9)	30 (1.6)	1.05 (0.57–1.95)		1.54 (0.60–3.92)	
Forceps extraction	19 (1.1)	35 (1.8)	1.07 (0.61–1.89)		2.21 (1.00–4.85)	

Table 8. (cont'd)

Variables	Antibiotic Prophylaxis		OR (95% CI)	p [a]	aOR (95%CI)[†]	p [a]
	Yes	No				
Intrapartum temperature ≥ 38 °C	48 (2.8)	56 (2.9)	0.96 (0.65–1.42)	0.85	0.71 (0.43–1.17)	0.18
PROM	991 (57.6)	44 (2.3)	58.37 (42.65–79.89)	<0.0001	117.78 (83.28-166.58)	<0.0001
Maternal leukocyte count >15,000/mm^3	474 (28.0)[d]	528 (28.1)[e]	0.99 (0.84–1.17)	0.93	--	--
Antepartum hemorrhage	62 (3.6)	0 (0)	145.87 (8.82–>999)	0.0005	309.93 (18.17 –>999)	<0.0001
Foul-smelling amnion fluid	17 (1.0)	18 (0.9)	1.06 (0.54–2.07)	0.86	--	--

[a] p-values from logistic regressions †Adjusted for maternal age, education, place of residence, birth attendant, previous abortion, gestational age, mode of delivery, PROM, intrapartum temperature, maternal leukocyte count, antepartum hemorrhage, foul-smelling amniotic fluid; [b]missing 167; [c]missing 5; [d] missing 118; [e] missing 257.

The factors relating to the choice of using ampicillin and ceftriaxone, the most frequently used prophylactic antibiotics at the study site, were also analyzed. The use of ceftriaxone was more likely to be suggested by Ob/Gyns than by residents in training (aOR 1.57, 95% CI 1.16–2.14). Patients with C-sections with leukocyte counts > 15,000/mm^3, with an intrapartum temperature >38 °C, and with foul-smelling amniotic fluid were also more likely to be given ceftriaxone ((aOR 2.08, 95% CI 1.59–2.74), (aOR 1.34, 95% CI 1.03–1.75), (aOR 2.11, 95% CI 1.04–1.75)) and (aOR 5.93, 95% CI 1.36–25.83), respectively). However, patients with PROM were more likely to be given ampicillin than ceftriaxone (aOR 2.73, 95% CI 2.12–3.53).

Referring to the local guidelines on prophylactic antibiotic administration in delivery, of the 3657 patients who had deliveries, 2725 (74.5%) had at least one indication for prophylactic antibiotic administration, and 932 (25.5%) had no indication. Among the 2725 patients who had an indication for prophylactic antibiotics, 1654 (60.7%) received prophylactic antibiotics, while

1071 (39.3%) did not. Among the 932 mothers who had no indications for prophylactic antibiotics, 67 (7.2%) still received antibiotic prophylaxis. Over the years, the proportion of patients who received prophylactic antibiotic without indication decreased from 11.7% to 5.9%. On the other hand, the proportion of patients who had indication but did not received prophylactic antibiotic increased from 26.6% to 51%. The overall adherence to prophylactic antibiotic use guidelines was achieved in 68.9% of all deliveries. However, over the years, the proportion of adherence significantly decreased, decreasing from 77.2% in 2016 to 71.2% in 2017 and to 60.1% in 2018 ($p < 0.0001$).

The number of indications for prophylactic use was also associated with guideline adherence ($p < 0.001$). The highest adherence was noted in patients who had three indications (93.2%) followed by zero indications (89.6%) and two indications (77.3%), and the lowest level of adherence was in patients with one indication (59.3%).

Multiple logistic regression showed that clinicians were more likely to adhere to the guidelines when the patient had PROM (aOR 27.88, 95% CI 17.17–45.26), antepartum hemorrhage (aOR 194.81, 95% CI 11.46 to >999.99), and foul-smelling amniotic fluid (aOR 3.65, 95% CI 1.26–10.58). However, adherence was significantly lower in more recent years (both 2017 (aOR 0.57, 95% CI 0.45–0.74) and 2018 (aOR 0.39, 95% CI 0.31–0.50) compared to the year of 2016) with preterm deliveries (aOR 0.37, 95% CI 0.25–0.54), with forceps extraction compared to spontaneous vaginal delivery (aOR 0.41, 95% CI 0.19–0.87), with maternal fever (aOR 0.52, 95% CI 0.29–0.95), and with a maternal leukocyte count > 15,000/mm^3 (aOR 0.23, 95% CI 0.18–0.28), and it was the lowest when the patient only had one indication for prophylactic antibiotics (aOR 0.36, 95% CI 0.24–0.54) (Table 9).

Table 9. Adherence to local prophylactic antibiotic prescribing guidelines.

Variables	Adherence (n = 2519)	Non-Adherence (n = 1138)	OR (95% CI)	p [a]	aOR (95% CI) [†]
Year of admission					
2016	839 (33.3)	248 (21.8)	Ref	<0.0001	Ref
2017	873 (34.7)	354 (31.1)	0.73 (0.60–0.88)		0.57 (0.45–0.74)
2018	807 (32.0)	536 (47.1)	0.45 (0.37–0.55)		0.39 (0.31–0.50)
Maternal age group					
<17 years old	13 (0.5)	10 (0.9)	0.58 (0.25–1.33)	0.39	--
17–35 years old	1976 (78.4)	881 (77.4)	Ref		
>35 years old	530 (21.1)	247 (21.7)	0.96 (0.81–1.14)		
Mother Education[b]					
No formal education	23 (1.0)	10 (0.9)	1.15 (0.54–2.49)	0.04	1.04 (0.34–3.11)
Less than high school	244 (10.1)	141 (12.9)	0.87 (0.65–1.16)		0.94 (0.63–1.39)
High school graduate	1867 (77.2)	796 (73.0)	1.18 (0.95–1.46)		1.19 (0.89–1.58)
College or higher	285 (11.8)	143 (13.1)	Ref		Ref
Mother's place of residence[c]					
Resident	1628 (64.8)	705 (62.0)	Ref	0.1	0.95 (0.78–112)
Non-resident	886 (35.2)	433 (38.0)	0.89 (0.77–1.02)		Ref
Birth attendant					
Ob/Gyn	375 (14.9)	167 (14.7)	1.02 (0.84–1.24)	0.87	--
Resident	2144 (85.1)	971 (85.3)	Ref		
Parity					
<5	2467 (98.1)	1116 (98.1)	Ref	0.95	--
≥5	48 (1.9)	22 (1.9)	0.99 (0.59–1.64)		
Previous abortion					
≤1	2449 (97.2)	1082 (97.4)	Ref	0.81	--
>1	70 (2.8)	30 (2.6)	1.06 (0.68–1.63)		

Table 9. (cont'd)

Variables	Adherence (n = 2519)	Non-Adherence (n = 1138)	OR (95% CI)	p [a]	aOR (95% CI) [†]
Gestational age < 37 weeks	450 (17.9)	386 (33.9)	0.42 (0.36–0.50)	<0.0001	0.37 (0.25–0.54)
Multiple birth	52 (2.1)	33 (3.5)	0.68 (0.44–1.06)	0.09	1.66 (0.90–3.03)
Mode of delivery					
C-section	1040 (41.3)	595 (52.3)	0.63 (0.54–0.72)	<0.0001	1.34 (0.09–2.00)
Spontaneous vaginal delivery	1414 (56.1)	508 (44.6)	Ref		Ref
Vacuum extraction	33 (1.3)	13 (1.1)	0.91 (0.48–1.75)		0.88 (0.37–2.05)
Forceps extraction	32 (1.3)	22 (1.9)	0.52 (0.30–0.91)		0.41 (0.19–0.87)
Intrapartum temperature ≥ 38 °C	48 (1.9)	56 (4.9)	0.38 (0.25–0.56)	<0.0001	0.52 (0.29–0.95)
PROM	991 (39.3)	44 (3.9)	16.13 (11.81–22.03)	<0.0001	27.88 (17.17–45.26)
Maternal leukocyte count > 15,000/mm^3	549 (20.9)[d]	482 (28.0)[e]	0.34 (0.29–0.41)	<0.0001	0.23 (0.18–0.28)
Antepartum hemorrhage	62 (2.5)	0 (0)	3.31 (1.42–7.70)	0.006	194.81 (11.46–>999.99)
Foul-smelling amnion fluid	28 (1.0)	7 (0.6)	57.92 (3.50–958.43)	0.005	3.65 (1.26–10.58)
Numbers of indications for antibiotic prophylaxis in a patient					
None	873 (34.7)	312 (33.3)	Ref	<0.0001	Ref
One indication	924 (36.7)	638 (56.1)	0.52 (0.44–0.61)		0.36 (0.24–0.54)
Two indications	612 (24.3)	180 (15.8)	1.22 (0.98–1.50)		0.64 (0.31–1.29)
Three or more indications	110 (4.4)	8 (0.7)	4.91 (2.37–10.19)		0.63 (0.17–2.29)

[a] *p*-values from logistic regressions †Adjusted for admission year, maternal education, place of residence, multiple births, gestational age, mode of delivery, PROM, intrapartum temperature, maternal leukocyte count, antepartum hemorrhage, foul-smelling amniotic fluid, number of indications for antibiotic prophylaxis; [b]missing 167; [c]missing 5; [d] missing 266; [e]missing 109.

3.4 Discussion

This study demonstrated that the prevalence of prophylactic antibiotic use in all deliveries was 47.1%. The overall prevalence of prophylactic antibiotic use during delivery in the current study was higher than in higher-income countries. Stockholm et al., through the Danish Copenhagen Prospective Study on Asthma in Childhood ($COPSAC_{2010}$) in Denmark, reported that prophylactic antibiotic use during delivery was 33%, similar to the prevalence in the USA and Canada (30% and 39%, respectively) (116,161,162). Compared to other studies from low- to middle-income countries, the current findings on prevalence were higher than those reported from Ghana, at 28%, but were much lower than those found in another study at a tertiary level hospital in India, where 994 of 1077 (92.3%) deliveries during the 2008–2010 period presented with indications that required the prescription of prophylactic antibiotics during and after delivery (61,156). The main difference between the study in India and the others is that the study site in India had not yet implemented a general policy on antibiotic prescription, thereby showing the need for specific, well-defined, and evidence-based antibiotic prescribing guidelines in healthcare institutions to reduce inappropriate antibiotic use. The present study site is a top referral hospital, and the moderately high use of prophylactic antibiotics may reflect the admission of more complicated cases that were referred from other hospitals.

More than 60% of the prophylactic antibiotic use was in C-section deliveries. We observed that despite the strong recommendation in the guidelines for prophylactic antibiotic use for C-section, the prevalence of the practice was nearly 65%, which was lower than expected. The main possible reason for this situation is that some patients who had C-section deliveries were already being treated with antibiotics for therapeutic purposes, such as for urinary tract infections or for intraamniotic infections. Therefore, the administered antibiotics were not

considered prophylactic antibiotics and were beyond the scope of this study. Another possible explanation, although less likely, was the possibility of inadequate patient management documentation, including prophylactic antibiotic administration, in the medical record. Despite the significant improvement in medical data recording, inaccurate and incomplete medical records remain a worldwide problem (163–166).

Our study also showed a significant decrease in prophylactic antibiotic use, which decreased from 59.2% in 2016 to 38.1% in 2018. However, this decline was followed by a significant decrease in the rate of clinician adherence to the guidelines. Therefore, the reduction in prophylactic antibiotic use may reflect an increased rate of non-adherence to the local guideline. Over the years, the proportion of patients who were suggested by the guideline to receive prophylactic antibiotic but did not received antibiotic increased. However, the proportion of patients who receive antibiotic without indication decrease, showing that in later years the clinician in study site tends to be more cautious to give prophylactic antibiotics. Even though guidelines are believed to represent the best evidence and judgments, they are not fixed, mandatory protocols for healthcare providers. The decision to follow a guideline is independently based on the healthcare provider's clinical judgment. The local guidelines recommend prophylactic antibiotics in C-section deliveries, PROM, cases of antepartum hemorrhage related to placenta previa, a maternal intrapartum temperature $\geq$ 38 °C, a maternal leukocyte count >15,000/mm^3, and preterm deliveries (gestational age < 37 weeks) (160). The local guidelines were developed by an independent committee by adopting other guidelines published by national and international health professional associations and Cochrane reviews (37,56,159,160,167–171). In addition, adjustments were also made based on local data and took into account some local expert opinions. C-sections and PROM are conditions that are also

recommended for prophylactic antibiotics by the American College of Obstetricians and Gynecologists (ACOG), as supported by consistent scientific evidence (56). Antepartum hemorrhage related to placenta previa was recommended for prophylactic antibiotics because of the possibility of causing maternal infection (170). Although our study site does not routinely implement culture-based screening for GBS due to resource constraints, intrapartum antibiotic prophylaxis (IAP) is advocated in preterm deliveries and for intrapartum temperature ≥ 38 °C (37,160). At our study site, the administration of IAP is not limited to the prevention of GBS infection but is also performed to prevent other possible pathogenic infection in newborns. Therefore, in line with the local guidelines from the hospital's pediatric department and the World Health Organization's recommendation for preventing neonatal sepsis, prophylactic antibiotics are also recommended for women with maternal leukocyte counts > 15,000/mm^3 (44,167).

This study found that, among the conditions recommended for prophylactic antibiotic administration, only C-sections, antepartum hemorrhage, and PROM were persistently associated with an increased risk of prophylactic antibiotic use. Maternal fever and isolated increased leukocyte counts were not associated with an increased risk of prophylactic antibiotic use but were significantly associated with non-adherence. These latter conditions were mainly adopted in the guidelines as part of a risk-based approach for neonatal GBS infection prevention (37). In some countries, the risk-based approach is no longer used and has been replaced by culture-based screening to determine antibiotic use (172,173). Previous studies have shown that the incidence of neonatal sepsis due to GBS infection is low in most Asian countries; therefore, this may be the reason why clinicians at our study site did not consistently administer prophylactic antibiotics to mothers solely based on the presence of one of these risk factors (11,12,174). The

same observation was also found in preterm birth cases, but our study did not find an increased risk of prophylactic antibiotic use, and clinician adherence was significantly low. This may reflect the fact that some inconsistent evidence still exists regarding the benefits of prophylactic antibiotics. Multiple clinical trials did not support routine prophylactic antibiotic administration to women in preterm labor with intact membranes in the absence of overt signs of infection. However, some studies have suggested that prophylactic antibiotics may prevent preterm births [23,47,48].

This study found that cefazoline, the type of antibiotic recommended by the guidelines for C-sections, was rarely used. Ceftriaxone was one of the most used antibiotics for prophylaxis in the current study setting. Drug sustainability may explain the choice of ceftriaxone compared to cefazoline in the pharmacy, as the supply of the former tends to be more stable and affordable. Ceftriaxone was also more commonly used due to common knowledge among practitioners that this antibiotic has excellent bioavailability and effectiveness, a low toxicity profile, and a long half-life (175).

This study showed that the healthcare providers had only moderate adherence to the guidelines, at 68%, regarding conditions indicated for prophylactic antibiotic. In general medical practices, a wide variation exists in terms of the guideline adherence rate, from 20% to nearly 100%. This rate varies according to the purpose of the guidelines, the definition of adherence employed, and the location of the study (176,177). As expected, in our study, the proportion of adherence was higher for cases that had multiple conditions recommended by the guidelines for prophylactic antibiotic administration. This arguably shows that the health practitioners' clinical judgment had a significant role in the decision-making process if the patient had only one risk factor for infection.

Given that the antibiotic prescribing guideline was formulated to minimize the emergence of antibiotic resistance and optimized patient treatment, the hospital's stakeholders need to review the guideline implementation on a regular basis. Local healthcare providers should be involved in guideline development and review processes. In settings where guidelines are not strictly followed, factors that determine the drug prescription in LMICs include economic incentives, stable supplies, fear of unfavorable outcomes, peer norms, and local medical culture (178). Consultation with targeted physicians would improve guideline adherence (179).

A significant association was observed between prophylactic antibiotic use and maternal education level, birth attendant, and history of multiple abortions. Our study had a high proportion of high school graduates, which may have lessened the power of our estimation; however, our study indicated that prophylactic antibiotics were given more frequently to high school graduates than to college graduates. A study by Stokholm et al. also found that education level was related to antibiotic use during pregnancy (161). Higher education levels may enhance mother's capacity to obtain and understand the importance of prenatal care and to receive important reproductive health services. In addition, a higher education level may indicate a better general health status or may influence the healthcare-seeking behavior of certain groups. This study found that this prophylactic antibiotic association was limited to high school graduate mothers and was not apparent in women with lower education levels. However, the collected data did not allow analysis of whether those with lower educational levels were receiving antibiotics for purposes other than prophylaxis.

In this study, women with deliveries assisted by Ob/Gyns specialists were more likely to be given prophylactic antibiotics than women whose deliveries were assisted by residents. As the study site is a teaching hospital, most of the deliveries are assisted by a resident under the

supervision of an Ob/Gyn specialist. If the delivery is too complicated and beyond the resident's medical competency, the Ob/Gyn specialist then directly assists in the delivery. Therefore, this may explain the finding that Ob/Gyn specialists used more prophylactic antibiotics compared to residents. Previous studies have suggested that a physician's experience may influence adherence to guidelines (180,181), but our study did not reveal this association. In a teaching hospital, residents rarely make independent decisions about patient treatment, so the specialists were more likely to be influencing prescription choices.

Our findings suggest an association between a history of multiple abortions and prophylactic use. Pregnancy with a history of multiple abortions is frequently considered as a high-risk pregnancy due to its association with a higher risk of preterm birth, C-section deliveries, post-partum hemorrhage, PROM, and congenital malformation (182–184); however, not enough evidence supports the need for prophylactic antibiotics in deliveries with a history of multiple abortions and no other signs of infection. Further studies may be needed to evaluate this association.

This study had several study limitations. The first limitation was that the data were obtained by reviewing medical records, and the documentation may have been incomplete, thereby leading to a possibility, although not necessary, for some information bias. However, we were able to minimize information bias given that most of the collected data in the medical records were complete. In addition, this study involved data collection on a large sample and covered over 90% of the total deliveries in the study period; therefore, it arguably represents the study site's overall situation. A second limitation was the retrospective nature of the study, as this precluded any further elaboration on the association of the clinician's characteristics regarding adherence. However, the main objective of this study was to describe the practice of

prophylactic antibiotic use in general and not to focus on clinician adherence. A further limitation was that the results of this study reflect the conditions in a single center in Indonesia and may not be generalizable. However, considering the large amount of data that we were able to collect from three consecutive years and given the fact that our study site is a large hospital that serves all primary, secondary, tertiary care from multiple neighboring cities, to some extent, our study could represent other teaching and public hospitals in Indonesia. However, it may not represent other private hospitals that mostly serve the wealthier proportion of the Indonesian population. The prophylactic antibiotic practices between teaching and private hospitals may differ substantially.

3.5 Conclusions

Our study demonstrated a moderate to high prevalence of prophylactic antibiotic use in our hospital. Maternal education level, the birth attendant, multiple abortions, C-sections, PROM, and antepartum hemorrhage were associated with prophylactic antibiotic use. Individual clinical judgment plays a vital role in the decision for prophylactic use and may lead to a low rate of clinician adherence to antibiotic prescribing guidelines. Therefore, clinicians, local stakeholders, and policymakers should be actively involved to ensure the development of guidelines that are based on the best and most recent scientific evidence and incorporate local data to ensure successful guideline implementation.

CHAPTER 4 MANUSCRIPT 3 – RISK FACTORS OF NEONATAL SEPSIS AND THE IMPACT OF PERINATAL ANTIBIOTIC PROPHYLAXIS ON NEOANTAL SEPSIS: A PROSPECTIVE COHORT STUDY

4.0 Abstract

4.0.1 Background & Aim:

While antibiotic prophylaxis has become a common practice to prevent maternal and early neonatal infection, sepsis remains a top cause of neonatal morbidity and mortality. The estimates of neonatal sepsis burden vary by setting. Given the widespread use of perinatal antibiotic prophylaxis, a better understanding of the changing neonatal sepsis epidemiology is needed. This study aimed to assess factors associated with neonatal sepsis and the impact of perinatal antibiotic prophylaxis exposure on neonatal sepsis incidence and prevention.

4.0.2 Methods:

We conducted a prospective cohort study in two referral hospitals in Indonesia. Mother–viable newborn pairs that were admitted for delivery were enrolled. Newborns were followed either until the age of 28 days or until sepsis was observed. Maternal and neonatal characteristics and medical intervention data were collected at enrollment and during follow-up. The adjusted relative risk (aRR) and average treatment effect (ATE) of antibiotic prophylaxis for neonatal sepsis were estimated.

4.0.3 Results:

The neonatal sepsis cumulative incidence was 10.4 per 100 live births. Premature rupture of the membrane > 18 hours (aRR 2.43, 95% confidence interval [CI]: 1.11–5.32), foul-smelling amniotic fluid (aRR 1.97, 95% CI: 1.58–6.36), high maternal leukocyte count (aRR 1.70, 95% CI: 1.01–2.88), birth weight < 2,500 grams (aRR 3.33, 95% CI: 1.56–6.96), exclusive infant

formula feeding or in combination with breastmilk (aRR 7.35, 95% CI: 1.99–27.16 and aRR 3.19, 95% CI: 1.50–6.78, respectively), and receiving nothing per mouth over 24 hours (aRR 14.17, 95% CI: 6.40–31.37) were found to be strongly associated with neonatal sepsis. During the perinatal period, 72% of the studied newborns were exposed to prophylactic antibiotics. The estimate of the average causal effect of perinatal antibiotic prophylaxis on neonatal sepsis was 0.10 ($p < 0.0001$), and this causal effect was stronger for postnatal antibiotic prophylaxis exposure alone or with maternal exposure (ATE 0.14, $p = 0.10$ and ATE 0.16, $p < 0.0001$, respectively).

4.0.4 Conclusions:

Our study indicates that neonatal sepsis is associated with a high rate of perinatal antibiotic prophylaxis. Further studies are needed to revisit the risks and benefits of perinatal antibiotic prophylaxis to improve its efficacy in preventing neonatal sepsis.

4.1 Introduction

Neonatal sepsis remains a significant cause of morbidity and mortality among newborns. A comprehensive review of 1,270 studies of sepsis incidence from 1957 to 2016 estimated that the global population-based sepsis incidence was 22 per 1,000 live births, which is equivalent to nearly 3 million cases annually and a mortality rate between 11% and 19% (2). In 2018, the World Health Organization (WHO) estimated that neonatal sepsis was responsible for more than 1,000 deaths per day globally, accounting for 15% of all neonatal deaths (5). Despite recent advances in neonatal care, the impact of neonatal sepsis remains marked in LMICs, with an incidence rate that is 2 to 4-fold times higher than in HICs, and the risk of death due to neonatal sepsis being 34 times higher (3,4). Identifying the dominant maternal risk factors and infant clinical indicators in different geographical contexts, especially in countries where high-quality evidence of neonatal sepsis prevention and reliable surveillance systems remains lacking, is crucial to optimizing neonatal care.

Multiple prevention strategies have been designed and implemented, mainly to prevent early-onset sepsis. The main strategy includes maternal and neonatal antibiotic prophylaxis administration during the perinatal period to prevent neonatal bacterial infection. In 1996, the Centers for Disease Control and Prevention (CDC) introduced the maternal screening and risk-based approach for Group B Streptococcus (GBS) neonatal sepsis prevention and intrapartum antibiotic prophylaxis (IAP). This policy has significantly reduced early-onset neonatal sepsis in HICs (119). The WHO recommends giving postnatal prophylactic antibiotics to neonates with documented risk factors for infection (44). These strategies have been adopted worldwide with varying degrees of consistency. In most LMICs, prophylactic antibiotic administration relies heavily on clinical risk factors and personal clinical judgment. In these countries, the goal of IAP

is not limited to preventing GBS infection; it also aims to prevent other pathogenic bacterial infections in neonates, including gram-negative bacteria, such as those that require neonatal antibiotic prophylactics, as recommended by the WHO (38).

The worldwide adoption of IAP and neonatal antibiotic prophylaxis has been increasing the percentage of neonates exposed to antibiotics during the perinatal period. Combined with the routine use of prophylactic antibiotics during C-section deliveries, it is estimated that > 40% of neonates are exposed to some type of antibiotic that was given to their mother immediately before delivery (59). This proportion is likely much higher if the use of prophylactic antibiotics in neonates born to mothers with risk factors for infection is also included.

Given that prophylactic antibiotic treatment has become a routine practice during the birthing process, concerns about overuse during the perinatal period have been growing. The concerns are greater in low- and middle-income countries, where antibiotic use and inappropriate antibiotic prescriptions tend to be higher but not well characterized (158). Although some of these prevention strategies help reduce short-term maternal and neonatal complications, there is mounting evidence that the wide use of prophylactic antibiotics during the perinatal period might contribute to the rising incidence of late-onset sepsis, gram-negative neonatal sepsis, and antibiotic-resistant neonatal sepsis (63,64,75,77,79). Therefore, a better understanding of the changing neonatal sepsis epidemiology is needed. This study aimed to assess the risk factors of neonatal sepsis in Palembang, Indonesia, and assess the impact of perinatal antibiotic prophylaxis exposure on neonatal sepsis incidence and prevention.

4.2 Methods

4.2.1 Study setting and population

This study was conducted at Mohammad Hoesin Hospital and Palembang Bari Hospital, tertiary- and secondary-level hospitals, respectively, in Palembang, Indonesia, from September 2019 to March 2021. Both hospitals have nearly 1,500 beds and serve populations of more than 2,000,000 people in their catchment areas. Participants were enrolled in the study upon presenting for delivery. The inclusion criteria in this study were mother–viable newborn pairs admitted to the participating hospitals for delivery, where the legal guardian agreed to participate in the study and resided within the city. If the mother had consumed or received antibiotics 72 hours before admission, the mother received antibiotics for therapeutic purposes, or her newborn was diagnosed with a congenital gastrointestinal malformation, then the infant was excluded from the study.

4.2.2 Study procedure and follow-up

The recruitment of subjects began before delivery until approximately two hours after each delivery. Maternal antibiotic prophylaxis data were collected upon enrollment in the study. The antibiotic prophylaxis administration, type of antibiotics, dose, interval, and duration were documented from hospital medical records. Other relevant clinical data on mothers, such as gestational age, parity, intrapartum temperature, history of rupture of membrane, mode of delivery, antepartum hemorrhage, presence of foul-smelling amniotic fluid, and maternal leukocyte count, were extracted. In addition, a questionnaire was administered during the mothers' hospitalization, which collected more detailed demographic, behavioral, and general health history data.

After birth, the newborns were followed up every six hours either until discharge or until they were diagnosed with neonatal sepsis. Administration of postnatal antibiotic prophylaxis, vital signs, any clinical symptoms of neonatal sepsis, and other treatments given to the newborn were recorded. If the newborn was discharged without developing sepsis, observation continued until the baby reached the age of 28 days. The first follow-up was conducted one week after the newborn was discharged, following the recommended well-baby visit schedule of the participating hospitals. Subsequent follow-ups were conducted weekly by phone; the mother or guardian was interviewed to assess any illness and advised to visit the study hospital if necessary. Other information, such as the baby's general condition and oral intake, was collected during the weekly interview. The mother or guardian also had direct access to a contact person, which enabled them to report any illness the newborn experienced between the scheduled phone calls.

4.2.3 Operational definition and case ascertainment

In this study, perinatal antibiotic prophylaxis was defined as antibiotics received by the mother and/or newborn during hospital admission for prophylaxis purposes. According to the local guidelines, all C-section deliveries (elective and emergency) and cases with antepartum hemorrhage due to placenta previa were recommended for prophylactic antibiotics (159). In addition, although maternal screening for GBS is not routinely implemented, IAP is recommended to mothers at risk for infection, which includes premature rupture of the membrane (PROM), a maternal intrapartum temperature $\geq$ 38 ºC, and a maternal leukocyte count > 15,000/mm^3 (160,168). For newborns, the local guideline recommends postnatal antibiotic prophylaxis for neonates with a documented risk factor for infection, which includes

membrane rupture > 12 hours, a maternal intrapartum temperature ≥ 38 °C, a maternal leukocyte count > 15,000/mm^3, and the presence of foul-smelling or purulent amniotic fluid [18].

The on-duty physician made the diagnosis of neonatal cases according to the standard guidelines. To be diagnosed with neonatal sepsis, a newborn must have at least one of the following clinical criteria: non-specific signs, such as lethargy, feeding intolerance, weight loss, temperature instability, neurological symptoms, respiratory instability, gastrointestinal symptoms, skin and subcutaneous lesions, cardiovascular instability, and hematologic abnormalities. In addition, the newborn must have at least two laboratory findings that include abnormal white blood cell counts (< 5,000/ml or > 34,000/ml), an immature to total neutrophil ratio > 0.2, an abnormal platelet count, an Erythrocyte Sedimentation Rate > 15 mm/hour, and a C-reactive protein > 9 mg/L (167). Neonatal sepsis will then be categorized by the time of onset and microbiology results. Early-onset sepsis (EOS) cases are defined as those occurring within the first 72 hours of life, and late-onset sepsis (LOS) cases are those occurring after 72 hours of life (1).

As part of standard medical care in both hospitals, all newborns diagnosed with sepsis will have a sample of blood taken for culture and will be treated appropriately per standard guidelines. At the study site, blood cultures were performed following the BD BACTEC™ automated blood culture systems protocol. All cultures were incubated aerobically at 37 °C for 18–24 hours, and negative cultures were incubated for up to 5 days for bacteria and 9 days for fungi before being reported as negative. All isolates' identification and antimicrobial susceptibility testing were performed using an automated method from VITEX-2 Compact (Biomérieux) following the Clinical and Laboratory Standard Institute's guidelines [20]. Based

on the microbiology results, neonatal sepsis was also classified as either culture-negative or culture-positive.

4.2.4 Statistical analysis

Descriptive characteristics of both the mother and the newborn were summarized as frequencies and proportions for categorical variables. Comparisons between groups were assessed with χ^2 and Fisher's exact tests, and 95% confidence intervals (CIs) are presented for differences in proportions. Tests of hypotheses were two-sided, with statistical significance declared for a p-value < 0.05. The crude and adjusted relative risks (aRR) were calculated to assess potential risk factors associated with neonatal sepsis incidence. Initially, each risk factor was tested individually in univariable models. All maternal and neonatal variables with a p-value < 0.20 and other conditions that were recommended for antibiotic prophylaxis by the local guidelines were included in the final model to estimate the adjusted relative risk. If two or more individual variables were highly correlated, then the variable with either the largest point estimate or the one with fewer levels of categories was included in the final model. Other outcomes, including EOS, LOS, and culture-negative sepsis, followed a similar analytic strategy. The relative risks were approximated by odds ratios (OR) for culture-proven sepsis because of the low rate of culture-proven sepsis in this population.

Following the analysis of risk factors associated with sepsis, we continued examining the causal effect of perinatal antibiotic prophylaxis (the treatment) on each sepsis outcome. Given that in this study population antibiotic prophylaxis were mostly given by indication listed in the local guideline, therefore, a potential problem of confounding by indication arise. To balance the treatment (exposure) and comparison groups regarding the observed preintervention

characteristics of the study population and to adjust the confounding problems, we used propensity score model and logistic regression model for perinatal antibiotic prophylaxis. The propensity score supplied the inverse-probability weights (IPW) that were used to estimate the average treatment effect (ATE). The ATE estimate provided the average difference in the sepsis incidence probabilities between the population who were exposed to perinatal antibiotic prophylaxis and to the unexposed (185). PROC CAUSALTRT in SAS was adopted for these analyses (186).

Variables that were included in the propensity score model were (a) all conditions that were recommended by the local guidelines to be given prophylactic antibiotics and (b) other preintervention variables that were found to be significantly associated with each outcome. Statistical analyses were conducted with SAS Software, version 9.4 (SAS Institute Inc., Cary, NC).

4.3 Results

From 1,002 mother–newborn pairs enrolled in the study; 904 pairs were analyzed. The remaining 98 pairs did not complete the 28 days of follow-up and were thus not included in the final analysis. The median maternal age was 30 years old (interquartile range [IQR] 25–35); most mothers were within the age group 20–35 years (75.1%), and most mothers and their spouses were high school graduates (51.8% and 55.1%, respectively). In this study, the median number of persons per household was 4 (IQR 2–5), and most of the families (74.6%) lived above the poverty threshold (Table 10).

Table 10. Crude and adjusted relative risk (95% confidence intervals) for the association between maternal and neonatal risk factors with neonatal sepsis.

Variable	Sepsis (+) (n=94)	Sepsis (-) (n=810)	N = 904	*p*-value	Crude RR (95% CI)	aRR (95% CI)
Maternal risk factors						
Maternal age group (%)						
< 20 years old	6 (6.4)	32 (4.0)	38 (4.2)	0.30	1.77 (0.72, 4.39)	--
20–35 years old	65 (69.2)	614 (75.8)	679 (75.1)		Ref	
> 35 years old	23 (24.5)	164 (20.3)	187 (20.7)		1.32 (0.80, 2.20)	
Mother Education (%)						
Less than high school	42 (44.7)	297 (36.7)	339 (37.5)	0.06	3.29 (1.15,9.41)	3.37 (0.76, 14.93)
High school graduate	48 (51.1)	420 (51.9)	468 (51.8)		2.66 (0.94, 7.55)	3.06 (0.70, 13.43)
College or higher	4 (4.3)	93 (11.5)	97 (10.7)		Ref	Ref
Spouse Education (%)						
Less than high school	36 (38.3)	265 (32.7)	301 (33.3)		1.29 (0.62, 2.70)	--
High school graduate	48 (51.1)	450 (55.6)	498 (55.1)	0.55	1.01 (0.50, 2.07)	
College or higher	10 (10.6)	95 (11.7)	105 (11.6)		Ref	
Person per household						
Less than 5	67 (71.3)	541 (66.8)	608 (67.3)	0.38	Ref	--
≥ 5	27 (28.9)	269 (33.2)	296 (32.7)		0.81 (0.51, 1.30)	

Table 10. (cont'd)

Variable	Sepsis (+) (n=94)	Sepsis (-) (n=810)	N = 904	*p*-value	Crude RR (95% CI)	aRR (95% CI)
Household Income						
Above poverty level	77 (81.9)	597 (73.7)	674 (74.6)	0.08	Ref	Ref
Below poverty level	17 (18.1)	213 (26.3)	230 (25.4)		0.62 (0.36, 1.07)	0.73 (0.36, 1.48)
Smoke	0 (0)	0 (0)	0 (0)	--	--	--
Diabetes / Gestational Diabetes	0 (0)	4 (0.5)	4 (0.4)	0.99	0.95 (0.04, 25.05)	--
Pre-pregnancy BMI						
Underweight	14 (15.1)	99 (12.3)	113 (12.5)	0.72	1.29 (0.69, 2.41)	--
Normal weight	56 (60.2)	512 (63.4)	568 (62.8)		Ref	
Overweight and obese	23 (24.8)	196 (24.3)	219 (24.3)		1.07 (0.64, 1.79)	
Missing	1	3	4			
Pregnancy weight gain						
Below recommendation	65 (69.9)	438 (54.3)	503 (55.9)	0.01	2.12 (1.20, 3.76)	1.70 (0.87, 3.33)
Within recommendation	16 (17.2)	229 (28.4)	245 (27.2)		Ref	Ref
Above recommendation	12 (12.9)	120 (14.9)	152 (16.9)		1.23 (0.56, 2.67)	1.24 (0.51, 2.98)
Missing	1	3	4			
Had at least one antenatal check-up	85 (90.4)	721 (89.0)	806 (89.2)	0.68	1.17 (0.57, 2.40)	--

Table 10. (cont'd)

Variable	Sepsis (+) (n=94)	Sepsis (-) (n=810)	N = 904	*p*-value	Crude RR (95% CI)	aRR (95% CI)
Had ≥ 4 antenatal check-ups.	37 (39.4)	425 (52.5)	462 (51.1)	0.02	0.59 (0.38, 0.91)	0.67 (0.40, 1.11)
Parity						
< 5	94 (98.9)	796 (98.3)	889 (98.3)	0.63	Ref	--
≥ 5	1(1.1)	14 (1.7)	15 (1.7)		0.61 (0.08, 4.70)	0.56 (0.28, 1.16)
Vaginal exams ≥ 4	16 (17.0)	190 (23.5)	206 (22.8)	0.16	0.67 (0.38, 1.17)	
Mode of delivery						
Vaginal birth	34 (36.2)	315 (38.9)	349 (38.6)	0.61	Ref	--
C-section	60 (63.8)	495 (61.1)	555 (61.4)		1.12 (0.72,1.75)	
Birth assistant						
Midwife	27 (28.7)	187 (23.1)	214 (23.7)	0.003	1.00 (0.61, 1.63)	0.93 (0.53, 1.61)
Birth assistant						
Resident	13 (13.8)	250 (30.9)	263 (29.1)		0.36 (0.19, 0.67)	0.30 (0.09, 1.03)
Ob/Gyn	54 (57.4)	373 (46.1)	427 (47.2)		Ref	Ref
Pregnancy						
Singleton	89 (91.5)	784 (96.8)	858 (96.6)	0.36	Ref	
Twin	5 (5.3)	26 (3.2)	31 (3.4)		1.69 (0.63, 4.52)	
PROM/PPROM	17 (18.1)	179 (22.1)	208 (23.1)	0.06	1.57 (0.98, 2.51)	--

Table 10. (cont'd)

Variable	Sepsis (+) (n=94)	Sepsis (-) (n=810)	N = 904	*p*-value	Crude RR (95% CI)	aRR (95% CI)
Rupture of membrane > 12 hours	29 (30.9)	84 (10.4)	101 (11.2)	0.02	1.91 (1.08, 3.38)	--
Rupture of membrane > 18 hours	12 (12.8)	34 (4.2)	46 (5.1)	0.002	3.34 (1.66, 6.70)	2.43 (1.11, 5.32)
Antepartum hemorrhage	1(1.1)	6 (0.7)	7 (0.8)	0.54	1.44 (0.17, 12.10)	--
Foul-smelling amniotic fluid	24 (25.5)	83 (10.3)	107 (11.8)	<0.0001	3.00 (1.79, 5.03)	1.97 (1.09, 3.55)
Maternal leukocyte count >15000/mm^3	30 (37.5)[a]	135 (21.5)[b]	165 (23.3)[c]	0.001	2.20 (1.24, 3.59)	1.70 (1.01, 2.88)
Intrapartum temperature ≥ 38ºC	0 (0)	7 (0.9)	7 (0.8)	0.999	0.57 (0.03, 12.3)	0.66 (0.02,19.25)
Neonatal risk factor						
Gender						
Male	55 (58.5)	401 (50.5)	456 (50.4)	0.10	Ref	Ref
Female	39 (41.5)	409 (49.5)	448 (49.6)		0.70 (0.45, 1.07)	0.79 (0.46, 1.34)
Gestational age						
< 32 weeks	8 (8.5)	27 (3.3)	35 (3.9)	< 0.001	4.35 (1.86, 10.16)	--
32 – 36 weeks	43 (45.7)	151 (18.6)	194 (21.5)		4.19 (2.65, 6.62)	
≥ 37 weeks	43 (45.7)	632 (78.0)	675 (74.7)		Ref	

Table 10. (cont'd)

Variable	Sepsis (+) (n=94)	Sepsis (-) (n=810)	N = 904	*p*-value	Crude RR (95% CI)	aRR (95% CI)
Prematurity (<37 weeks)	51 (54.3)	176 (22.0)	229 (25.3)	<0.0001	4.21 (2.72, 6.53)	1.32 (0.63, 2.83)
Birthweight						
< 1000 grams	2 (2.1)	3 (0.4)	5 (0.6)	<0.0001	11.98 (1.94, 73.98)	--
1000 – 1499 grams	12 (12.8)	16 (2.0)	28 (3.1)		13.45 (6.93, 30.61)	
1500 – 2499 grams	44 (46.8)	144 (17.8)	188 (20.8)		5.49 (3.41, 8.84)	
> 2500 grams	36 (38.3)	647 (79.9)	683 (75.5)		Ref	
Low birth weight (< 2,500 grams)	58 (61.7)	163 (20.1)	221 (24.5)	<0.0001	6.40 (4.08, 10.03)	3.30 (1.56, 6.96)
Early initiation of breastfeeding	8 (8.6)	250 (30.9)	258 (28.6)	< 0.0001	0.21 (0.10, 0.44)	1.12 (0.46, 2.72)
Apgar score in 1st minute						
< 7	38 (40.4)	88 (10.9)	126 (13.9)	<0.0001	5.58 (3.49, 8.89)	1.25 (0.63, 2.47)
≥ 7	56 (59.6)	722 (89.1)	778 (86.1)		Ref	Ref
Apgar score in 5th minute						
< 7	24 (25.5)	39 (4.8)	63 (7.0)	<0.0001	6.78 (3.86, 11.92)	2.11 (0.96, 4.65)
≥ 7	70 (74.5)	771 (95.2)	841 (93.0)		Ref	Ref
Respiratory support						
None	31 (33.0)	708 (87.4)	739 (81.8)	<0.0001	Ref	Ref
Non-invasive	59 (62.8)	96 (11.9)	155 (17.2)		14.04 (8.65, 22.78)	2.01 (0.91, 4.44)

Table 10. (cont'd)

Variable	Sepsis (+) (n=94)	Sepsis (-) (n=810)	N = 904	*p*-value	Crude RR (95% CI)	aRR (95% CI)
Respiratory support						
Invasive	4 (4.3)	6 (0.7)	10 (1.1)		15.23 (4.09, 56.73)	1.38 (0.24, 7.93)
First days of life oral intake						
Breast milk	27 (28.7)	619 (76.4)	646 (71.5)	<0.0001	Ref	Ref
Breastmilk infant formula	15 (16.0)	151 (18.6)	166 (18.4)		2.27 (1.18, 4.39)	3.19 (1.50, 6.78)
Infant formula	6 (6.4)	8 (1.0)	14 (1.6)		17.19 (5.56, 53.04)	7.35 (1.99, 27.16)
Nothing per oral	46 (49.0)	32 (4.0)	78 (8.6)		32.96 (18.21,59.65)	14.17 (6.40, 31.37)

[a] missing 14 [b] missing 181 [c] missing 195.

During the study, 94 newborns were diagnosed with neonatal sepsis. The cumulative incidence of neonatal sepsis in this study population was 10.4 per 100 live births, while the neonatal sepsis case fatality rate was 5.4%. In the univariable analysis, pregnancy weight gain, the mother receiving at least four antenatal check-ups by a skilled provider, birth attendant, prolonged PROM, foul-smelling amniotic fluid, an elevated maternal leukocyte count, gestational age, birth weight, early initiation of breastfeeding, 1st- and 5th-minute Apgar scores, the need for respiratory support, and oral intake during the first days of life were associated with the risk of sepsis (Table 10). After adjustment, rupture of the membrane for > 18 hours (aRR 2.43, 95% CI: 1.11–5.32), foul-smelling amniotic fluid (aRR 1.97, 95% CI: 1.09–2.88), lower birth weight (aRR 3.33, 95% CI: 1.56–6.96), maternal leukocyte count > 15,000/mm^3 (aRR 1.70, 95% CI: 1.012.88), and newborns receiving other than breast milk during the first days of life for more than 24 hours remained significantly associated with a higher risk of neonatal

sepsis (aRR 3.19, 95% CI:1.50, 6.78 for breastmilk mixed with infant formula; aRR 7.35, 95% CI: 1.99, 27.16 for infant formula; and aRR 14.17, 95% CI: 6.40, 31.37) .

In this study, there were 46 (48.9%) EOS cases and 48 (51.1%) LOS cases. After adjustment, factors that increased the risk of EOS included PROM > 18 hours, foul-smelling amniotic fluid, low birthweight, low 5th-minute Apgar score, and newborns receiving milk other than breast milk during the first days of life for more than 24 hours. By contrast, factors associated with a lower risk of EOS included a C-section birth and deliveries assisted by Ob/Gyn residents (Table 11). Conditions found to significantly increase the risk of LOS were elevated maternal leukocyte count, when newborns received non-invasive respiratory support, and when newborns received nothing orally during their first days of life for more than 24 hours.

Table 11. Crude and adjusted relative risk (95% CIs) for the association between maternal and neonatal risk factors with EOS and LOS compared to non-sepsis cases

Variable	EOS (n=46)			LOS (n=48)		
	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Maternal risk factors						
Maternal age group (%)						
< 20 years old	3 (6.5)	1.40 (0.37, 5.29)	--	3 (6.3)	1.98 (0.61, 6.37)	--
20–35 years old	32 (69.6)	Ref	--	33 (68.8)	Ref	--
> 35 years old	11 (23.9)	1.28 (0.64, 2.61)	--	12 (25.0)	1.39 (0.71, 2.73)	--

Table 11. (cont'd)

Variable	EOS (n=46)			LOS (n=48)		
	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Mother Education (%)						
Less than high school	20 (43.5)	2.09 (0.61, 7.18)	--	22 (45.8)	4.71 (0.88, 25.23)	3.89 (0.49, 31.13)
High school graduate	23 (50.0)	1.70 (0.50, 5.78)	--	25 (52.1)	3.78 (0.71, 20.42)	2.59 (0.32, 20.78)
College or higher	3 (6.5)	Ref	--	1 (2.1)	Ref	Ref
Spouse Education (%)						
Less than high school	17 (37.0)	0.76 (0.32, 1.82)	--	19 (39.6)	2.81 (0.73, 10.75)	--
High school graduate	21 (45.7)	0.55 (0.24, 1.29)	--	27 (56.3)	2.33 (0.62, 8.73)	--
College or higher	8 (17.4)	Ref	--	2 (4.2)	Ref	--
Person per household						
Less than 5	31 (80.4)	Ref	--	36 (75.0)	Ref	--
≥ 5	9 (19.6)	0.97 (0.52, 1.83)	--	12 (25.0)	0.69 (0.36, 1.33)	--
Household Income						
Above poverty level	37 (80.4)	Ref	--	40 (83.3)	Ref	Ref
Below poverty level	9 (19.6)	0.68 (0.32, 1.44)	--	8 (16.7)	0.56 (0.26, 1.22)	0.67 (0.24, 1.86)

Table 11. (cont'd)

Variable	EOS (n=46)			LOS (n=48)		
	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Household Income						
Smoke	0 (0)	--	--	0 (0)	--	--
Diabetes / Gestational Diabetes	0 (0)	1.92 (0.07, 51.21)	--	0 (0)	1.85 (0.07, 49.07)	--
Pre-pregnancy BMI						
Underweight	7 (15.2)	1.29 (0.55, 3.04)	--	7 (14.9)	1.29 (0.55, 3.04)	--
Normal weight	28 (60.9)	Ref	--	28 (66.7)	Ref	--
Overweight and obese	11 (23.9)	1.03 (0.50, 2.10)	--	12 (25.5)[b]	1.12 (0.56, 2.25)	--
Pregnancy weight gain						
Below recommendation	33(71.7)	2.46 (1.07, 5.66)	2.21 (0.82, 5.94)	32 (68.1)	1.86 (0.87, 3.96)	1.24 (0.51, 3.03)
Pregnancy weight gain						
Within recommendation	7 (15.2)	Ref	Ref	9 (19.2)	Ref	Ref
Above recommendation	6 (13.0)	1.40 (0.46, 4.26)	1.46 (0.40, 5.26)	6 (12.8)	1.10 (0.38, 3.13)	1.08 (0.34, 3.45)
Had at least one antenatal check-up	42 (91.3)	1.30 (0.45, 3.70)	--	43 (88.6)	1.06 (0.41, 2.75)	--

Table 11. (cont'd)

Variable	EOS (n=46)			LOS (n=48)		
	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Pregnancy weight gain						
Had ≥ 4 antenatal check-ups.	18 (39.1)	0.58 (0.32, 1.07)	0.69 (0.35, 1.35)	19 (39.6)	0.59 (0.33, 1.08)	0.57 (0.28, 1.17)
Parity						
< 5	45 (97.8)	Ref	--	48 (100)	Ref	--
≥ 5	1 (2.2)	1.26 (0.16, 9.82)		0 (0)	0.56 (0.03, 10.63)	
Vaginal exams ≥ 4	12 (26.1)	1.15 (0.58, 2.27)	--	4 (8.3)	0.30 (0.11, 0.84)	0.37 (0.12, 1.14)
Mode of delivery						
Vaginal birth	24 (52.2)	Ref	Ref	10 (20.8)	Ref	Ref
C-section	22 (47.8)	0.58 (0.32, 1.06)	0.15 (0.03, 0.73)	38 (79.2)	2.42 (1.19, 4.92)	9.13 (0.23, 355.63)
Birth assistant						
Midwife	18 (39.1)	1.50 (0.79, 2.83)	0.20 (0.04, 1.04)	9 (18.8)	0.60 (0.78, 1.29)	4.76 (0.12, 186.95)
Resident	4 (52.2)	0.25 (0.09, 0.73)	0.06 (0.01, 0.59)	9 (18.8)	0.45 (0.21, 0.96)	0.63 (0.13, 2.97)
Ob/Gyn	24 (8.7)	Ref	Ref	30 (62.5)	Ref	Ref
Pregnancy						
Singleton	16 (34.8)	Ref	--	47 (97.2)	Ref	
Twin	4 (8.7)	2.87 (0.96, 8.61)		1 (2.1)	0.64 (0.09, 4.83)	--

Table 11. (cont'd)

Variable	EOS (n=46)			LOS (n=48)		
	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Pregnancy						
PROM/PPROM	19 (41.3)	1.54 (0.81, 2.95)	--	15 (31.3)	1.60 (0.86, 3.02)	--
Rupture of membrane > 12 hours	9 (19.6)	2.10 (0.98, 4.51)	--	8 (16.7)	1.73 (0.78, 3.82)	--
Rupture of membrane > 18 hours	7 (15.2)	4.10 (1.71, 9.82)	2.73 (1.04, 7.16)	5 (10.4)	2.66 (0.99, 7.13)	2.43 (0.82, 7.27)
Antepartum hemorrhage	0 (0)	1.33 (0.06, 30.17)	--	1 (2.1)	3.91 (0.57, 26.67)	--
Foul-smelling amniotic fluid	14 (30.4)	3.83 (1.40, 7.47)	2.26 (1.05, 4.86)	10 (20.9)	2.31 (1.11, 4.80)	1.83 (0.80, 4.21)
Maternal leukocyte count >15000/mm^{3*}	12 (29.27)[a]	1.51 (0.75, 3.05)	1.08 (0.50, 2.30)	18 (20.8)[c]	3.14 (1.62, 6.05)	2.59 (1.29, 5.19)
Intrapartum temperature ≥ 38ºC	0 (0)	1.15 (0.05, 24.93)	1.05 (0.03, 33.52)	0 (0)	1.11 (0.05, 23.89)	--
Neonatal risk factor						
Gender						
Male	25 (54.4)	Ref	--	30 (62.5)	Ref	Ref
Female	21 (45.7)	0.82 (0.45, 1.50)		18 (37.5)	0.59 (0.33, 1.07)	0.60 (0.29, 1.23)

Table 11. (cont'd)

Variable	EOS (n=46)			LOS (n=48)		
	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Gestational age						
< 32 weeks	3 (6.5)	2.60 (0.75, 9.11)	--	5 (10.4)	7.31 (2.50, 21.44)	--
32 – 36 weeks	16 (34.8)	2.48 (1.30, 4.72)	--	27 (56.3)	7.06 (3.71, 13.44)	--
≥ 37 weeks	27 (58.7)	Ref	--	16 (33.3)	Ref	--
Prematurity	19 (41.3)	2.50 (1.36, 4.60)	0.65 (0.25, 1.71)	32 (66.7)	7.10 (3.81, 13.24)	2.73 (0.98, 7.62)
Birthweight						
< 1000 grams	1 (2.2)	10.78 (1.07, 108.26)	--	1 (2.1)	13.48 (1.33, 136.73)	--
1000 – 1499 grams	2 (4.4)	4.04 (0.87, 18.78)	--	10 (20.8)	25.27 (9.94, 64.24)	--
1500 – 2499 grams	23 (50.0)	5.17 (2.76, 9.66)	--	21 (43.8)	5.90 (3.00, 11.58)	--
> 2500 grams	20 (43.5)	Ref		16 (33.3)	Ref	--
Low birth weight	26 (56.5)	5.16 (2.81, 9.48)	4.04 (1.58, 10.38)	32 (66.7)	7.94 (4.26, 14.82)	2.65 (0.94, 7.47)
Early initiation of breastfeeding	4 (8.7)	0.21 (0.08, 0.60)	1.15 (0.33, 4.07)	4 (8.3)	0.20 (0.07, 0.57)	1.28 (0.36, 4.51)
Apgar score in 1st minute						
< 7	16 (34.8)	4.38 (2.29, 8.35)	0.93 (0.38, 2.28)	22 (45.8)	6.94 (3.77, 12.77)	1.44 (0.62, 3.36)
≥ 7	30 (65.2)	Ref	Ref	26 (54.2)	Ref	

Table 11. (cont'd)

Variable	EOS (n=46)			LOS (n=48)		
	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Apgar score in 5th minute						
< 7	12 (26.1)	6.98 (3.35, 14.52)	2.84 (1.00, 8.08)	12 (25.0)	6.59 (3.18, 13.65)	1.76 (0.67, 4.59)
≥ 7	34 (73.9)	Ref	Ref	36 (75.0)	Ref	Ref
Respiratory support						
None	17 (37.0)	Ref	Ref	16 (33.3)	Ref	Ref
Non-invasive	27 (58.7)	11.71 (6.16, 22.28)	1.59 (0.50, 5.10)	6 (12.5)	16.86 (8.68, 32.72)	2.70 (1.02, 7.18)
Invasive	2 (4.4)	13.88 (2.61, 73.82)	0.86 (0.08, 8.81)	3 (6.3)	16.86 (3.12, 90.94)	1.48 (0.16, 13.20)
First 24 hours of life oral intake						
Breast milk	11 (23.9)	Ref	Ref	16 (33.3)	Ref	Ref
Breastmilk + infant formula	9 (19.6)	3.34 (1.37, 8.24)	4.10 (1.47, 11.42)	6 (12.5)	1.54 (0.59, 3.99)	2.66 (0.88, 7.98)
Infant formula	3 (6.5)	21.10 (4.93, 90.38)	9.54 (1.74, 52.27)	3 (6.3)	14.51 (3.52, 59.82)	4.78 (0.92, 24.80)
Nothing per oral	23 (50.0)	40.45 (18.15, 90.15)	20.22 (6.51, 62.82)	23 (47.2)	27.81 (13.40, 57.72)	12.05 (4.34, 31.34)

[a] missing data 5, [b]missing data 1, [c]missing data 9

Among EOS cases, 11 (23.9%) were culture-proven sepsis, and coagulase-negative staphylococci (CoNS) was the most common pathogen (6/11). Among LOS cases, 13 (29.2%) were culture-proven, with *K. pneumoniae* the leading cause (6/14), followed by CoNS (5/11). Among 25 positive blood cultures, 13 were gram-positive bacteria, and 12 were gram-negative

bacteria. The gram-negative pathogen was more predominant in LOS compared to EOS cases (57.1% and 36.4%, respectively). Nineteen of 25 (76%) isolated pathogens were multi-drug resistant (MDR). In this study, MDR pathogens were more frequently found in EOS cases and gram-positive neonatal sepsis cases (81.8% and 64.3%, 81% and 75%, respectively) (Table 12).

Table 12. Isolated pathogens in culture-proven neonatal sepsis (n = 25)

Isolated pathogen	EOS (MDR) n = 11	LOS (MDR) n = 14
Gram Negative		
Acinetobacter baumanii	1 (1)	1 (1)
Enterobacter spp.	1 (1)	1 (1)
Klebsiella pneumoniae	2 (2)	6 (3)
Gram Positive		
Coagulase-negative staphylococci (CoNS)	6 (4)	5 (3)
Staphylococcus aureus	1 (1)	1 (1)

Table 13 shows the risk factors of culture-proven and culture-negative neonatal sepsis. An adjusted analysis showed that younger maternal age, antepartum hemorrhage, foul-smelling amniotic fluid, low birth weight, a low 5th-minute Apgar score, receiving respiratory support, and receiving oral intake other than breast milk were significantly associated with pathogen growth in the blood culture. After adjustment, multiple pregnancies, foul-smelling amniotic fluid, elevated maternal leukocyte count, and newborns receiving other than breast milk during the first 24 hours of life were associated with culture-negative sepsis.

Table 13. Crude and adjusted odds ratio (95% CIs) for the association between maternal and neonatal risk factors with culture-proven sepsis

Variable	Culture-proven sepsis (n=25)			Culture-negative sepsis (n=69)		
	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Maternal risk factors						
Maternal age group (%)						
< 20 years old	3 (12.0)	3.58 (1.07, 12.00)	4.29 (1.23, 14.94)	3 (4.4)	1.22 (0.36, 4.15)	--
20–35 years old	18 (72.0)	Ref	Ref	47 (68.1)	Ref	--
> 35 years old	4 (16.0)	0.91 (0.32, 2.59)	0.82 (0.29, 2.31)	19 (27.5)	1.51 (0.86, 2.65)	--
Mother Education (%)						
Less than high school	15 (60.0)	9.74 (0.57, 166.68)	5.35 (0.37, 78.12)	27 (39.1)	2.11 (0.72, 6.20)	--
High school graduate	10 (50.0)	4.67 (0.27, 81.50)	3.60 (0.29, 2.31)	38 (53.6)	2.10 (0.73, 6.04)	--
College or higher	0 (0)	Ref	Ref	4 (5.8)	Ref	--
Spouse Education (%)						
Less than high school	11 (44.0)	1.18 (0.35, 4.02)	--	25 (36.2)	1.28 (0.54, 3.06)	--
High school graduate	11 (44.0)	0.70 (0.21, 2.36)	--	47 (53.6)	1.12 (0.48, 2.58)	--
College or higher	3 (12.0)	Ref	--	7 (10.1)	Ref	--
Person per household						
Less than 5	19 (76.0)	Ref	--	48 (69.6)	Ref	--
≥ 5	6 (24.0)	0.67 (0.27, 1.65)	--	21 (30.4)	0.88 (0.52, 1.50)	--

Table 13. (cont'd)

Variable	Culture-proven sepsis (n=25)			Culture-negative sepsis (n=69)		
	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	Frequency (%)	Crude RR (95% CI)	Adjusted RR (95% CI)
Household Income						
Above poverty level	20 (80.0)	Ref	--	57 (82.6)	Ref	Ref
Below poverty level	5 (20.0)	0.75 (0.30, 1.95)	--	12 (17.4)	0.59 (0.31, 1.12)	0.74 (0.33, 1.66)
Smoke	0 (0)	--	--	0 (0)	--	--
Diabetes / Gestational Diabetes	0 (0)	3.52 (0.13, 64.35)	--	0 (0)	1.29 (0.05, 34.14)	--
Pre-pregnancy BMI						
Underweight	2 (8.3)	1.20 (0.31, 4.69)	0.82 (0.22, 3.02)	12 (17.4)	1.51 (0.77, 2.98)	--
Normal weight	15 (62.5)	Ref	Ref	41 (59.4)	Ref	--
Overweight and obese	7 (29.2)	1.52 (0.35, 6.53)	1.25 (0.52, 3.03)	16 (23.2)	1.02 (0.56, 1.86)	--
Missing	1					
Pregnancy weight gain						
Below recommendation	18 (75.0)	2.77 (0.87, 8.79)	--	47 (68.1)	0.53 (0.28, 1.00)	1.41 (0.69, 2.86)
Within recommendation	3 (12.5)	Ref	--	13 (18.8)	Ref	Ref
Above recommendation	3 (12.5)	1.63 (0.36, 7.32)	--	9 (13.0)	0.60 (0.29, 1.25)	0.96 (0.36, 2.51)
Missing	1					
Had at least one antenatal check-up	21 (84.0)	0.59 (0.21, 1.68)	--	64 (92.8)	1.58 (0.62, 4.03)	--
Had antenatal ≥ 4 check-ups.	8 (32.0)	0.44 (0.19, 1.01)	0.59 (0.27, 1.31)	29 (42.0)	0.66 (0.40, 1.08)	0.72 (0.41, 1.26)
Parity						
< 5	25 (100.0)	Ref	--	68 (98.6)	Ref	--
≥ 5	0 (0)	1.08 (0.06, 20.47)	--	1 (1.5)	0.84 (0.11, 6.45)	

Table 13. (cont'd)

Variable	Culture-proven sepsis (n=25)			Culture-negative sepsis (n=69)		
	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Parity						
Vaginal exams ≥ 4	3 (12.0)	0.51 (0.16, 1.59)	--	13 (18.8)	0.76 (0.41, 1.42)	--
Mode of delivery						
Vaginal birth	12 (48.0)	Ref	--	22 (31.9)	Ref	--
C-section	13 (52.0)	0.69 (0.31, 1.51)	--	47 (68.1)	1.36 (0.80, 2.30)	
Birth assistant						
Midwife	10 (40.0)	1.82 (0.77, 4.28)	1.19 (0.50, 2.82)	17 (24.6)	0.79 (0.44, 1.42)	0.61 (0.35, 1.23)
Resident	11 (44.0)	0.58 (0.19, 1.76)	0.51 (0.15, 1.74)	9 (13.0)	0.31 (0.15, 0.65)	0.29 (0.09, 1.00)
Ob/Gyn	4 (16.0)	Ref	Ref	43 (62.3)	Ref	Ref
Pregnancy						
Singleton	25 (100.0)	Ref	--	64 (92.8)	Ref	Ref
Twin	0 (0)	0.58 (0.03, 10.32)		5 (7.3)	2.36 (0.88, 6.34)	3.02 (1.04, 8.76)
PROM/PPROM	10 (40.0)	2.38 (1.09, 5.32)	1.79 (0.77, 4.17)	19 (24.6)	1.34 (0.77, 2.33)	--
Rupture of membrane > 12 hours	5 (20.0)	2.31 (0.87, 6.10)	--	12 (17.4)	1.82 (0.94, 3.53)	--
Rupture of membrane > 18 hours	3 (12.0)	3.50 (1.06, 11.51)	--	9 (13.0)	3.42 (1.57, 7.47)	0.93 (0.43, 2.00)
Antepartum hemorrhage	1 (4.0)	7.58 (1.09, 52.73)	25.03 (2.84, 220.59)	0 (0)	0.89 (0.04, 20.10)	--
Foul-smelling amniotic fluid	7 (28.0)	3.53 (1.46, 8.53)	2.55 (1.02 6.36)	17 (24.6)	2.86 (1.58, 5.18)	2.17 (1.08, 4.35)

Table 13. (cont'd)

Variable	Culture-proven sepsis (n=25)			Culture-negative sepsis (n=69)		
	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Maternal leukocyte count >15000/mm^{3*}	6 (30.0)[a]	1.64 (0.64, 4.22)	--	24 (40.0)[b]	2.44(1.41, 4.23)	2.11 (1.18, 3.79)
Intrapartum temperature ≥ 38ºC	0 (0)	2.10 (0.10, 45.97)	--	0 (0)	1.30 (0.06, 27.89)	--
Neonatal risk factor						
Gender						
Male	17 (68.0)	Ref	Ref	38 (55.1)	Ref	--
Female	8 (32.0)	0.48 (0.21, 1.09)	0.45 (0.17, 1.22)	31 (45.0)	0.80 (0.49, 1.31)	--
Gestational age						
< 32 weeks	4 (16.0)	9.00 (2.80, 28.96)	--	4 (5.8)	2.93 (0.97, 8.87)	--
32 – 36 weeks	10 (40.0)	3.81 (1.62, 8.98)	--	33 (47.8)	4.32 (2.57, 7.24)	--
≥ 37 weeks	11 (44.0)	Ref	--	32 (46.4)	Ref	--
Prematurity	14 (56.0)	4.47 (2.02, 9.87)	0.53 (0.16, 1.77)	37 (53.6)	4.11 (2.49, 6.78)	1.84 (0.81, 4.19)
Birthweight						
< 1000 grams	0 (0)	1.33 (0.04, 42.46)	--	2 (2.9)	13.91 (2.24, 86.32)	--
1000 – 1499 grams	5 (20.0)	3.11 (1.02, 9.51)	--	7 (10.1)	9.13 (3.50, 23.81)	--
1500 – 2499 grams	15 (60.0)	0.08 (0.03, 0.21)	--	29 (42.0)	4.20 (2.46, 7.20)	--
> 2500 grams	5 (20.0)	Ref		31 (44.9)	Ref	--
Low birth weight	20 (80.0)	14.76 (5.67, 38.47)	16.19 (4.24, 61.86)	38 (55.1)	4.87 (2.94, 8.06)	1.95 (0.85, 4.51)
Early initiation of breastfeeding	2 (8.0)	0.24 (0.06, 0.89)	3.89 (0.70, 21.55)	6 (8.7)	0.21 (0.09, 0.50)	0.85 (0.31, 2.30)

Table 13. (cont'd)

Variable	Culture-proven sepsis (n=25)			Culture-negative sepsis (n=69)		
	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	Frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Apgar score in 1st minute						
< 7	13 (52.0)	8.81 (3.95, 19.67)	1.15 (0.37, 3.54)	25 (36.2)	4.66 (2.72, 7.99)	1.80 (0.76, 4.25)
≥ 7	12 (48.0)	Ref	Ref	44 (63.8)	Ref	Ref
Apgar score in 5th minute						
< 7	8 (32.0)	9.49 (3.91, 23.02)	4.61 (1.26, 16.89)	16 (23.2)	5.97 (3.13, 11.38)	1.79 (0.76, 4.25)
≥ 7	17 (68.0)	Ref	Ref	53 (76.8)	Ref	Ref
Respiratory support						
None	4 (16.0)	Ref	Ref	27 (39.1)	Ref	Ref
Non-invasive	20 (80.0)	33.45 (11.78, 94.99)	7.07 (1.69, 29.58)	39 (56.5)	10.65 (6.24, 18.19)	1.58 (0.66, 3.81)
Invasive	1 (4.0)	36.33 (4.39, 300.40)	4.61 (1.26, 16.89)	3 (4.4)	13.11 (3.11, 55.24)	1.08 (0.16, 7.14)
First 24 hours of life oral intake						
Breast milk	6 (24.0)	Ref	Ref	21 (30.4)	Ref	Ref
Breastmilk + infant formula	3 (12.0)	2.21 (0.59, 8.20)	7.48 (1.51, 36.92)	12 (17.4)	2.34 (1.13, 4.87)	2.70 (1.20, 6.06)
Infant formula	1 (4.0)	16.82 (2.31, 122.60)	2.32 (0.20, 27.03)	5 (7.3)	18.42 (5.55, 61.10)	9.70 (2.48, 37.87)
Nothing per oral	15 (60.0)	45.45 (16.96, 121.80)	15.34 (4.51, 52.21)	31 (44.9)	28.55 (14.79, 55.14)	14.07 (5.78, 34.21)

In a subgroup analysis that included sepsis cases only, unadjusted logistic regression showed that culture-negative sepsis was associated with low birth weight (OR 3.26, 95% CI: 1.1–9.70). After adjustment, the point estimate remained high, but the association was no longer statistically significant (OR 2.8, 95% CI: 0.92–8.67). Culture-negative sepsis was not associated with other maternal and neonatal factors, including the level of care and study site differences.

4.3.1 Maternal and postnatal antibiotic prophylaxis exposure and neonatal sepsis

Of the 904 deliveries, 519 (57.5%) mothers received antibiotic prophylaxis before delivery, and 394 (43.6%) neonates received postnatal antibiotic prophylaxis for early neonatal infection. Overall, among the 904 neonates, 651 (72.0%) were exposed to at least one type of antibiotic prophylaxis. Of those 651 neonates, 257 (29.9%) were exposed to maternal antibiotic prophylaxis only, 132 (14.6%) received postnatal antibiotic prophylaxis, and 262 (28.9%) were exposed to both antibiotic prophylaxes. The maternal and newborn characteristics "exposed" and "not exposed to perinatal antibiotic prophylaxes" are shown in Table 14.

Table 14. Maternal and newborn characteristics based on perinatal antibiotic exposure (n = 904)

Variable	Antibiotic prophylaxis (+)	Antibiotic prophylaxis (-)	*p*-value	Crude RR (95% CI)	Adjusted RR (95% CI)
	n=651	n = 253			
Sociodemographic and maternal factors					
Maternal age group (%)					
< 20 years old	26 (4.0)	12 (4.7)	0.39	0.79 (0.39, 1.61)	--
20–35 years old	497 (76.3)	182 (71.9)		Ref	
> 35 years old	128 (19.7)	59 (23.3)		0.79 (0.56, 1.13)	

Table 14. (cont'd)

Variable	Antibiotic prophylaxis (+)	Antibiotic prophylaxis (-)	*p*-value	Crude RR (95% CI)	Adjusted RR (95% CI)
	n=651	n = 253			
Mother Education (%)					
Less than high school	286 (43.9)	53 (21.0)	<0.0001	3.95 (2.40, 6.50)	2.35 (1.16, 4.75)
High school graduate	309 (47.5)	159 (62.9)		1.42 (0.91, 2.22)	1.24 (0.69, 2.23)
College or higher	56 (8.6)	41 (16.2)		Ref	Ref
Spouse Education (%)					
Less than high school	253 (38.9)	48 (19.0)	<0.0001	3.95 (2.41, 6.48)	1.74 (0.87, 3.47)
High school graduate	338 (51.9)	160 (63.2)		1.58 (1.03, 2.44)	1.38 (0.78, 2.42)
College or higher	60 (9.2)	45 (17.8)		Ref	Ref
Person per household					
Less than 5	440 (67.6)	168 (66.4)	0.73	Ref	--
≥ 5	211 (32.4)	85 (33.6)		0.95 (0.70, 1.29)	
Household Income					
Above poverty level	504 (77.4)	170 (67.2)	0.002	Ref	Ref
Below poverty level	147 (22.6)	83 (32.8)		0.60 (0.43, 0.82)	0.69 (0.46, 1.03)
Smoke	0 (0)	0 (0)	--	--	--
Diabetes/Gestational Diabetes	4 (0.6)	0 (0)	0.58	3.53 (0.13, 93.05)	--
Pre-pregnancy BMI					
Underweight	87 (13.4)	26 (10.3)	0.01	1.54 (0.96, 2.47)	1.19 (0.79, 1.73)

Table 14. (cont'd)

Variable	Antibiotic prophylaxis (+)	Antibiotic prophylaxis (-)	*p*-value	Crude RR (95% CI)	Adjusted RR (95% CI)
	n=651	n = 253			
Pre-pregnancy BMI					
Normal weight	389 (60.3)	179 (71.0)		Ref	Ref
Missing	3	1			
Overweight and obese	172 (26.5)	47 (18.7)		1.68 (1.17, 2.43)	1.50 (0.97, 2.32)
Pregnancy weight gain					
Below recommendation	380 (58.6)	123 (48.8)	0.02	1.61 (1.20, 3.76)	1.17 (0.79, 1.73)
Within recommendation	161 (24.9)	84 (33.3)		Ref	Ref
Above recommendation	107 (16.5)	45 (17.9)		1.24 (0.80, 1.92)	0.89 (0.53, 1.48)
Missing	3	1			
Had at least one antenatal check-up	581 (89.3)	225 (88.9)	0.89	1.03 (0.65, 1.64)	--
Had ≥ 4 antenatal check-ups.	316 (48.5)	146 (57.7)	0.01	0.69 (0.52, 0.93)	0.87 (0.62, 1.22)
Birth assistant					
Midwife	172 (26.4)	42 (16.6)	<0.0001	0.59 (0.38, 0.92)	0.47 (0.23, 0.75)
Resident	373 (57.3)	54 (21.3)		0.10 (0.07, 0.14)	0.12 (0.08, 0.18)
Ob/Gyn	106 (16.3)	157 (62.1)		Ref	Ref
Obstetrics and neonatal factors					
Parity					
< 5	636 (97.7)	253 (100)	0.10	Ref	Ref
≥ 5	15 (2.3)	0 (0)		12.37 (0.67, 228.13)	4.75 (0.22, 104.59)
Vaginal exams ≥ 4	142 (21.8)	64 (25.3)	0.26	0.82 (0.29, 1.16)	--
Mode of delivery					
Vaginal birth	217 (33.3)	132 (52.2)	<0.0001	Ref	Ref
C-section	434 (66.7)	121 (47.8)		2.18 (1.62, 2.93)	2.92 (1.95, 4.38)

Table 14. (cont'd)

Variable	Antibiotic prophylaxis (+)	Antibiotic prophylaxis (-)	*p*-value	Crude RR (95% CI)	Adjusted RR (95% CI)
	n=651	n = 253			
Pregnancy					
Singleton	452 (69.4)	250 (98.8)	0.02	Ref	Ref
Twin	28 (4.3)	3 (1.2)		3.74 (1.13, 12.41)	2.71 (0.63, 11.75)
PROM/PPROM	199 (30.6)	9 (3.6)	< 0.0001	11.93 (6.01, 23.68)	12.29 (5.41, 27.91)
Rupture of membrane > 12 hours	552 (84.8)	2 (0.8)	< 0.0001	22.5 (5.51, 91.95)	--
Antepartum hemorrhage	7 (1.1)	0 (0)	0.2	5.90 (0.28, 126.10)	3.58 (0.12, 104.57)
Foul-smelling amniotic fluid	102 (15.7)	5 (2.0)	< 0.0001	9.21 (3.70, 22.89)	4.69 (1.82, 12.05)
Maternal leukocyte count >15000/mm^3*	141 (25.1)[a]	24 (16.3)[b]	0.03	1.72 (1.07, 2.77)	1.16 (0.69, 1.96)
Intrapartum temperature ≥ 38ºC	7 (1.1)	0 (0)	0.2	5.90 (0.28, 126.10)	3.45 (0.09, 136.80)
Gender					
Male	331 (49.2)	125 (49.4)	0.70	Ref	--
Female	320 (50.8)	128 (50.6)		0.94 (0.71, 1.26)	
Gestational age					
< 32 weeks	31 (4.8)	4 (1.6)	0.08	3.15 (3.15, 9.03)	0.53 (0.13, 2.24)
32 – 36 weeks	140 (21.5)	54 (21.3)		1.05 (0.74, 1.50)	0.88 (0.48, 1.61)
≥ 37 weeks	480 (73.7)	195 (77.1)		Ref	Ref

Table 14. (cont'd)

Variable	Antibiotic prophylaxis (+) n=651	Antibiotic prophylaxis (-) n = 253	*p*-value	Crude RR (95% CI)	Adjusted RR (95% CI)
Gestational age					
Prematurity	171 (26.3)	58 (22.9)	0.31	1.20 (0.85, 1.69)	--
Birthweight					
< 1000 grams	5 (0.8)	0 (0)	0.01	4.73 (0.20, 113.33)	2.03 (0.04, 100.26)
1000 – 1499 grams	26 (4.0)	2 (0.8)		4.55 (1.20, 17.21)	2.40 (0.32, 17.79)
1500 – 2499 grams	142 (21.8)	46 (18.2)		1.32 (0.91, 1.91)	0.84 (0.45, 1.60)
> 2500 grams	478 (73.4)	205 (81.0)		Ref	Ref
Low birth weight	173 (26.6)	48 (19.0)	0.02	1.54 (1.07, 2.20)	--
Apgar score in 1st minute					
< 7	109 (16.7)	17 (6.7)	< 0.001	2.73 (1.61, 4.63)	2.96 (1.21, 7.25)
≥ 7	542 (83.3)	236 (93.3)		Ref	Ref
Apgar score in 5th minute					
< 7	56 (8.6)	7 (2.8)	0.002	3.12 (1.43, 6.82)	0.42 (0.14, 1.27)
≥ 7	595 (91.4)	246 (97.3)		Ref	Ref

[a]missing data 89, [b]missing data 106

Per the local guidelines, the frequency of maternal and neonate conditions that were considered indications for prophylactic antibiotics are listed in Table 15. The types of antibiotics used for maternal antibiotic prophylaxis included ceftriaxone (83.8%), ampicillin (9.4%), cefazoline (5.8%), and others (1%). For postnatal prophylaxis, the types of antibiotics included gentamycin (22.6%) and ampicillin combined with gentamycin (77.4%).

Table 15. Indications for prophylactic antibiotic use*

	Frequency
Maternal (n=519)	
C-section	358
PROM/PPROM	199
Intrapartum temperature ≥ 38° C	6
Leukocyte count > 15,000/mm^3	109
Antepartum hemorrhage	6
No documented indication for antibiotic prophylaxis	54
Neonate (n=394)	
Foul-smell amniotic fluid	98
Rupture of membrane ≥ 12 hours	90
Intrapartum temperature ≥ 38° C	6
Maternal leukocyte count >15000/mm^3	130
No documented indication for antibiotic prophylaxis	173

*Subjects may have more than one indication.

The incidence of neonatal sepsis in newborns exposed to perinatal prophylaxis was 13.8 per 100 live births, which was higher than the incidence of neonatal sepsis in newborns who were not exposed to perinatal prophylaxis (1.6 per 100 live births, $p < 0.001$). The incidence among those exposed by the mother only was 1.6 per 100 live births. It was 29.6 per 100 live births among those who received postnatal prophylaxis and 17.9 per 100 live births among those exposed via both treatment avenues. Based on sepsis onset, the incidence of EOS in newborn who were exposed to perinatal antibiotic prophylaxis was 6.61 per 100 live birth; while the incidence in unexposed newborns were 1.19 per 100 live birth. On the other hand, the incidence of LOS in perinatal antibiotic prophylaxis exposed newborn were 7.22 per 100 live birth. The incidence of LOS in the unexposed newborns were 0.40 per 100 live birth.

4.3.2 Average treatment effect of perinatal antibiotic prophylaxis

In this study, factors that were significantly associated with prophylactic antibiotic administration (treatment assignment) included maternal education level, birth attendant, mode of delivery, PROM, foul-smelling amniotic fluid, and 1st-minute Apgar score (Table 14). For the prediction of treatment assignment (propensity score), all indications for prophylactic antibiotics listed in the local guidelines and factors that were significantly associated with the outcomes were considered for the IPW calculation.

In the group where the newborns were exposed to any type of perinatal antibiotic prophylaxis, 10% more newborns developed sepsis, and the coefficient was statistically significant, controlling for other covariates. For EOS, the ATE estimate was -0.05, indicating that giving prophylactic antibiotics is, on average, effective in preventing the development of EOS compared to not giving prophylactic antibiotics. However, in LOS, all types of prophylactic antibiotics, on average, increased the incidence of LOS. For both culture-proven and culture-negative sepsis, the estimated ATE also indicated that prophylactic antibiotics increased the risk of sepsis. The ATEs of each treatment and outcome are reported in Table 16.

Table 16. Average treatment effect of perinatal antibiotic prophylaxis for neonatal sepsis

	ATE	95% CI	*p-value*
Sepsis			
ATE of perinatal exposure	0.10	0.06 to 0.13	< 0.0001
Maternal	0.01	-0.03 to 0.05	0.59
Postnatal	0.14	0.03 to 0.26	0.01
Maternal + Postnatal	0.16	0.10 to 0.22	< 0.0001
Early-onset sepsis			
ATE of perinatal exposure	-0.05	-0.08 to -0.01	0.02
Maternal	-0.01	-0.03 to 0.01	0.28
Postnatal	0.01	-0.07 to 0.08	0.87
Maternal + Postnatal	-0.04	-0.09 to 0.01	0.16

Table 16. (cont'd)

	ATE	95% CI	*p-value*
Late-onset sepsis			
ATE of perinatal exposure	0.06	0.04 to 0.09	< 0.0001
Maternal	0.01	-0.02 to 0.04	0.41
Postnatal	0.17	0.08 to 0.26	0.0003
Maternal + Postnatal	0.08	0.04 to 0.12	<0.0001
Culture-proven sepsis			
ATE of perinatal exposure	0.04	0.02 to 0.05	< 0.0001
Maternal	0.01	-0.003 to 0.02	0.18
Postnatal	0.05	0.01 to 0.09	0.02
Maternal + Postnatal	0.04	0.02 to 0.06	0.0004
Culture-negative sepsis			
ATE of perinatal exposure	0.06	0.02 to 0.10	0.001
Maternal	- 0.01	- 0.05 to 0.03	0.62
Postnatal	0.19	0.07 to 0.30	0.001
Maternal + Postnatal	0.13	0.06 to 0.19	0.001

In our study, 197 mother–newborn pairs have no documented indications of antibiotic prophylaxis based on the local guidelines. Overall, 41.6% of the neonates were exposed to at least one type of antibiotic prophylaxis. Of that 41.6%, 22.8% were exposed to maternal prophylaxis, 16.2% were given postnatal antibiotic prophylaxis, and 2.4% were exposed to both. In this subgroup, 12 newborns developed sepsis, 8 developed EOS, and 4 developed LOS. Further analysis found that preintervention factors associated with sepsis were prematurity, the presence of a birth attendant, multiple pregnancies, low birth weight, the mother having had at least four antenatal check-ups, and gender. After including these pre-intervention factors in the propensity score model, the ATE estimate was 0.09 (95% CI: 0.02–0.16, $p = 0.02$), which indicated that perinatal antibiotic exposure in average increased the probability neonatal sepsis development by 9%. For LOS, the ATE estimate was 0.04 (95% CI: 0.01–0.08, $p = 0.02$), while for EOS, the ATE estimate was 0.07, but it was not statistically significant ($p = 0.07$).

4.4 Discussion

At present, Indonesia lacks national data on neonatal sepsis incidence. Like other low- and middle-income countries, in Indonesia, most available data are on the prevalence of neonatal sepsis admissions and come from single-center, typically retrospective studies. Several studies have reported that the neonatal admission prevalence in some hospitals in Indonesia, including one of the sites in this study, varies between 5% and 25% (11–13). Studies from other LMICs in Asia have shown admission prevalence ranging from 20.5% to 45.9% (9,10). This study found that the incidence of neonatal sepsis during the study was 10.4% of delivery admission. In 2016, a large prospective study in India reported that the incidence of neonatal sepsis was 14.3% of neonatal intensive care unit (NICU) admissions (16). This number is much higher than what has been reported by most HICs (6–9 per 1,000 live births), affirming that neonatal sepsis continues to be a significant health problem in low- and middle-income countries (7).

The proportions of EOS and LOS in this study were similar (48.9% and 51.1%, respectively). Previous studies have shown a higher EOS burden in low- and middle-income countries than in high-income countries (127,128). Multiple strategies that have been implemented in labor and delivery to prevent infections, including prophylactic antibiotics, have significantly reduced the incidence of EOS in high-income countries; however, in low- and middle-income countries, the incidence of EOS remains high. EOS is frequently associated with colonization of the newborn by vertical transmission from the maternal genital tract, unhygienic birth practices during labor, and ultra-early horizontal transmission from the delivery room or neonatal care units. These problems are more common in LMICs (7,129). LOS tends to reflect community or nosocomial infection and is strongly associated with infant prematurity. Improvements in premature infant survival due to advances in neonatal intensive care in low-

income countries have led to increases in LOS incidence (7,129,130). The comparable proportion of LOS and EOS observed in this study may indicate that basic obstetric practices aimed at preventing vertical infection from mothers to newborns are still inadequate, despite improvements in overall neonatal care.

Among 94 cases of neonatal sepsis, the proportion of culture-proven sepsis in this study was 26.6% (incidence 2.8 per 100 live births). In a study in India, the incidence of culture-proven sepsis was 6.2% of NICU admissions (16). In most high-income countries, the incidence of culture-confirmed neonatal sepsis has been around 0.4–0.8 cases per 1,000 live-born term infants, with a range of culture-proven versus culture-negative sepsis ratios between 1:6 and 1:12 (18). The reasons for the large proportion of culture-negative sepsis cases remain unclear. The low blood volume obtained from newborns and a low level of bacteremia are likely reasons. Anaerobic blood cultures are not routinely performed in most hospitals. In addition, blood cultures obtained after antibiotic initiation in newborns and maternal antibiotic treatment before and during delivery are also possible explanations because they mask the detection of bacteremia in newborns. Conventional microbiological methods may frequently fail to identify pathogens because of technical issues or intrinsic traits that limit sepsis detection. Although new diagnostic approaches such as the use of metagenomics have been developed to replace conventional methods, implementation in LMICs will be challenging due to the lack of resources (42,137,187). In addition, it is hard to know how these more sophisticated tools can differentiate between the presences of pathogen signatures and disease. However, the possibility of sepsis over-diagnosis among non-infected infants still needs to be considered as one of the explanations for the high rate of culture-negative sepsis.

In high-income countries, the most common causes of both EOS and LOS are GBS and *Escherichia coli* (*E. coli*) (188,189). However, this study found that, overall, the predominant pathogen was CoNS. Based on sepsis onset, CoNS was the leading cause of EOS and *K. pneumoniae* in LOS. Studies from other low- and middle-income countries, such as Brazil, Peru, Egypt, and India, also reported that CoNS is the leading cause of sepsis (10,71,138,139). A 14-year study from Turkey reported that 64.4% of neonatal sepsis cases were caused by CoNS (43). While the determination of CoNS as a true pathogen or contamination in neonatal sepsis is still debatable, consistent findings from multiple studies that reported CoNS as the most causative pathogen in neonatal sepsis cases should indicate that CoNS has an important role in neonatal sepsis. CoNS infection should be considered if the case is supported by clinical sepsis signs and other abnormal laboratory findings. Consistent with previous studies from LMICs reporting that GBS infections were rarely found, this study also did not detect any GBS neonatal sepsis cases (7,16). However, given that this study has a high rate of culture-negative sepsis cases and maternal GBS culture screening was not performed in the sites, a definite causative agent comparison between countries may have masked the true bacteriological profile. Therefore, interpreting these results should be done cautiously.

This study's results are consistent with the high degree of antimicrobial resistance pathogen documented in previous studies from other low- and middle-income countries (9,16). In high-income countries, MDR neonatal sepsis accounts for less than 20% of cases, whereas this proportion can reach 40%–80% in low-income countries (16,140–142). Factors that were reportedly responsible for the surge in MDR in LMICs include empirical antibiotics and IAP overuse, the non-existence of antibiotic prescription guidelines, over-the-counter sales of

antibiotics, poor sanitary conditions, a lack of basic facilities and practices, and a lack of surveillance regarding organisms that cause infections (16,146,147).

In general, this study found that PROM for > 18 hours, foul-smelling amniotic fluid, a maternal leukocyte count > 15,000/mm^3, low birth weight, and newborns receiving other than exclusive breast milk during their first days of life were factors that increased the development of sepsis. Foul-smelling amniotic fluid and a high maternal leukocyte count were documented by the WHO and the local prescribing guidelines as risk factors for neonatal infections (44,167). Rupture of the membrane is commonly associated with the occurrence of neonatal sepsis due to the threat of ascending infection (190–192). This study showed that the association was stronger with a longer duration of the membrane rupture.

Prior studies have identified low birth weight as a risk factor for neonatal sepsis (190,193). This study found that newborns with a birth weight of less than 2,500 grams were 3 times more likely to develop sepsis than newborns weighing at least 2,500 grams. Newborns with low birth weight are prone to heat loss and have a low store of glucose, which leads to a higher risk of developing hypoglycemia and thus the likelihood of infection. In addition, most low-birth-weight newborns who are premature have immature immune systems and have not received transplacental acquired maternal immunoglobulin G antibodies or have it in a lower level than term newborns(194). Preterm and low-birth-weight newborns also often require prolonged hospitalization with intravenous access, respiratory support, and other invasive procedures that provide entry or impair the barrier mechanism, which increases the risk of infection (15,195). Although not statistically significant, this study found that premature newborns were more likely to develop sepsis, mostly LOS.

In terms of the association between breast milk and neonatal sepsis, it has been suggested that breast milk protects newborns from infection, mostly late-onset infection (196,197). Introducing exclusive breast milk to newborns in the first hours of life will facilitate the colonization of their naive gut with beneficial bacteria (i.e., *lactobacilli* and *Bifidobacteria*) that are known to be critical for the immune system and mucosal barrier function development, gut motility, and digestive function (197,198). Maternal breast milk also contains lactoferrin, a glycoprotein with anti-infective and immune-modulating effects. Lactoferrin prevents infection by modulating bacterial growth in the gastrointestinal tract, which promotes intestinal cell proliferation, differentiation, and maturation. These functions may decrease intestinal permeability, prevent bacterial translocation from the gut to the bloodstream, and regulate the host–immune response (197). In addition, infant formula feeding has a higher risk in causing infection due to product contamination or inappropriate milk preparation while infectious disease transmission through breastfeeding is very rare (199).

Based on the sepsis onset, this study found that EOS was highly associated with PROM, foul-smelling amniotic fluid, low birth weight, C-Section, the presence of a birth attendant, and low 5th-minute Apgar score. However, having a high maternal leukocyte count lost statistical significance, most likely due to the small sample size. Deliveries that were assisted by Ob/Gyn residents and C-section deliveries were found to be less likely to develop EOS. One of the study sites is a teaching hospital where residents assist the majority of deliveries and do so under the supervision of an Ob/Gyn specialist. If the delivery is complicated and beyond the resident's medical competency, including some with a higher risk of infection, the OB/GYN specialist then directly assists the delivery. Therefore, this may explain the finding that deliveries assisted by Ob/Gyn residents were less likely to develop-EOS because their cases were low-risk deliveries.

A possible explanation for C-sections' association with a lower incidence of EOS is that one mechanism of bacterial transmission in birth is associated with maternal infection and colonization. During vaginal delivery, newborns have direct contact with the bacterial flora in the vaginal canal and perineum, which may lead to acquired infections through the mouth, umbilicus, or a minor skin lesion (200,201). However, C-sections are not recommended as a means of preventing EOS because they do not eliminate the risk of EOS, and they pose other risks for both mothers and newborns (37). In line with previous studies, this study found that neonates with low 5th-minute Apgar scores were at a higher risk of developing EOS (202). A lower Apgar score indicated perinatal asphyxia, which led to immunological insult and increased the risk of infection. However, studies also suggested that intra-uterine infection can also cause low Apgar score (203).

Late-onset sepsis can be acquired from the environment, with preterm infants' involvement mainly due to their immune systems' immaturity. Recent advances in neonatal care have resulted in significant survival, which has led to prolonged hospitalization, increased use of invasive procedures and devices, and subsequently an increased risk of infections (130). This study showed that newborns who had respiratory support and received no oral feeding during the first 24 hours of life were at a significantly higher risk of developing LOS. After adjustment, preterm newborns were found to be three times more likely to develop LOS. However, this association was not statistically significant (aRR 2.73, 95% CI 0.98–7.62), which was possibly due to the sample size.

Based on the culture results, the mother's age, antepartum hemorrhaging, foul-smelling amniotic fluid, low birth weight, low 5th-minute Apgar score, respiratory support, and receiving other than whole breast milk during their first days of life were associated with a higher risk of

culture-proven sepsis. Conversely, a higher risk of culture-negative sepsis was associated with multiple pregnancies, foul-smelling amniotic fluid, a high maternal leukocyte count, and newborns receiving other than exclusive breast milk. A low maternal education level has been reported to be associated with neonatal sepsis (204). A high maternal education level is expected to enhance the mother's capacity to obtain and understand the importance of prenatal care and obtaining reproductive health services as well as benefit linked to breastfeeding. A higher education level may also indicate a better status of general health or may influence the healthcare-seeking behavior of certain groups. Multiple pregnancies are often related to low birth weight and prematurity, which may explain the increased risk of neonatal sepsis. The reason for the risk factors' difference related to culture-proven and culture-negative neonatal sepsis has not been well established. Most literature has reported that the occurrence of culture-negative sepsis is more related to the technique of blood collection and previous exposure to antibiotics (18,81). Among sepsis cases, only culture-negative sepsis was more frequently found in newborns with low birth weight. However, whether this association was related to the difficulty of obtaining sufficient blood volume for blood culture in smaller newborns or due to the possibility that newborns with low birth weight who develop sepsis have a lower level of bacteremia than sepsis newborns of normal birth weight cannot be determined.

In this study, the prevalence of maternal prophylactic antibiotic use in all deliveries was 57.5%. The prevalence of prophylactic antibiotic use in newborns that was given to prevent early infection was nearly 45%. Therefore, more than 70% of the newborns in this study were exposed to prophylactic antibiotics immediately before and after birth. This finding is higher than what was reported by a study in Canada, where 40% of the newborns were exposed to maternal antibiotics, and only 6% of the newborns were directly exposed to antibiotics after

birth (116). Other studies from high-income countries have estimated that 40% of women are exposed to antibiotics before birth (64,161). In low- to middle-income countries, studies have shown that maternal antibiotic exposure before birth varies from 28% to 92% (61,156). Although the WHO recommends giving antibiotic prophylaxis to newborns with documented risk factors for infection, data on the rate of antibiotic prophylaxis use in newborns is quite limited (44). Most available data on antibiotic use in newborns are from high-income countries, where antibiotic use is not for prophylaxis purposes but for therapeutic. This fact is concerning, considering that multiple reports have shown that antibiotic use and inappropriate antibiotic prescriptions tend to be much higher in low-income countries than in high-income countries (158,205).

Antibiotics prophylaxes were more likely to be given to mothers with lower educational levels and when an Ob/Gyn specialist assisted the birth. Mothers with higher-level education had better maternal health, and Ob/Gyn specialists attended higher-risk deliveries. In newborns, a low 1st-minute Apgar score was also related to antibiotic use. Given that the Apgar score provides information regarding the cardiovascular, respiratory, and neurological status of the newborn, compromise in any of these components may potentially lead to a negative impact on the evolving immune system of the newborn and thus increase their susceptibility to infection (202). This may drive the physician to give antibiotic prophylaxis to newborns with low Apgar scores.

Among all the conditions recommended by the local guidelines for prophylactic antibiotic administration, only C-sections, PROM, and foul-smelling amniotic fluid were persistently associated with an increased risk of prophylactic antibiotic use. Maternal fever and isolated increased leukocyte counts were not associated with an increased risk of prophylactic

antibiotic use. These latter conditions were mainly adopted in the guidelines as part of the risk-based approach to neonatal GBS infection prevention (37). In high-income countries, the risk-based approach is no longer used and has been replaced by culture-based screening to determine antibiotic use (172,173). Previous studies have shown that the incidence of neonatal sepsis due to GBS infection in most Asian countries is low. Therefore, this may why clinicians at our study site did not consistently administer prophylactic antibiotics to mothers solely based on the presence of one of these risk factors (11,12,174). The same observation was made for antepartum hemorrhage. Although recommended by the guidelines, this study did not find an increased risk of prophylactic antibiotic use in deliveries with antepartum hemorrhage. Although this may be due to a small number of deliveries with antepartum hemorrhage in the study, few studies have shown the benefits of prophylactic antibiotics in deliveries with antepartum hemorrhage.

In terms of the impact of perinatal antibiotic prophylaxis on neonatal incidence and prevention, two main findings deserve discussion. First, in this study, the ATE of both maternal and neonatal antibiotic prophylaxis can be negligible in the effort to prevent EOS. Second, postnatal antibiotic prophylaxis provided a notable positive ATE in the incidence of overall sepsis, LOS, and culture-negative sepsis.

The main goal of the IAP policy published by the CDC is to prevent EOS due to GBS. Previous studies have shown that in most low- and middle-income countries, the incidence of neonatal sepsis due to GBS infection is low (11,12,174). Despite this evidence, the adoption of IAP as one of the strategies to prevent EOS by low- and middle-income countries continues. The use of IAP in these countries is to prevent any type of early pathogenic bacterial infection, which is similar to the goal of giving antibiotic prophylaxis to newborns with documented risk factors recommended by the WHO. For ethical reasons, few randomized clinical trials have

been conducted to determine whether prophylactic antibiotics decrease the incidence of neonatal sepsis in newborns born to mothers with risk factors for neonatal infection. A Cochrane review concluded that there were insufficient randomized controlled trial data to guide the clinical practice of antibiotic prophylaxis to prevent culture-proven sepsis or any systemic infection (206). In addition, there is some concern that perinatal antibiotic prophylaxis may result in a change in the epidemiology of neonatal infection due to the selection of resistant pathogens present that may be present in low densities. Although there is still some inconsistency, multiple studies have suggested that antibiotic exposure in early life may increase the incidence of LOS, gram-negative sepsis, and antibiotic-resistant sepsis. An early study in the US that evaluated neonatal mortality from sepsis before and after the IAP recommendation release found an increasing trend in LOS mortality (69). Another more recent study in the US also showed that antibiotic exposure in early life in preterm and very low-birth-weight infants (VLBW) is significantly associated with an increased risk of late-onset sepsis, necrotizing enterocolitis (NEC), or death. A study from Korea also showed the same trend (68), while one conducted at 15 neonatal centers in the Neonatal Research Network of the National Institute of Child Health and Human Development (NICHD) found an increase in *E. coli* sepsis, from 3.2 to 6.8 per 1,000 live births, after introducing IAP (121). Bizzarro et al.'s study of neonatal *E. coli* sepsis at Yale New Haven Hospital from 1979 to 2006 observed that in VLBW (< 1,500 g) newborns, there was an increase in *E. coli* EOS and ampicillin-resistant *E. coli* infection (188). A study from Taiwan also reported an increase in EOS due to *E. coli*, from 40.9% to 70% (77). Regarding antibiotic resistance, in an analysis of San Francisco and Atlanta data for the CDC Active Bacterial Core surveillance, the rate of ampicillin resistance in EOS attributable to *E. coli* in preterm newborns increased from 29% in 1998 to 84% in 2000 (79). The large proportion of

culture-negative sepsis may also be linked to high rate of antibiotic prophylaxis usage. Antibiotic prophylaxis use in some of these cases could have decrease the density of pathogenic microbes including GBS, impairing the ability of culture based methods to detect these pathogens.

Although the mechanism is debatable, it was proposed that antibiotic exposure during the perinatal period may alter a newborn's microbiota, leading to increased susceptibility to bacterial infection and expansion of antibiotic resistant bacterial population. The intestinal microbiota is believed to play a major role in immune system development, particularly in the early postnatal period (207,208). Previous studies have strongly suggested that a newborn's microbiome composition influences a host's immune system development, particularly in neutrophils, which play a crucial role in a host's defense against microbial infections. Microbial colonization in the early days after birth is influenced by several factors, including the mode of delivery, the maternal microbiota of the intestine, vagina, and skin, gestational age, the type of infant feeding (breast or formula), and antibiotic exposure during the early days of life (83). Antibiotic use is known to have a strong impact on host-associated microbial communities (122). Broad-spectrum antibiotic exposure affects the acquisition and development of a newborn's microbiota. Imbalances in the microbiome's composition can cause the immune system to become deregulated, which leads to a failure to prevent the occurrence of infection, including neonatal sepsis (104).

This study showed a significant positive ATE of perinatal antibiotic prophylaxis on LOS and culture-negative sepsis. The positive ATE estimate indicated that in average perinatal antibiotic prophylaxis exposure is increasing the possibility the occurrence LOS and culture-negative sepsis. The effect was mainly driven by neonatal antibiotic prophylaxis. Maternal

prophylaxis was not associated with the incidence of sepsis. The proportion of culture-proven sepsis in this study was too small to assess the impact of antibiotic prophylaxis on the incidence of gram-negative and antibiotic-resistant sepsis. Because of this data limitation, further studies that characterize the causal effect of antibiotic exposure to pathogen-specific and antibiotic-resistant neonatal sepsis are needed. In addition, exploration of how antibiotic exposure affects a newborn's gut microbiome and whether the microbiome mediates antibiotic exposure's impact on sepsis risk are also needed.

A limitation of this study is that, consistent with other observational studies, investigating the treatment effects was confounded by indication. Conceptually, newborns at greatest risk for sepsis are likely to be the same infants exposed to perinatal antibiotic prophylaxis. However, this study aimed to minimize the impact of confounding by using propensity scores as the statistical approach to equalize the treatment and non-treatment groups before evaluating the association with the outcome and thus mimicking a randomized clinical trial. Although this strategy may not balance the groups' unobserved confounders, it should be effective in balancing the observed confounders (209–211). Multiple studies using different strategies to adjust for confounding have reported similar outcomes after perinatal antibiotic exposure. Moreover, the clinical findings are biologically plausible with the increasing understanding of antibiotic-driven disruptions of the normal intestinal bacterial microbiome.

Another limitation was the possibility of selection bias due to the pandemic. The pandemic started in the middle of the study period. The number of admissions for deliveries decreased during the pandemic, and fewer eligible participants were enrolled in the study due to increasing hesitancy to participate in medical-related studies. However, the number of losses of

follow-up during the pandemic decreased. Inverse probability weighting was used as the analytical strategy to adjust the bias (212).

4.5 Conclusion

Despite advances in maternal and neonatal healthcare, this study confirmed the high burden of neonatal sepsis in low- and middle-income countries. Culture-negative sepsis was involved in a large proportion of the sepsis cases. A low-cost alternative method for pathogen identification is needed to supplement traditional microbiological techniques to improve neonatal sepsis management and mitigate antimicrobial resistance This study also highlighted the benefit of breast milk as a strong protective factor against all types of sepsis and confirmed that the use of antibiotic prophylaxis during the perinatal period increases the risk of LOS. Although this study did not find a strong indicator that maternal antibiotic prophylaxis prevents the occurrence of sepsis and there is an indication that perinatal antibiotic prophylaxis increased the risk of late-onset neonatal sepsis, the benefit of maternal antibiotic prophylaxis for EOS prevention in high-income countries may not yet be challenged, considering that the evidence for it has been long established by multiple literatures. However, for LMICs a different approach may need to be consider in the effort to effectively prevent neonatal sepsis. Extensive prospective population-based studies are urgently required to provide an accurate assessment of the main drivers of neonatal sepsis occurrence in LMIC. More studies that continuously evaluates the benefits and potential negative consequences of perinatal antibiotic prophylaxis are also needed. If LMICs continue to adopt IAP and postnatal antibiotic prophylaxis to prevent EOS, the use culture-based GBS colonization screening in LMIC may be need to be encourage to mitigate antibiotics'

overuse; or, as GBS vaccine is already under development, the deployment of GBS vaccine in this region may also be relevant once it is available (213).

CHAPTER 5 MANUSCRIPT 4 – The Influence of Perinatal Antibiotic Prophylaxis on Newborns' Intestinal Microbiomes and its Impact on Neonatal Sepsis

5.0 Abstract

5.0.1 Background & Aim:

Antibiotic exposure has been correlated with intestinal microbial ecosystem disruption in newborns. Mounting evidence suggests that dysbiosis influences newborns' susceptibility to infection, including neonatal sepsis. The existence of a causal sequence between antibiotic exposure, dysbiosis, and neonatal sepsis has not yet been determined. This study investigates the effect of perinatal antibiotic prophylaxis exposure on the infant gut microbiome and asks whether the microbiome feature mediates the association between perinatal antibiotic prophylaxis and neonatal sepsis.

5.0.2 Methods:

A nested case-control study was conducted in two referral hospitals in Palembang, Indonesia. Fifty-three newborns who were diagnosed with sepsis were matched to 102 healthy infants by mode of delivery. Newborns' gut microbiomes from meconium and a follow-up stool specimens that were collected approximately 7 days after birth were profiled with 16S ribosomal RNA sequencing.

5.0.3 Results:

The meconium and follow-up stool specimens in newborns with sepsis had a significantly lower alpha diversity than the controls. Compared to newborns who were not exposed to perinatal antibiotic prophylaxis, the meconium of newborns exposed to perinatal antibiotic prophylaxis showed a distinct gut microbiome community structure (Bray-Curtis dissimilarity PERMANOVA, $p = 0.03$). Although there were multiple negative effects in the pathway across

perinatal antibiotic prophylaxis, microbiome features, and neonatal sepsis, mediation analysis did not reveal significant mediating effects for the infants' meconium microbiome features in the association between antibiotic prophylaxis exposure and neonatal sepsis.

5.0.4 Conclusion:

This study suggests that perinatal antibiotic exposure may impact the diversity of newborns' intestinal diversity, and also that microbiome features of newborns with sepsis differ from newborns without sepsis. However, we did not find evidence that newborns' intestinal microbiomes mediate the association between perinatal antibiotic prophylaxis and neonatal sepsis.

5.1 Introduction

The intestinal microbiota in early life is now known to have an essential role in the infant's immune training and metabolic programming (214,215). Microbial colonization patterns in immune naive infants may lay the groundwork for disease risk from immune modulation. Intestinal dysbiosis which is refer as a perturbation to the structure of complex commensal communities has been linked with a higher incidence of various long-term and short-term diseases (111) . The long-term diseases include obesity, chronic inflammatory bowel diseases, diabetes, allergies, and cancers (105,106,216). In the short term, dysbiosis has been associated with necrotizing enterocolitis, diarrhea, colic, and sepsis, including antibiotic-resistant bacterial sepsis (105–107,217). In terms of systemic bacterial infection, it is suggested that a symbiotic host-microbiome relationship prevents bacterial infections by maintaining and strengthening the intestinal mucosal barrier, preventing disruption of the mucosal barrier, which could lead to translocation of luminal pathogenic contents (218). In addition, microbiotas influence the development of immune cells, particularly neutrophils, which play a crucial role in the defense against microbial infection (104).

Intestinal bacterial colonization starts once the fetus is still in the lower uterus and is established after birth. The early life period is a critical window for establishing the infant gut microbiota, which is influenced by multiple factors, such as maternal factors, mode of delivery, feeding type, gestational age, and medication exposure, particularly to antibiotics (219,220). It is strongly suggested that antibiotic exposure during early life affects the acquisition and development of a newborn's microbiota (91,106). Antibiotics impact early microbial colonization via several mechanisms. First, maternal antibiotic administration reaches the neonatal bloodstream via the umbilical cord at least ten hours after administration [13,14]. The second

mechanism, maternally administered antibiotics, alters both maternal vaginal and intestinal microbiome and influences vertical transmission (221). Furthermore, neonates may also receive antibiotics for suspected early-onset infection, directly altering the newborn's microbiome composition (116).

Considerable evidence indicates that both long or short regimens of antibiotics before, during, and after birth also disrupt the natural microbiome assembly (84). Intrapartum antibiotic use has been associated with decreased bacterial diversity of the neonate's first stool (86,87) and lower abundance of lactobacilli and *Bifidobacterium* in the neonatal gut (87–90). IAP administration was also correlated with a reduction in *Actinobacteria* and *Bacteroidetes* and an increase in *Proteobacteria* (91,220). Studies also reported that in the microbiome from newborns' meconium who received antibiotic immediately after birth were less diverse with higher proportion of Poroteobacteria compared to those who were free from antibiotic exposure (90,98).

In line with studies showing that newborns exposed to antibiotics have a less diverse microbiome, bacterial-profiling studies also found that newborns with sepsis have lower bacterial diversity than those without sepsis. Studies observed gut dysbiosis in neonatal sepsis cases with a preponderance of *Proteobacteria* and *Firmicutes* abundance, similar to newborns exposed to IAP (109,110). Stewart et al., who studied over 600 stool samples from newborns with and without sepsis, reported that *Bifidobacteria* were found only in stool samples from healthy newborns. These findings showed possible indications that antibiotic prophylaxis exposure in neonates influences microbial assembly in the neonates' gut which may lead to the occurrence of neonatal sepsis.

Despite the growing evidence on the association between early-life antibiotic exposure, dysbiosis, and diseases including bacterial infections, antibiotics (prophylactics and non-prophylactics) remain among the most prescribed medications during delivery and right after birth. More than 40% of newborns are exposed to perinatal antibiotics in high-income countries, which are given either directly to the newborn or indirectly through their mothers (116,161). In terms of prophylaxis purposes, professional societies from all over the world have published their own national or local guidelines that recommend prescribing prophylactic antibiotics for C-section deliveries and prevention of neonatal Group B *Streptococcus* (GBS) infection (37,56). In low- and middle-income countries, antibiotic prophylaxis is not limited to preventing early GBS infection but is also given to prevent other early-onset infections due to other known pathogenic bacteria (44). Due to the wide adoption of antibiotic prophylaxis recommendations, prophylactic antibiotic administration has become a routine practice during the birthing process and right after birth, consequently increasing the antibiotic exposure of the newborn.

This study aimed to evaluate the impact of maternal and newborn antibiotic prophylaxis use during the perinatal period on the newborn's microbiome, explore the association between newborns' intestinal microbiome features, and investigate whether microbiome features mediate the association between perinatal antibiotic prophylaxis and neonatal sepsis.

5.2 Methods

5.2.1 Study population and sampling frame

This study was approved by Michigan State University's Biomedical and Health Institutional Review Board, the Health Research Review Committee of Mohammad Hoesin Hospital, and the Faculty of Medicine, Sriwijaya University. The study was conducted at

Mohammad Hoesin Hospital and Palembang Bari Hospital, a tertiary and secondary level hospital, in Palembang, Indonesia, from September 2019 to March 2021. Both hospitals have nearly 1,500 beds and serve more than 2,000,000 people in their catchment area. Participants were enrolled in the study on presentation for delivery. Participants were eligible for inclusion in the study cohort if they were mother-viable newborn pairs admitted to the participating hospitals for delivery. The mother agreed to participate in the study and resided within the city. If the mother consumed or received antibiotics 72 hours before admission, the mother received antibiotics for therapeutic purposes, or her newborn was diagnosed with a congenital gastrointestinal malformation that would cause the absence of defecation, then the infant was excluded from the study.

5.2.2 Study procedure and follow-up

Recruitment of subjects began either before delivery or approximately two hours after each delivery. Maternal antibiotic prophylaxis data were collected upon enrollment in the study. The antibiotic prophylaxis administration, type of antibiotics, dose, interval, and duration were documented from hospital medical records. Other relevant clinical data on mothers were extracted. In addition, a questionnaire was administered during mothers' hospitalization that collected more detailed demographic, behavioral, diet, and general health history data.

After birth, newborns were followed up every six hours until discharge or until being diagnosed with neonatal sepsis. Administration of antibiotic prophylaxis, vital signs, clinical symptoms of neonatal sepsis, and other treatments given to the newborn were recorded. If the newborn was discharged without developing sepsis, the newborn was observed until the baby reached 28 days. The first follow-up was conducted one week after the newborn was discharged,

following the recommended well-baby check visit schedules of participating hospitals. Subsequent follow-ups were conducted weekly by phone; the mother or guardian was interviewed to assess any illness and advise them to visit the study hospital if necessary. Other information, such as the baby's general condition and oral intake, was collected during the weekly interview. The mother or guardian directly contacted a contact person, enabling them to report any illness the newborn experienced between scheduled phone calls.

5.2.3 Operational definition and case ascertainment

In this study, perinatal antibiotic prophylaxis was defined as antibiotics received by the mother and/or newborn during hospital admission for prophylaxis purposes. According to the local guidelines, all C-section deliveries (elective and emergency) and cases with antepartum hemorrhage due to placenta previa were recommended for prophylactic antibiotics (159). In addition, although maternal screening for GBS is not routinely implemented, IAP is recommended to mothers who have risk factors for infection, which include premature rupture of membrane (PROM), a maternal intrapartum temperature $\geq 38^{o}$ C, and a maternal leukocyte count > 15,000/mm^3 (160,168). For newborns, the local guideline recommends postnatal antibiotic prophylaxis for neonates with a documented risk factor for infection, which includes membrane rupture > 12 hours, mothers with an intrapartum temperature $\geq 38°C$, a maternal leukocyte count > 15,000/mm^3, and the presence of foul-smelling or purulent amniotic fluid (167).

The on-duty physician made the diagnosis of neonatal cases according to the standard guidelines. The diagnosis was then confirmed by the neonatologists, who were part of the study team. To be diagnosed with neonatal sepsis, a newborn must have at least one of the following clinical criteria: non-specific signs such as lethargy, feeding intolerance, weight loss, temperature

instability, neurological symptoms, respiratory instability, gastrointestinal symptoms, skin and subcutaneous lesions, cardiovascular instability, or hematologic abnormalities. In addition, the newborn must have at least two laboratory findings that include abnormal white blood cell counts (<5,000/ml or >34,000/ml), an immature to total neutrophil ratio (I/T) greater than 0.2, an abnormal platelet count, ESR >15 mm/hr, and C-reactive protein >9 mg/L (167). Neonatal sepsis will then be categorized by the time of onset and microbiology results. Early-onset sepsis (EOS) cases are defined as those occurring within the first 72 hours of life, and late-onset sepsis (LOS) cases are those occurring after 72 hours (1).

5.2.4 Case and control patients

Because sepsis incidence is relatively low and fecal microbiota analysis was too costly to be performed on the entire cohort, we used a nested case-control design to reduce the sample size needed for the sequencing to address our study objective. Potential case patients were newborns in the cohort who were diagnosed with sepsis. Control patients were selected randomly from the remaining newborns in the cohort without sepsis and matched 1:2 for each case patient based on the mode of delivery.

5.2.5 Biological sample collection, processing, and fecal microbiota analysis

Stool samples were collected from all newborns in the cohort study. The first stool (meconium) was collected soon after birth by a nurse. The second stool sample was collected between 7–10 days after delivery. If a newborn was discharged before the stool specimen collection, the newborn's mother or guardian would collect the specimen. The guardian collected diapers containing the stool and transferred each diaper with the stool to a provided plastic storage bag to be stored in a household refrigerator. The sample collection will be prearranged so

that the research staff can retrieve the stool sample at the subject's home no more than 24 hours after collection and deliver it to the laboratory in an icebox. All stool samples were aliquoted and stored at -80^{0}C until DNA extraction. Genomic DNA was extracted from selected subjects' stool samples using QIAamp Fast DNA Stool Mini Kit and QIAamp PowerFecal Pro DNA Kit and run on QIAGEN QIAcube Connect, according to the manufacturer's instructions. All extracted DNA was stored at -80^{0}C until shipped to MSU for 16S ribosomal RNA (rRNA) gene amplification.

The DNA amplicons were used to construct the sequencing libraries for high throughput on an Illumina Miseq platform. Briefly, the V4 hypervariable region of the 16S rRNA gene was amplified using Illumina compatible, dual indexed primers 515f/806 following the protocol developed in the Schloss lab (222). PCR products were batch normalized using the Norgen Biotek NGS Normalization Kit, and products recovered were pooled. The pool was cleaned up and concentrated using AmpureXP magnetic beads. The pool was QC'd and quantified using a combination of Qubit dsDNA HS, Agilent 4200 TapeStation HS DNA1000, and Kapa Illumina Library Quantification qPCR assays. This pool was loaded onto the MiSeq v2 Standard flow cell, and sequencing was carried out in a 2x250bp paired-end format using a MiSeq v2 500 cycle reagent cartridge. Custom sequencing and index primers complementary to the 515f/806r oligomers were added to appropriate wells of the reagent cartridge. Base-calling was performed by Illumina Real-Time Analysis (RTA) v1.18.54, and the output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.20.0.

5.2.6 Bioinformatic analysis

After the sequences were optimized, Quantitative Insights Into Microbial Ecology 2 (QIIME2) software was used to analyzed the 16S rRNA gene sequences from the meconium and follow-up specimen (223). Sequences were demultiplexed with CASAVA 1.8.2 and denoised with DADA2 (224). Operational taxonomic units (OTU) were clustered with VSEARCH at a 97% similarity level. SATé-enabled phylogenetic placement (SEPP) was used to generate phylogenetic trees of the 16S rRNA sequences [39]. Statistical and microbial ecology analyses were performed in the R and SAS software environments. Phyloseq v.1.24.2 and Vegan v.2.5-6 were used to estimate the OTU richness and composition with the Shannon and Chao1 indices for alpha-diversity and Bray-Curtis dissimilarity and Weighted Unifrac distances for beta-diversity (225,226). The non-parametric Wilcoxon test and permutational multivariate analysis of variance (PERMANOVA) were used to compare categorical groups' alpha and beta diversity. The bacterial taxonomic composition and relative abundance were estimated with QIIME. The gut microbial communities of different conditions were further compared using LDA Effect Size (LEfSe). An LDA score cutoff of > 2 was used to discriminate bacterial taxon. The differential features were identified at the phylum to genus levels (227).

Regression models of the Shannon and Chao1 indexes (higher versus lower values based on a median cutoff) were performed against sepsis status. To determine confounding factors, preintervention factors that have been previously found to be related to the pattern of the newborn’s microbiome were assessed with the Chi-Square test. To permit microbiome feature analysis that can predict sepsis occurrence, follow-up specimen that was collected after the development sepsis was excluded from the analysis. Given that the study design was a matched case-control study, we modeled the effect of antibiotic prophylaxis use on the risk of neonatal

sepsis using conditional logistic regression. Variables with a p-value < 0.05 were included in the regression. If two variables were highly correlated, then one variable with the highest point of estimate was chosen to be included in the final model. Mediation analysis was applied to address the structure of types of perinatal antibiotic prophylaxis as the exposure, sepsis occurrence as the outcome, and microbiome profiles as the mediator. Microbiome features examined in the analysis included α diversity (i.e., the Shannon and Chao1 indexes), the relative abundance of four phyla (i.e., Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria), and the ratio of Firmicutes/Bacteroidetes and relative abundance of Proteobacteria/Actinobacteria. All mediators were categorized into tertiles. Mediation analysis per mediator variable was conducted using the Hayes PROCESS macro in SPSS version 26.0 to examine the indirect associations. Bootstrapping was used to generate 95% confidence intervals in mediation models.

5.3 Results

5.3.1 Study population

This study included 155 newborns, 53 cases, and 102 control individuals. Of 53 cases of neonatal sepsis, 26 (49.1%) were late-onset sepsis, and 15 (28.3%) were culture-proven sepsis. Among all culture-proven sepsis, eight were gram-positive sepsis, and seven were gram-negative sepsis. There were significant differences between the sepsis and control groups in maternal leukocyte count, gestational age, birth weight, and whether early initiation of breastfeeding was implemented or not. The characteristics of the newborns with sepsis and non-sepsis newborns are shown in Table 17.

Table 17. Background characteristics of study subjects

Variable	Sepsis **(n=53)**	Non-Sepsis **(n=102)**	***p*-value**
Rupture of membrane > 18 hours	1 (1.9)	0 (0)	0.99
Foul-smelling amniotic fluid	5 (9.4)	0 (0)	0.08
Maternal leukocyte count >15000/mm^3	12 (29.3)[a]	9(10.7)[b]	0.0004
Intrapartum temperature ≥ 38° C	0 (0)	0 (0)	-
Gender			
Male	30 (56.6)	53 (52.0)	0.58
Female	23 (43.4)	49 (48.0)	
Gestational age			
< 32 weeks	4 (7.6)	5 (4.9)	<0.0001
32 – 36 weeks	36 (67.9)	12 (11.8)	
≥ 37 weeks	13 (24.5)	85 (83.3)	<0.0001
Prematurity (<37 weeks)	31 (58.5)	17 (16.7)	<0.0001
Birthweight			
< 1000 grams	0 (0)	0 (0)	<0.0001
1000 – 1499 grams	10 (18.9)	4 (3.9)	
1500 – 2499 grams	26 (49.1)	14 (13.7)	
> 2500 grams	17 (32.1)	84 (82.3)	
Low birth weight (< 2,500 grams)	38 (67.9)	18 (17.7)	<0.0001
Early initiation of breastfeeding	7 (13.2)	38 (37.3)	0.002

In total, 36 (23.2%) newborns were not exposed to any perinatal antibiotic prophylaxis, while 119 (76.8%) were exposed to at least one type of perinatal antibiotic. Among the 119 newborns exposed to antibiotic prophylaxis, 55 were exposed to maternal antibiotic prophylaxis, 30 received a postnatal antibiotic prophylaxis, and 34 were exposed to maternal and postnatal antibiotic prophylaxis. Between groups, there were significant differences in the mode of

delivery, gestational age, birth weight, and whether early initiation of breastfeeding was implemented or not (Table 18).

Table 18. Characteristics of study subjects based on antibiotic exposure

Variable	No antibiotics	Perinatal antibiotic prophylaxis	*p*	Maternal prophylaxis only	Postnatal prophylaxis only	Maternal and postnatal prophylaxis	
	n = 36	n = 119		n = 55	n = 30	n = 34	
Maternal age group (%)							
< 20 years old	2 (5.6)	5 (4.2)	0.50	1 (1.8)	3 (10.0)	1 (2.9)	0.41
20–35 years old	24 (66.7)	91 (76.5)		44 (80.0)	23 (76.7)	24 (70.6)	
> 35 years old	10 (27.8)	23 (19.3)		10 (18.2)	4 (13.3)	9 (26.5)	
Smoke	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	-
Diabetes / Gestational Diabetes	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	-
Pre-pregnancy BMI							
Underweight	4 (11.1)	19 (16.0)	0.57	9 (16.4)	3 (10.0)	7 (20.6)	0.09
Normal weight	24 (66.7)	69 (58.0)		31 (56.4)	24 (80.0)	14 (41.2)	
Overweight and obese	7 (19.4)	30 (25.2)		15 (27.3)	3 (10.0)	12 (35.3)	
Missing	1 (2.8)	1 (0.8)		0 (0)	0 (0)	1 (2.9)	

Table 18. (cont'd)

Variable	No antibiotics	Perinatal antibiotic prophylaxis	*p*	Maternal prophylaxis only	Postnatal prophylaxis only	Maternal and postnatal prophylaxis	
	n = 36	n = 119		n = 55	n = 30	n = 34	
Pregnancy weight gain							
Below recommendation	20 (55.6)	69 (52.1)	0.85	30 (54.6)	20 (66.7)	19 (55.9)	0.63
Within recommendation	9 (25.0)	29 (24.4)		12 (21.8)	8 (26.7)	9 (26.5)	
Above recommendation	6 (16.7)	20 (16.8)		13 (23.6)	2 (6.7)	9 (26.5)	
Missing	1 (0.8)	1 (0.8)		0 (0)	0 (0)	1 (2.9)	
Daily prenatal vitamin consumption	18 (50.0)	57 (47.9)	0.83	31 (56.4)	15 (50.0)	11 (32.4)	0.18
Daily dairy product consumption during pregnancy	27 (75.0)	96 (80.7)	0.60	46 (83.6)	24 (80.0)	26 (76.5)	0.70
History of diarrhea in the 3rd trimester of pregnancy	7 (19.4)	43 (36.1)	0.06	23 (41.8)	7 (23.3)	13 (38.2)	0.09
History of systemic antibiotic consumption in the 3rd trimester of pregnancy	3 (19.4)	15 (12.6)	0.77	8 (14.6)	4 (13.3)	3 (8.8)	0.78
Mode of delivery							
Vaginal birth	17 (47.2)	36 (30.3)	0.02	14 (25.5)	15 (50.0)	7 (20.6)	0.04
C-section	19 (52.8)	43 (69.8)		41 (74.6)	15 (50.0)	27 (79.4)	

Table 18. (cont'd)

Variable	No antibiotics	Perinatal antibiotic prophylaxis	*p*	Maternal prophylaxis only	Postnatal prophylaxis only	Maternal and postnatal prophylaxis	
	n = 36	n = 119		n = 55	n = 30	n = 34	
Gender							
Male	19 (52.8)	64 (53.8)	0.92	24 (43.6)	18 (60.0)	22 (64.7)	0.22
Female	17 (47.2)	55 (46.2)		31 (56.4)	12 (40.0)	12 (35.3)	
Gestational age							
< 32 weeks	1 (2.8)	8 (6.7)	0.22	2 (3.6)	4 (13.3)	2 (5.9)	<0.0001
32 – 36 weeks	6 (16.7)	33 (27.7)		6 (10.9)	16 (53.3)	11 (32.4)	
≥ 37 weeks	29 (80.6)	78 (65.6)		47 (85.5)	10 (33.3)	21 (61.7)	
Prematurity	7 (19.4)	41 (34.5)	0.09	8 (14.6)	20 (66.7)	13 (38.2)	<0.0001
Birthweight							
< 1000 grams	0 (0)	0 (0)	0.30	0 (0)	0 (0)	0 (0)	<0.0001
1000 – 1499 grams	1 (2.8)	13 (10.9)		1 (1.8)	10 (33.3)	2 (5.9)	
1500 – 2499 grams	9 (25.0)	31 (26.1)		6 (10.9)	12 (40.0)	13 (38.2)	
> 2500 grams	26 (72.2)	75 (34.5)		48 (87.3)	8 (26.7)	19 (55.9)	
Low birth weight	10 (27.8)	44 (37.0)	0.31	7 (12.7)	22 (73.3)	15 (44.1)	<0.0001
Early initiation of breastfeeding	17 (47.2)	28 (23.5)	0.01	23 (41.8)	3 (10.0)	2 (5.9)	<0.0001

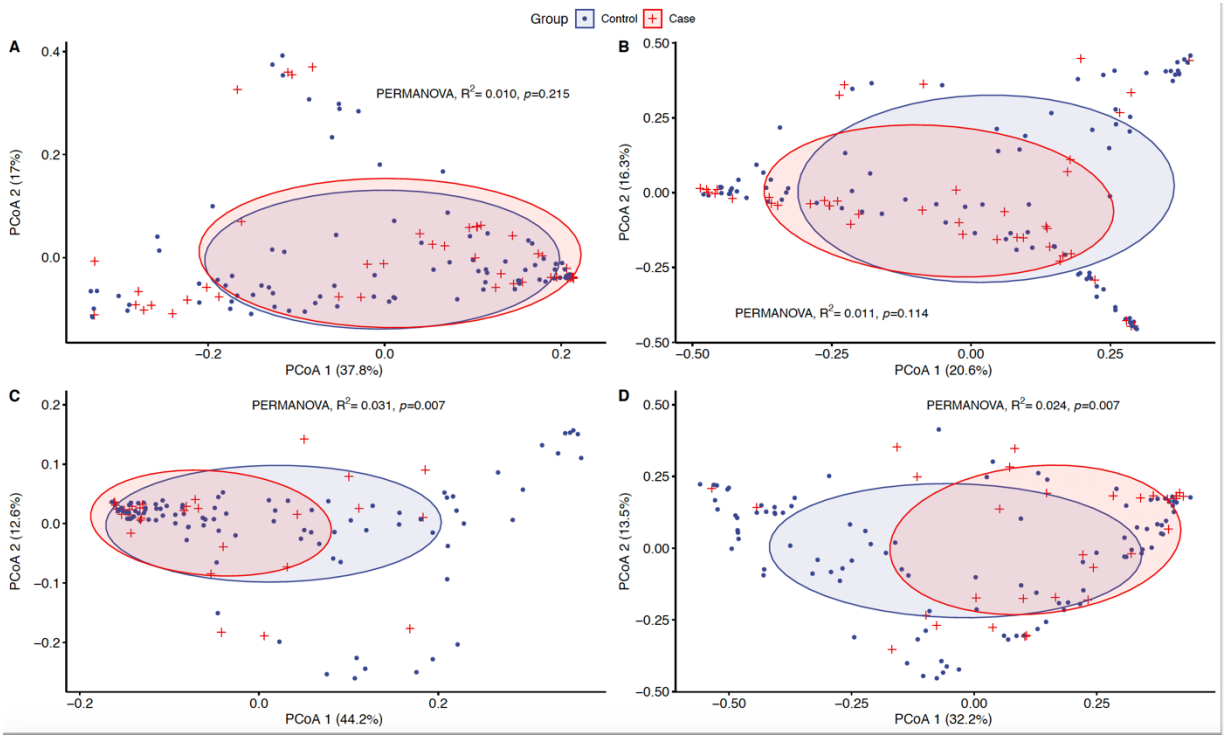
The study collected 287 stool samples, 152 meconium samples, and 135 follow-up samples from 155 subjects. 16s rRNA gene sequencing data were available in 142 meconium samples and 135 follow-up samples. Among 53 cases of neonatal sepsis, there were 50 meconium samples and 36 follow-up samples. From 102 controls, there were 92 meconium samples and 99 follow-up samples. Bacteria were not detected in 10 meconium samples from the non-sepsis group.

Based on exposure to perinatal antibiotic prophylaxis, 31 meconium and 35 follow-up samples were collected from the unexposed group. Furthermore, from the exposed group, there were 111 meconium and 100 follow-up stool samples. Of 111 meconium samples collected from the exposed group, 50 were exposed to maternal antibiotics only, 29 were exposed to postnatal antibiotics only, and 32 were exposed to both. Meanwhile, from the 100 follow-up samples, 52 were exposed to maternal antibiotics only, 23 were exposed to postnatal antibiotics only, and 25 were exposed to both.

5.3.2 Association of neonatal sepsis with microbial diversity

This study found that, when measured by weighted-UniFrac and Bray-Curtis dissimilarity, the microbiome β diversity of meconium did not differ by the neonate's sepsis status ($R^2 = 0.01$, $p = 0.22$ and $R^2 = 0.01$, $p = 0.114$). However, for the follow-up samples, which excludes cases that had developed sepsis before the follow-up stool specimen was collected, the beta diversity differed by sepsis status ($R^2 = 0.031$, $p = 0.007$ for weighted-UniFrac; $R^2 = 0.024$, $p = 0.007$ for Bray-Curtis dissimilarity) (Figure 4).

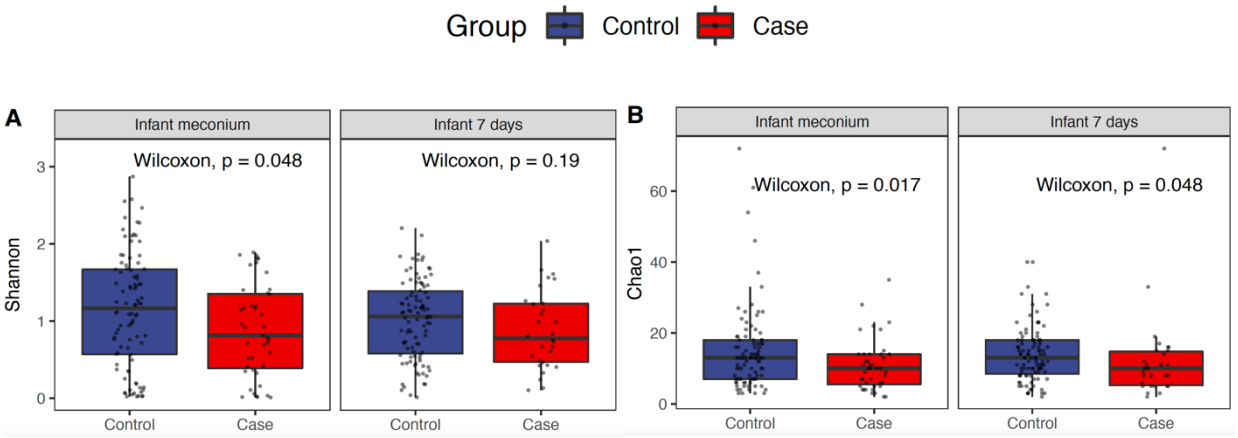
Figure 4. Microbiome beta diversity between sepsis cases and non-sepsis.



A) Weighted UniFrac ordination of meconium samples, B) Bray-Curtis dissimilarity of meconium samples, C) Weighted UniFrac ordination of follow-up samples, D) Bray-Curtis dissimilarity of follow-up samples.

Both meconium and follow-up stool specimens from infants with sepsis had a significantly lower species diversity and richness relative to the non-sepsis group (Shannon and Chao1 indexes, Figure 5). In the meconium samples and after adjusting for the infant's gestational age, early initiation of breastfeeding status, and maternal leukocyte count, the statistical support for the differences in richness remains strong in the (Chao1 index, $p = 0.03$) but not for differences in richness (Shannon index, $p = 0.18$). Similar differences were observed for the follow-up specimens ($p = 0.07$ for the Shannon index and $p = 0.03$ for the Chao1 index).

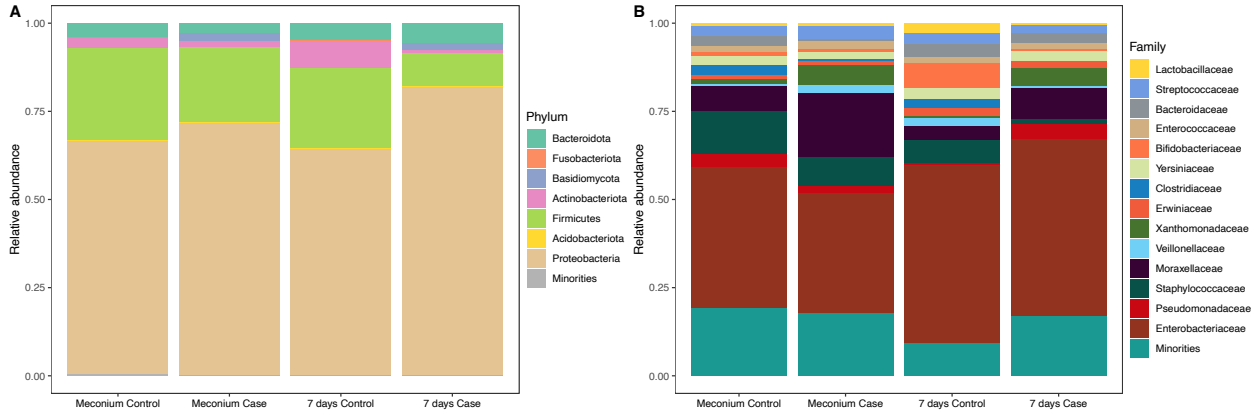
Figure 5. Microbiome alpha diversity between sepsis cases and non-sepsis.



A) Shannon index, B) Chao index

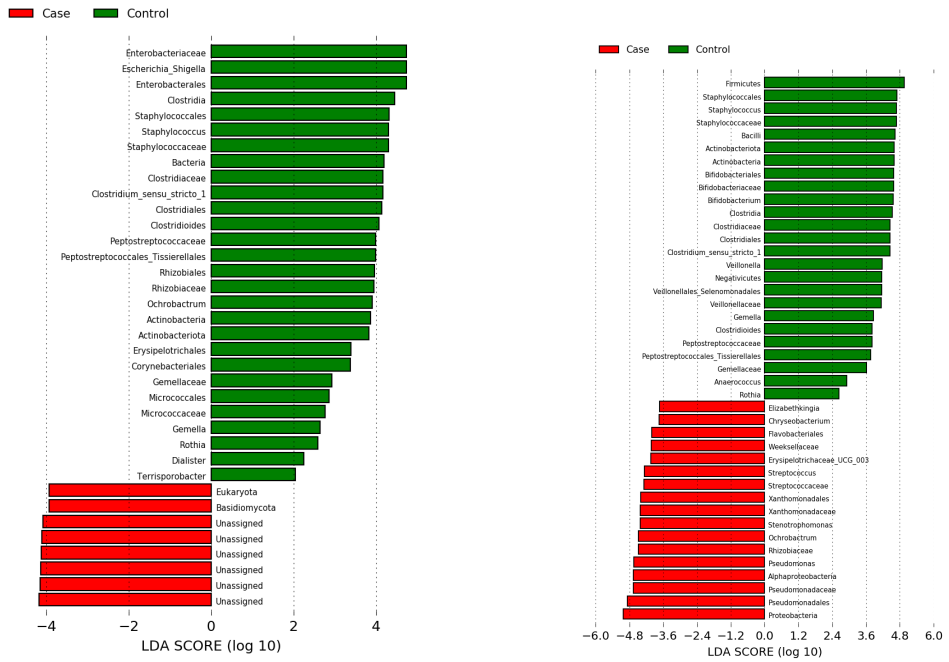
In both the meconium and follow-up samples, the predominant phyla were Proteobacteria followed by, Firmicutes, Bacteroidetes, Basidiomycota, and Actinobacteria. Proteobacteria were more abundant in the sepsis group compared to the non-sepsis group. In contrast, Firmicutes were more abundant in the non-sepsis group. At the family level, in all groups of specimens, the predominant families were Enterobacteriaceae, Moraxellaceae, and Staphyloccaceae (Figure 6).

Figure 6. The relative abundance of bacterial phyla and family in meconium and a follow-up specimen according to sepsis and non-sepsis.



The LEFse analysis revealed that in the meconium specimens, Basidiomycota and Eukaryota were significantly higher in infants with sepsis relative to the non-sepsis infants. In the follow-up specimens, infants with sepsis had a higher abundance genera compromising pathogenic microbes such as *Pseudomonas* and *Streptococcus* (Figure 7).

Figure 7. Differentially abundant taxa between sepsis and non-sepsis in (A) meconium and (B) follow-up 7-day-old samples. LDA scores were calculated with LEfSe.

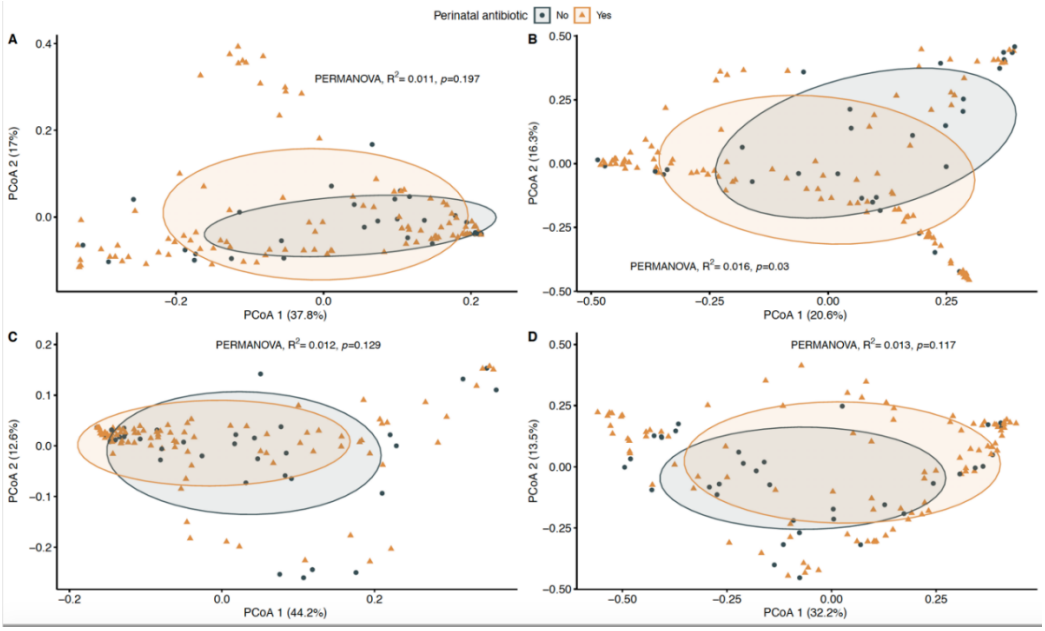


A. Meconium B. Follow-up

5.3.3 Association of perinatal antibiotic exposure with microbial diversity

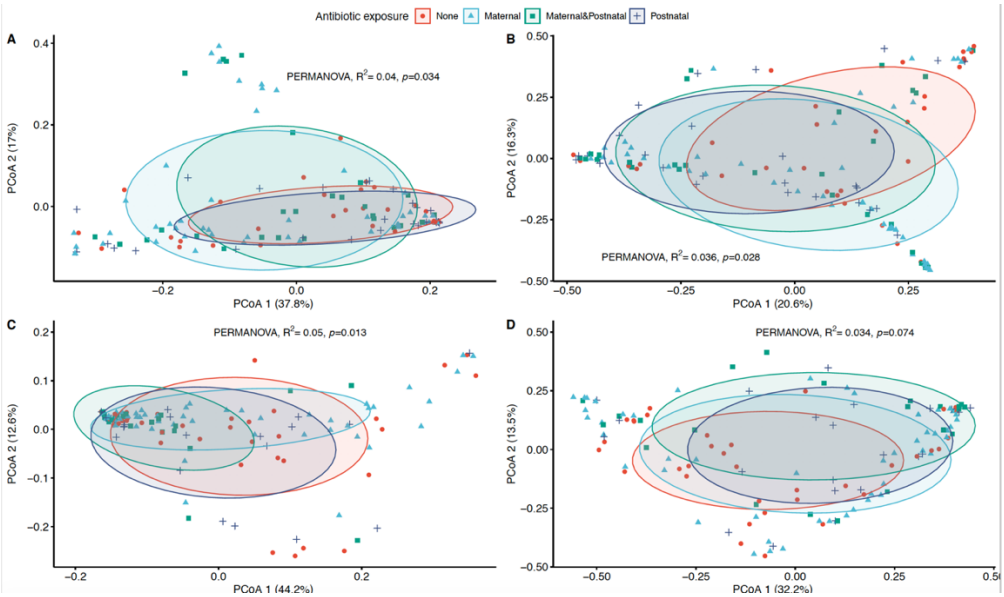
Overall, the beta diversity of infants exposed to perinatal antibiotic prophylaxis did not differ from the unexposed, except for the meconium specimens measured by Bray Curtis dissimilarity (Figure 8). However, when categorized by type of antibiotic exposure, significant differences were found in the meconium samples by both measurements and the follow-up samples measured by Weighted UniFrac (Figure 9).

Figure 8. Microbiome beta diversity between infants who were exposed and unexposed to the perinatal antibiotic.



A) Weighted UniFrac ordination of meconium samples, B) Bray-Curtis dissimilarity of meconium samples, C) Weighted UniFrac ordination of follow-up samples, D) Bray-Curtis dissimilarity of follow-up samples.

Figure 9. Microbiome beta diversity between infants who were exposed and unexposed to the perinatal antibiotic.



A) Weighted UniFrac ordination of meconium samples, B) Bray-Curtis dissimilarity of meconium samples, C) Weighted UniFrac ordination of follow-up samples, D) Bray-Curtis dissimilarity of follow-up samples.

Overall, the Chao1 index indicates a significant difference for the follow-up specimen between infants exposed and unexposed to perinatal antibiotic prophylaxis. A difference of microbiome diversity was also shown by the Shannon index also not statistically significant (Figure 10). In addition, there was a trend that the follow-up samples in the exposed group were slightly less diverse compared to the meconium (Figure 10 and Figure 11).

Figure 10. Microbiome alpha diversity comparison by perinatal antibiotic exposure with Shannon and Chao 1 indexes

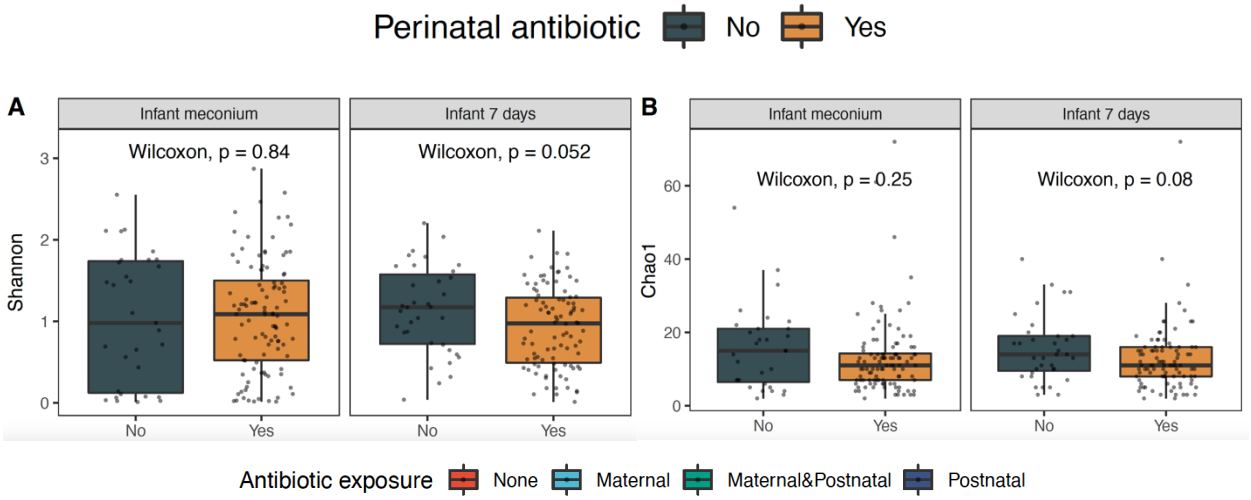
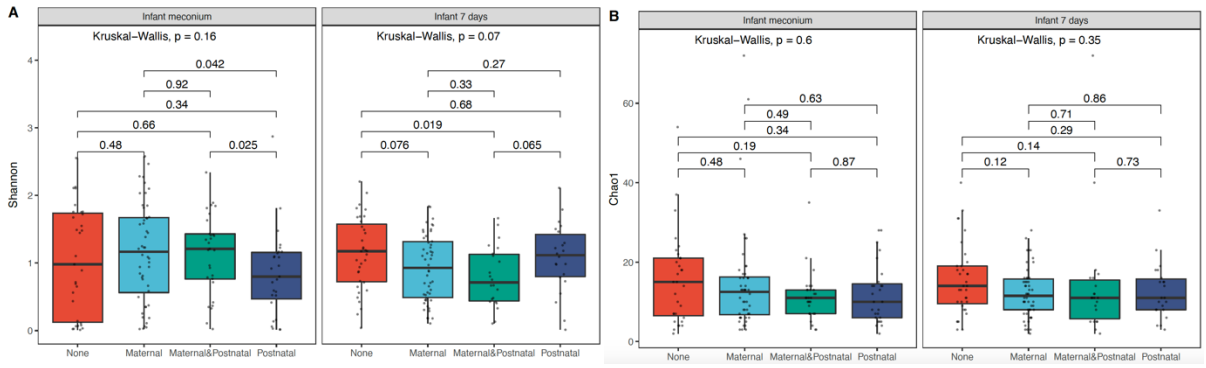


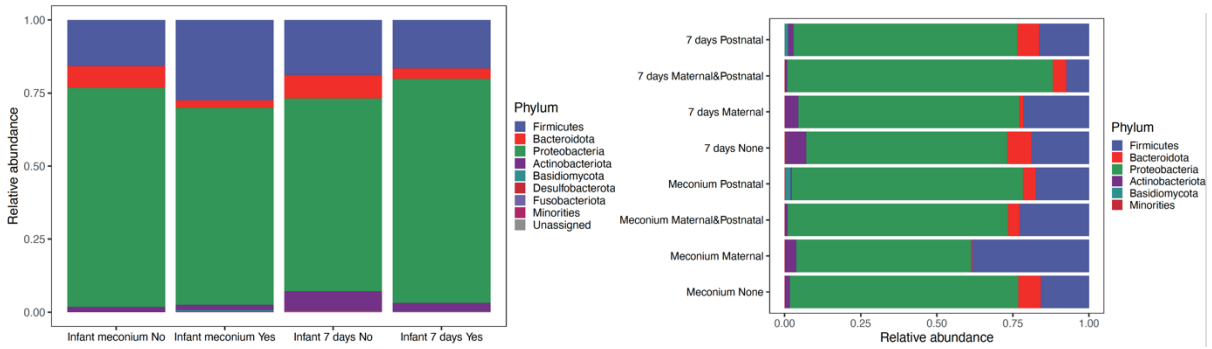
Figure 11.Microbiome alpha diversity comparison by four groups of perinatal antibiotic exposure with Shannon and Chao1 indexes



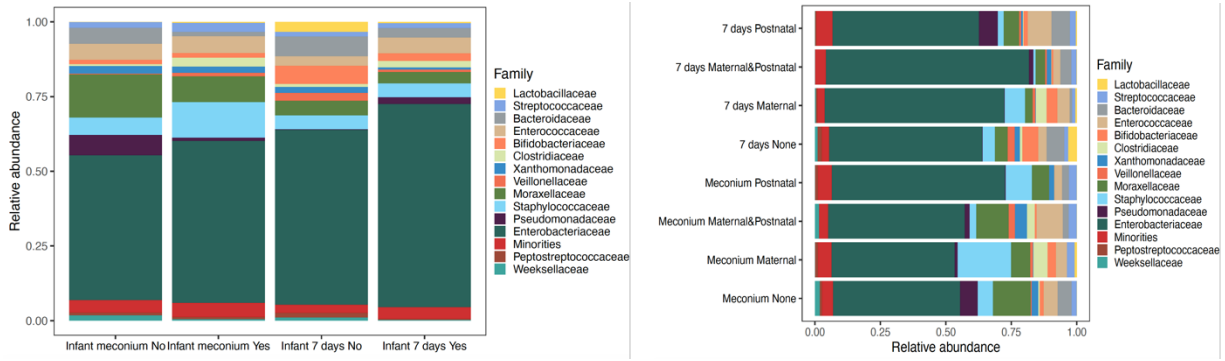
Proteobacteria are relatively more abundant in the unexposed group in the meconium specimens, while Firmicutes are relatively more abundant in the exposed group. In the follow-up specimen, we observed a switch showing that Proteobacteria is relatively more abundant in the exposed group, while Firmicutes is slightly more abundant in the unexposed group. Breaking it down to the type of antibiotic prophylaxis, Proteobacteria and Firmicutes remained the two most predominant phyla. In the follow-up specimen, we observed the greatest increment of Proteobacterium phylum in the group exposed to maternal antibiotics and the combination of maternal and postnatal antibiotics. On the other hand, compared to the meconium specimen, the abundance of Firmicutes was lower in the follow-up specimen in the maternal and maternal plus postnatal antibiotic exposed group. The predominant families found in all groups of specimens were *Entrobactericaeae, Staphylococcaeae, Moraxeleceae*, and *Lactobacilaceae* (Figure 12).

Figure 12.The relative abundance of bacterial phyla in meconium and follow-up specimens according to prophylactic antibiotic exposure.

A. Phylum level

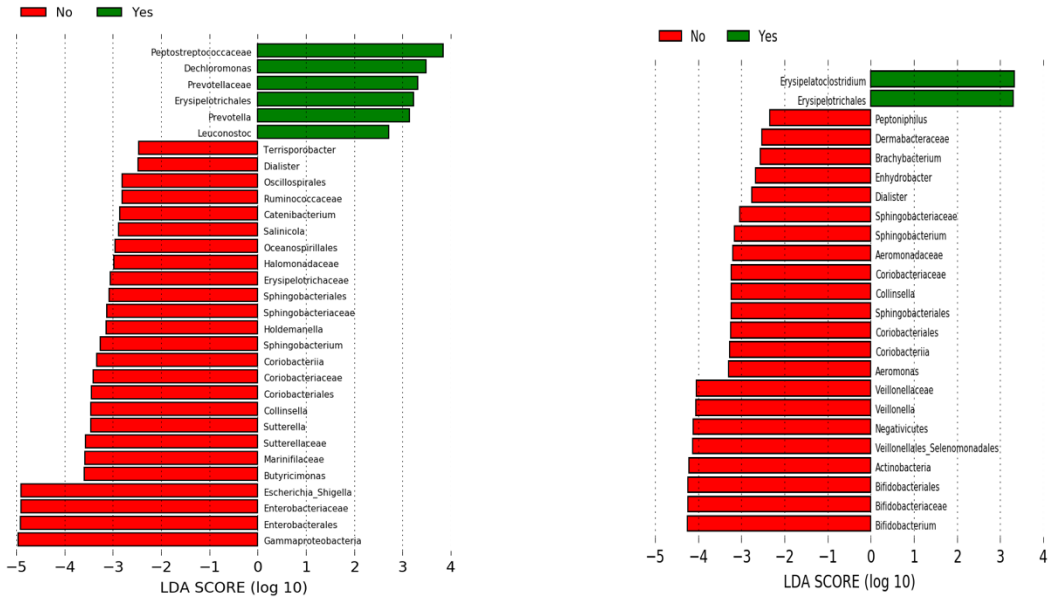


B. Family level



LEfSe analysis showed that in both specimens, Erysipelotrichales were overrepresented in the exposed group. In the follow-up specimen, we observed that the genus Bifidobacterium was more abundant in the unexposed group (Figures 13A and 13B).

Figure 13.The difference in the relative abundance of bacterial taxa between infants exposed and not exposed to all types of perinatal antibiotic prophylaxis

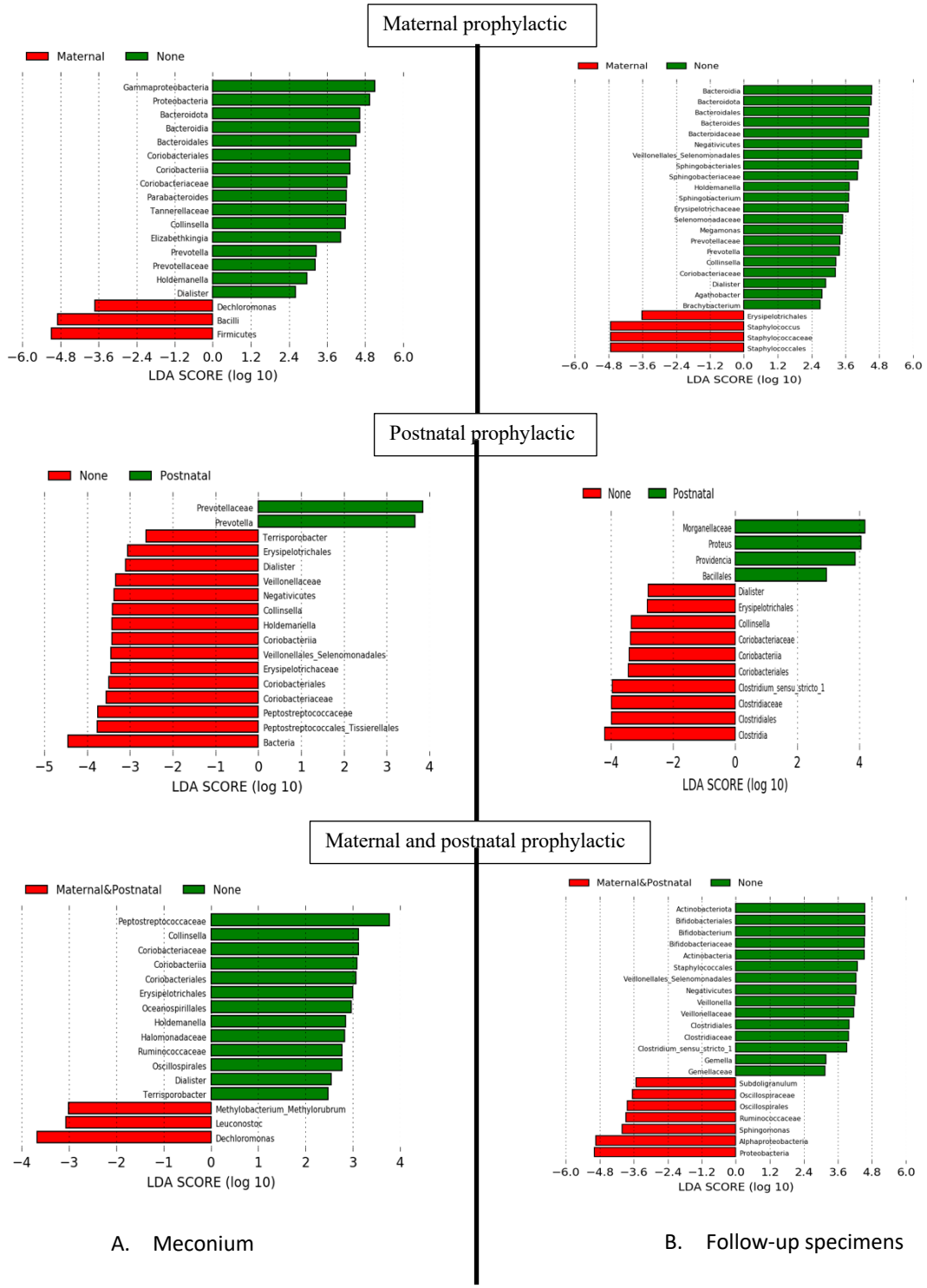


A. Meconium specimens

B. Follow-up specimens

Stratified to the types of perinatal antibiotic prophylaxis, we found that infants exposed to maternal antibiotics had members of the phylum Firmicutes, e.g., class Bacilli, order Erysipelotrichales, order Staphylococcales, family *Staphylococcaceae*, and genus *Staphylococcus*, at a rate at least three-fold higher than non-exposed infants. In the meconium specimen, the genus *Dechloromonas* from the phylum Proteobacteria was higher in infants exposed to maternal antibiotics. Infants exposed to postnatal antibiotics had a higher abundance of Proteobacteria phylum members, e.g., family *Prevotellaceae*, family *Morganellaceae*, genus *Prevotella*, genus *Proteus*, and genus *Providencia*. In the follow-specimen, the order Bacillales was also found to be higher in the exposed group. In the follow-up specimens, we found that Proteobacteria members such as *Alphaproteobacteria* and *Sphingomonas* were higher in the exposed group than in the unexposed group (Figure 14).

Figure 14.The difference in the relative abundance of bacterial taxa between infants exposed and not exposed to antibiotic prophylaxis stratified by types



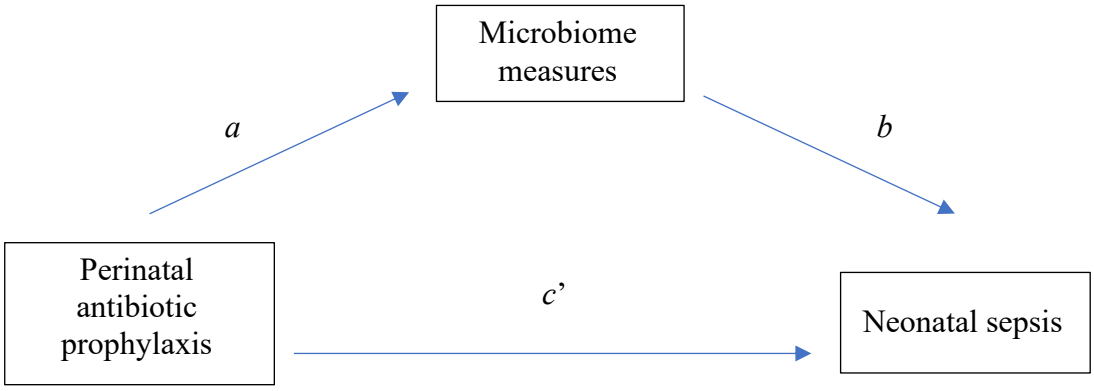
5.3.4 Impact of perinatal antibiotic prophylaxis on neonatal sepsis

After adjustment for maternal leukocyte count, gestational age, and implementation of early breastfeeding initiation, conditional logistic regression showed that infants exposed to any type of perinatal antibiotic prophylaxis had an increase in neonatal sepsis (aOR 5.31, 95% CI 1.17 to 24.04). Based on the type of antibiotic prophylaxis, this study found that maternal prophylaxis decreased the risk of neonatal sepsis (aOR 0.19, 95% CI 0.01 to 3.26). On the other hand, postnatal and the combination of maternal and postnatal prophylaxis increased the risk of neonatal sepsis (aOR 33.98, 95% CI 1.48 to 778 and aOR 38.58, 95% CI 3.08 to 483.64, respectively).

5.3.5 Mediation effect of microbiome measures for the relationship between perinatal antibiotic exposure and neonatal sepsis

The mediation analysis path diagram is illustrated in Figure 15; *a* is the estimated effect of perinatal antibiotic exposure on each microbiome measure, *b* is the estimated effect of each microbiome measure on neonatal sepsis incidence, and *c'* is the estimate of the total effect of perinatal antibiotic exposure on neonatal sepsis incidence. Due to the small sample size, the microbiome measures data from the follow-up stool specimens were not included in the mediation analysis.

Figure 15.The mediation analysis path diagram.



All mediation models showed significant positive direct associations (path *c*') of perinatal antibiotic prophylaxis in general, postnatal, and a combination of maternal and postnatal antibiotic prophylaxis with neonatal sepsis. The results indicated that neonates exposed to perinatal antibiotic prophylaxis, specifically postnatal and the combination of postnatal and maternal antibiotic prophylaxis, were more likely to develop sepsis. On the other hand, although statistically insignificant, the path from maternal antibiotic prophylaxis to neonatal sepsis was negative. On paths a and b, most of the relationships between antibiotic prophylaxis exposures with microbiome measures and between microbiome measures with neonatal sepsis were negative but insignificant. There was a significant association between postnatal prophylaxis and the Shannon index, maternal prophylaxis and relative abundance of Firmicutes, and relative abundance of Actinobacteria and Firmicutes and neonatal sepsis incidence. A positive effect was observed on the relationship between maternal prophylaxis and relative abundance of Firmicutes, while the remaining relationship that was found statistically significant was negative. In terms of indirect effect, the indirect effect was predominantly positive, except for the indirect effect of the relative abundance of Firmicutes and Proteobacteria. However, this study did not find a

statistically significant indirect effect of all types of antibiotic prophylaxis exposure on neonatal sepsis via each evaluated microbiome measure (Table 19).

Table 19. Direct and indirect effects of perinatal antibiotic prophylaxis on neonatal sepsis mediated by microbiome measures.

	Estimate of *a*	**Estimate of *b***	**Estimate *c'* (Direct effect)**	**Indirect effect (Bootstrap 95% CI)**
Shannon index				
Overall	-0.31[a]	-0.28	1.49*	0.09 (-0.05, 0.32)
Maternal	-0.22	-0.47	-0.96	0.10 (-0.21, 0.63)
Postnatal	-0.50*	-0.24	2.45*	0.12 (-0.35, 0.73)
Maternal + Postnatal	-0.29	0.12	3.05*	-0.03 (-0.41`, 0.27)
Chao1 index				
Overall	-0.18	-0.42[a]	1.52*	0.08 (-0.08, 0.34)
Maternal	-0.13	-0.61	-0.91	0.08 (-0.23, 0.55)
Postnatal	-0.19	-0.47	2.56*	0.09 (-0.16, 0.55)
Maternal + Postnatal	-0.26	-0.58	3.00*	0.15 (-0.14, 0.69)
Actinobacteria				
Overall	-0.06	-0.59*	1.59*	0.03 (-0.18, 0.27)
Maternal	-0.18	0.03	-0.85	0.005 (-0.43, 0.55)
Postnatal	-0.11	-0.75[a]	2.65*	0.08 (-0.34, 0.61)
Maternal + Postnatal	-0.37[a]	0.38	2.92*	0.14 (-0.24, 0.70)
Bacteroidetes				
Overall	-0.26	-0.07	1.55*	0.02 (-0.12, 0.26)
Maternal	-0.32	-0.53	-0.99	0.17 (-0.22, 5.11)
Postnatal	-0.28	-0.50	2.53*	0.14 (-0.17, 0.63)
Maternal + Postnatal	-0.15	-0.06	3.00*	0.01 (-0.22, 0.26)

Table 19. (cont'd)

	Estimate of *a*	Estimate of *b*	Estimate *c'* (Direct effect)	Indirect effect (Bootstrap 95% CI)
Firmicutes				
Overall	0.21	-0.53*	1.72*	-0.11 (-0.35, 0.05)
Maternal	0.38*	-0.85	-0.57	-0.32 (-8.60, 1.05)
Postnatal	-0.11	-0.33	2.55*	0.04 (-0.17, 0.43)
Maternal + Postnatal	0.22*	-0.52	3.22*	-0.11 (-0.68, 0.16)
Proteobacteria				
Overall	-0.12	0.50	1.67*	-0.06 (-0.32, 0.10)
Maternal	-0.30	0.30	-0.75	-0.09 (-0.69, 0.30)
Postnatal	0.15	0.28	2.54*	0.04 (-0.16, 0.44)
Maternal + Postnatal	-0.10	0.89[a]	3.39*	-0.09 (-0.73, 0.35)
Bacteriodetes/Firmicutes ratio				
Overall exposure	-0.32[a]	0.10	1.78*	-0.03 (-0.24, 0.15)
Maternal	-0.32	-0.15	-0.59	0.07 (-0.64, 7.82)
Postnatal	-0.30	-0.53	2.66*	0.16 (-0.19, 0.72)
Maternal + Postnatal	-0.14	0.12	3.15*	-0.02 (-0.40, 0.25)
Actinobacteria/Proteobacteria ratio				
Overall exposure	-0.10	-0.44[a]	1.56*	0.04 (-0.11, 0.23)
Maternal	0.06	0.05	-0.85[c]	0.003 (-0.23, 0.43)
Postnatal	-0.04	-0.37	2.59*	0.01 (-0.29, 0.36)
Maternal + Postnatal	-0.40[a]	-0.55[a]	2.91*	0.22 (-0.17, 0.87)

*p<0.05 [a]p≥0.05 - ≤ 0.10

5.4 Discussion

This study found the microbiomes' β-diversity of sepsis and non-sepsis was similar in the meconium specimens but differed in the follow-up specimens. However, the meconium's α-diversity was already found to be significantly lower in newborns with sepsis and continued to decrease in the follow-up specimens. These findings demonstrated that the microbiome patterns in meconium were similar but less diverse in the earlier days of life and became more apparent as the infection process in newborns with sepsis developed. Comparing meconium's microbiome features between different studies is a great challenge due to limited published studies and the heterogeneous methods used for specimen collection and analysis. Our findings were similar to a study reported by Madan et al. that reported the microbiome of newborns with sepsis was less diverse than newborns without sepsis. However, other studies did not find a significant difference in the alpha diversity of sepsis and non-sepsis cases, although a difference in the composition and characteristics of the meconium microbiome was reported (218,228).

In addition, although not supported by strong statistical support due to our small sample size, supporting previous studies, this study indicated that perinatal antibiotic exposure was associated with the feature of infants' early life gut microbiome (63,84,90,98). In the meconium beta-diversity analysis, we observed a significant difference between the exposed and unexposed groups when using the Bray-Curtis metrics but not the Unifrac metric. The non-difference in the Unifrac metric may be a sign that the OTUs that differ between groups were phylogenetically close to each other. Therefore, there is a high level of shared branch length in the community, which does not significantly differ when using the Unifrac distance. The alpha diversity also showed that the microbiome in the exposed group tended to be less diverse than that in the unexposed group. Previous studies have also indicated that both long and short regimens of

antibiotics before, during, and after birth also disrupt the natural microbiome assembly (84). Intrapartum antibiotic use has been associated with decreased bacterial diversity in the neonate's stool (86,87). Similar associations have been observed after administering antibiotics to the neonate directly after birth (90,98). The two-time stool collection enables us to show that there was a trend of less alpha diversity in the follow-up specimen. Nogacka et al. and Tapiainen et al. reported that the impact of IAP on gut colonization was observed by the age of two days (93,229,230). Our findings support that even brief exposure to antibiotic prophylaxis before and right after birth may reach the fetus' circulation, leading to changes in the meconium microbiome composition, and the impact continues for a certain length of time.

Consistent with previous studies, our study also found that the most abundant phyla characterized across groups were Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes (91–93). When we compared the relative phylum level abundance of meconium and the follow-up specimen, we found that in the sepsis group there was a decrease in the abundance of Firmicutes and an increase in the abundance of Proteobacteria. Meanwhile, in the non-sepsis group, the alteration of the relative abundance of Proteobacteria and Firmicutes was found in the opposite direction. . Given that most genera in the Proteobacteria phylum are more harmful bacteria and the majority genera in the Firmicutes phylum are more beneficial bacteria, increasing Proteobacteria and decreasing Firmicutes may be a sign of an unstable microbial community that could lead to an infection process (231,232). Similar results were found when we compared the relative abundance of Proteobacteria and Firmicutes in infants who were exposed and not exposed to antibiotic prophylaxis.

Although the proportion of newborns who were breastfed was higher in the group of infants who were exposed to antibiotic prophylaxis (74.2% vs 25.8%), our study observed that

infants unexposed to any antibiotic prophylaxis had a significantly high *Bifidobacterium* in the follow-up specimens but not in the exposed group. *Bifidobacterium* is an intestinal protective bacterium which is found to be the predominant components of the intestinal flora in breast-fed infants. This may be a strong indication that perinatal antibiotic exposure inhibits the growth of *Bifidobacterium. Bifidobacterium* and *Bacteroidetes* have a role in restoring intestinal micro-ecological balance, repairing the intestinal membrane barrier, improving intestinal colonization, and inhibiting opportunistic pathogens (233). Although we found that several members of Proteobacteria were increased in the group exposed to antibiotic prophylaxis, we did not find a significant increase in the members of the class *Gammaproteobacteria*, such as *Klebsiella* and *Escherichia,* which are frequently related to neonatal infection. We found that *Gammaprotecbateria* was significantly higher in the unexposed group compared to those exposed to maternal antibiotics in the meconium specimen. This could indicate that the maternal antibiotic prophylaxis being used may be able to inhibit the growth of some pathogenic gram-negative bacteria. We also found that in the exposed group, there was a significantly higher abundance of order *Erysipelotrichales*, which was reported to be highly immunogenic. It has been reported that broad-spectrum antibiotics can make the growth of *Erysipetotrichales* flourish. *Erysipelotrichales* has also been associated with the inflammation process (234). . However, further investigation is still needed to determine its role during the neonatal period

Stratified by sepsis cases and non-sepsis, our study did not find a member of the predominant genus that was significantly higher in infants with sepsis. Reports with a smaller sample size than our study reported a predominance of *Staphylococcus, Paenibacillus*, and *Caulobacter*. Further research is required to explain these findings (109,228). In the healthy infants' meconium, several Proteobacteria, Firmicutes, and Actinobacteria members were found

to be significantly high. Although increased of Proteobacteria is usually related to cases with sepsis, this may show that in the meconium the microbiome composition may still be similar and start to alter as the infection developed. Consistent with the study by Stewart et al., our study also found that the intestinal protective microbiome, i.e., class *Bacilli* and genus *Bifidobacterium*, was significantly higher in healthy infants (235). Meanwhile, in infants with sepsis, several bacteria general containing species linked to disease, such as *Pseudomonas* and *Streptococcus*, were more abundant.

Our cohort study demonstrated that perinatal antibiotic exposure is associated with increased neonatal sepsis incidence in the earlier chapter. This nested case-control study design also showed that neonates who developed sepsis were significantly more likely to be exposed to perinatal antibiotic prophylaxis than those without sepsis. After stratifying by antibiotic type, we found that the positive association was strongest in neonates exposed to postnatal or to a combination of maternal and postnatal antibiotic prophylaxis. It has been suggested that antibiotic exposure can promote pathogen-predominant microbiota that are associated with sepsis due to the predominant pathogen in the microbiome(109). Therefore, we proceed with this study by exploring whether the neonates' gut microbiome features are involved in the pathway that links perinatal antibiotic prophylaxis exposure and neonatal sepsis. Due to the small number of cases, we limited the analysis to the meconium specimens. Our mediation analysis did not show a significant mediating association for the infants' meconium microbiome features in the association between antibiotic prophylaxis exposure and neonatal sepsis. This may be related to the small sample size. However, we observed several negative effects in the path of exposure, some mediators, and the outcome, mainly in the path of postnatal antibiotic prophylaxis. These negative effects could indicate that antibiotic prophylaxis may reduce alpha diversity and the

relative abundance of Actinobacteria and Bacteroidetes. Sequentially, higher alpha diversity and a higher abundance of Actinobacteria, Bacteroidetes, and Firmicutes may act as suppresser factors for the occurrence of sepsis. A few trials have evaluated the use of some strains from the Actinobacteria and Firmicutes phyla to prevent neonatal sepsis.

The trials that used *Bifidobacterium* strains as probiotics did not find a reduction in neonatal sepsis (114,115). In contrast, a trial in India observed a 40% reduction in newborn sepsis among those who received a combination of *Lactobacillus plantarum* and fructooligosaccharide (52). We did not reach the same findings in the path that included Proteobacteria. This may be related to the fact that phylum Proteobacteria contains more pathogenic species compared to the other predominant phyla (231). We also conducted a mediation analysis on Firmicutes/Bacteroidetes and Actinobacteria/Proteobacteria ratios. The ratios of these phyla have been widely accepted to have an important influence on maintaining normal intestinal homeostasis (236,237). However, our study did find a significant mediation association for these two ratios.

In addition, we also observed some possible inconsistent mediation in the mediation path of Firmicutes and Proteobacteria. Although the overall effect of perinatal antibiotic prophylaxis increased the risk of sepsis, these particular mediational paths had the opposite effect. Firmicutes and Proteobacteria may act as suppressor factors in the development of neonatal sepsis. In this situation, there could be other unmeasured mediators at play that would then explain the positive indirect variance of exposure in the development of sepsis.

Although several studies have addressed the impact of antibiotic exposure on the microbiome and neonatal sepsis, our study is the first to assess the sequential pathway of perinatal antibiotic prophylaxis, microbiome features, and neonatal sepsis. Our study was nested

in a prospective cohort study that enabled us to demonstrate the trend of gut colonization. We were also able to compare the impact of three types of antibiotic prophylaxis exposure in newborns. The main limitation of this study is the relatively small sample size and low rate of culture-proven sepsis, which limit the ability to conduct a rigorous mediation analysis by sepsis onset, mode of delivery, and microbiological results, and include other factors that may be involved in the incidence of neonatal sepsis. Applying metagenomics analysis may be able to better define the differences between study groups. A further limitation is that the meconium samples generally had a low abundance of bacteria, and there may be a potential for contamination.

5.5 Conclusion

Perinatal antibiotic prophylaxis and neonatal sepsis are associated with lower microbiome diversity in meconium and newborns' stool. Although there may be an indication that perinatal antibiotic exposure reduces microbiomes' alpha diversity, while the higher relative abundance of Actinobacteria, Bacteroidetes, and Firmicutes may reduce the risk of neonatal sepsis, there was not enough statistical evidence to show that microbiome composition mediates the effect of perinatal antibiotic prophylaxis on neonatal sepsis. A more extensive cohort study that includes maternal specimens to provide more robust evidence is worth further investigation.

CHAPTER 6 CONCLUSION AND FUTURE DIRECTION

Neonatal sepsis is an ongoing major public health challenge, particularly in low- and middle-income countries (LMICs). Among the available approaches to prevent the occurrence of neonatal sepsis, the perinatal antibiotic prophylaxis strategy adopted from high-income countries is one of the most frequently implemented strategies in LMICs. However, it is essential to underline that the disease burden in LMICs and high-income countries is different. The implementation of perinatal antibiotic prophylaxis in LMICs varies by country and even by health center. Considering the lack of studies that address the practice of perinatal antibiotic prophylaxis in LMICs and its impact on neonatal sepsis occurrence, it is essential to assess how antibiotic prophylaxis influences the epidemiology of neonatal sepsis.

By conducting a retrospective study and a prospective cohort study, this dissertation estimated the prevalence and incidence of neonatal sepsis, risk factors associated with neonatal sepsis, and the prevalence and predictors for antibiotic prophylaxis use during delivery and right after birth in Palembang, Indonesia. This dissertation also assessed the impact of antibiotic prophylaxis usage during the perinatal period on the occurrence of neonatal sepsis. Furthermore, using a nested case-control study, this dissertation investigated the effect of perinatal antibiotic prophylaxis exposure on infants' gut microbiome and whether infants' gut microbiome mediated the impact of perinatal antibiotic prophylaxis on neonatal sepsis.

Our study confirmed that, as in other LMICs, the admission prevalence and incidence of neonatal sepsis in Indonesia remains high. Unlike in high-income countries where LOS are more predominant than EOS, we found that the proportion of EOS and LOS was comparable. Culture-negative sepsis accounts for a significant proportion of neonatal sepsis cases. Culture-negative and culture-proven neonatal sepsis and EOS and LOS contribute to an equivalent proportion of

neonatal sepsis fatalities. Among all culture-proven sepsis, the proportion of gram-positive and gram-negative was similar. The most frequently found isolated pathogen was coagulase-negative staphylococci. Our study highlighted the high prevalence of multidrug-resistant pathogens. More than 50% of the isolated pathogens were resistant to at least one agent in three or more antimicrobial classes. In our analyses, we observed a stronger association with neonatal sepsis for deliveries with the presence of more than 18 hours of premature membrane rupture, the presence of foul-smelling amniotic fluid, high maternal leukocyte count, newborns with low birth weight, newborns receiving oral feeding other than exclusive breastfeeding, and newborns receiving nothing per mouth over 24 hours. We also found that low 5th minute Apgar scores increase EOS risk, while C-Section deliveries lower the risk of EOS. In addition, newborns who received non-invasive respiratory support were more likely to develop LOS.

We estimated that more than half of the newborns were exposed to prophylaxis antibiotics at least once during delivery. The highest exposure was from maternal prophylactic antibiotics, followed by the combination of maternal and postnatal prophylactic antibiotics and postnatal prophylactic antibiotics. Ceftriaxone, ampicillin, and gentamycin were the most frequently used prophylactic antibiotics during delivery and right after birth. In terms of maternal prophylactic antibiotics, in the retrospective review, there was a declining trend of prophylactic antibiotic use during the three consecutive years, from 59.2% to 46.2% and 38.1%. We observed that in the same study site, the tertiary level hospital, the declining trend continues to 24.6%. However, in our second study site, the secondary level hospital, the proportion of maternal prophylactic antibiotic use was significantly higher, 78.9%. Given that tertiary level hospitals serve more complicated pregnancy and deliveries cases compared to lower level referral hospital and the ratio of the numbers of tertiary level hospitals to secondary level hospitals in Indonesia is 6 to

43 we believe that to some extent, the description of prophylactic use in our second study site may be a better representation of how it is being used in a larger population in Indonesia (238).

By implementing a double estimator, our study found that, first, the Average Treatment Effect (ATE) of maternal and the combination of maternal and postnatal antibiotic prophylaxis can be negligible in the effort to prevent EOS. Second, postnatal antibiotic prophylaxis provided a notable positive ATE in the incidence of overall sepsis, LOS, and culture-negative sepsis. These findings indicate that although antibiotic prophylaxis may prevent some cases of early-onset sepsis, there is a strong indication that perinatal antibiotic prophylaxis may increase the risk of late-onset sepsis.

In terms of exploring the sequential association between perinatal antibiotic prophylaxis, gut microbiome, and neonatal sepsis incidence, our study observed that, in general, microbiome diversity in newborns with sepsis and newborns who were exposed to antibiotic prophylaxis tends to be less diverse compared to newborns without sepsis or who were not exposed to antibiotic prophylaxis. Overall, the predominant phyla were Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. We also demonstrated that in follow-up, in the exposed and sepsis group, the abundance of Proteobacteria tended to increase while the abundance of Firmicutes tended to decrease. In contrast, among the unexposed and non-sepsis group, the abundance of Firmicutes tended to increase while the abundance of Proteobacteria increased. The mediation analysis did not show a significant mediating association for the infants' meconium microbiome features in the association between antibiotic prophylaxis exposure and neonatal sepsis. However, in the sequential pathway, some possible adverse effects were observed, indicating that higher alpha diversity, higher abundance of Bacteroidetes, and Firmicutes may act as suppresser factors in the development of neonatal sepsis.

6.0 Future direction

The findings of this dissertation provide insights into the epidemiological situation of neonatal sepsis and the use of antibiotic prophylaxis during delivery and after birth in mothers and newborns in a low-middle-income country, Indonesia. This study affirms that the burdens and challenges in the management of neonatal sepsis in LMIC differ from those in high-income countries. Considering that most literature on neonatal sepsis epidemiology comes from high-income countries, but the highest burden of the disease is in low- and middle-income countries, it may be necessary to advocate a global collaborative effort to standardize neonatal sepsis surveillance to better understand the magnitude of neonatal sepsis and to fill the knowledge gaps on the disease. This will inform strategies at all levels of neonatal sepsis prevention and management efforts. We observed a high proportion of culture-negative neonatal sepsis along with a high proportion of multidrug-resistant neonatal sepsis. Given that antimicrobial resistance represents a significant threat to human health and has important global economic and security implications, there is an urge to develop an alternative method for pathogen identification that can add to traditional microbiological techniques to better manage neonatal sepsis and prevent injudicious antibiotic use. It will be an added value if the alternative diagnostic method is feasible to implement in LMICs, where the disease burden is highest.

Although the results of our study indicate that antibiotic prophylaxis given before and right after birth may increase the risk of late-onset sepsis, our study does not aim to challenge the existing clinical guidelines for giving IAP and prophylactic antibiotics to mother and neonates with documented risk factors for infection to prevent early-onset sepsis. However, our study supports the ongoing efforts to discover a non-antibiotic alternative strategy for neonatal sepsis

prevention to avoid the potentially harmful effects of perinatal antibiotics on subsequent health conditions, including intestinal microbiome alteration.

Our study may pioneer the implementation of causal mediation analysis to disentangle the role of microbiome features in the perinatal antibiotic exposure-neonatal sepsis relationship. More extensive cohort studies that document microbiota composition before and after antibiotic prophylaxis exposure and before, during, and after an episode of sepsis using more sophisticated next generation sequencing methods are needed to identify those commensals that protect against sepsis. Similarly, these tools could help identify those microbial population that are linked to increased susceptibility and worse outcomes. Conducting a larger human prospective study that includes an assessment of maternal microbiome (intestinal, vaginal, and skin), for instance, will allow us to better define the role of other confounding factors' roles in the path of antibiotic exposure, microbiome, and neonatal sepsis. These insights could enable us to provide more robust evidence to determine whether the overall microbiome composition or specific strains have a significant mediation role, and can help identify bacterial groups associated with immunological resilience. These groups, could be harnessed as potential biomarkers of susceptibility to neonatal sepsis or other infection. Being able to pinpoint the context in which gut commensals can drive protection against neonatal sepsis could help develop another intriguing and relatively new area of intervention research targeting microbiota restoration to prevent neonatal sepsis.

APPENDICES

APPENDIX A

Supplemental Table

Table 20. Antimicrobial resistance patterns among isolated pathogens in neonatal sepsis cases with antimicrobial susceptibility test (AST) results (n = 139)

	Resistance/Tested	Not tested
Gram-Positive		
Coagulase-negative staphylococci (n = 43)		
Methicillin	37/41	2
Vancomycin	1/36	7
Extended-spectrum cephalosporin	22/27	16
Extended-spectrum penicillin	21/23	20
Enterococcus sp. (n = 3)		
Methicillin	2/3	0
Vancomycin	0/3	0
Extended-spectrum cephalosporin	1/1	2
Extended-spectrum penicillin	1/1	2
Kocuria sp. (n = 1)		
Vancomycin	0/1	0
Extended spectrum cephalosporin	1/1	0
Extended-spectrum penicillin	0	1
Non-beta hemolytic streptococcus (n = 10)		
Extended spectrum cephalosporin	7/7	3
Extended-spectrum penicillin	7/7	3
Staphylococcus aureus (n = 5)		
Methicillin	3/5	0
Vancomycin	1/5	0

Table 20. (cont'd)

	Resistance/Tested	Not tested
Gram-Negative		
Acinetobacter sp. (n = 15)		
Extended spectrum cephalosporin	14/14	1
Extended spectrum penicillin	12/13	2
Carbapenem	7/12	3
Fluoroquinolones	9/11	4
Aminoglycosides	6/15	0
Enterobacter sp. (n = 10)		
Extended spectrum cephalosporin	8/10	0
Extended spectrum penicillin	7/8	2
Carbapenem	8/10	0
Aminoglycosides	0/10	0
Fluoroquinolones	3/10	0
Escherichia coli (n = 5)		
Extended-spectrum cephalosporin	1/3	1
Extended-spectrum penicillin	2/2	1
Carbapenem	0/1	3
Aminoglycosides	1/3	2
Fluoroquinolones	2/3	2
Klebsiella pneumoniae (n = 27)		
Extended spectrum cephalosporin	16/26	1
Extended spectrum penicillin	18/21	6
Carbapenem	3/23	1
Aminoglycosides	7/26	1
Fluoroquinolones	6/26	1

Table 20. (cont'd)

	Resistance/Tested	Not tested
Pantoea sp. (n = 5)		
Extended-spectrum cephalosporin	5/5	0
Extended-spectrum penicillin	4/4	1
Carbapenem	4/5	0
Aminoglycosides	0/5	0
Fluoroquinolones	1/5	0
Pseudomonas aeruginosa (n = 11)		
Extended-spectrum cephalosporin	9/10	1
Extended-spectrum penicillin	0/11	0
Carbapenem	7/11	0
Aminoglycosides	6/11	0
Fluoroquinolones	3/7	1
Serratia sp. (n = 4)		
Extended-spectrum cephalosporin	1/4	0
Extended-spectrum penicillin	4/4	0
Carbapenem	1/4	0
Aminoglycosides	1/4	0
Fluoroquinolones	0/4	0

Extended-spectrum cephalosporin (any one of ceftriaxone, ceftazidime, or cefotaxime): extended-spectrum penicillin (piperacillin); carbapenems (meropenem or imipenem); fluroquinolone (ciprofloxacin or levofloxacin); and aminoglycoside (gentamycin or amikacin).

APPENDIX B

IRB Determination

MICHIGAN STATE
UNIVERSITY

Initial Study APPROVAL
Pre-2018 Common Rule

April 2, 2019

To: Lixin Zhang

Re: **MSU Study ID:** STUDY00001738
IRB: Biomedical and Health Institutional Review Board
Principal Investigator: Lixin Zhang
Category: Expedited 3, 5, 7
Submission: Initial Study STUDY00001738
Submission Approval Date: 4/1/2019
Effective Date: 4/1/2019
Study Expiration Date: 3/31/2020

Title: Intrapartum antibiotic prophylaxis and gram-negative neonatal sepsis in Palembang, Indonesia

This submission has been approved by the Michigan State University (MSU) Biomedical and Health Institutional Review Board. The submission was reviewed by the Institutional Review Board (IRB) through the Non-Committee Review procedure. The IRB has found that this study protects the rights and welfare of human subjects and meets the requirements of MSU's Federal Wide Assurance (FWA00004556) and the federal regulations for the protection of human subjects in research (e.g., pre-2018 45 CFR 46, 28 CFR 46, 21 CFR 50, 56, other applicable regulations).

Office of Regulatory Affairs
Human Research Protection Program

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How to Access Final Documents
To access the study's final materials, including those approved by the IRB such as consent forms, recruitment materials, and the approved protocol, if applicable, please log into the Click™ Research Compliance System, open the study's workspace, and view the "Documents" tab. To obtain consent form(s) stamped with the IRB watermark, select the "Final" PDF version of your consent form(s) as applicable in the "Documents" tab. Please note that the consent form(s) stamped with the IRB watermark must typically be used.

Continuing Review: IRB approval is valid until the expiration date listed above. If the research continues to involve human subjects, you must submit a Continuing Review request at least one month before expiration.

Modifications: Any proposed change or modification with certain limited exceptions discussed below must be reviewed and approved by the IRB prior to implementation of the change. Please submit a Modification request to have the changes reviewed. If changes are made at the time of continuing review, please submit a Modification and Continuing Review request.

MICHIGAN STATE
UNIVERSITY

Continuing Review APPROVAL
Pre-2018 Common Rule

February 11, 2020

To: Lixin Zhang

Re: **MSU Study ID:** STUDY00001738
IRB: Biomedical and Health Institutional Review Board
Principal Investigator: Lixin Zhang
Category: Expedited 3, 5, 7
Submission: Continuing Review CR00001049
Submission Approval Date: 2/7/2020
Effective Date: 2/7/2020
Study Expiration Date: 2/6/2021

Title: Intrapartum antibiotic prophylaxis and gram-negative neonatal sepsis in Palembang, Indonesia

This submission has been approved by the Michigan State University (MSU) Biomedical and Health Institutional Review Board.The submission was reviewed by the Institutional Review Board (IRB) through the Non-Committee Review procedure. The IRB has found that this study protects the rights and welfare of human subjects and meets the requirements of MSU's Federal Wide Assurance (FWA00004556) and the federal regulations for the protection of human subjects in research (e.g., pre-2018 45 CFR 46, 28 CFR 46, 21 CFR 50, 56, other applicable regulations).

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How to Access Final Documents
To access the study's final materials, including those approved by the IRB such as consent forms, recruitment materials, and the approved protocol, if applicable, please log into the Click™ Research Compliance System, open the study's workspace, and view the "Documents" tab. To obtain consent form(s) stamped with the IRB watermark, select the "Final" PDF version of your consent form(s) as applicable in the "Documents" tab. Please note that the consent form(s) stamped with the IRB watermark must typically be used.

Continuing Review: IRB approval is valid until the expiration date listed above. If the research continues to involve human subjects, you must submit a Continuing Review request at least one month before expiration.

Modifications: Any proposed change or modification with certain limited exceptions discussed below must be reviewed and approved by the IRB prior to implementation of the change. Please submit a Modification request to have the changes reviewed. If changes are made at the time of continuing review, please submit a Modification and Continuing Review request.

MICHIGAN STATE
UNIVERSITY

Continuing Review APPROVAL
Pre-2018 Common Rule

January 13, 2021

To: Lixin Zhang

Re: **MSU Study ID:** STUDY00001738
IRB: Biomedical and Health Institutional Review Board
Principal Investigator: Lixin Zhang
Category: Expedited 3, 5, 7
Submission: Continuing Review CR00001493
Submission Approval Date: 1/11/2021
Effective Date: 1/11/2021
Study Expiration Date: 1/10/2022

Title: Intrapartum antibiotic prophylaxis and gram-negative neonatal sepsis in Palembang, Indonesia

This submission has been approved by the Michigan State University (MSU) Biomedical and Health Institutional Review Board. The submission was reviewed by the Institutional Review Board (IRB) through the Non-Committee Review procedure. The IRB has found that this study protects the rights and welfare of human subjects and meets the requirements of MSU's Federal Wide Assurance (FWA00004556) and the federal regulations for the protection of human subjects in research (e.g., pre-2018 45 CFR 46, 28 CFR 46, 21 CFR 50, 56, other applicable regulations).

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Institutional restrictions to in-person human subject research activities conducted by MSU employees, MSU students, or agents of MSU are in place, but MSU is phasing in human research that has the potential for in-person interactions with participants, using a Tier approach. Restrictions to in-person interactions with human research participants by MSU employees, MSU students, or agents of MSU are in place until the activity is permitted under a Tier and a Human Research Plan for a Safe Return is approved. Visit http://hrpp.msu.edu/COVID-19/index.html for the restrictions, Tiers, forms, and the process.

How to Access Final Documents
To access the study's final materials, including those approved by the IRB such as consent forms, recruitment materials, and the approved protocol, if applicable, please log into the Click™ Research Compliance System, open the study's workspace, and view the "Documents" tab. To obtain consent form(s) stamped with the IRB watermark, select the "Final" PDF version of your consent form(s) as applicable in the "Documents" tab. Please note that the consent form(s) stamped with the IRB watermark must typically be used.

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