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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BLOOD CULTURE ISOLATES OF  
NEONATES WITH SEPSIS, HO MUNICIPALITY

BY

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## DECLARATION

I Fortress Yayra Aku, declare that this dissertation is my original work, except for duly referenced articles, and that no form of it has been presented elsewhere for another degree.

.....  
DATE.....

FORTRESS YAYRA AKU  
(CANDIDATE)

.....  
DATE.....

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(ACADEMIC SUPERVISOR)



## **DEDICATION**

This study is dedicated to the memory of my late father Rev. Wilson Komla Aku.



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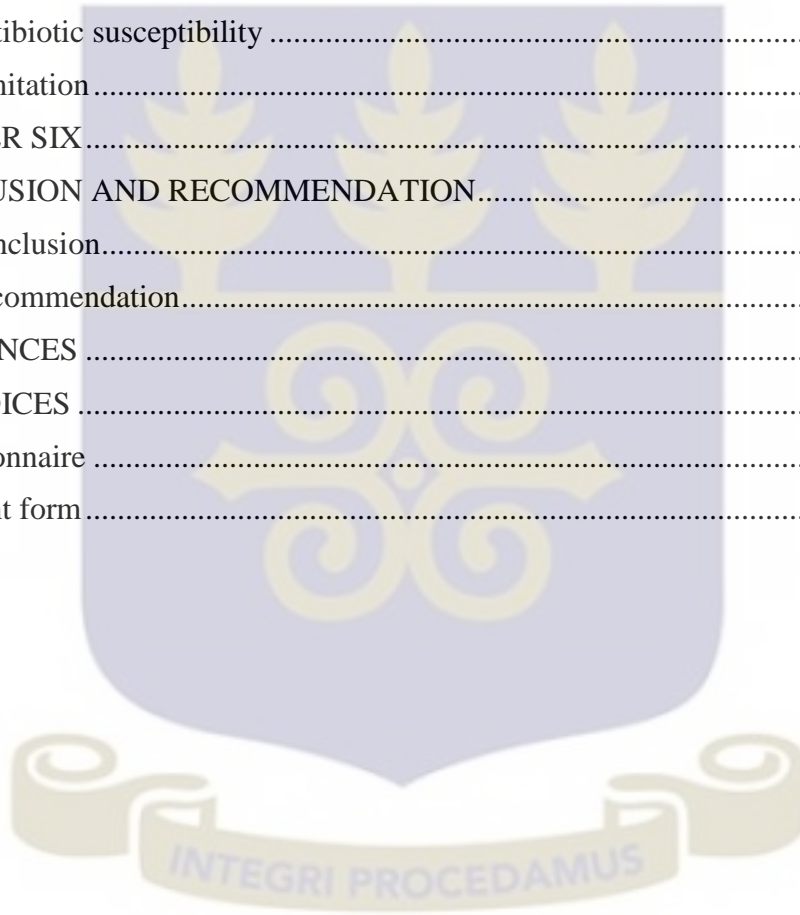
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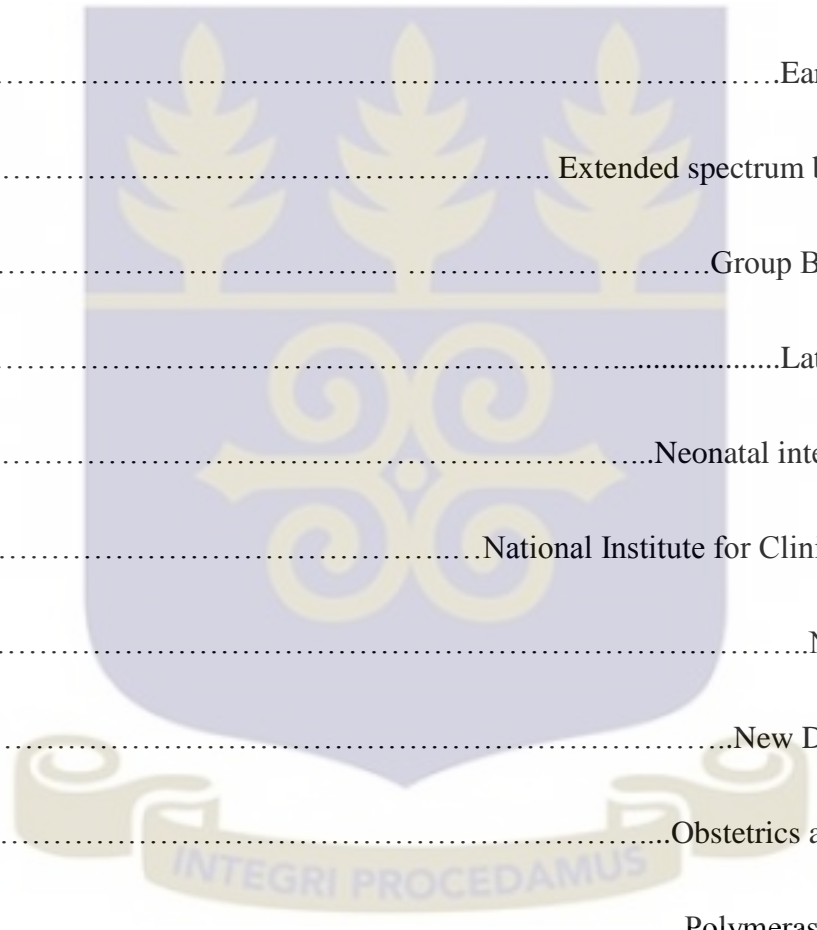
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## LIST OF ABBREVIATIONS



BNFC.....	British National Formulary for Children
CoNS.....	Coagulase negative Staphylococcus
CRP.....	C - reactive protein
EOS.....	Early onset sepsis
ESBL.....	Extended spectrum beta-lactamase
GBS.....	Group B streptococcus
LOS.....	Late Onset sepsis
NICU.....	Neonatal intensive care unit
NICE.....	National Institute for Clinical Excellence
NS.....	Neonatal sepsis
NDM-1.....	New Delhi-metallo 1
O & G.....	Obstetrics and gynecology
PCR.....	Polymerase chain reaction
SCN.....	Special care nursery
VLBW.....	Very low birth weight
WHO.....	World Health Organization

## ABSTRACT

**Background:** Globally, 4 million neonatal deaths occur annually, with one-third of such deaths occurring as a result of infection. In 2011, there were 7.2million deaths in children below 5years globally, and a proportion of 40% of these deaths occurred in neonates. Sepsis was reported to account for one-third of these deaths. Presently, multi drug antibiotic resistance is rapidly increasing in Neonatal Intensive Care Units (NICUs), particularly in developing countries and poses a threat to public health. Blood culture of neonatal sepsis cases in Tamale Teaching Hospital showed 44% positive cases of all samples and *Acinetobacter* and *Klebsiella* species showed 100% resistance to ampicillin. The change in these organisms has been reported to vary across regions, between health facilities and even within the same facility. Continuous surveillance is required to inform antibiotic choice for neonatal sepsis management. This study aims at identifying the common causative organisms of neonatal sepsis and their antibiotic susceptibility pattern in the Ho municipality.

**Method:** A cross sectional study was conducted, in the Ho municipality from January to May, 2016. A semi-structured questionnaire was used to collect socio-demographic data from mothers of neonates clinically suspected of sepsis. Clinical data of both mothers and neonates were extracted from case notes. Blood was also taken from neonates for culture and antibiotic susceptibility pattern determined. Data was computed into proportions, frequencies and odds ratio using STATA 13.0 software.

**Results:** Out of the 150 clinically suspected neonatal sepsis cases, 91 (60.7%) were males. The mean gestational age was 37 weeks (standard deviation  $\pm 3$ weeks) and mean

birth weight was 2855.28g (standard deviation  $\pm 740.43$ g). The prevalence of culture positive sepsis was 17.3% of the 150 suspected cases. Gram positive organisms, had a preponderance (69%) over gram negative organisms (31%). *Staphylococcus epidermidis* was the most common (53.8%) isolate identified. There was a single isolate (4%) each of *Proteus mirabilis* and *Escherichia coli* identified. All the isolates identified showed 100% resistance to ampicillin.

**Conclusion:** The prevalence of culture proven sepsis was 17.3% and *Staphylococcus epidermidis* was the most common isolate identified. There was a high resistance rate among the pathogens isolated. There is need for ampicillin to be reviewed as first line drug for empirical treatment in neonatal sepsis.



### Definition of Term

**Neonatal sepsis:** For the purpose of this study, is defined as both term and preterm neonates clinically diagnosed with sepsis and presenting with one or more of the following features: presence of fever ( $\geq 38^{\circ}\text{C}$ ) or hypothermia ( $\leq 36^{\circ}\text{C}$ ), convulsions, lethargy, difficulty to feed, difficulty to breath, hypoglycaemia, vomiting, bulging fontanel, respiratory distress, jaundice and signs of infection on the skin and umbilical pus discharge or hypereamia.

**Early onset sepsis:** The onset of signs and symptoms of sepsis in neonates within 72hours of life.

**Late onset sepsis:** The onset of signs and symptoms of sepsis in neonates after 72hours of life.



## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

Neonatal sepsis (NS) refers to a clinical syndrome that is marked by signs and symptoms of infection in the first 28 days of life, with or without isolation of a pathogen (Verma et al., 2015). NS is a significant contributor of morbidity and mortality in the newborn (Al-Shamahy et al., 2012) and constitutes different systemic infections in the newborn such as septicaemia, meningitis, pneumonia, osteomyelitis, urinary tract infection and arthritis. A normal fetus is sterile until shortly before birth since placenta and amniotic sac serve as effective protection against infection. However at birth, the newborn is exposed to the microbial environment. Neonatal sepsis can be categorized as early onset sepsis (EOS) and late onset sepsis (LOS). EOS is defined as onset of signs and symptoms of infection within 72hours of life and may be associated with pathogen isolation or not. In the LOS, signs and symptoms present after 72hours of life (Shane & Stoll, 2013). The categorization of EOS and LOS is to show the varying causes and pathophysiology of common isolates related to the time of onset of the condition. Premature infants stand a higher risk of invasive infection because of their impaired innate immunity. Since fetal immune response begins at 24weeks and develops until term, premature neonates are disadvantaged with incomplete immune system development. This makes them more susceptible to infections compared to term neonates (Shane & Stoll, 2013). Neonatal infections defined as bacteraemia/sepsis, pneumonia and meningitis are responsible for 23.4% of neonatal mortality globally each year (Liu et al., 2012) and estimates indicate

that sepsis is the major cause of mortality, accounting for 30%-50% of neonatal deaths yearly worldwide especially in developing countries (Sriram, 2011). It has also been reported that 42% of the deaths caused by sepsis occur in the first week of life (Liu et al., 2012). Data shows that infections are the leading causes of neonatal mortality, followed by asphyxia and congenital abnormality (Lawn, Cousens, & Zupan, 2005). More than 76% of under 5 mortality is caused by infectious disease in Africa (Liu et al., 2012). The possibility of new forms of antibiotic resistance in sepsis, to spread fast is great considering the recent global proliferation of New Delhi metallo-lactamase-1 (NDM-1) producing *enterobacteriaceae*. The gene which confers resistance to carbapenems started in India in 2009 and since 2010 has been recorded in North America, Europe and Asia (Kumarasamy et al., 2010). Several factors are responsible for the steady rise in antibiotic resistance in developing countries including misuse of antibiotics, over-the-counter and parallel market access, and counterfeit or poor quality drugs, in addition to suboptimal hygiene and living conditions (Kelesidis et al., 2007). The World Health Organization (WHO) pocket book of Hospital Care for Children outlines guidelines for clinical diagnosis and management of suspected bacterial infections in children in resource-limited settings (WHO, 2005). The generic recommendation gives a list of 8 major antibiotics for treating severe bacterial infections among neonates and children (Le Doare et al., 2014). In line with this recommendation, when a neonatal sepsis is suspected, an empirical antimicrobial treatment is given until culture and antimicrobial susceptibility results are ready that would set up a specific management (Ganatra & Zaidi, 2010).

## 1.2 Burden

It is estimated that globally 4million newborns die yearly with one-third of the deaths caused by infections (Ganatra & Zaidi, 2010). Sepsis and meningitis are one of the major causes of these deaths particularly among low birth weight newborn infants (Puopolo et al., 2011). Various conditions contribute to the high neonatal mortality especially in Sub-Saharan Africa of which neonatal sepsis accounts for approximately 26% (Lawn et al.,2005). In 2011, 7.2million deaths occurred in children under 5years, 40% of which occurred within the first 28days of life. Estimates show that severe infection accounts for one-third of these deaths especially in developing countries (Lozano et al., 2011). However, little attention is given to long-term morbidity related to neonatal sepsis especially in resource-limited settings (Seale et al., 2013). Data on direct-cause-of-death indicates that sepsis could account for 15% of neonatal deaths. Estimates show that 30-40% of infections result in neonatal sepsis, and transmissions occur at the time of birth (Ganatra & Zaidi, 2010). Records indicate that morbidity of NS varies from country to country, with incidence ranging from 1 to 5 cases per 1000 live births in the developed countries (Thaver & Zaidi, 2009). In developing countries, where neonatal mortality disproportionately occurs, neonatal sepsis accounts for approximately one-third of neonatal deaths (Ganatra & Zaidi, 2010) and incidence is higher compared to developed countries, ranging from 49 to 170 per 1000 live births (Thaver & Zaidi, 2009). In 2008 in Ghana, there were 22, 672 deaths estimated to have occurred among neonates with neonatal sepsis causing 4,923 deaths (21.7%) (Black et al., 2010) Estimates indicate that infections account for at least 32% of newborn deaths in Ghana and a substantial number

of these infections are transmitted from the mother to the newborn (Enweronu-Laryea et al., 2011). In a similar study conducted in the Kassena-Nankana district of northern Ghana, infections was reported to account for 59% of late neonatal deaths with almost 46% of deaths occurring within the first three days of life (Welaga et al., 2013). Antimicrobial resistance is an emerging global public health problem as far as neonatal sepsis is concerned. In a study done in China on 116 blood cultures obtained from neonates with sepsis, antibiotics showed resistance to isolates. For EOS category, the resistance rate for *Staphylococcus aureus*, coagulase negative staphylococcus (CoNS), *Klebsiella pneumoniae* and *Escherichia coli* was 100%, 90.9%, 85.7%, and 75% respectively while for the LOS category, the resistance rate for *Staphylococcus aureus* and *Klebsiella pneumoniae*, CoNS and *Escherichia coli* was 100%, 100%, 92.6% and 85.7% respectively (Li et al., 2013). A similar study done in India found that Gram-negative bacteria were susceptible to meropenem (80%) and piperacillin-tazobactam (75%) followed by cefotaxime (50%), imipenem (50%) and chloramphenicol (50%) while all were resistant to ofloxacin and least sensitive to amikacin (25%). Of three isolates of *Staphylococcus aureus*, one was found to be methicillin resistant (Jajoo et al., 2015). The WHO recommends clean delivery practices as an important strategy in a bid to reduce sepsis-related deaths through infection prevention (WHO, 2005).

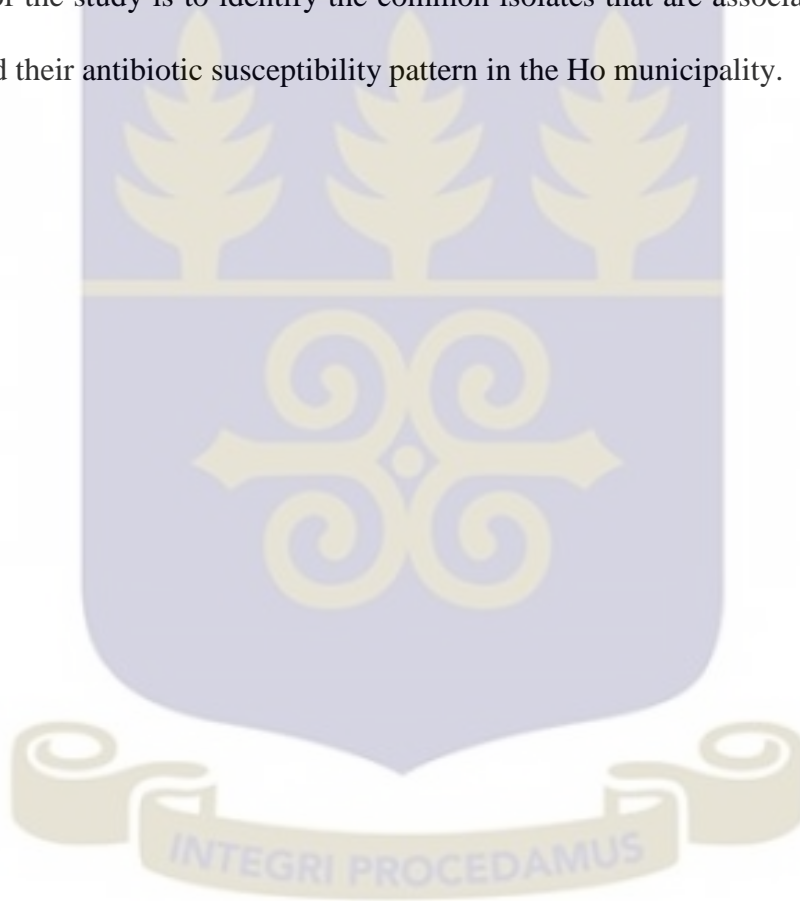
### **1.3 Problem statement**

Reports show that the range of microorganisms responsible for neonatal sepsis vary from time to time and differ from region to region (Sheth et al., 2012). Over the period, the

causative organisms of neonatal sepsis have changed; therefore health facility based surveys are important for prevention and treatment of neonatal invasive infections (Shim et al., 2011). Neonates in neonatal intensive care units (NICUs) worldwide are often exposed to antibiotics. However, differences exist in this practice between NICUs (Schulman et al., 2015). Exposure to these antibiotics may be linked to change in microbial environment and susceptibility to infections, which may lead to adverse outcomes (Wang et al., 2010). In addition, regulatory system on antibiotic use is weak, with porous surveillance system and limited reporting of treatment failures (Gyansa-Lutterodt, 2013).

In a study done in a NICU in India, 67.8% of isolated strains from blood cultures have shown multiple resistance, of which *Staphylococcus aureus* and *Streptococcus epidermidis* were the most common strains showing resistance (Sheth et al., 2012). Extended beta-spectrum-lactamase (EBSL)- *Enterobacteriaceae* showed resistance to penicillin, narrow-spectrum and extended-spectrum cephalosporins, except the cephamycins and aztreonam (Sharma, 2013). However, there are few reported studies on the problem of EBSL in Africa and Ghana particularly. Reports on drug resistance in Ghana has come mainly from the two teaching hospitals in Accra and Kumasi; Korle-Bu Teaching Hospital and Komfo Anokye Teaching Hospital respectively and rarely any report from the regional and district hospitals (Opintan & Newman, 2007). A greater proportion of neonates are admitted in the NICUs of the Volta Regional Hospital and Ho Municipal Hospital on account of neonatal sepsis.

The consequence of neonatal sepsis and antibiotic resistance in sepsis are graveous. It could result in high morbidity, high mortality and long term effects such as neuro developmental impairments. Also, there will be longer hospitalization of neonates, and families and health care systems will incur additional costs (Obiero et al., 2015). The purpose of the study is to identify the common isolates that are associated with neonatal sepsis and their antibiotic susceptibility pattern in the Ho municipality.



### 1.4 Conceptual framework

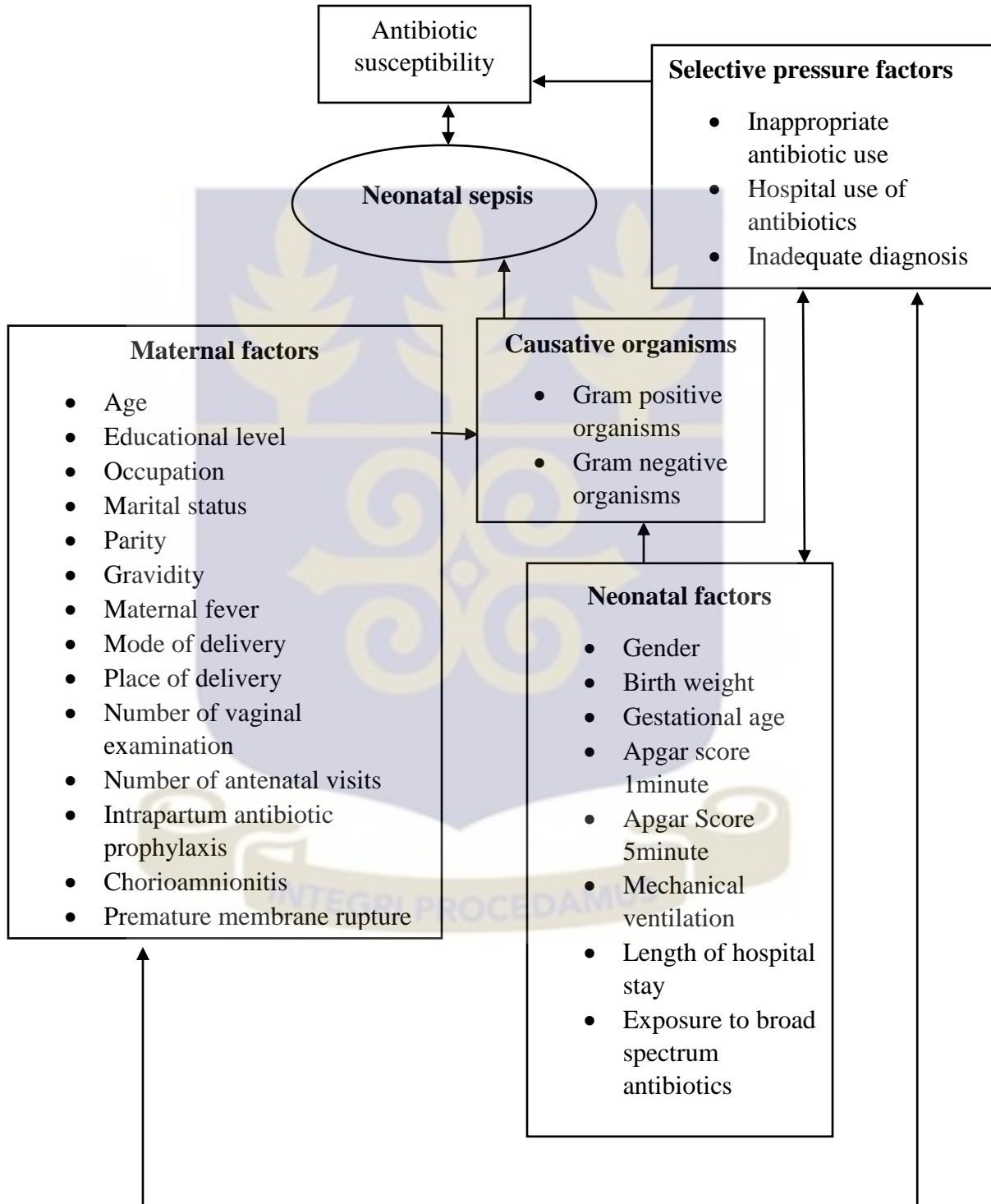


Figure 1: Conceptual framework of risk factors and causative organisms of neonatal sepsis and their inter-relationship with antibiotic susceptibility.

The factors influencing transmission of pathogens causing neonatal sepsis are interrelated with selective pressure factors that affect antibiotic susceptibility in neonatal sepsis (Figure 1). This selective pressure factors enhance transmission of resistant organisms and hence affect effectiveness of antibiotics. Critically ill neonates may require antibiotics, however the heavier use of antibiotics in hospital may select for resistant organisms that would affect antibiotic susceptibility. In a bid to satisfy insistent mothers of ill neonates, health care workers may initiate antibiotics without a complete laboratory diagnosis of infection. They may give broad spectrum antibiotics when a specific one may be more appropriate. This practice enhances selective pressure for transmission of resistant organisms causing neonatal sepsis.

The occurrence of neonatal sepsis is influenced indirectly by maternal and neonatal factors as described in the conceptual framework (Figure 1). Microbiological organisms that cause neonatal sepsis have a direct relationship with neonatal sepsis. This is because they are either transmitted vertically from the mother or horizontally from the hospital environment or the community to the neonate. The susceptibility pattern of these causative organisms to commonly used antibiotics is influenced by selective pressure and affects treatment outcome of neonatal sepsis. The inter relationship of these factors are represented in a conceptual framework in Figure 1.

Maternal factors that affect neonatal sepsis could be considered in two parts: maternal socio- demographic and maternal obstetric factors. The age of a mother has been identified to affect neonatal sepsis. Women of advanced ages are more prone to infections in pregnancy due to gradual deterioration of the immune system. Infections during pregnancy in these women are more likely to be transmitted to neonates. Also, women of younger ages during pregnancy are equally prone to infections that may be transmitted during delivery due to the fact that, their physiological system may not be ready enough for pregnancy and its associated risks.

The educational level of a woman could indirectly affect a neonate developing sepsis. Since some of the causative agents for neonatal sepsis are transmitted from a mother whose genital tract is colonized with pathogens, an educated pregnant woman may seek health care in the event of symptoms of vaginal infection. On the other hand, a less educated woman may seek other sources of treatment that could eventually transmit infections to the baby. Also the hygienic practices of an educated woman soon after delivery could be more informed and healthier to prevent infection in the newborn as compared to a less educated woman.

When a woman is married, decisions regarding health care as well as hygienic practices during pregnancy and soon after delivery could be influenced by the husband's knowledge, beliefs and decisions. A man who is knowledgeable on healthy practices during pregnancy, would have a positive impact on the woman, hence preventing possible practices that may lead to infection in the newborn. On the other hand, a man whose knowledge and beliefs regarding healthy practices during pregnancy is not

adequate and yet makes the decisions in the home may negatively impact the pregnant woman's health, and consequently influence infection in the newborn. For a woman who is not married, decisions on healthy practices during pregnancy may depend on information obtained from health care workers, friends and family from whom she gets support. Therefore based on her decision, the adequate or inadequate knowledge would be used. This could lead to hygienic or unhygienic practices that would positively or negatively influence infection in the neonate. Usually, the educational level of women interplays with practices they engage in during pregnancy and postnatally; irrespective of their marital status.

When women have regular source of income, they are able to better take care of themselves during pregnancy and after delivery. This is because pregnancy and babies bring with them peculiar economic demands. Hence a woman who is gainfully employed, will not hesitate to access health care during pregnancy and after delivery in order to ensure healthy practices to keep their babies safe from infection. On the contrary, women who are not gainfully employed may not be able to access health care adequately and engage in practices that would keep their babies safe from infection. Therefore, whether a woman has an occupation or not, could influence practices that lead to transmission of infection to newborns.

The obstetric factors of a mother also determine the possibility of infection acquisition by newborns. Premature rupture of membranes has been identified as a predisposing factor to sepsis in the neonate. A woman who has premature membrane rupture, is more prone to infection that could be transferred to the newborn rather than a woman who has an

intact membrane and ruptures at the appropriate time. Chorioamnionitis has been linked with neonatal sepsis. It is reported that, neonates born to women who experience chorioamnionitis, stand a greater risk of developing sepsis. When membranes and the chorion of the placenta are inflamed, neonates become exposed to infections and could develop sepsis.

When fever is reported in pregnant women, it is a sign of infection. Therefore, neonates are predisposed to these infections by vertical transmission from their mothers. Usually, mothers are given intrapartum antibiotic prophylaxis, on account of a suspected or a confirmed infection. This suggests that maternal fever and women who receive intrapartum antibiotic prophylaxis are risk factors of neonatal sepsis. Increased vaginal examination before delivery has been associated with neonatal sepsis. More vaginal examination is identified as a predictor of neonatal sepsis. The more women attend antenatal care clinics, the greater the chance, that they may receive more education on good practices to prevent neonatal sepsis. Also, more antenatal visits may ease the chances of identifying risk factors of sepsis among pregnant women by health staff for timely intervention. It is noted that, there is a link between parity and gravidity of women and neonatal sepsis. In addition, the place of birth has an influence on neonates developing sepsis. If a mother gives birth at a health facility, chances are that, delivery practices would be safer to prevent sepsis. On the other hand, in home deliveries sub-standard hand and environmental hygiene could serve as predictors of infection in the neonate.

Neonates are delivered either by spontaneous vaginal delivery, caesarean section or assisted delivery. It is noted that, when a mother's vaginal tract is colonized by infectious organisms, there could be vertical transmission to neonates during delivery to result in neonatal sepsis. On the other hand, transmission of infectious organisms to neonates during caesarean section, or assisted delivery could be due to delivery practices or from health care personnel.

A baby born prematurely has a greater risk of developing sepsis, compared to a baby born at term. Preterm babies are disadvantaged in terms of development of their immune system and other organs like the skin that serve as barriers against infection. Hence in the event of an infection, term babies may be able to subdue, while preterm babies may be susceptible to these infections. Low birth weight has also been identified as a predictor to neonatal sepsis. The same explanation has been given as in the case of premature neonates. Incomplete development of neonates' physiological and structural make up affects a neonate's susceptibility to infections. Generally, male neonates have been identified as more susceptible to sepsis than their female counterparts. The reason given is the possible defect in the X-linked immune regulatory genes in males, which has no defect in females.

Neonates that have undergone invasive and non-invasive hospital procedures such as mechanical ventilation, intravenous cannula, central venous catheter, parenteral ventilation and assisted ventilation have a higher risk of developing sepsis. The manipulations of these devices by health care workers may predispose these devices to colonization of pathogens. When these devices are colonized with pathogens from the

health care system, neonates can develop sepsis. However, neonates that are not exposed to these manipulations, have a lower risk of developing sepsis.

Exposure to broad spectrum antibiotics may suggest that a neonate is clinically diagnosed with infection, without a known specific causative organism. Broad spectrum drug administration, may make a neonate more susceptible to sepsis, due to breakdown of the immune system. Infection with sepsis causing organisms in an immune compromised neonate also renders treatment difficult if not impossible because of resistance. Reports also indicate that neonates with lower Apgar scores at one and five minutes are more likely to develop sepsis. The Apgar score, which is rated on a scale of ten is a general assessment of the performance of a neonate after birth.

The length of stay in a Neonatal Intensive Care Unit (NICU) is related to neonatal sepsis development. Neonates who have longer hospital stay, are predisposed more to sepsis, as compared to those with shorter hospital stay. The longer a neonate stays in the NICU, the higher their risk of acquiring infection from the hospital environment and handling by health care staff, if they are colonized with pathogens. Additionally, when other neonates are colonized with sepsis causing pathogens, a neonate that is hospitalized longer, has a greater risk of acquiring infection as the same health care workers will be handling all the neonates despite infection control practices. Shorter hospital stay of neonates is inversely related to greater risk of developing sepsis.

Neonatal sepsis development is directly influenced by microorganisms. These causative agents are transmitted vertically from the mother or horizontally from the health care

system (nosocomial infections) or the community. They are usually gram positive and gram negative organisms. The vertical transmission from the mother may be due to colonization of the genital tract with normal flora, which becomes harmful in the neonate upon transmission. The horizontal transmission from the hospital environment or the community may be due to unhygienic practices of both family and health care system. Depending on the type of organisms that are transmitted, antibiotic susceptibility in neonatal sepsis may be challenging. Infection with resistant strains may result in prolonged treatment or treatment failure.

### **1.5 Justification**

At the end of the study, the causative organisms of neonatal sepsis in the Volta Regional Hospital and Ho Municipal Hospital will be identified. The antibiotic susceptibility pattern of these organisms will also be determined. Since reports indicate that causative agents for neonatal sepsis change over time even within the same health facility, this study may add to knowledge on the current pathogens causing neonatal sepsis in the Volta Regional Hospital and Ho Municipal Hospital Neonatal Intensive Care Units (NICUs). Hence this may improve on the preventive strategies against neonatal sepsis in the two facilities. Awareness of the most common causative agents of neonatal infection may be important in establishing effective measures to prevent and treat neonatal infection (Dagnew et al., 2012). Findings from a study showed that, to develop adequate measures of prevention, the mechanism through which newborns acquire infections must be understood better (Chan et al., 2013). The findings on antibiotic susceptibility pattern of isolates may inform clinicians on the choice of the most effective drugs for empirical

treatment of neonatal sepsis. This is because when empirical treatment is not based on current causative agents, treatment of sepsis may not be successful and may increase cost. In the absence of surveillance records of antibiotic susceptibility, empirical treatment could fail at the same time be expensive (Newman, Frimpong, Donkor, Opintan, & Asamoah-Adu, 2011). Findings from this study may also add to knowledge on factors that are associated with culture proven sepsis.

## **1.6 Objective**

### **1.6.1 General Objective**

- To assess antibiotic susceptibility pattern of blood culture isolates of neonates with sepsis in the Ho municipality.

### **1.6.2 Specific Objectives**

1. To determine the prevalence of culture proven neonatal sepsis in the Ho municipality.
2. To determine common isolates associated with neonatal sepsis in the Ho municipality.
3. To determine antibiotic susceptibility pattern of isolates associated with neonatal sepsis in the Ho municipality.
4. To determine factors associated with culture proven sepsis in the Ho municipality.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Neonatal sepsis

Neonatal sepsis (NS) is described as a clinical condition of bacteraemia that is characterized by systemic signs and symptoms of infection in the first month of life. When pathogenic organisms enter the bloodstream, they may result in enormous infection with or without much localization (Paolucci, Landini, & Sambri, 2012). This is a serious condition that may lead to death, if effective antibiotic therapy with supportive care is not given (Zaidi et al., 2011). Usually, the signs and symptoms that NS presents with are not specific and include cyanosis and apnea, feeding difficulties, lethargy or irritability, hypotonia, seizures, bulging fontanel, poor perfusion, bleeding problems, abdominal distension, hepatomegaly, guaiac-positive stools or unexplained jaundice. Occurrence of NS is low among asymptomatic neonates however, it cannot be overlooked (Polin, 2012); while term neonates were reported to be more likely to react to bacterial infections with fever, preterms on the other hand, were more likely to react with hypothermia. Depending on the time a neonate presents with infection, NS can be categorized into early onset sepsis (EOS) and late onset sepsis (LOS) (Mhada, Fredrick, Matee, & Massawe, 2012). The classification into early and late onset neonatal sepsis is crucial in prevention and treatment due to aetiological variation. EOS and LOS categorization especially between time of birth and clinical onset of infection differ between (or even within) countries, with the timeline for early-onset ranging from <48hours to <1week after delivery (Guerti et al., 2011).

Early onset sepsis (EOS) is still a complication in the post-delivery period and refers to infection occurring within the first three (3) days of life and is a major cause of morbidity and mortality (Weston et al., 2012). The predictors of EOS include prematurity, associated immunologic immaturity, maternal group B streptococcal colonization, rupture of membranes greater than 18hours and maternal intra-amniotic infection (Stoll et al., 2011).

Recent reviews of causative agents associated with infants with sepsis in the developing world revealed that in EOS, gram negative organisms predominated in the ratio of 2:1 with *Escherichia coli* being the most commonly isolated pathogen. The authors were of the view that the reason behind the predominance of gram negative organisms in EOS was due to its acquisition from the environment during unhygienic birth practices (Zaidi et al., 2011). Previously, group B streptococcus (GBS) has been shown to be rare in developing countries. However, GBS colonization rates in mothers and neonates in over nine African countries, showed results that were comparable to rates in countries with documented high disease burden (Dagnew et al., 2012). EOS GBS disease usually manifests early in life with majority of cases occurring within 24 to 48hours and the rest within 7days after birth (Black, Margarit, & Rappuoli, 2013). However, recent systematic reviews revealed that studies that indicated the use of intrapartum antibiotic prophylaxis (IAP) are associated with lower incidence of EOS GBS disease (Edmond et al., 2012). More recent studies in Africa have shown higher rates of early onset neonatal GBS infection (Gray et al., 2007).

Late onset sepsis (LOS) occurs after 72 hours, and the causative agents may be acquired at birth during delivery when a mother's genital tract is colonized with these causative agents, or from the hospital environment (Hornik et al., 2012). The main causative agent of LOS in neonatal intensive care unit has been reported to be coagulase negative staphylococcus (CoNS). Factors shown to be associated with its occurrence include extreme prematurity and very low birth weight (VLBW) (Venkatesh et al., 2006). Reasons given for the association between LOS and both factors especially very low birth weight infants include underdeveloped immune systems, need for central venous access and prolonged hospitalization (Wynn et al., 2012). Since LOS is usually a nosocomial infection, its occurrence and prevention is greatly considered in the design and function of neonatal intensive care units (NICU) and special care nurseries (SCN). In developed countries, the occurrence of late onset sepsis (LOS) is estimated to be 0.8% of live births and in 3-7% of NICU and SCN patients (Jones et al., 2012). Other risk factors that expose infants in the NICU to LOS include lower gestational age (Stoll et al., 2010), central venous catheterization, hyperalimantation, invasive procedures and mechanical ventilation (Boghossian et al., 2013).

A ten-year survey in a tertiary NICU in India reported *Enterobacter aerogenes* as the major cause of LOS during the first five years, while *Staphylococcus aureus* was reported as the leading cause of LOS in the latter five years (Sundaram et al., 2009). In a study done in the Maxeke Johannesburg Academic Hospital neonatal unit, they found 0.93 episodes of EOS and 13.5 episodes of LOS per 1000 live births. Both gram positive and gram negative infections were prevalent in LOS, with coagulase negative

staphylococcus being the most isolated pathogen (Ballot et al., 2012). A similar study done in the Aga Khan University Hospital NICU Nairobi Kenya, on 152 neonates who presented with sepsis, found that 58 (38.2%) of them had LOS; and coagulase-negative staphylococcus was the most common isolate both in EOS and LOS (Kohli-Kochhar et al., 2011). High mortality is associated with LOS and ranges from 7% among near-term to 39% among preterm neonates infected with Gram-negative organisms (Cohen-Wolkowicz et al., 2009). In spite of improvement in perinatal care, neonatal sepsis remain a significant public health problem especially in developing countries (Leal et al., 2012).

## **2.2 Diagnosis of Neonatal Sepsis**

Diagnosing neonatal sepsis (NS) depends on a combination of clinical presentations; the use of nonspecific markers, including C-reactive protein and procalcitonin (where available); blood cultures; and the use of molecular methods, including Polymerase chain reaction (PCR) (Simonsen et al., 2014) have proven to be useful in diagnosing NS. Clinically, a suspected neonatal sepsis is based on poor feeding, feeding difficulty, respiratory distress, jaundice, convulsion, fever or hypothermia (Naher, Afroza, Roy, Nahar, & Kundu, 2013) among others. Since there are no pathognomonic signs and symptoms for sepsis in neonates, it is necessary to carry out investigations in a well set up laboratory that has the capacity to do so (Kayange, Kamugisha, Mwizamholya, Jeremiah, & Mshana, 2010). The main method of diagnosing sepsis is the isolation of causative agents from blood cultures (Sriram, 2011). In a study done in Tanzania, clinical signs such as difficulty in feeding, lethargy, convulsion, increase in respiratory rate and

cyanosis had a strong association with culture proven early onset sepsis, while hypothermia, chest indrawing, umbilical redness and jaundice were related to late onset form of sepsis (Kayange et al., 2010). Timely diagnosis for effective treatment is essential in order to avoid deaths and complications that result from neonatal sepsis (NS). This timely diagnosis is concerned with identifying the specific pathogen that is causing the disease. Given the sensitivity of the case definition for NS, timely recognition of neonatal sepsis poses a challenge to clinicians including: i) identifying neonates at a high risk of sepsis promptly and starting antimicrobial therapy; ii) differentiating “high risk” healthy looking infants or infants with clinical signs who do not require treatment; and iii) halting antimicrobial therapy once sepsis is deemed unlikely (Polin, 2012). Though culture is the “gold standard”, it is however not without errors, since it can be falsely sterile as indicated by postmortem cultures, or due to low levels caused by inadequate sample volumes, intermittent or low bacteraemia concentration (Connell et al., 2015). Additionally, when empirical treatment is started, before blood culture is done, it further decreases the diagnostic performance of blood culture (Connell et al., 2015). Another challenge faced by resource-limited settings, especially in rural health care settings is unavailability of diagnostic infrastructure, which results in unavailability of data on culture-proven neonatal sepsis in some settings (Viswanathan et al., 2012).

### **2.2.1 Blood Culture**

A single blood culture in adequate blood volume is required for all neonates with suspected sepsis. Data indicates that 1ml of blood should be the minimum volume drawn for culture when using a single pediatric bottle (Polin et al., 2014). Since inadequate

blood volume obtained from neonates have been associated with reduced positive culture result, it is essential to obtain adequate blood volumes, as it is twice as likely to give a positive result (Bhandari, 2014). When blood volume is less than 1ml, it is thought not to be reliable in detecting a positive blood culture of neonatal sepsis probably due to the low-level bacteremia in some neonates. Studies have also indicated that, blood drawn from umbilical vein during delivery or drawn through umbilical artery catheter shortly after placement for other clinical conditions is a dependable alternative to blood taken from the ante-cubital vein (Polin et al., 2014). A positive blood culture and the susceptibility of isolates serve as a basis for antibiotic therapy; hence the protocol for obtaining blood should be adhered to in order to prevent contamination. The site should be cleaned with isopropyl alcohol of 70% concentration in a circular manner moving outward from the centre of the site for venipuncture. It is followed by cleaning with povidine-iodine and allowed to dry for 1minute. Blood is then collected from the peripheral vein and dispensed into blood culture broths in a 1:10 dilution and transported to the laboratory. Trypticase soy broth, thioglycollate broth or brain heart infusion broth is used for manual procedures, while paediatric culture bottles for Bactec blood culture system are used in the automated systems.

### **2.3 Factors affecting neonatal sepsis**

The two main factors leading to neonatal sepsis are maternal and neonatal factors. Among maternal factors identified, ethnicity; maternal age and parity; marital status; education; premature rupture of membranes and chorioamnionitis had an association with early onset sepsis in neonates. A study indicated that 56% of the infants who developed

early onset sepsis, had a proven maternal chorioamnionitis (Strunk et al., 2012). In another study, maternal age >35; mother with affixed occupation; mother of urban residence; caesarean section delivery; and parity were found to influence early onset neonatal sepsis in a univariate analysis (Jiang & Ye, 2013). A number of maternal factors such as intrapartum fever, prolonged rupture of membranes, chorioamnionitis, maternal group B streptococcus (GBS) colonization, urinary tract infection, and neonatal factors such as low birth weight and prematurity have been identified as neonatal sepsis risk factors in studies conducted (Nizet, 2011).

Multiple risk factors relating to the neonate, also permit the occurrence and spread of such sepsis-causing pathogens. This is because neonates in intensive care units are critically ill, which presupposes that they have an impaired immunity, hence their vulnerability to infection (Hatcher, Dhillon, & Azadian, 2012). In addition, some medical procedures that are performed on neonates in the intensive care units could serve as mediums for acquiring sepsis causing organisms. Some of these procedures include intubation, catheterization and mechanical ventilation. In a study done in Ethiopia, Apgar score in the fifth minute, baby crying immediately after birth and place of delivery were other factors that had significant association with sepsis development in a neonate (Gebremedhin, Berhe, & Gebrekirstos, 2016). In a study conducted at King Edward Memorial Hospital in Western Australia, it was also found that neonates who were born at a lower gestational age ( $26\text{weeks} \pm 1.8$ ) and those who had a lower birth weight ( $848\text{g} \pm 240\text{g}$ ) developed sepsis (Strunk et al., 2012). In the same study, infants who developed sepsis, had assisted ventilation (median of 297 hours at  $p < 0.005$ ) and needed parenteral

nutrition of longer duration (16days  $p < 0.05$ ) (Strunk et al., 2012). A study conducted on 9,575 low gestational age infants showed that 36% of them developed late onset sepsis, which is indicative that prematurity is a risk factor for neonatal sepsis (Stoll et al., 2010).

Several factors have been identified to predispose a neonate to sepsis. One major risk factor category of sepsis is neonatal factor, which includes the following: longer duration of NICU stay, assisted ventilation, presence of central venous catheter, lower gestational age and prior antibiotic exposure among others (Hornik et al., 2012). Another study identified maternal factors including; the socioeconomic status and age of the mother, premature rupture of membranes, prolonged time of rupture of membranes, maternal peripartum infection and traumatic delivery to be associated with a neonate developing sepsis (Satar & Ozlu, 2012). Other internal and external factors during the antenatal, intrapartum and neonatal period in the developing countries, also predispose a neonate to infections (Satar & Ozlu, 2012).

#### **2.4 Common isolates of laboratory specimens of neonatal sepsis**

Pathogens commonly isolated from culture of specimens of neonatal sepsis include both gram negative and gram positive pathogens. Of 20 different studies done on aetiological agents of NS, *Staphylococcus aureus* was reported in all except two, giving 3% to 63% of pathogens. Other isolates reported included *Klebsiella* species in 16 of the studies varying from 8% to 66% of pathogens. *Escherichia coli* was reported in 14 of the studies ranging from 5% to 23% of pathogens (Huynh et al., 2015). A similar study done in Tanzania, found that *Staphylococcus aureus* was the most common organism isolated from blood culture and pus swab followed by *Klebsiella* species and *Escherichia coli* (Mhada et al.,

2012). In China, blood culture was performed on 116 neonates diagnosed with sepsis, 83.3% of gram positive bacteria were isolated from early onset sepsis and 70% from late onset sepsis; coagulase negative *Staphylococcus* accounted for the major gram positive isolates in both early onset sepsis and late onset sepsis (Li et al., 2013). A study was also done on outborn neonates that were admitted at a neonatal intensive care unit in India. Blood was obtained for culture and the pathogens responsible for sepsis were isolated. In this study, 5 (36%) *Klebsiella pneumoniae*, 3 (21%) *Staphylococcus aureus* and 2 (14%) *Escherichia coli* were the common isolates responsible for sepsis.

Outbreaks of EBSL have been reported in several countries in Africa including Tanzania, South Africa, Tunisia and Nigeria (Adeyankinnu et al., 2014). A study done in a NICU in Yemen, isolated pathogens in 57% of 158 blood culture performed. *Klebsiella* species, *Enterobacter* species, and *Pseudomonas* species among others were identified and showed varied resistance from 10% to 100% (Al-Shamahy et al., 2012). A similar study done in the Korle-Bu Teaching Hospital in Ghana to determine the prevalence of EBSL found that the overall antibiotic susceptibility of isolates indicate a high drug resistance prevalence to many available drugs, of which substantial EBSL prevalence was higher in neonates and the elderly (Obeng-Nkrumah et al., 2013). Also, blood culture performed for neonates with sepsis in Tamale Teaching Hospital showed 44.4% of cases to be culture positive, while isolates such as *Acinetobacter* species and *Klebsiella* species were 100% resistant to ampicillin (Acquah et al., 2013).

## **2.5 Management of neonatal sepsis**

Current recommendations for empirical therapy of NS consider the possible mode of acquisition of infection, with EOS associated with maternal flora and LOS associated with nosocomial infection (Blackburn, Verlander, Heath, & Muller-Pebody, 2014). The clinical guideline for empirical therapy for neonatal sepsis in the UK have been given by the British National Formulary for Children (BNFC) and recently the National Institute of Health and Clinical Excellence (NICE) introduced protocol for early onset sepsis (EOS) (Stoll et al., 2010). The two guidelines support empirical treatment with penicillin in combination with gentamicin for EOS; however, there is difference in timelines described by both institutions. Whereas BNFC uses an earlier end-point of 48hours to define EOS, NICE uses 72hours. BNFC also recommends that flucloxacillin combined with gentamicin should be given for late onset sepsis (LOS) (i.e. neonates aged  $\geq 48$ hours) (Black et al., 2013).

Since symptoms of neonatal sepsis usually start as nonspecific, delay in their recognition and hence their treatment may involve risk of preventable mortality. On the contrary, presumptive antimicrobial treatment may suggest an over treatment (Naher et al., 2013). A greater proportion of infants with suspected sepsis recover when given supportive care. This may or may not include starting an antimicrobial therapy (Polin, 2012).

Even though isolating a causative agent in culture is required for proven bacterial sepsis, laboratory results are not ready until at least 48-72hours. Commencing antibiotic therapy early and appropriately in high-risk neonates before laboratory results are ready is referred to as “empirical antibiotic therapy”(Sivanandan et al., 2011). Majority of infants

admitted to the NICU are given empirical treatment when actually the incidence of culture proven EOS is only between 1 and 4.6 cases per 1000 live births. Since symptoms of NS are non-specific, the danger of missing diagnosis is undesirable because of high morbidity and mortality associated with sepsis (Sivanandan et al., 2011). There is a wide difference between centres concerning the length of empirical treatment for suspected EOS should blood culture be negative. The World Health Organization (WHO) Integrated Management of Childhood Illness (IMCI) outlined the algorithm for early detection of severe diseases including severe bacterial infection (WHO, 2005). The algorithm indicates that clinicians should look out for the following as risk factors for serious neonatal bacterial infections: maternal fever (temperature  $> 37.9^{\circ}\text{C}$  before delivery or during labour); membranes ruptured more than 24hours before delivery; and foul smelling amniotic fluid. The signs of infection in neonates should be observed for which include: severe abdominal distension; deep jaundice; reduced movement; painful joint; joint swelling; many or severe skin pustules; umbilical redness extending to the periumbilical skin; and bulging fontanelle. If any of these signs are present, treatment should begin by first admitting the neonate into hospital. A blood culture should be obtained if available before antibiotics are initiated. Ampicillin (or penicillin) must be given for any of the signs that present, however, if there are extensive skin pustules or abscesses give cloxacillin. The antibiotic therapy must be given for at least 10days and if there is no improvement between 2 to 3 days, the therapy may need to be changed or the baby referred (WHO, 2005). According to a systematic review, the most common pathogens of community acquired neonatal sepsis (NS) in low-middle-income-countries

(LMIC) were *Staphylococcus aureus* (26% of blood culture isolates), *Klebsiella* (21%) and *Escherichia coli* (8%), while group B streptococcus was uncommon (Ballot et al., 2012), though differences exist between regions (Zaidi et al., 2011). Presently, the recommended first line treatment drugs for NS are injectable gentamycin and procaine benzylpenicillin for ten days and ceftriaxone for ten days as second line drugs. These antibiotics are currently on the WHO Model List of Essential Medicines for Children and are marked as Life-Saving Commodities for women and Children to lower mortality rates (Coffey et al., 2012). The access to antibiotics requires special consideration in neonates, infants and children because of their specialized dosing, drug formulation, delivery routes, risk profile, physiology, monitoring needs.

## **2.6 Antimicrobial resistance in neonatal sepsis**

Antimicrobial resistance is a global emerging public health problem. It has been shown that multi drug antibiotic resistance is steadily rising in neonatal intensive care units (NICU) especially in developing countries (Sheth et al., 2012). Moreover the causative organisms of neonatal sepsis change over time and varies across regions (Sheth et al., 2012). Continuous surveillance for antibiotic susceptibility, rational use of antibiotic and measures of antibiotic recycling are strategies required to provide answers to the emerging antimicrobial resistance (Shrestha, Adhikari, Rai, & Shrepaili, 2010). Timely review of antimicrobial sensitivity patterns in individual health facilities will serve as a guide against development of antimicrobial resistance of microbial agents to first line and higher class drugs (Sheth et al., 2012).

In a study conducted in Tanzania, isolates of both blood and swab cultures of neonates clinically diagnosed of sepsis had the highest resistance against ampicillin and cloxacillin which are two of the three first line antibiotics recommended by WHO for the management of neonatal sepsis. All the *Klebsiella* species isolates and 92.9% of *Escherichia coli* isolates from blood were resistant to ampicillin, 77.0% of *Klebsiella* species and 42.9% of *Escherichia coli* isolated from blood were resistant to gentamycin. A proportion of 81.5% of *Staphylococcus aureus* isolated from blood were resistant to cloxacillin (Mhada et al., 2012). In Pakistan, blood culture samples obtained from 103 neonates showed positive results in 68.9% and majority of the gram negative rods showed 100% resistance to the available and commonly used antibiotics; in the case of gram positive isolates, over 50% of coagulase negative *staphylococcus* and *Staphylococcus aureus* showed resistance to penicillin, cotrimoxazole and clindamycin (Hannan et al., 2013). In Ghana, out of a total of 63 culture samples collected from neonates with sepsis, pathogens were isolated from 44% of them. *Salmonella* species and *Acinetobacter* species were 100% resistant to ampicillin, tetracycline and cotrimoxazole; *Klebsiella* species were 100% resistant to ampicillin and cotrimoxazole; and most coagulase positive *staphylococcus* and coagulase negative *staphylococcus* were resistant to ampicillin and penicillin (Acquah et al., 2013).

EOS and LOS have been said to be related to specific maternal or hospital pathogens, which have been observed to change over time as a result of changing trend in antibiotic use (Mehtar et al., 2013). It is also reported that these pathogens are increasingly developing resistance to the antibiotics used in the neonatal intensive care units (NICUs).

This is partly because of antibiotic overuse, in up to 95% neonates admitted in the NICU, despite that a positive blood culture turn out may be 1-5% (Tziella, Borghesi, Pozzi, & Stronati, 2015). Other reasons that have been suggested for antibiotic resistance development include indiscriminate use of antibiotics and use of broad spectrum antibiotics (Bell, Schellevis, Stobberingh, Goossens, & Pringle, 2014). Intrapartum antibiotic prophylaxis which works effectively against transmission of GBS from colonized mother to a neonate has otherwise been found to lead to apparent shifts in causative agents and their susceptibility resulting in antibiotic resistance. When neonates contract infections either from the mother, from the health care system or from the community, it is related to treatment failure when empiric treatment is not effective against causative organisms, higher morbidity, mortality and increased cost. It becomes more devastating, when infections are due to resistant strains. Increasing rate of antimicrobial resistance also poses treatment challenges of bacteraemia in children particularly in low-and-middle-income countries (Le Doare et al., 2014). The burden of antibiotic resistance is devastating particularly in low resource settings since it leads to additional financial cost, prolonged hospital stay and increased morbidity and mortality (Hannan et al., 2013).

## **2.7 Summary of review**

According to literature, the predictors of neonatal sepsis are in two broad categories; maternal risk factors and neonatal risk factors. Maternal risk factors that have been identified include socio-demographic factors such as age, ethnicity, educational level, parity and gravidity among others. Clinical factors of mothers that are shown to have

association with neonatal sepsis include, premature rupture of membrane; chorionamnionitis; intrapartum antibiotic administration and history of previous neonate with sepsis. When a neonate is born preterm, has a low birth weight, has undergone some medical procedure like mechanical ventilation, parenteral nutrition and central venous catheterization, they have a greater risk of developing neonatal sepsis. Studies have shown that causative organisms for early onset sepsis mainly are transmitted from colonized genital tracts of mothers, while late onset sepsis are caused by organisms acquired from the community or hospital environment (nosocomial infections). The pathogens that are usually isolated from culture samples are gram positive and gram negative organisms

Studies have also shown that these causative organisms change over time between regions and even within the same facility. For this reason, isolation of pathogens and antibiotic susceptibility must be put under surveillance in order to determine antibiotics that are effective against neonatal sepsis causing organisms at a point in time.

The literature reviewed was able to highlight the gap of data availability from regional and district hospitals on antibiotic resistance, despite the fact that many hospitals have the capacity to monitor the incidence of the resistance. This therefore presents this research with the task of showing the antibiotic susceptibility pattern of blood culture isolates of neonates with sepsis in a municipality of Ghana –Ho municipality specifically. This would help to provide needed data required to improve management plan for neonatal sepsis and lay down an evidence base that will guide further research on preventive strategies for neonatal sepsis.

## CHAPTER THREE

### METHOD

#### 3.1 Study Design

A hospital based analytical cross sectional study was conducted to collect both qualitative and quantitative data on neonates admitted at the Neonatal Intensive Care Units (NICU) of the Ho Municipal Hospital and Volta Regional Hospital and, their mothers. Blood sample was taken from neonates for culture, to determine the common isolates causing sepsis and antibiotic susceptibility pattern of the isolates. The study included neonates clinically suspected of sepsis and was conducted between January and May, 2016.

#### 3.2 Study Site

The study was conducted at the Ho Municipal Hospital and Volta Regional Hospital, located in Ho Municipality.

##### 3.2.1 Profile of the Ho Municipality

The Ho Municipality is one of five municipalities in the Volta Region. It is located between latitudes 6° 20'N and 6° 55'N and longitudes 0° 12'E and 0° 53'E. The Municipality shares boundaries with Adaklu and Agotime-Ziope districts to the south, Ho West district to the north and west and the Republic of Togo to the east. It has a total land area of 2,660 square kilometres. The general relief of the municipality is made up of both mountainous and lowland areas. The mountainous areas are mostly to the north and northeast which are part of the Akuapim-Togo range with height between 183-853metres above sea level. The lowland areas are to the south of the municipality and are between

60-152metres above sea level. The annual mean rainfall figures are between 20.1mm and 192mm, whilst the annual mean temperature ranges from 16.5°C to 37.8°C.

### **3.2.2 Demography of Ho Municipality**

In 2015, the total population was 202,165 with a proportion of 52.5% being females. Out of the total population in 2015 for the municipality, 6,948 (3.4%) were children <1year. The proportion of women in fertility age (WIFA) for 2015 was 53371, (26.4%).

The target set for skilled delivery in 2015 was 98%, of which 94.5% was achieved. There was a target of 15% for caesarean section, of which 20.3% was achieved. Neonatal death reduction was targeted at 1.3%, of which 1.2% was achieved. Out of a total of 291,653 outpatient department (OPD) attendance for 2015, the proportion of <1year was 4.49%. A total of 17,309 admissions were made for children aged <1year, with female proportion of 63.9%.

The municipality is divided into five sub municipalities, which are organized along traditional/electoral lines and include: Ho central, Hokpeta, Norvisi, Sokode/Akrofu and Dutasor. Although an urban area, agriculture is the major economic activity in the municipality. The municipality's economy is also characterized by a large number of small scale commercial and industrial activities including petty trading and construction. These activities are concentrated in the city centre, making it the business hub of the municipality. The road network within the municipality is quite motorable both in the mountainous and lowland areas, making health care quite accessible. People are also in various forms of employment both in the public and private sector. The Ewe ethnic group

predominates in the municipality. The most predominant religion in the municipality is Christianity (91.9%), followed by Islam (3.2%), Traditional religion (2.2%) and other religions constitute less than one percent.

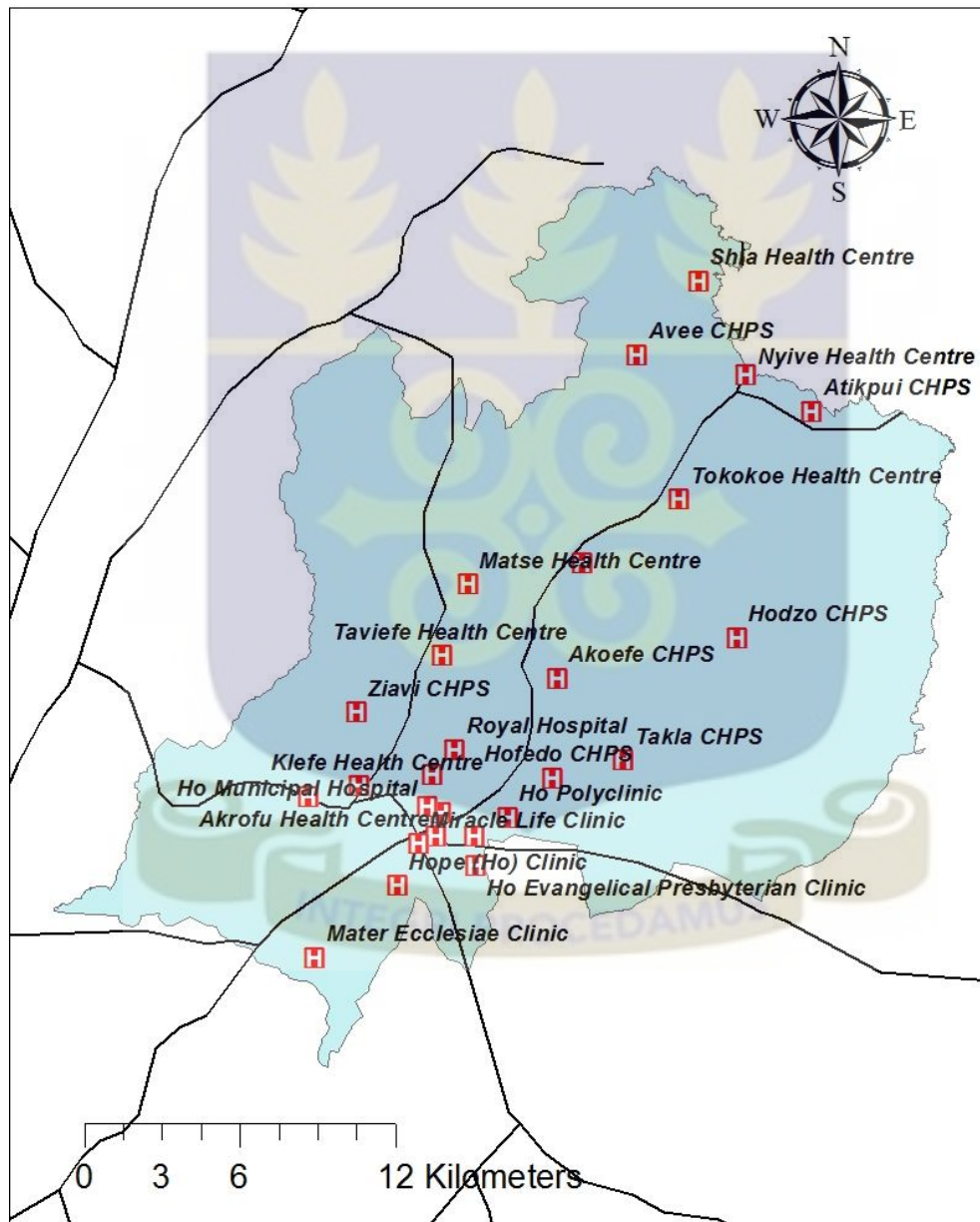


Figure 2: Map showing various health facilities in Ho municipality

### 3.2.3 Health Services

The municipality has 29 health facilities at various levels of health care. The following are the number and levels of facilities:

**Table 1: Distribution of health facilities, Ho municipality**

Health facility	Number
Regional Hospital	1
Municipal Hospital	1
7MRS Hospital	1
Polyclinic	1
Health centre	10
CHPS compound	5
Clinic	5
Private Hospital	3
Health unit	2

According to the levels of health facilities listed above, neonatal sepsis can be managed in the regional and municipal hospitals, which are government owned in addition to the three private owned hospitals in the municipality. With regards to laboratory testing on neonatal sepsis, only the regional hospital laboratory has the microbiological capacity to conduct this test in the municipality. Data was collected in the Ho Municipal Hospital

and Volta Regional Hospital. Even though there are laboratories in both hospitals, the regional hospital laboratory is the one that has the capacity to run microbiological tests in the municipality, including blood culture of neonates with sepsis.

### **3.2.4 Profile of Volta Regional Hospital**

The Volta Regional Hospital is a referral hospital in the region, apart from the primary health care it offers to the citizens in and around Ho. It is a 240 bed capacity hospital, and was established in 1998. The hospital is government owned and has several departments including: surgery, medicine, obstetrics and gynaecology, diagnostics, psychiatry and paediatric departments. The paediatric department has a Neonatal Intensive Care Unit (NICU) where neonatal cases are managed and treated. One of the commonest clinical diagnosis made in the NICU is neonatal sepsis. The head of the paediatric department is a paediatrician specialist, who works with other staff. In 2015, the total outpatient department (OPD) attendance was 100,557 whiles, 10,945 cases were admitted. Sepsis was among the top ten causes of admission. A total of 1,392 spontaneous deliveries were done in 2015, whiles 532 caesarean sections were carried out. On the average, the NICU admits 50 neonates in a month, with sepsis being the second highest cause of admission.

### **3.2.5 Profile of Ho Municipal Hospital**

The Ho Municipal Hospital is the second most attended government owned hospital in the municipality. It is also the oldest government hospital in the municipality that serves the people of Ho and surrounding towns and villages. It has the following departments: medical department, obstetrics and gynaecology department, pharmacy, laboratory, Neonatal Intensive Care Unit (NICU) and other departments. A medical doctor and other

clinical staff manage the NICU. The first line drug of choice for empirically treating neonatal sepsis cases is a combination of ampicillin and gentamicin. In 2015, a total of 42,511 cases were OPD attendants, while 6063 admissions were done. The total number of spontaneous vaginal deliveries that were made in 2015 was 1713, with 202 being caesarean delivery. Of the 140 neonatal admissions made in 2015, the greater proportion 47 (33.6%) was on account of sepsis.

### **3.3 Sampling**

#### **3.3.1 Study Population**

The study population included neonates admitted at the Volta Regional Hospital and Ho Municipal Hospital Neonatal Intensive Care Units. Data were collected for neonates seen between January, and May, 2016.

#### **3.3.2 Inclusion criteria**

All neonates admitted at the Volta Regional Hospital and Ho Municipal Hospital Neonatal Intensive Care Units, who were clinically diagnosed of neonatal sepsis by a clinician during the study period, and whose mothers or caretakers consented to be part of the study were included in the study.

#### **3.3.3 Exclusion criteria**

All neonates who were clinically diagnosed of sepsis, by a clinician at the Ho Municipal Hospital and Volta Regional Hospital Neonatal Intensive Care Units within the study period, but died immediately upon arrival before blood culture sample could be obtained,

or neonates who were referred to a tertiary facility immediately after assessment were excluded.

### 3.4 Variables

The dependent variable is clinically diagnosed neonatal sepsis. The various independent variables of interest are categorized into maternal and neonatal factors and include:

#### 1. Maternal factors

- Maternal age
- Educational level
- Ethnicity
- Occupation
- Residence
- Mode of delivery
- Membrane rupture time
- Parity
- Gravidity
- Intrapartum antibiotic prophylaxis
- Chorioamnionitis
- Number of vaginal examination
- Number of antenatal visit

#### 2. Neonatal factors

- Birth weight
- Sex
- Gestational age
- Apgar score at 1minute
- Apgar score at 5minutes
- Length of hospital stay
- Mechanical ventilation

Data was also collected on the pathogens isolated after blood culture and the antibiotic susceptibility of these isolates as follows.

#### 1. Causative agents

- Gram positive organisms
- Gram negative organisms

#### 2. Susceptibility of causative agents

- Resistance pattern of causative organisms
- Sensitivity pattern of causative organisms

**Table 2a: Definition and scale of measurement for variables**

Variable	Operational definition	Scale of measurement	Data collection technique
Maternal age	Mothers age at delivery	Categorical (ordinal) 1. ≤20years 2. 21-30years 3. ≥31years	Structured questionnaire/interview
Educational level	Highest level of education	Categorical (ordinal) 1. No formal education 2. Primary 3. JSS 4. SSS 5. Tertiary	Structured questionnaire/interview
Occupation	Occupation in any paid or unpaid work	Categorical 1. Unemployed 2. Unemployed	Structured questionnaire/interview
Religion	Faith mother professes	Categorical 1. Christianity 2. Islam 3. African traditional belief	Structured questionnaire/interview
Ethnicity	Ethnic origin of mother	Categorical 1. Ewe 2. Akan 3. Northern descent	Structured questionnaire/interview
Residence	Place of habitation of mother	Categorical 1. Within Ho municipality 2. Outside Ho municipality	Structured questionnaire/interview

**Table 2b: Definition and scale of measurement for variables**

Variable	Operational Definition	Scale of measurement	Data collection technique
Marital status	Description of mothers relation status	Categorical 1. Married 2. Unmarried	Structured questionnaire/interview
Mode of delivery	Means of child birth	Categorical 1. Spontaneous vaginal delivery 2. Caesarean section	Review of case notes
Membrane rupture time	Time when membrane is no more intact to time when labour starts	Categorical 1. $\leq 18$ hours before labour 2. $\geq 18$ hours before labour	Review of case notes
Gravidity	Number of times a woman has been pregnant	Categorical (Ordinal) 1. Primigravida 2. Multigravida	Review of case notes
Parity	Number of times mother has delivered	Categorical (ordinal) 1. Primiparous 2. Multiparous	Review of case notes
Intrapartum antibiotic prophylaxis	Administration of any antibiotic during pregnancy	Categorical 1. Yes 2. No	Review of case note
Number of antenatal visit	Number of times mother attended antenatal clinic for care	Categorical (ordinal) 1. $\leq 3$ times 2. $\geq 4$ times	Review of antenatal record book
Number of vaginal examination	Number of times the vagina of the mother was examined during labour	Categorical 1. $\leq 3$ times 2. $\geq 4$ times	Interview of mother
Chorionamnionitis	Infection of chorion amniotic membrane	Categorical 1. Yes 2. No	Review of case note
Birth weight	Weight at birth of the neonate	Categorical 1. $< 2500$ g 2. $\geq 2500$ g	Review of case notes
Gender	Sex of neonate	Categorical 1. Male 2. Female	Review of case notes
Gestational age	Age of pregnancy at which delivery occurred	Ordinal 1. $\leq 36$ weeks 2. 37-40 weeks 3. $\geq 41$ weeks	Review of case note
Apgar score at 1 minute	Score on apgar test at 1 minute after delivery	Categorical (Ordinal) 1. $\leq 5$ 2. $\geq 6$	Review of case notes
Apgar score at 5 minutes	Score on apgar test at 5 minutes after delivery	Categorical 1. $\leq 6$ 2. $\geq 7$	Review of case notes
Mechanical ventilation	Use of device to assist neonate in respiration	Categorical 1. Yes 2. No	Review of case notes

**Table 2c: Definition and scale of measurement for variables**

Variable	Operational definition	Scale of measurement	Data collection technique
Causative organism		Categorical	Laboratory result
Gram positive organism	Isolation and identification of gram positive organism	1. Present 2. Absent	
Gram negative organism	Isolation and identification of gram negative organism	1. Present 2. Absent	Laboratory result

### 3.5 Sample size

The sample size was calculated using;

$$n = \frac{Z^2 p (1-p)}{d^2}$$

Where,

n= the sample size

p=the proportion of the main outcome

d=the margin of error

z= the normal deviate to provide the 95% confidence level

l= constant

Using a prevalence of 11% of neonatal sepsis, a confidence level of 95% and a margin of error of 5%, the sample size was determined by;

$$\begin{aligned} n &= \frac{(1.96^2 * 0.11 * 0.89)}{0.05^2} \\ &= 150.04 \approx 150 \end{aligned}$$

Hence the total sample size = 150

### **3.6 Sampling method**

Neonates that were clinically diagnosed of sepsis and admitted in the Neonatal Intensive Care Units (NICU), of the Volta Regional Hospital and Ho Municipal Hospital were serially recruited into the study until the sample size of 150 was reached.

#### **3.6.1 Data collection method**

Data was collected using a structured questionnaire and this was done in two parts: A structured questionnaire was used to collect maternal socio-demographic data, while case notes of neonates diagnosed with sepsis and their mothers were reviewed by the researcher and research assistants, to collect clinical data on neonates and their mothers as well as laboratory results of blood culture of neonates. Data on the pathogens isolated and their antibiotic susceptibility pattern were also collected after laboratory test was done.

#### **3.6.2 Sample collection and laboratory investigation**

A clean tourniquet was applied to the upper arm of the neonate, to locate an antecubital vein. The antecubital fossa was then cleaned aseptically using 70% alcohol. The cleaning was done twice to ensure that, the skin was free from any contamination for venipuncture. A 2ml volume of venous blood was then taken aseptically, dispensed into a 20ml mixture of thioglycollate fluid broth and tryptone soy broth in a universal bottle for culture, labelled and transported to the laboratory. Culture bottles were placed in the incubator at 37°C overnight and sub-cultured into commercially prepared blood,

chocolate and MacConkey agar. The plated chocolate agar was placed in a candle jar with a lit candle to create an anaerobic environment for fastidious organisms to grow, while the blood and MacConkey agar were placed in an aerobic environment for aerobic organisms to thrive. All three agars were then incubated at 37°C for 24 hours. The plated agars were observed for growth the following day.

Plated agars that showed significant growth were selected, pure colonies of isolates were picked and gram stain performed on them to identify them as gram positive or gram negative organisms. Pure colonies were emulsified in a drop of saline on a slide to make a smear. The smear was then fixed on the slide by passing it over a Bunsen flame and stained with crystal violet for 1 minute. The smear was washed with water, and stained with Gram's iodine for 1 minute. It was washed again with water, and decolorized with 95% ethyl alcohol for 30 seconds. After washing the smear with water, it was counter stained with dilute carbol fuchsin. The slide was allowed to air dry for 10-15 minutes and observed under the microscope using x100 objective. Gram positive organisms appeared as cocci and retained the purple colour of the primary stain-crystal violet. Gram negative organisms appeared as rods and took up the red colour of the counter stain-dilute carbol fuchsin.

Pure colonies were then identified to know the specific organism using standard biochemical tests. For gram positive organisms, the biochemical tests done to confirm them included catalase and coagulase tests. To confirm the various gram negative organisms, the biochemical tests included oxidase test, triple sugar iron test, citrate test, urea test and indole test. The positivity or negativity of these tests identified the specific

organism that was isolated. All negative blood culture samples were incubated at 37°C and kept for at least 7 days. During these 7 days, each blood culture sample was sub-cultured intermittently and observed for growth until it was finally discarded on the 7<sup>th</sup> day as negative culture. All blood culture samples were decontaminated by autoclaving at 121°C for 1 hour. Then they were transported to the main hospital waste disposal unit for incineration and disposal.

### **3.6.3 Antibiotic susceptibility testing**

The antibiotic susceptibilities of isolated pathogens to the selected antimicrobial agents were determined, using the Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standard Institute (CLSI, 2007). Pure colonies of isolates were emulsified in 2ml sterile saline to reach turbidity that can be compared to 0.5 McFarland standard. A sterile cotton bud dipped into the inoculum tube was pressed firmly against the sides of the tube above the inoculated saline to remove excess fluid. The swab was used to streak the dried surface of Muller Hinton agar in three directions over the entire surface of the agar for uniformity. The multi disc of antimicrobials was then placed on the streaked agar and incubated overnight. It was observed for zones of inhibition and the diameter of these zones of inhibition were measured with a rule in millimetres. Measurements were compared to a chart to determine the breakpoints. The breakpoints determined whether isolated organisms were resistant, susceptible or intermediate to the antibiotics. The chart comprised of the common antibiotics used in treating infections and measurements in millimetres at which microorganisms could be considered as resistant, intermediate or

susceptible to a given antibiotic according to the clinical and laboratory standards institute (CLSI, 2007).

### **3.6.4 Laboratory quality control**

Routine quality control to test against all media types and antibiotics to be used was performed according to the clinical and laboratory standards institute (CLSI) guideline. This was achieved by growing known isolates on specific media that were required for their growth and observed to see if the media supported their growth. When there was significant growth, the media were considered to be of good quality; however, when there were no growths, or growths were not significant, media was considered to be of poor quality. Susceptibility testing was controlled with American Type Culture Collection (ATTC) strains: *Staphylococcus aureus* ATTC 25923, *Escherichia coli* ATTC 25922 and *Pseudomonas aeruginosa* ATTC 27853.

### **3.6.5 Data collection quality control**

#### **Training for data collection**

The research assistants were trained a week before the start of the data collection. The purpose of the training was to ensure that research assistants understood the study, were familiar with the data collection tools and were able to give the exact interpretation of the questions on the data collection tool. The research assistants were asked to simulate the data collection procedure repeatedly in order to ensure that they were conversant with and were consistent with the use of the questionnaire in collecting data.

The questionnaire to be used was pilot tested at Hohoe Municipal Hospital, Hohoe. This was to ensure that questions asked were appropriate enough to collect the required variables for analysis. Each questionnaire was given a unique identification number (ID), which had run through the identification of blood culture samples. Mothers or caretakers of neonates were interviewed alone in separate rooms, to prevent the bias of mothers or caretakers giving same responses to questions.

### **3.7 Data processing and analysis**

Data collected was checked for completeness and double entry was done. Microsoft Excel software was used in data cleaning which included crosschecking for missing data, duplicates and outliers. Case notes were reviewed again and interviews conducted to correct these anomalies if detected.

#### **3.7.1 Data management**

All variables collected were given unique numbers (coded) and entered into Microsoft excel. Analysis of data was done using Microsoft excel and STATA 13.0 software.

#### **3.7.2 Statistical analysis**

**Descriptive statistics:** Descriptive analysis was done using frequencies and proportions. Continuous variables obtained from neonates and mothers are presented using means and standard deviation. Categorical variables obtained from neonates and mothers are presented as proportions and frequencies in tables and graphs. Pathogens isolated from the laboratory investigation are presented as frequencies and proportions. In addition, antibiotic susceptibility of the isolates is presented in frequencies and proportions.

**Inferential statistics:** Univariable and multivariable analysis was done using logistic regression and computed as odds ratio. Significant association of the independent variable with the dependent variable was determined at p value of 0.05.

### **3.8 Ethical consideration**

#### **Ethical Approval**

Before data collection, ethical approval was sought from the Ghana Health Service Ethical Review Committee of the Research and Development Division of the Ghana Health Services.

Research permission was sought from the Volta Regional Health Directorate, the management of the Volta Regional Hospital and the management of the Ho Municipal Hospital.

#### **Potential risks/benefits**

Both the target population and the health facility would stand to benefit from the study. Findings pertaining to pathogens isolated from blood culture of neonates and their antibiotic susceptibility pattern may inform clinicians to improve management plan for neonates. However in drawing blood, participants may feel some pain, discomfort or experience some swelling at the site of venipuncture.

#### **Privacy/confidentiality**

All interviews were conducted in an enclosed place to ensure privacy. Each respondent was given a unique identifier such that data gathered could not be traced back to

respondents. Unauthorized persons were not allowed access to the information collected on respondents except the research team, and every information collected from respondents was kept under lock and key. These were done to ensure the confidentiality and anonymity of respondents. Results are reported in the aggregate and hence no one will be able to trace the results back to any particular respondent.

**Voluntary consent**

A signed consent was sought from all respondents before data was collected from them. Participation was fully voluntary. Respondents were given the option to refuse answering any question they deemed uncomfortable, end the interview any time they wanted to or opted out of the study.

**Conflict of interest**

Apart from the academic and public health importance of the study, the researcher declares no other personal interest in the study.



## CHAPTER FOUR

### RESULTS

#### 4.1 Prevalence of culture proven sepsis

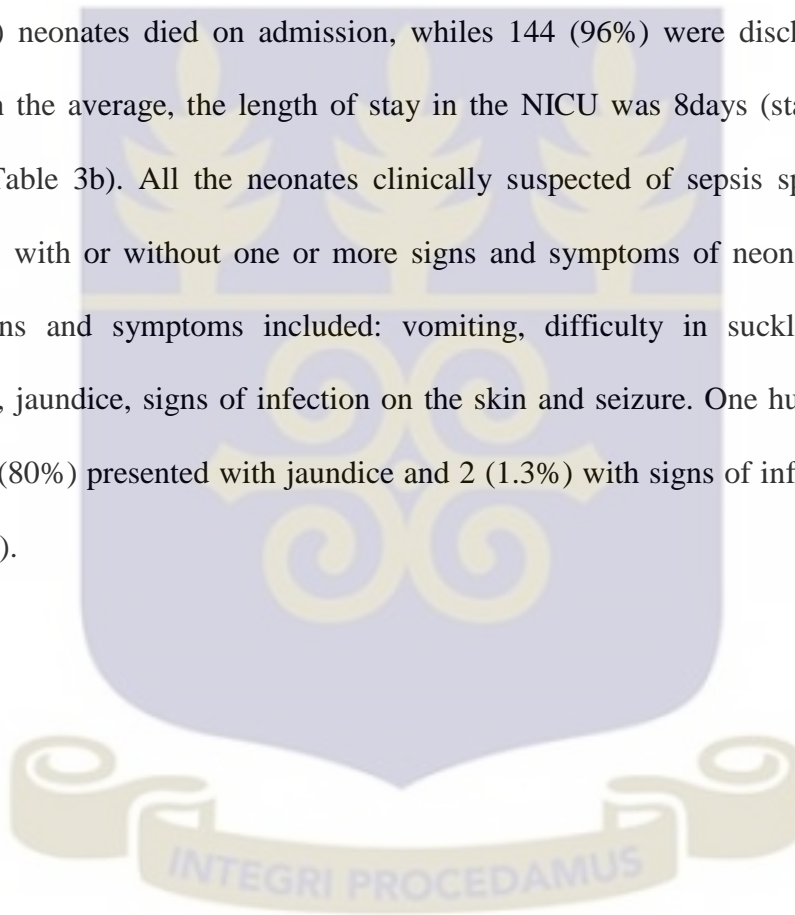
Of the 150 suspected neonatal sepsis cases recruited into the study, 26 (17.3%) was positive for culture. Thus the prevalence of culture proven sepsis was 17.3%.

#### 4.2 Neonatal characteristics

Out of the 150 neonates that were suspected of sepsis and recruited into the study during the study period, 91 (60.7%) were males and 59 (39.3%) females (Table 3a). Majority, 87 (58%) of the mothers of these neonates were delivered by caesarean section, while 63 (42%) was by spontaneous vaginal delivery. The mean gestational age was 37 weeks (standard deviation $\pm$ 3weeks) with a range from 28weeks to 43weeks; and mean birth weight was 2855.28g (standard deviation $\pm$  740.43g) with a range from 800g to 4300g. The mean Apgar score at one minute for the participants was 7.09 (standard deviation $\pm$ 1.87) with a range from 1 to 9 and 8.5 (standard deviation $\pm$ 1.65) with a range from 2 to 10 at five minutes. Most of the deliveries, 109 (72.7%) were either at the Volta Regional Hospital or Ho Municipal Hospital; with 41(27.3%) being outside the study facilities (Table 3a). Of the 41 born outside the study facilities, 30 (73.1%) were referred from other health facilities and 11 (26.8%) were home deliveries. Overall, antibiotic therapy was initiated in 66 (44%) neonates before blood sample was taken for culture, including 15 (10%) neonates that venipuncture was attempted before antibiotic administration but proved initial difficulty. The mean time before obtaining blood sample for culture in the cases that empiric therapy was initiated prior to sample collection was

24hours. Out of the 30 neonates referred from other health facilities, all (100%) had initiated antibiotic therapy before arrival at the referred hospitals. There was low blood volume obtained in 15 (10%) neonates during venipuncture for laboratory testing (Table 3b).

Six (4%) neonates died on admission, while 144 (96%) were discharged during the study. On the average, the length of stay in the NICU was 8days (standard deviation $\pm$  4days) (Table 3b). All the neonates clinically suspected of sepsis spiked temperature ( $\geq 38.0^{\circ}\text{C}$ ) with or without one or more signs and symptoms of neonatal sepsis. These other signs and symptoms included: vomiting, difficulty in suckling, difficulty in breathing, jaundice, signs of infection on the skin and seizure. One hundred and twenty neonates (80%) presented with jaundice and 2 (1.3%) with signs of infection on the skin (Table 3b).



**Table 3a: Characteristics of neonates with suspected sepsis on admission at NICU, Ho municipality, 2016**

Variable	Count (N)	Percent (%)
<b>Sex</b>		
Male	91	60.7
Female	59	39.3
<b>Birth weight</b>		
<2500g	35	24.5
≥2500g	108	75.5
<b>Place of birth</b>		
Within study facilities	109	72.7
Outside study facilities	41	27.3
<b>Sepsis category</b>		
Early onset sepsis	81	54
Late onset sepsis	69	46
<b>Gestational age</b>		
≤36	41	27.3
37-40	94	62.7
≥41	15	10
<b>Length of hospital stay</b>		
≤7days	77	51.3
≥8days	73	48.7
<b>Delivery mode</b>		
SVD	63	42
C/S	87	58

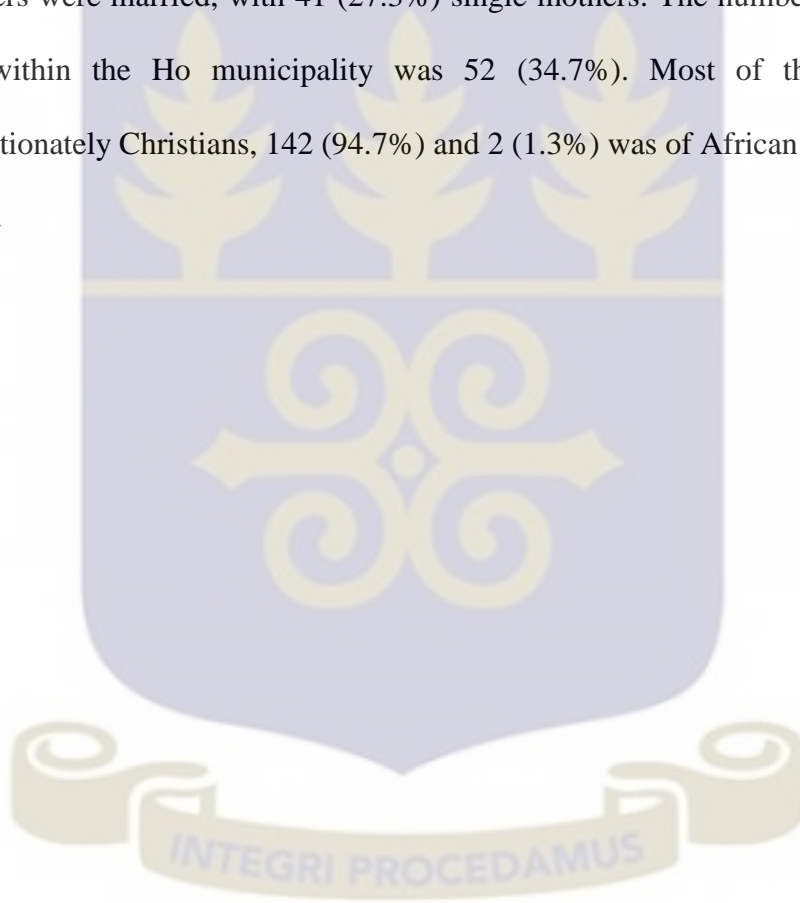
\* SVD: spontaneous vaginal delivery; C/S: caesarean section

**Table 3b: Characteristics of neonates with suspected sepsis on admission at NICU, Ho municipality, 2016**

Variable	Count (N)	Percent (%)
<b>Outcome</b>		
Discharged	144	96
Died	6	4
<b>Signs and Symptoms</b>		
Fever	100	66.7
Jaundice	120	80
Difficulty in suckling	60	40
Difficulty in breathing	50	33.3
Vomiting	40	26.7
Seizure	20	13.3
Signs of infection on skin	2	1.3
<b>Antibiotic administration</b>		
Before taking blood	66	44
After taking blood	84	56
<b>Ventilation</b>		
No mechanical ventilation	142	94.7
Mechanical ventilation	8	5.3
<b>Blood volume for culture</b>		
Adequate	135	90
Low volume	15	10

### 4.3 Maternal characteristics

The maternal characteristics of neonates enrolled into the study are described in Table 4. The mean age of mothers of the neonates included in the study was 28years (standard deviation $\pm$ 6years) with a range from 16years to 41years. One hundred and eight (72%) of the mothers were married, with 41 (27.3%) single mothers. The number of mothers who resided within the Ho municipality was 52 (34.7%). Most of the mothers were disproportionately Christians, 142 (94.7%) and 2 (1.3%) was of African Traditional belief (Table 4).



**Table 4: Distribution of maternal socio-demographic characteristics**

Variable	Count (N) 150	Percent (%)
<b>Age</b>		
≤20	20	13.3
21-30	71	47.3
≥31	59	39.3
<b>Marital status</b>		
Married	108	72
Single	41	27.3
Divorced	1	0.7
<b>Residence</b>		
Within Ho Municipality	52	34.7
Outside Ho Municipality	98	65.3
<b>Ethnicity</b>		
Ewes	136	90.7
Akan	9	6
Northern descent	5	3.3
<b>Religion</b>		
Christianity	142	94.7
Islam	6	4
Traditional African Belief	2	1.3
<b>Educational level</b>		
No formal education	12	8
Primary	18	12
JSS	59	39.3
SSS	35	23.3
Tertiary	26	17.3
<b>Employment</b>		
Employed	122	81.3
Unemployed	28	18.7

\*JSS: Junior secondary school; SSS: senior secondary school

Fifty-nine (39.3%) mothers of the neonates included in the study had Junior secondary education as their highest level of education, followed by 35 (23.3%) mothers who attained Senior secondary level, while 12 (8%) had no formal education (Table 4). The mothers were predominantly Ewes, 136 (90.7%), followed by 9 (6%) Akans. In relation to the occupation of mothers, 122 (81.3%) were employed, followed by 28 (18.7%) unemployed mothers (Table 4).

#### **4.4 Common isolates identified**

Equal proportions of microorganism were isolated in both early and late onset sepsis. The gram positive isolates identified were *Staphylococcus aureus* and *Staphylococcus epidermidis*. Gram negative organisms include: *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter* species and *Proteus mirabilis* (Table 5). Overall, the commonest microorganism isolated was *Staphylococcus epidermidis* 14 (53.9%), with the greater proportion 9 (69.2%) in early onset sepsis (Table 5).



**Table 5: Distribution of isolates of blood culture of neonates with sepsis**

Isolate	Early Onset Sepsis		Late Onset Sepsis		Total Count (N)
	Count	Percentage (%)	Count	Percentage (%)	
Gram Positive organism					
<i>Staphylococcus epidermidis</i>	9	69.2	5	38.5	14
<i>Staphylococcus aureus</i>	1	7.7	3	23.1	4
Gram Negative organism					
<i>Escherichia coli</i>	1	7.7	0	0	1
<i>Pseudomonas aeruginosa</i>	2	15.4	2	15.4	4
<i>Enterobacter</i> species	0	0	2	15.4	2
<i>Proteus mirabilis</i>	0	0	1	7.7	1
Total	13		13		26

This was followed by *Pseudomonas aeruginosa*, 4 (15.4%) in equal proportions among early and late sepsis. *Staphylococcus aureus* had the same proportion as *Pseudomonas aeruginosa*, 4 (15.4%) however, the greater number, 3 (75%) was isolated among late onset sepsis cases. The two *Enterobacter* species, and the single *Proteus mirabilis* identified in the study were isolated in late sepsis (Table 5). A single *Escherichia coli* was also identified in early sepsis. All the microorganisms isolated during the study period are shown in (Table 5).

#### 4.5 Antibiotic susceptibility

Based on the antibiotic susceptibility testing of the gram positive organisms isolated from the blood culture, *Staphylococcus epidermidis* showed 100% resistance to ampicillin, 92.9% resistance to tetracycline, 76.9% resistance to erythromycin, 57% resistance to gentamicin and, 42.9% resistance to ciprofloxacin, (Table 6).

**Table 6: Percentage resistance of gram positive organisms isolated from blood culture**

Isolate	Count (N)	AMP	GEN	CRX	COT	TET	CIP*	ERY	PEN	FLU
<i>Staphylococcus epidermidis</i>	14	100	57	64	100	92.9	42.9	76.9	100	100
<i>Staphylococcus Aureus</i>	4	100	50	75	50	75	50	100	100	-

\*AMP: ampicillin; GEN: gentamicin; CRX: cefuroxime; COT: cotrimoxazole; TET: tetracycline; CIP: ciprofloxacin; ERY: erythromycin; PEN: penicillin; FLU; flucloxacillin; CIP: ciprofloxacin  
antibiogram was available for half the total number of both of the gram positive organisms.

Of the four *Staphylococcus aureus* organisms isolated, there was a 100% resistance to ampicillin, 100% resistance to penicillin 75% resistance to tetracycline and 50% resistance to gentamicin, (Table 6).

A total of eight gram negative organisms were identified in this study, namely *Pseudomonas aeruginosa* (4), *Escherichia coli* (1), *Enterobacter* species (2) and *Proteus mirabilis* (1). *Pseudomonas aeruginosa* showed 100% resistance to ampicillin, 75% resistance to cefuroxime, 50% resistance to cefotaxime, 25% resistance to gentamicin and, 0% resistance to ciprofloxacin (Table 7).

**Table 7: Percentage resistance of gram negative organisms isolated from blood culture**

Isolate	Count (N)	AMP	GEN	CRX	COT	CTR	TET	CHL	CTX	CIP
<i>Pseudomonas aeruginosa</i>	4	100	25	75	75	25	75	75	50	0
<i>Escherichia Coli</i>	1	100	0	0	100	0	100	I*	0	-
<i>Enterobacter species</i>	2	100	50	50	50	50	100	50	100	-
<i>Proteus mirabilis</i>	1	100	100	100	100	0	100	100	0	-

\*I: Intermediate susceptibility; AMP; ampicillin, GEN; gentamicin, CRX; cefuroxime, COT; cotrimoxazole, CTR; ceftriaxone, TET; tetracycline, CHL; chloramphenicol, CTX; cefotaxime, CIP; ciprofloxacin. I; intermediate

The percentage resistance of *Escherichia coli* was 100% to ampicillin, 100% to cotrimoxazole, 0% to gentamicin, 0% to ceftriaxone and, 0% to cefotaxime (Table 7). *Enterobacter* species showed 100% resistance to ampicillin, 100% resistance to cefotaxime 50% resistance to gentamicin, 50% resistance to ceftriaxone and, 50% resistance to chloramphenicol (Table 7). The resistance of *Proteus mirabilis* was 100% to ampicillin, 100% to cefuroxime, 100% to tetracycline, 100% to chloramphenicol and, 0% to cefotaxime (Table 7)

#### **4.6 Factors associated with culture proven neonatal sepsis**

In a univariate logistic analysis, none of the maternal socio-demographic factors was significantly associated with culture proven sepsis (Table 8)

**Table 8: Univariate logistic analysis of maternal socio-demographic factors associated with culture proven sepsis**

Variable	COR	P value	95% CI
Maternal age		0.77	
≤20	Ref		
21-30	1.39		0.36 5.42
≥31	1.02		0.25 4.21
Marital status		0.20	
Unmarried	Ref		
Married	0.56		0.23 1.35
Educational status		0.53	
None	Ref		
Primary	3.14		0.31 32.28
JSS	1.73		0.20 15.24
SSS	2.28		0.25 21.12
Tertiary	4.05		0.44 37.43
Occupation		0.25	
Unemployed	Ref		
Employed	0.55		0.21 1.48

\*JSS: Junior secondary school; SSS: Senior secondary school; COR: crude odds ratio

In a univariate logistic analysis, gravidity (p value=0.02) and number of antenatal visits of a mother (p value= 0.04) showed a significant association with culture proven sepsis (Table 8).



**Table 9: Univariate logistic analysis of pregnancy related factors associated with culture proven sepsis**

Variable	COR	P value	95% CI
Parity Multiparous Primiparous	Ref 2.31	0.054	0.98 5.46
Gravidity Multigravida Primigravida	Ref <b>2.81</b>	<b>0.02</b>	<b>1.17 6.74</b>
Maternal fever No Yes	Ref 0.95	0.95	0.20 4.62
Number of vaginal examination ≤3 ≥4	Ref 0.72	0.57	0.23 2.28
Number of antenatal visit ≥4 ≤3	Ref <b>3.0</b>	<b>0.04</b>	<b>1.12 8.03</b>
Delivery mode SVD C/S	Ref 0.68	0.37	0.29 1.58
Intrapartum antibiotic prophylaxis Yes No	Ref 0.78	0.59	0.31 1.94

The odds of neonates whose mothers were primigravida was 2.81 higher, compared to neonates whose mothers were multigravida, to have a culture proven sepsis (OR=2.81; 95% CI 1.17 6.74) (Table 9). The odds of neonates whose mothers attended antenatal clinic ≤3 times was 3.0 higher, compared to neonates whose mothers attended antenatal clinic ≥4times, to have culture proven sepsis (OR=3.0; 95% CI 1.12 8.03 ) (Table 9).

**Table 10: Univariate logistic analysis of neonatal factors associated with culture proven sepsis**

Variable	COR	P value	95% CI
Sex		0.92	
Male	Ref		
Female	0.96		0.40 2.28
Birth weight		0.85	
<2500g	Ref		
≥2500g	0.90		0.33 2.50
Gestational age		0.09	
≤36weeks	Ref		
37-40weeks	1.02		0.36 2.88
≥41weeks	3.89		1.01 14.97
Apgar score 1minute		0.18	
≤5	Ref		
≥6	0.44		0.14 1.38
Apgar score 5minutes		0.29	
≤6	Ref		
≥7	0.49		0.14 1.71
Ventilation		0.57	
No mechanical ventilation	Ref		
Mechanical ventilation	1.64		0.31 8.61
Length of hospital stay		0.25	
≤7days	Ref		
≥8days	0.61		0.25 1.44
Birth weight		0.07	
Within study facilities	Ref		
Outside study facilities	2.29		0.95 5.54

Even though gestational age showed no significant association with culture proven sepsis (p value= 0.09), the odds of neonates who were delivered at gestational age  $\geq 41$  weeks was 3.89 higher, compared to neonates who were delivered at gestational age  $\leq 36$  weeks, to have a culture proven sepsis (OR= 3.89; 95% CI 1.01-14.97) (Table 10). Mechanical ventilation was not significantly associated with culture proven sepsis (p value=0.57). However, the odds of neonates who underwent mechanical ventilation was 1.64 higher, compared to neonates who did not undergo mechanical ventilation to, have a culture proven sepsis (OR=1.64; 95% CI 0.31 8.61) (Table 10). The odds of neonates who were

delivered outside the study facilities was 2.29 higher, compared to neonates who were delivered within the study facilities, to have a culture proven sepsis (OR= 2.29; 95% CI 0.95 5.54) (Table 10).

**Table 11: Multivariate logistic analysis of factors associated with culture proven sepsis**

Variable	AOR	P value	95% CI
<b>Gravidity</b>			
Multigravidous	Ref		
Primigravidous	2.74	0.03	1.12 6.68
<b>Number of antenatal visits</b>			
≥4	Ref		
≤3	2.90	0.04	1.06 7.96

AOR: adjusted odds ratio

In a multivariate logistic analysis, gravidity and the number of antenatal visits of a mother showed significant association with culture proven sepsis (Table 11). The odds of neonates whose mothers were primigravida was 2.74 higher compared to neonates whose mothers had multiple gravidity, to have culture proven sepsis (OR= 2.74; 95% CI 1.12 6.684). (Table 11). The odds of neonates whose mothers attended antenatal clinic ≤3 times was 2.9 higher, compared to neonates whose mothers attended antenatal clinic ≥4 times, to have culture proven sepsis (OR= 2.90; 95% CI 1.06 7.96) (Table 11).

## CHAPTER FIVE

### DISCUSSION

#### 5.1 Prevalence of neonatal sepsis

Culture results in this study shows that 26 (17.3%) of the suspected neonatal sepsis cases were positive.

The prevalence in this study, is not consistent with the prevalence of 38.5% obtained in a study done in India (Nayak et al., 2014), and the 21.8% prevalence of positive blood culture reported in Uganda (John, David, Mathias, & Elizabeth, 2015). It also does not agree with the 43.1% prevalence found in a similar study done in Nigeria (West, Peterside, Ugwu, & Eneh, 2012). The negativity of blood culture results however, does not rule out neonatal sepsis. The low prevalence of culture proven sepsis obtained in this study, could be due to the inability to take blood sample for culture before the initiation of antimicrobial therapy in some neonates. All referred neonates to the study sites had started antibiotic therapy before arrival. In addition, empiric therapy was started in some neonates born at the study facilities before blood sample was obtained due to initial difficulty. The mean time of 24hours between initiation of antibiotics and collection of blood sample for culture in some neonates could have interfered with sepsis causing organisms, to affect culture positivity; hence a low prevalence. Low concentrations of bacteria in blood, combined with insufficient blood volume for culture could also result in low culture positivity rate. The insufficient blood volume obtained in some neonates, could also account for the low prevalence in this study.

## 5.2 Common isolates

In this study, culture results indicate that, gram positive organisms (69%) has a preponderance over the gram negative organisms (31%). This suggests that majority of the infections were transmitted from handling by health care personnel and family members. Since *Staphylococcus epidermidis* and *Staphylococcus aureus* are the major normal flora located on the skin and in the nose respectively, suboptimal hand hygiene by persons who handle neonates could contribute to the acquisition of these bacteria. Gram negative organisms on the other hand are mostly located in the natural environment and the gut, with small proportions in moist areas on the skin. It implies that, infection by gram negative organisms could be acquired from the hospital environment, the community, or handlers of neonates who may not observe optimal hand hygiene. Findings from this study, does not correspond to a study done in a Neonatal Intensive Care Unit (NICU) in Bangladesh, where they identified gram negative organisms (78%) to be the most common pathogen of neonatal sepsis (Naher et al., 2013). However, in a similar study in a NICU in China, they found that gram positive organisms were responsible for a greater proportion of early onset sepsis (83.3%) and late onset sepsis (70%) compared to gram negative organisms (Li et al., 2013), which corroborates with findings from this study. Another study that was carried out in Dhaka found that 51.1% of isolates obtained from 88 positive blood cultures in a NICU were gram positive bacteria while 48.8% were gram negative organisms (Begum et al., 2013), which supports findings from this study that, gram positive bacteria was the commonest cause of neonatal sepsis as compared to gram negative bacteria. In a similar study in India,

gram positive organisms predominated (51.1%) over gram negative organisms (48.9%) in determining bacteriological profile of isolates from blood culture of neonates (Debnath & Das, 2015), confirming the results from this study. Findings of this study is also similar to the 52.6% proportion of gram positive organisms reported from blood culture of neonates with sepsis in Nigeria (Peterside, Pondei, & Akinbami, 2015).

*Staphylococcus epidermidis* was the most common isolate (54%) followed by equal proportions of *Staphylococcus aureus* (15%) and *Pseudomonas aeruginosa* (15%). The proportion of *Enterobacter* species was (8%), with *Escherichia coli* and *Proteus mirabilis* representing 4% each. A greater number of *Staphylococcus epidermidis* 9 (64%), with 1(8%) *Staphylococcus aureus*, 1 (8%) *Escherichia coli* and, 2 (15%) *Pseudomonas aeruginosa* were isolated in early onset sepsis (EOS). Since reports indicate that, organisms causing EOS are mostly transmitted vertically from the colonized genital tract of mothers, or sometimes through the delivery process, the findings suggest that EOS causing organisms could be transmitted by these means.

The multivariate logistic analysis to determine factors associated with culture proven sepsis in this study reveals that, neonates whose mothers attended antenatal clinic for  $\leq 3$  times have a higher odds (2.9) to have culture proven sepsis. This could be due to the fact that mothers who attended antenatal clinic  $\leq 3$  times, may not receive sufficient education on the healthy practices to adopt in order to prevent transmission of these organisms to their neonates, or the insufficient antenatal visits by the mothers may not give the health personnel the opportunity to screen and disinfect affected mothers. Also, the same

mothers may not have adequate interaction with the health care personnel to be able to identify and report risk factors of acquiring neonatal sepsis causing organisms. Hence neonates born to such mothers are more prone to have culture proven sepsis, which is evident by the isolation of these organisms. Neonates whose mothers were primigravidae show greater odds of developing culture proven sepsis. The change in physiology of mothers in pregnancy and more especially for mothers who get pregnant for the first time could predispose neonates to some adverse outcomes including sepsis. First time pregnant women also stand a greater chance of reporting to hospital before, during and after delivery. Hence, when a neonate becomes septic, chances of isolating causative organisms may be higher.

Additionally, the immature immunity of neonates makes them susceptible, irrespective of the minutest infection they may be predisposed to. Findings therefore suggest that, intrapartum and delivery practices need improvement for better neonatal care and protection of neonates from sepsis.

In a study in Nepal, results revealed that *Staphylococcus epidermidis* accounted for the greater proportion (57.3%), followed by (28.1%) of *Escherichia coli*, (11.2%) of *Staphylococcus aureus* and (1.1%) of *Pseudomonas aeruginosa* that were isolated in EOS (Adhikari, Shah, Acharya, & Vaidya, 2014), as observed in this study. In another study to determine the common isolates of neonatal sepsis in India, (Shah, Mulla, & Revdiwala, 2012) found that coagulase negative staphylococcus (which includes *Staphylococcus epidermidis*) was the most common gram positive pathogen (27%), followed by *Staphylococcus aureus* (13%). The finding supports results from this study that

*Staphylococcus epidermidis* is the most common (64%) gram positive causative organism of neonatal sepsis, followed by *Staphylococcus aureus* (8%). Similar reports were given in a study done in Tamale Teaching Hospital in Ghana, where 8 (53.3%) of *Staphylococcus* species, 1 (6.7%) *Escherichia coli* (Acquah et al., 2013) and other gram negative organisms of a total of 15 isolates were identified in EOS. A study was carried out in six countries of the Asian and African continent (including Ghana) to determine bacteriological profile of organism causing sepsis among neonates and infants, *Staphylococcus aureus* was found to dominate (48%) the organisms isolated in early onset sepsis, followed by (9%) *Escherichia coli* bacteria (Hamer et al., 2015), which is not consistent with this study.

In the late onset sepsis (LOS) cases, majority of the bacteria identified were *Staphylococcus epidermidis* and *Staphylococcus aureus*. Acquisition of these bacteria could be due to suboptimal hand hygiene by caretakers or family members of neonates or the environment. This is comparable to a study done in Uganda which showed that neonates born outside the health facility had a higher odds of acquiring culture proven sepsis, than those born within the health facility though statistically insignificant (John et al., 2015). This is because majority of LOS causing bacteria are acquired from the community. In addition, most of the gram negative organisms (*Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterobacter* species) in this study were isolated in LOS, which is in accordance with literature that, a greater number of gram negative organisms are found in the environment and the gut of humans, with some proportion in moist areas on the skin.

Another possible source of these organisms in this study, could be the mechanical ventilation procedure that some neonates underwent. Though insignificant, neonates who underwent mechanical ventilation were more prone to have organisms isolated from their samples upon culture, compared to those who did not undergo that procedure. Both invasive and noninvasive procedures such as mechanical ventilation have been identified as risk factors for sepsis, and indications are that, manipulation of the instruments used in these procedures by health personnel could introduce sepsis causing organisms and make them colonized; thereby putting the neonate at risk. Hence isolation of these organisms in this study, could be attributed to mechanical ventilation, even though not significant. In the case of neonates born outside the study facilities in this study, findings are evident that they have a higher odds of acquiring culture proven sepsis. With regards to home deliveries specifically, suboptimal hand hygiene from family and friends before handling neonates could predispose them to such infections. When infection control measures in the home environment in which neonates are delivered are not favourable, neonates could acquire sepsis causing organisms leading to infection. It is also possible that transmission of these organisms could occur trans-placentally, or through the birth canal during delivery, which later manifests as sepsis in neonates. Studies have attributed late onset sepsis to be prominent among low birth weight neonates. As observed in this study, neonates with birth weight  $>2500\text{g}$  had lower odds of 0.79 compared to neonates of birth weight  $\leq 2500\text{g}$ , to have a culture proven sepsis though insignificant. This is consistent with a study done in Ghana, which found that neonates with birth weight  $<2500\text{g}$  had higher odds of acquiring culture proven sepsis compared to neonates with birthweight

≥2500g which was statistically significant (Siakwa, Kpikpitse, Mupepi, & Semuatu, 2014).

In a study by Hamer et al, *Enterobacter* sp. and *Acinetobacter* sp. were the majority of gram negative organisms isolated in late onset sepsis (LOS), followed by *Klebsiella* species and *Escherichia coli* (Hamer et al., 2015), which is not comparable to findings of this study. Reports in Nepal, also indicate that *Enterobacter* species (15%), *Acinetobacter* species (12%) and, *Escherichia coli* (12%) were the commonest isolated gram negative organisms in LOS (Ansari, Gautam, Shrestha, Neopane, & Chapagain, 2015), which contradicts the findings of this study. Additionally, a study done in South Africa, identified *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Escherichia coli* as the predominant gram negative bacteria, together with few *Pseudomonas aeruginosa* and *Enterobacter* species (Ballot et al., 2012), which is contrary to findings in this study. The difference in the type and proportions of organisms in the studies could be due to the duration of the studies. This study had a shorter duration. Considering also that, there is variation of causative organisms of neonatal sepsis, between geographic regions and facilities, the difference in findings in this study could be a confirmation. Bacteriological profile of neonatal sepsis causing organisms may vary among countries (Yunanto, Margareta, Indah, & Pratiwi, 2014) and, composition of these organisms have changed over the last century because changing trend of antibiotic use and life style (Marchant, Boyce, Sadarangani, & Lavoie, 2013).

### 5.3 Antibiotic susceptibility

Results of antibiotic susceptibility in the present study indicate that, *Staphylococcus epidermidis*, shows 100% resistance to ampicillin, penicillin, flucloxacillin and cotrimoxazole. This is alarming considering that, either ampicillin or penicillin in combination with gentamicin is recommended as first line drugs for empiric treatment of neonatal sepsis. Though *Staphylococcus epidermidis* shows an approximated average (43%) sensitivity to gentamicin, it indicates that its treatment by first line drugs poses a threat to management of neonates. Neonates may spend longer days on antibiotics and stay longer in hospital as well. It confirms reports in an antibiotic susceptibility test carried out on blood cultures from neonates that, Coagulase Negative *Staphylococcus* including *Staphylococcus epidermidis* showed poor sensitivity (13.5%) to ampicillin (Begum et al., 2013). In another study, *Staphylococcus epidermidis* showed 100% resistance to ampicillin, penicillin and cotrimoxazole (Debnath & Das, 2015), which corresponds to the findings of this study. Findings from a similar study in India revealed that *Staphylococcus epidermidis* showed 0% and 88.9% resistance to gentamicin and penicillin respectively (Sheth, Patel & Tripathi, 2012). This is not comparable to findings in this study. However, results in this study could serve as evidence of increasing resistance to commonly used antibiotics.

*Staphylococcus aureus*, the second gram positive isolate in this study is 100% resistant to ampicillin, penicillin and erythromycin. The overall high resistance rate exhibited could be attributed to the frequent and unrestricted use of the commonly used antibiotics. Consequently, this may limit future antibiotic choice for treating neonatal infections

thereby, affecting survival of septic neonates. In Ghana, resistance rate of 96.4%, 96.4% and 64.3% to ampicillin, penicillin and erythromycin respectively was reported (Acquah et al., 2013); this confirms high resistance rate to these antibiotics identified in this study. In the same study by Acquah et al, (2013), 75% resistance was shown to cefuroxime, which agrees to the 75% resistance to cefuroxime reported in this study. In Nepal, *Staphylococcus aureus* however, showed 50% resistance to gentamicin, in a study (Adhikari et al., 2014), which supports findings in this study.

There is 100% resistance by *Pseudomonas aeruginosa* to ampicillin, but a lower resistance (25%) to ceftriaxone and gentamycin. High sensitivity to gentamicin and ceftriaxone by *Pseudomonas aeruginosa* is good for neonatal care. It therefore means that, the use of both drugs in empiric treatment of neonatal sepsis will be effective against *Pseudomonas aeruginosa*. However, the complete resistance to ampicillin, as exhibited by other organisms in this study is devastating. As observed in a study done in India, *Pseudomonas aeruginosa* was 25% resistant to gentamycin, (Minal, Vegad, Shah, & Soni, 2015) which is comparable to this study.

This study also reveals that *Enterobacter* species show a multi drug resistance (100%) to ampicillin, cefotaxime and tetracycline. The lowest resistance (50%) shown is to gentamycin, cefuroxime, cotrimoxazole, ceftriaxone and chloramphenicol. Multi drug resistance in the sick newborn is not a desirable experience for the neonate, family or clinical management staff. The reason is that, it leads to increased cost in terms of money and productive time spent at the hospital by family. Additionally, multi drug resistance of

infectious organisms could result in unsuccessful treatment leading to death. Though sensitivity to gentamicin and the third generation cephalosporins (cefuroxime and ceftriaxone) are fairly high, it calls for caution and restriction in its use. This is to prevent development of further resistance to these antibiotics. As observed in Nepal, *Enterobacter* species had a high multi drug resistance rate (100%) to antibiotics including ampicillin and cefotaxime (Ansari et al., 2015), which is consistent with findings in this study.

The only *Escherichia coli* isolated also exhibits a multi drug resistance to ampicillin, cotrimoxazole and tetracycline. It however, is susceptible to gentamicin, and the cephalosporin (cefuroxime, ceftriaxone and cefotaxime) that are used. *Escherichia coli* also exhibits multi drug resistance like *Pseudomonas aeruginosa* in this study, which poses a threat to neonatal care in this era of increasing antibiotic resistance. Susceptibility to gentamicin and the cephalosporin however, suggests that they must be prescribed under strict guidelines to further antibiotic resistance development. In a similar study done in Indonesia, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* species and non-fermenting gram negative bacilli were the gram negative organisms isolated; it was reported that, all of them exhibited high resistance to ampicillin, gentamicin and cefotaxime (Viswanathan et al., 2012). These results are partly consistent with this study, and partly disagrees with it.

The single *Proteus mirabilis* organism identified, exhibits a high level of multi drug resistance. It however, is susceptible to two of the cephalosporin. They are ceftriaxone and cefotaxime. The high level of antibiotic resistance observed is in line with the

emerging threat of global antibiotic resistance. One factor accounting for this, could be the indiscriminate use of available antibiotics in the presumptive treatment of neonatal infection. Also, the tendency of clinicians initiating antibiotic therapy before performing blood culture could result in antimicrobial resistance. As observed in this study, blood was obtained from 66 (44%) neonates after the initiation of antibiotic therapy. Thus, it could be a contributing factor to high rates of antibiotic resistance exhibited in this study. In developing countries, especially in low-resource areas where blood culture facility may be unavailable or difficult to access, physicians may be pressurized to treat neonates empirically without specific antibiotics, hence high levels of resistance to antibiotics could be observed.

Findings from this study reveal that, all the common isolates of neonatal sepsis show 100% resistance to ampicillin. In addition, all gram positive organisms show 100% resistance to penicillin. However, organisms show appreciable sensitivity to gentamicin, ceftriaxone and cefuroxime. This indicates that “ampicillin/penicillin +gentamicin” combination as the first line of drugs for empiric therapy, needs to be reviewed in terms of ampicillin and penicillin.

#### **5.4 Limitation**

This study did not determine the selective pressure factors influencing antibiotic susceptibility, but only determined antibiotic susceptibility. Thus factors associated with high antibiotic resistance in this study are not known. The initiation of antibiotic therapy in some neonates prior to obtaining blood culture sample could have reduced culture positivity, hence affecting prevalence of culture proven sepsis in the study.

## CHAPTER SIX

### CONCLUSION AND RECOMMENDATION

#### 6.1 Conclusion

The prevalence of culture proven neonatal sepsis in this study is 17.3%. Gram positive organisms were the prevalent neonatal sepsis causing organisms in this study. Of the gram positive organisms, *Staphylococcus epidermidis* was the most common isolate, followed by *Staphylococcus aureus*. *Pseudomonas aeruginosa* was the most common isolate among the gram negative organisms, with single isolates each of *Escherichia coli* and *Proteus mirabilis*. Both gram positive organisms and gram negative organisms show high rates of antibiotic resistance. Gram positive organisms show high resistance to ampicillin, penicillin, erythromycin and tetracycline. Sensitivity is fairly high to ciprofloxacin and gentamicin. Generally, gentamicin and ceftriaxone exhibit a better susceptibility rate to gram negative bacteria, while gram positive organisms are more sensitive to gentamicin and ciprofloxacin. Gentamicin as the first line antibiotic for neonatal sepsis is still quite effective. However, the microorganisms isolated in this study demonstrate an increasing resistance rate. All neonatal sepsis causing organisms were completely resistant to ampicillin and penicillin and hence the need to review the empirical treatment for neonatal sepsis. Gravidity and number of antenatal visits show significant association with culture proven sepsis. Based on the findings from this study, the following recommendations are being made for consideration.

## **6.2 Recommendation**

### **Clinicians**

- Clinicians in the Ho Municipality need to review the use of ampicillin and penicillin as first line drugs for empiric treatment of neonatal sepsis.
- Clinicians should ensure where possible, that blood sample for culture is collected before initiating antibiotic therapy in suspected neonatal sepsis cases.
- A combination of gentamicin and ceftriaxone should be opted for, in the choice of drugs for empiric therapy for neonatal sepsis.

### **Health care staff**

- Health care staff should ensure that, they continuously engage in good hand washing practices to reduce contamination.
- Health care staff should give family members continuous education on good hand washing practices before handling neonates to prevent infection.
- Health care staff should improve on infection prevention control during good delivery practices in order to prevent possible transmission of infection to neonates during this process.

### **Hospital management of the study sites**

- Hospital management should intensify and improve infection control practices in the health facilities to reduce predictors of hospital acquired infections among neonates.
- Laboratory based continuous surveillance is required in order to determine bacteriologic profile and antibiotic susceptibility pattern of common pathogens of neonatal sepsis.

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## APPENDICES

### Questionnaire

Antibiotic susceptibility pattern of blood culture of neonates with sepsis, Ho municipality

Date.....

Form number

Interviewer code

#### 1.0 Personal details (mother):

What is your contact number? .....

1.1 How old are you? (Complete age in years).....

1.2 Where do you stay?

Within Ho municipality  Outside Ho municipality

1.3 What is your marital status?

Single  Married  Divorced

1.4 How many times have you been pregnant?

Once  2 or more times

1.5 How many times have you given birth?

Once  2 or more times

1.6 Which ethnic group do you belong to?

Akan  Ewe  Ga  Northern descent  others

Specify.....

1.7 What is your religion?

Christianity  Islamic  Traditional African belief  Others

Specify.....

1.8 What is your highest level of education?

None  Primary  JHS/Middle school  SHS/Tech/Voc/Sch

Tertiary

1.9 Do you work ?

Yes  No

**2.0 Clinical details (mother) (Review case note):**

2.1 Has she had antenatal care?

Yes  No

2.2 If yes, how many times has she attended antenatal care? .....

2.3 What was her mode of delivery?

Spontaneous vaginal delivery  Assisted delivery  Caesarean section

2.4 How many vaginal examinations has the mother had before delivery.....

2.5 Did you loose liquor before labour started?

Yes  No

2.6 If yes, how many hours did the membrane rupture before labour?

≤18hours  >18hours

2.7 Was an intrapartum antibiotic prophylaxis given to her?

Yes  No

2.8 If yes, what antibiotic was given? .....

2.9 If yes, when was it given?

Before labour  During labour  After labour

2.10 Has she had a previous baby with sepsis?

Yes  No

2.11 Did she have any fever during labour?

Yes  No

**3.0 Neonatal details (Review case note):**

3.1 What is the baby's sex?

Male  Female

3.2 How old is the baby? (in days) .....

3.3 What antibiotics have been prescribed for the baby since diagnosis was made? .....

3.4 What is the baby's birth weight? (in grams) .....

3.5 At what gestational period was the baby born? (in weeks) .....

3.6 What was the baby's Apgar score at 1minute? .....

3.7 What was the baby's Apgar score at 5minutes? .....

3.8 Was the baby resuscitated?

Yes  No

3.9 Which of the following procedures has the baby had/is having?

Intravenous cannula  Parenteral nutrition

Mechanical ventilation  Central venous catheter

3.10 When was the baby admitted for sepsis? .....

**4.0 Laboratory details (blood culture):**

4.1 Is there a significant growth?

Yes  No

4.2 If yes, what pathogen has been isolated? .....

Antibiotic susceptibility pattern (tick as appropriate)

Name of antibiotic	Resistance	Intermediate	Susceptibility

## **Consent form**

Study title: Antibiotic susceptibility pattern of blood culture of neonates with sepsis, Ho municipality

Principal investigator: Fortress Yayra Aku

Qualification: M.Phil Applied Epidemiology and Disease Control

Address: School of Public Health

Informed Consent Form -participant

## **PART I: Information sheet**

### **Introduction**

I am..... a graduate student of the University of Ghana. As partial fulfilment of the requirements of the University, I am doing a study on “Antibiotic susceptibility pattern of blood culture of neonates with sepsis, Volta Regional Hospital”. I am going to give you information and invite you to be part of this study. You may choose to talk to anyone you feel comfortable with concerning the research before you decide to participate in the study. There may be some words that you do not understand, please feel comfortable to stop me as we go through the information and I will take time to explain to your understanding. In case you have questions later, do not hesitate to ask me.

### **Purpose of the research**

When babies are born with some diseases, they are given medicines to treat these diseases. Some babies recover when they are given these medicines and others do not. The occurrence of some babies not recovering is increasing all over the world. The cause of these diseases have also been said to be different from one place to another. To avoid babies from getting these diseases and before medicines are given to babies with these diseases, the cause of the disease, the manner in which babies recover or not when the medicines are given to them and the things that make babies to get these diseases when they are born must be known. This study is aimed at identifying the cause of the disease,

the manner in which babies recover or not when the medicines are given to them and the things that make babies to get these diseases when they are born.

### **Participant selection**

I am inviting all mothers or caretakers of babies who have this disease at the Volta Regional Hospital.

### **Participation**

Your decision to participate in this study is entirely voluntary. Every help the hospital offers you will remain unchanged whether or not you agree to participate in the study.

### **Procedure/protocol**

I will ask mothers of the babies some questions about themselves and collect some information from the folders of the mothers and their babies. I will also collect information on results of the laboratory test that is done on the babies.

### **Duration**

The study will take place over a period of four months. However, when a baby is part of the study, the mother will be asked some questions only once for 5minutes. Some information on the mother and the baby will be collected from their folders and information on the laboratory results done on the baby will be collected.

### **Risks**

A small volume of blood (2ml) will be taken from your baby by a trained health personnel. This may cause some pain and discomfort or swelling at the site where the blood will be taken; but the study will not cause any problem with the way in which your baby is being treated for the disease.

### **Benefits**

The findings from the laboratory test will help the doctor to treat your baby better. Your taking part in this study will also help us to know the causes of this disease in babies and

it will give us information on how to prevent the disease and also what medicines should be given to babies that get this disease in this hospital.

**Confidentiality**

Every information that will be collected from you and the baby during the study will be kept away from unauthorized persons, except those doing the study. I will not share the information collected from you with anybody, but will keep it and use it only for the study. I will give a different number to every individual who is part of the study so that information collected from them cannot be traced to them. This information will be kept under lock and key.

**Who to contact**

In case of any questions, you may ask them now or later, or even after the study has begun. You may contact the following:

Principal investigator: Fortress Y. Aku

Telephone: 0246121421

Supervisor: Dr. Patricia Akweongo

Telephone: 0243138376

Ethical review contact person: Madam Hannah Frimpong

Telephone number: 0507041223

**Part II: Certificate of Consent**

I have read the above information, or it has been read to me. I have had the opportunity to ask questions concerning the study, and all questions I have asked have been answered to my satisfaction. I consent voluntarily to take part in this study.

Name of mother/caretaker of participant.....

Signature/thumbprint of mother/caretaker of participant.....

**Date**.....

I confirm that the participant was given the opportunity to ask questions regarding the study, and all questions asked by the participant were answered correctly and to the best of my ability. I confirm that no participant has been coerced into giving consent, and the consent has been given freely and voluntarily.

