NEONATAL SEPSIS, CYTOKINES AND THE ASSOCIATED FACTORS IN PATIENTS ADMITTED IN NAKURU COUNTY REFERRAL HOSPITAL, KENYA

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JULY, 2017
DECLARATION

This thesis is my original work and has not been submitted for degree or other awards to any other University

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Department of Zoological Sciences
Kenyatta University
DEDICATION

I dedicate this work to my family members; my dear husband Vincent Obwoge and lovely children Anthony Magoma, Andrew Nyachieo and Tracey Kemunto.
ACKNOWLEDGEMENTS

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# ABBREVIATIONS AND ACRONYMS

<table>
<thead>
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<th>Full Form</th>
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<tbody>
<tr>
<td>AAP</td>
<td>American Academy of Pediatricians</td>
</tr>
<tr>
<td>AMREF</td>
<td>African Medical and Research Foundation</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BH-I</td>
<td>Brain Heart Infusion</td>
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<tr>
<td>BSI</td>
<td>Blood Stream Infection</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control</td>
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<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standard Institute</td>
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<tr>
<td>CONS</td>
<td>Coagulase Negative Staphylococci</td>
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<td>CRP</td>
<td>C-reactive proteins</td>
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<td>CS</td>
<td>Caesarean Section</td>
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<td>CSF</td>
<td>Cerebral Spinal Fluid</td>
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<td>CMV</td>
<td>Cytomegalovirus</td>
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<td>E coli</td>
<td>Escherichia Coli</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay.</td>
</tr>
<tr>
<td>EOS</td>
<td>Early Onset Sepsis</td>
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<tr>
<td>ESR</td>
<td>Erythrocyte Sedimentation Rate</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>FI</td>
<td>Floresent Intensity</td>
</tr>
<tr>
<td>GBS</td>
<td>Group B Streptococci</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte Macrophage Colony Stimulating Factor</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>IAP</td>
<td>Intrapartum Antibiotic Programme</td>
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<tr>
<td>I/T</td>
<td>Immature to total Neutrophil ratio</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>LOS</td>
<td>Late Onset Sepsis</td>
</tr>
<tr>
<td>MSAF</td>
<td>Meconium-Stained Amniotic Fluid</td>
</tr>
<tr>
<td>NCRH</td>
<td>Nakuru County Referral Hospital</td>
</tr>
<tr>
<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer Cell</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonucleocyte</td>
</tr>
<tr>
<td>PROM</td>
<td>Preterm Rapture of Membranes</td>
</tr>
<tr>
<td>RT</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>SA-HRP</td>
<td>Streptavidin-Horseradish Peroxidase</td>
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<tr>
<td>SPSS</td>
<td>Statistical Programme for Social Sciences</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethylbenzidine</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>VD</td>
<td>Vaginal Delivery</td>
</tr>
<tr>
<td>VLBW</td>
<td>Very-Low-Birth-Weight</td>
</tr>
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<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Neonatal infections mostly sepsis and meningitis currently cause about 4.9 million deaths annually in developing countries. According to World Health Organization (WHO) estimates, there are about 5 million neonatal deaths that occur in the world per year due to sepsis, with 98% occurring in developing countries. Neonatal sepsis is the leading cause of neonatal mortality in Nakuru County Referral Hospital (NCRH) according to the hospital records, yet no study has been done in the hospital on this infection prior to the current research. The purpose of this study was to provide an overview of the characteristics of bacterial sepsis in the newborns in NCRH. The study focused on the bacterial pathogens mostly implicated in neonatal sepsis, their antibiotic susceptibility patterns and cytokine involvement in neonatal sepsis. The study also determined some of the factors associated with neonatal sepsis infection in NCRH. A sample of 104 neonates admitted in Nakuru County Referral Hospital with positive clinical diagnosis of neonatal sepsis and some with normal health was enrolled for the study and grouped according to sex, birth weight and gestational age at birth. Samples of blood for culture and cytokine assay were obtained by a phlebotomist from peripheral foot veins (2 ml per neonate) using aseptic method. After blood culture, antibiotic susceptibility testing was performed for identified bacteria by disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Cytokine measurement was done using commercially available kit for T helper-1 and T helper-2 cytokines. Enzyme Linked Immunosorbent Assay (ELISA) was used to measure cytokine (IL-1β, IL-6, IL-8, IL-10 and TNF-α) levels. Quantities of individual cytokine were computed using standard reference curve. The Statistical Programme for Social Sciences (SPSS) software version 22 was utilized for data analysis where paired t-test was used to compare continuous variables, Analysis of variance (ANOVA) was used to compare cytokine levels, Chi-square was used to compare categorical variables and Spearman rank correlation to show the relationship between inflammatory and anti-inflammatory cytokine responses in neonatal sepsis. Neonatal sepsis infection was more common in preterm and low birth weight neonates compared to term and normal birth weight neonates (P< 0.05). Escherichia coli was the most isolated pathogen in septic cases (44.4%) while Pseudomonas aeruginosa caused the least number of cases (16.7%). There was a significant relationship between gestational age of the neonates and sepsis type (r=0.6, P< 0.05) where P. aeruginosa was responsible for sepsis only in preterm neonates. The isolated bacteria pathogens (E. coli, S. aureus and P. aeruginosa) were 100% resistant to first line antibiotics (Ampicillin and Gentamycin) but more susceptible to Chloramphenical and Ciprofloxacine (>75%). The measured cytokines were elevated in the sick neonates when compared with the control group where a significant correlation was observed between IL-10 and TNF-α levels in the sick neonates (r=0.561, P< 0.05). The findings of this study also revealed a significant relationship between IL-10 levels and sex (P< 0.05) among the sick neonates. The findings from this study provides necessary information for clinicians to use in diagnosis and management of neonatal sepsis.
CHAPTER 1: INTRODUCTION

1.1 Background information

Neonatal sepsis refers to presence of bacterial blood stream infection in the setting of fever (Stoll, 2011). Neonatal septic infection occurs in infants younger than 90 days of age (Edwards et al., 2006). This infection is divided into two categories: early onset sepsis (EOS) and late onset sepsis (LOS). Early onset sepsis presents within seven days of life while late onset sepsis refers to presentation of sepsis from 8 to 89 days of life (Tallur et al., 2000). Neonatal sepsis is characterized by fever, breathing problems, diarrhea, low blood sugar, reduced suckling, slow heart rate, vomiting, swollen belly and jaundice (Verani et al., 2010).

The incidence of neonatal sepsis is higher in developing countries (Vergnano et al., 2005) where it is associated with over 50% of deaths that occur in infants (WHO, 2000). In neonatal sepsis death rate is dependent on the pathogen that is associated with the infection, where Gram negative organisms are the leading cause of neonatal deaths. The most common cause of neonatal sepsis in developed countries are group B Streptococci (GBS), Escherichia coli (E. coli) and Listeria monocytogenes (Isaac et al., 1999; Hyde et al., 2002) while Gram negative bacteria and coagulase negative Staphylococci (CONS) are the most common cause of neonatal sepsis in developing countries (Lim et al., 1995; Palazzo et al., 2006). The type of pathogen causing disease in newborns may vary from one country to another and also from one medical institution to the next. Hence there is need for studies that will establish the specific bacteria associated with the disease and their response to empirical antimicrobials.
(Desinor et al., 2004). This means that, for effective management of neonatal sepsis, studies should be carried out in the various countries, health institutions and even in different units within the hospitals independently without generalization (Alzwaini et al., 2000).

Exploration of immunological techniques such as assaying for C-Reactive proteins (CRP) may play an important role in the diagnosis of neonatal sepsis although they lack ability to detect specific bacterial pathogens. Blood culture to isolate the pathogen remains the gold standard method for diagnosis of sepsis (Lund et al., 2002). Knowledge of predictors of positive blood culture and antimicrobial susceptibility pattern of common pathogens in a given area is essential in guiding local empirical choice of antibiotics (Goosen, 2000). The objective of the present study was to determine the epidemiology of neonatal sepsis and the associated cytokines in Nakuru county referral hospital (NCRH).

1.2 Problem statement

Neonatal sepsis has been often associated with morbidity and deaths that occur in newborns despite the various therapeutic and curative measures being under taken especially in the neonatal intensive care units. This may be due to inadequate information on neonatal sepsis in terms of causes, sources, prevalence, drug sensitivity and immune responses. Diagnosis and treatment of neonatal sepsis is further complicated by the varied pathogens responsible for the infection. Failure to make proper diagnosis of neonatal sepsis is further complicated by commonly shared
symptoms between sepsis infection and other non-infectious diseases. Neonates are at high risk of developing bacterial infections due to their low production of immune cells, which are also insufficient in their immunologic functions especially the granulocytes.

1.3 Justification of the study
Neonatal sepsis is the single most important cause of neonatal deaths in many communities, accounting for over a half of the deaths that occur (WHO, 2000). In NCRH, 247 neonates were diagnosed with positive clinical signs of neonatal sepsis in the year 2013. In the course of treatment 48 of the neonates lost their lives. This calls for a study to establish the population of neonates with a higher risk of sepsis infection (Kuruvilla et al., 1999) in order to take precaution in their management and treatment. Studies aiming at identifying and describing specific bacterial pathogens causing sepsis in a community and describing their antibiotic susceptibility are necessary (Musoke and Revathi, 2000) in order to identify the appropriate antibiotics for treatment. There is also need to study the unique neonatal immunologic responses such as, determining the cytokines involved in neonatal sepsis since they are immunomodulators. Early diagnosis, proper management and treatment of neonatal sepsis based on bacterial culture results and cytokine profile will enable pediatricians to reduce mortality cases that result from neonatal sepsis infection.

1.4 Research questions
(i) What bacterial pathogens cause neonatal sepsis in Nakuru County Referral Hospital?
(ii) How do the bacterial pathogens respond to commonly used antibiotics in NCRH?

(iii) What cytokines are involved in neonatal sepsis infection in NCRH?

(iv) What factors are associated with neonatal sepsis in NCRH?

1.5 Hypotheses

(i) There is low prevalence of neonatal sepsis infections in Nakuru County Referral Hospital.

(ii) Bacteria associated with neonatal sepsis are not sensitive to the commonly used antibiotics.

(iii) There are no cytokines associated with neonatal sepsis.

(iv) There are no factors associated with neonatal sepsis in NCRH.

1.6 Objectives

1.6.1 General objective

To determine the prevalence of neonatal sepsis, cytokines and the associated factors in Nakuru County Referral Hospital.

1.6.2 Specific objectives

(i) To determine bacterial pathogens causing neonatal sepsis in Nakuru County Referral Hospital.

(ii) To determine bacterial sensitivity to drugs used in treatment of neonatal sepsis in NCRH.

(iii) To determine cytokines associated with neonatal sepsis in NCRH.

(iv) To determine factors associated with neonatal sepsis in NCRH.
1.7 Significance of the study

The establishment of the epidemiology of neonatal sepsis in NCRH and cytokines involved will be an important step in the control and management of neonatal sepsis because, an awareness of specific bacterial pathogens causing neonatal sepsis in a community, their sources, and drug interaction enables the medical personnel to correctly diagnose and manage the disease in early in order to reduce the rate of infection and also the rate of death in infants.
CHAPTER 2: LITERATURE REVIEW

2.1 Neonatal sepsis

The occurrence of neonatal sepsis infections in developed countries is quite low (2/1000) when compared with developing countries especially in Africa where the incidence is 42/1000 live births (WHO, 1996). According to WHO (2000) estimates, globally, there are about 5 million neonatal deaths a year, 98% occurring in developing countries, where neonatal infection, premature births and delivery related complications are the most contributory factors. Micro-organisms most commonly associated with neonatal sepsis include; group B Streptococci (GBS), E. coli, coagulase negative Staphylococcus, Haemophilus influenza, Listeria monocytogenes, Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas species.

In addition to microbial factors, host factors such as immature cellular immunity and humoral immunity predispose the newborn to sepsis. The most common risk factors associated with LOS include; maternal GBS colonization if untreated during labor, premature rapture of membranes (PROM), preterm rapture of membranes, prolonged rapture of membranes, colonization of the mothers vaginal tract by other pathogenic microorganisms and intra amniotic infections which result to prolonged labor (Kuruvilla et al., 1999).

Septic shock due to the effect of neonatal sepsis infection has frequently been reported where the pathogenic effects are said to be mediated by various immune factors such as tumor necrosis factor alpha (TNF- α), Interleukins, platelet activating factor and
activators of complement cascade (Zimmer et al., 1996). It has further been reported that other immune molecules that may play a role in neonatal sepsis immune response include heat shock proteins, adhesion molecules and polypeptides which are members of antacoid family (Hassan et al., 2013). The main mediator of neonatal sepsis is not clearly known but studies have reported TNF-α to be playing a major role following pathogenesis of the disease. Anti-inflammatory cytokines such as IL-4 and IL-10 play a major role in down regulating the effects of these mediators thus inhibiting their immunologic effects thus lowering the severity of the disease (Van Dissel et al., 1998).

The pathological damage of the inner lining of blood vessels during neonatal sepsis infection may be due to the continuous and repeated inflammatory responses by the immune cells. Finally, when the endothelial damage is intolerable, it cannot be reversed by the natural down regulators leading to a state of metabolic imbalance in which, the body of the neonate can no longer be able to regulate its various inflammatory responses. The symptoms of neonatal sepsis mostly are not specified but may include: lethargy, poor feeding, fever, jaundice, renal failure, chest reaction, seizures, high pitched cry, vomiting, neck-retract and heart rate above 160 among others (Stoll, 2011).

The symptoms of neonatal sepsis are not specific therefore necessitating more studies on immune responses in septic infections for proper management and treatment for risk factors associated with GBS infections according to the American Academy of Pediatricians (AAP) and Centers for Diseases Control and prevention (CDC).
2.2 Bacteria associated with neonatal sepsis

The causative organisms in neonatal sepsis vary from place to place and their frequency is different in different hospitals and even in the same hospital at different times (Desinor et al., 2004). In developed countries, group B *Streptococci* (GBS) is the pathogen mainly responsible for neonatal sepsis while it is very rare in developing countries. Therefore, ethnic and social economic differences may contribute to the varying differences in incidence of GBS infection among neonates in different populations (Stoll et al., 2011). The bacterial group commonly associated with neonatal sepsis is the Gram negative bacteria. This may be due to contamination by microbial flora from vaginal tract of the mother and also microbes from feecal matter of the mother at delivery. Bacterial infection may also result from the maternity and neonatal wards where the newborns are delivered (Polin and St Geme, 1992).

The most common pathogens in neonatal sepsis include: *Streptococcus pneumonia*, *Staphylococcus aureus*, *Enterococcus* spp., *Haemophilus influenza*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus viridans* (Bizzano et al., 2005; Weston et al., 2011). When studies were carried out on very low birth weight (VLBW) and preterm neonates, *E. coli* was established to be the major cause of early onset neonatal sepsis (> 50%) when compared with the other species of gram negative bacteria (Weston et al., 2011; Hornik et al., 2012).
Recent studies have reported *Staphylococcus aureus* to be causing more cases of neonatal sepsis when compared to GBS. These studies have further reported a lower rate of EOS due to GBS which is said to be associated with treatment and maternal care during antenatal period (Puopolo and Eichenwald, 2010; Bauserman *et al.*, 2013). In a recent health survey on neonatal sepsis infection, it was established that GBS causes less than 40% of neonatal sepsis cases in developing countries with a frequency of about 0.041% in neonates (Stoll *et al.*, 2011; Weston *et al.*, 2011).

In preterm neonates, gram negative bacteria was reported to be the leading cause of early onset sepsis with more than 60% of the cases while gram positive bacteria caused less than 30% of the cases and other pathogens were responsible for less than 2% of the septic cases (Klinger *et al.*, 2009). Recent studies have also established that *E. coli* is associated with over 80% of neonatal sepsis cases that affect gestational premature infants (Shane and Stoll, 2013). When VLBW newborns were considered alone, *E. coli* was the major cause of EOS accounting for 33.4% of all the cases in a large multicenter study (Hornik *et al.*, 2012).

The success of *Escherichia coli* as a pathogen has been associated with its varied antigenic structure, especially the possession of the K1 antigen, which enables it to evade the killing by the phagocytes (Xie *et al.*, 2004). The antigenic structure in *E. coli* is very closely related to the antigenic structure found in a group of bacteria that causes meningitis hence making it difficult to clinically distinguish between neonatal sepsis and meningitis in infants (McCracken and Sarff, 1974). The *E. coli* related infections
and meningitis cause the highest cases of neonatal deaths. Furthermore, the *E. coli* has also been reported to have the ability to invade the endothelium of the brain which further complicates the disease (Huang *et al*., 2000).

Neonatal sepsis caused by *Lesteria monocytogenes* in developing countries has been reported to be a very low occurrence. This may be related to the hygienic standards in these societies because the *Lesteria monocytogenes* basically habitat in the soil and their transmission to human being is through contaminated food. In case a pregnant mother is infected with listeriosis, there may be high risk of prenatal death of the fetus or neonatal sepsis (Niels le Souef and Walters, 1981). The *L. monocytogenes* infection is transmitted from the mother to fetus through the placenta and also through accidental intake of contaminated amniotic fluid. The invasive infection of the neonate with *L. monocytogenes* may be due to diminished cell-mediated immune responses of the premature infant, decreased interferon gamma (IFN-γ) and IL-12 production, immature chemotaxis and phagocytosis of the killing macrophages and decreased number and function of the natural killer (NK) cells (Wilson and Lewis, 1990).

The other Gram negative bacteria pathogens are less associated with EOS but very important causes of late onset sepsis (LOS). In the family of gram negative bacteria, *E. coli* and *Klebsiella* species are some of the common causes of sepsis in newborns. This may be due to their complex and virulent nature which enable them to evade the effects of neutralizing antibodies and also the phagocytic killings. These immune evasions are contributed by their encapsulation (Hunter and Bean, 2013). *Staphylococcus* species
are commonly associated with late onset neonatal sepsis mostly in very low birth weight infants (Andre et al., 2000). *Staphylococcus epidermidis* has been established to cause the highest percentage of sepsis infections in gestational premature newborns (D’Angio et al., 1989).

### 2.3 Antibiotic susceptibility in neonatal sepsis

Antibiotic resistance is a global problem and the antibiogram pattern differs from one country to the other depending upon the variability of the epidemiology of neonatal sepsis (Shaw et al., 2007). Studies have reported high rate of resistance to first line antibiotics (Ampicillin and Gentamycin) (Mustafa and Ahmed, 2014). Gram negative bacteria (predominantly *E. coli* and *Klebsiella* spp.) have shown high resistance to ampicillin (≥ 90.5%) and moderate sensitivity to gentamycin (64.4%) and third generation cephalosporins, ceftazidime (56.3%) and cofotaxime (57.63%) (Rao et al., 2015). A study conducted in India reported low resistance to Amikacin (13.6%), moderate resistance to gentamycin (45%) and a higher resistance to third generation cephalosporins such as cofotaxime (73%) (Vinodkumar et al., 2008). Increase in antibiotic resistance has also been reported in aminoglycosides and third generation cephalosporins yet they are mostly set up as empirical therapy for neonatal sepsis (Rao et al., 2015).

Gram negative bacteria have been reported to show susceptibility to Imepenem (86.13%), Meropenem (83.22%), Tazobactum (76%) and to Fluroquinolones (74.5%) which also was reported to have increased side effects. Gram positive bacteria (mostly
Staphylococcus aureus) have been reported to show high sensitivity to Amikacin (100%) (Arpita et al., 2012). Non-fermenting Gram negative bacteria such as Pseudomonas spp., Acinetobacter spp. and Stenotrophomonas maltophilia are among the opportunistic pathogens commonly associated with neonatal sepsis especially in neonatal intensive care units with high multidrug resistance (Vergnano et al., 2005).

Results from studies that were carried out in a Pakistan neonatal intensive care unit established a high antibiotic resistance where over 90% of gram negative bacteria were resistance to ampicillin and co-trimazole (Mahmood et al., 2002). More than 80% of these bacterial pathogens were resistant to gentamycin and about 70% were resistant to the third generation cephalosporins. However, more than 90% of these pathogens were susceptible to meropenem and amikacin. Report from another study that was done in a neonatal intensive care unit of Karachi, showed a higher resistance to ampicillin and third generation cephalosporins by gram negative bacteria (Anwar et al., 2000).

Gram positive bacteria such as Staphylococcus aureus were found to be resistant to ampicillin while they showed moderate resistance to methicillin (61.54%) but they were susceptible to amikacin and vancomycin. This may mean that vancomycin should be combined with amikacin or carbapenems in order to eradicate most of bacteria pathogens associated with neonatal sepsis (Mahmood et al., 2002). However, if these antibiotics are used in treatment of sepsis for a long period of time, this could lead to the development of new strains of bacteria that are resistant to them yet they are the last line of antibiotics in most hospitals. In order to prevent neonatal sepsis infections, there is
need to recognize the group of neonates that is at high risk of sepsis infection, restricting sepsis during labor and mass education of mothers on exclusive breast feeding during the early months of neonatal life. All these measures may lead to minimized neonatal infections (Bhutta and Yusuf, 1997).

2.4 Maternal-neonatal factors associated with neonatal sepsis

Preterm delivery and low birth weight are the most well established risk factors in neonatal sepsis both in developed countries like United States of America and in developing countries (Schuchat et al., 1994; Schrag et al., 2006). For pathogens like *E. coli*, low gestational age is the main risk factor with two-thirds of *E. coli* infections in the United States among preterm newborns (Schrag et al., 2006). Unfortunately, there are no effective strategies for preventing preterm deliveries although improved maternal nutrition and access to prenatal care may be important in reducing low birth weight.

The other neonatal associated risk factors include birth related abnormalities, mode of delivery, health status of the neonate at birth and the reduced ability of the neonate to respond to infectious agents which is associated with the reduced transfer of soluble antibody (IgG) from the mother to the neonate through the placenta (Benitz et al., 1999). The protective role of the skin and mucus membranes in newborns is decreased and it may even be totally impaired in very ill infants (Benitz et al., 1999). Furthermore, improper care given to the mother during pregnancy, low income endowment, malnourishment of the mother and abuse of drugs have also been considered as risk factors in neonatal sepsis infection (Stoll et al., 2011; Anderson-Berry, 2012).
Complications at delivery such as poor uterine contractions, preterm rapture of membranes and maternal infections account for maternal risk factors in sepsis (Schuchat et al., 1994; Adair et al., 2003). Low immunity of the mother has also been reported to be a common risk factor where soluble antibodies (IgG) from the mother have been established to be effective against group B Streptococci infections in their newborns. Studies have demonstrated an increased risk for GBS associated early onset sepsis in neonates delivered by mothers with low levels of IgG (Baker and Kasper, 1976).

In developing countries, there has been a significant association between meconium-stained amniotic fluid (MSAF) and culture confirmed early and late onset sepsis (Kayange et al., 2010). California health maintenance organization also reported a significant association between MSAF with sepsis. Meconium-stained amniotic fluid is mostly common among women delivering at term (Balchin et al., 2011). This is because the growth of bacteria pathogens associated with sepsis may be stimulated by meconium (Eidelman et al., 2002) leading to disorder of the neonates nervous system which may predispose the infant to late onset sepsis and may also lead to sepsis associated with birth asphyxia.

Primiparity has rarely been identified as a risk factor in sepsis yet women delivering a first birth usually have longer duration between membrane rapture and delivery which is an established risk factor in sepsis that is associated with women giving birth for the
first time (Soman et al., 1985). Studies comparing primiparous women with those that had previous births established that, those with first births were more likely to have more symptoms of sepsis associated infections and also high chances of having stillbirths in a multivariable analysis (Soman et al., 1985).

Some of the conditions that a pregnant mother experiences which include high blood pressure and prenatal Diabetes mellitus have been identified as some of risk factors associated with neonatal sepsis but they are not usually associated with neonatal death (Seale et al., 2009). The mode of delivery has been established as a risk factor in sepsis infection where greater percentage of sepsis cases are associated with vaginal delivery (over 70%). In contrast, emergency cesarean due to prior cesarean has been associated with very low cases of sepsis infection (≤ 1%) as compared to emergency cesarean due to other reasons such as maternal illness and other complications (Curtland et al., 2009).

Studies have reported that administration of antibiotics to the mother during pregnancy has no effects on preventing neonatal sepsis infections or preventing early neonatal deaths (Schuchat et al., 1994) although intrapartum antibiotic programme (IAP) has been associated with effective preventing early onset sepsis associated with GBS but with no effect on late onset invasive GBS sepsis or early onset E. coli associated sepsis (Jordan et al., 2008). Intra-amniotic infections together with elevation of body temperatures during pregnancy are also important maternal risk factors associated with sepsis in infants (Schrag et al., 2002). More studies are needed to establish the relationship between meconium-stained amniotic fluid and neonatal sepsis infection
since it has been identified as a risk factor in neonatal sepsis (Seale et al., 2009). Further studies to determine whether immunizing pregnant mothers could be used as a measure to prevent neonatal infections including sepsis are also required (Seale et al., 2009).

2.5 Immune responses

The key mediators of inflammatory response to host invasion include the immune cells and gene products such as pro-inflammatory cytokines and chemokines, complement activating factors, coagulation factors and immune active proteins are very important in ensuring the movement of immune cells to infection site and also directing their participation in bacteria killing (David et al., 2006). Cellular immune response involves polymorphonucleocytes (PMNs) which are very effective in bacteria eradication. Polymorphonucleocytes in neonates have low ability and low capacity for chemo attraction (George, 2006). These PMNs also fail to attach to the inner lining of arteries, veins and blood capillaries and this hinders their movement into body tissues where they are meant to release the antimicrobial cytotoxic molecules in response to microbial components. Neutrophil reserves are usually decreased due to the reduced ability of the bone marrow to produce more neutrophils mostly in preterm neonates. Monocytes levels in newborns are comparable to those of adults although their function is greatly reduced (Abul and Andrew, 2009).

Neonatal macrophages have significantly low ability to produce cytokines and this could be explained by their relatively low population of T cells. Most of these T cells are functionally immature hence have reduced response to infections and their number
tends to increase with increase in age of neonate (Romagnani, 2000). Neonatal T cells do not readily proliferate following antigenic stimulation, they also produce low levels of cytokines that are unable to stimulate the B cells and aid in their differentiation, and are also ineffective in granulocytes and monocytes proliferation. The memory T cells in neonates represent a very low population and this is what is responsible for delay in immune responses that require memory functions even after primary antigen stimulation has occurred. The number of these memory T cells tends to increase following a continued exposure to antigenic stimuli, and they can be up to 100% effective as those in adult.

Natural killer (NK) cells are few and also represent a functionally immature population leading to reduced production of the pro-inflammatory cytokine- interferon gamma (IFN-γ) following antigenic stimulation (Costello, 2001). This may increase severity of infections during neonatal period. Humoral immune response is by immunoglobulins which are acquired through non-specific placental transfer from the mother. Newborns have the ability to produce immunoglobulins when stimulated by antigens. However, their level of response is limited but it increases with neonatal age. Immunoglobulin M (IgM) is synthesized by the fetus within ten weeks of prenatal age, although the level still remains low even at birth but it rises in neonates who might have been exposed to antigen stimulation during pregnancy (David et al., 2006). Although IgG and IgE may be synthesized in utero the neonate acquires most of its IgG from the mother during the last trimester. Neonates acquire IgA mostly from breast feeding.
Neonatal response to the bacterial polysaccharide antigen is greatly reduced and it therefore increases with age up to the age of 2 years. Fetus is capable of producing complement proteins in utero within one and a half months of gestation. Complement deficiencies have however been reported in neonates and their inadequacy seems more in alternative pathway than in classical pathway of complement activation (Barrington et al., 2006). The terminal cytotoxic components of the complement cascade that leads to killing of micro-organisms, especially gram-negative bacteria are deficient. Effective complement activity is achieved in newborns by the age of two months. Antibodies in neonates have decreased opsonic level against bacteria pathogens including *E. coli*, GBS, and *Streptococcus* species. This may be due to their low levels of a protein in serum (fibronectin) that aid in adherence of neutrophils and opsonic properties (Van Dissel et al., 1998).

Cytokine response to neonatal sepsis involves both pro-inflammatory and anti-inflammatory cytokines (Kasai, 1997). Pro-inflammatory cytokines include IL-2, IL-6, IL-8, IFN-γ and TNF-α while anti-inflammatory cytokines include IL-4, IL-10 and transforming growth factor beta (TGF-β) (Romagnani, 2000). From experiments, both anti-inflammatory cytokines and pro-inflammatory cytokines were reported to increase significantly during infection. This concept seems important because, failure to reduce the effects of pro-inflammatory mediators over the course of sepsis was associated with higher mortality rates (Taniguchi et al., 1999). High plasma IL-6, IL-10 and TNF-α concentration and IL-10/TNF-α and IL-6/IL-10 ratios signify severe infection (Van Dissel et al., 1998; Marchant et al., 2013). Altered adaptive immune-system function
leaves the neonate mostly dependent on the innate immune system for defense against these bacterial infections.

Deficits exist in barrier integrity of the skin and mucus membrane, circulating complement components, expression of anti-microbial proteins and peptides, production of type 1-interferons and T-helper 1 polarizing cytokines (Kasai, 1997). Neonatal immune system has quantitative and qualitative impairments in neutrophil, monocyte, macrophage and dendritic cell function and decreased response to most Toll-like receptor antagonists. The net effect of these deficits is a functional immunocompromised state that leaves the premature neonate extremely susceptible to microbial invasion. Further studies aimed at characterizing the unique neonatal immunologic response and the capacity for positive immune modulation are necessary (Bang et al., 1999).

2.5 Diagnosis of neonatal sepsis
Neonatal sepsis can be classified according to the time of onset of the disease (Tallur et al., 2000). Early onset sepsis (EOS) is due to bacteria acquired before and within the course of delivery while late onset sepsis (LOS) is due to bacteria acquired a week after delivery (nosocomial or community sources). Bacteremia infection that occurs between one day and seven days after delivery is said to be early onset sepsis while late onset sepsis occurs from 8 days to 89 days. Diagnosis of neonatal sepsis can be done through direct method or indirect method. Direct method involves isolation of microorganisms from blood, cerebral spinal fluid (CSF), urine, pleural fluid or pus. Indirect method
involves variety of tests which are helpful for screening neonates with sepsis. They include white blood cells count and differential leucocyte count. An absolute neutrophil count of < 1800 per mm$^3$ of blood is an indicator of infection. Immature neutrophil to total neutrophil ratio (I/T) > 0.02 means that more than 20% of the neutrophil population in circulation are premature since they are forced into circulation to fight the bacteria infection by the bone marrow (Ahmed et al., 2005).

Platelets count of less than 100,000 per mm$^3$ of blood; toxic granules on peripheral smear and gastric aspirates smear showing more than 5 leucocytes per high power field are also useful indirect evidence of infection. The micro-Erythrocytes sedimentation rate (micro-ESR) may be elevated with sepsis and a fall of > 15 mm during first hour indicates infection (Elder and Denton, 1995). Acute phase reactants have been established to be quite reliable in the diagnosis of neonatal sepsis especially the C-reactive protein due to its unique high level of sensitivity, where positive test signifies infection. The C-reactive protein plays a major role in complement binding to foreign microbes and damaged cells in response to inflammation, hence rising to peak levels after fifty hours of infection. Along with other clinical symptoms of neonatal sepsis, CRP provides necessary information concerning diagnosis of sepsis (Kawamura et al., 1995; Ahmed et al., 2005).

Recent studies have found cytokines to be new biological markers that play important role in the diagnosis of neonatal sepsis (Mehr and Doyle, 2000; Gonzalez et al., 2003; Maamouri et al., 2006). These cytokines include: interleukin (IL)-2, IL-6, IL-8, IL-10,
IL-1beta, tumor necrosis factor-alpha (TNF-α), and interferon gamma (IFN-γ). However, systemic research and comparison of these markers in the diagnosis of neonatal sepsis are necessary since their available data is limited (Yuan et al., 2013). For complete diagnosis, lumbar puncture should be done for all LOS and symptomatic early onset sepsis since 10-15% of the cases have associated meningitis. Cerebral spinal fluid (CSF) screen showing increased cells and proteins is an implication for meningitis hence need for using antibiotics with a high CSF penetration (Palazzi et al., 2006).

2.6 Management of neonatal sepsis

Newborn infants are more prone to environmental or community related infections because of their naïve innate immune responses to infection and also due to the invasive procedures to which they are subjected to during and after delivery. Preventive measures such as hand washing with water and soap, early discharge from hospitals, minimized invasive procedures, skin preparation procedures and antiseptic use to disinfect birth canal need to be implemented in order to reduce nosocomial infections (Hyde et al., 2002). Once diagnostic tests are performed, treatment with suitable antibiotics should be started immediately in order to prevent immunosuppression of the neonate’s immune system by the bacterial pathogens. The antibiotics used in treatment should be based on the history of the mother on infection, the results obtained from the trends of microbial prevalence and on susceptibility of antibiotics in the various hospital units (Al-Harthi et al., 2000).
In developed countries such as United States of America, the current treatment of EOS includes: combined IV aminoglycoside and broad-spectrum of penicillin antibiotic therapy which provides a coverage for gram–positive organisms especially GBS and gram-negative bacteria such as *E. coli*. The antibiotics used in the management of late onset sepsis should be based on the bacterial contaminants implicated in the hospital wards and other neonatal units which include; *S. aureus*, *S. epidermidis* and *pseudomonas* species. Most strains of *Staphylococcus aureus* produce beta-lactamase which makes them resistant to penicillin G, ampicillin, carbenicillin and ticarcillin but susceptible to vancomycin (Karunasekera and Pathirana, 1999).

Zaidi (2004) compared the failure rates of 3 clinical – based antibiotic regimens in 0 to 59 day old infants with possible serious bacterial infections in Karachi, Pakistan. In a randomized study, the researcher found that outpatient therapy with injectable antibiotics is an effective alternative when hospitalization is not possible. Procaine penicillin/gentamicin was superior to oral trimethoprim-sulfamethoxazole. It may be difficult to manage neonatal sepsis in cases where antibiotics were administered to the mother before delivery immediately after delivery. Furthermore, this may lead to negative culture results in newborns who are still infected with neonatal sepsis (Moreno *et al.*, 1994). It is important to continue with sepsis treatment after a week even when the cultures remain negative following 72 hours of incubation.

It has been noted that antimicrobial resistance is currently a worldwide challenge and reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing particularly in intensive care units (Musoke and Revathi, 2000). *Klebsiella*
and *Enterobacter* species are often reported in this context (Weber *et al*., 2003). Spread of resistant organisms in hospitals is a recognized problem although babies born out of the hospital may be infected with pathogens that are resistant to antibiotics since there is a wide availability of these antibiotics over the counter in these communities.

Further studies are needed to compare levels of antibiotic resistance amongst the babies born in the community and those that are born in the hospital (Barlett *et al*., 1991; Bang *et al*., 1999). It is necessary that antibiotic programme is reviewed continuously based on pathogens implicated and on their susceptibility profiles (Goosen, 2000). There have been difficulties in comparing antibiotic resistance between regions and between countries because of the different pathogens associated with neonatal sepsis (Lim *et al*., 1995). Few studies compare antibiotics susceptibility overtime in the same unit and the available data show increasing resistance to commonly used antibiotics (Tallur *et al*., 2000). Most gram negative bacteria are reported to be resistant to ampicillin and cloxacillin while others are resistant to gentamicin. Coagulase-negative *Staphylococcus* is a leading cause of LOS (Adejuyigbe *et al*., 2001).

Additional therapies in the management and treatment of neonatal sepsis include: granulocyte transfusion, IV immunoglobulin (IV Ig) infusion, exchange transfusion and Recombinant cytokine administration. Although granulocyte transfusion is still at clinical trials, it has been shown to be effective in newborns with significantly low neutrophil population. The use of GM-CSF and GCSF has been studied in clinical trials but it has not been used in treatment of sepsis in newborns (Stoll, 2011).
2.7 Gaps necessitating the present study

From the previous studies reviewed in this chapter, there is still much to be done in most parts of the world in order to reduce the infant morbidity and mortality due to sepsis and also to reduce incidences of neonatal sepsis. There is urgent need for studies on simple and sustainable interventions to reduce the burden of neonatal infection, risk factors associated with neonatal sepsis, longitudinal surveillance to describe the varied pathogens causing neonatal sepsis as well as their changing antibiotic susceptibility profile, biological markers for use in diagnosis of neonatal sepsis infection, developing anti-sepsis vaccine for mothers and infants. Studies are also needed to compare patterns of antimicrobial resistance in babies born in and out of the hospital and on the impact of HIV infections on incidences of neonatal sepsis. Therefore, the present study aimed at determining the bacterial pathogens associated with neonatal sepsis and their antibiotic susceptibility patterns, cytokine involvement in neonatal sepsis and the factors associated with neonatal sepsis in NCRH.
CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study was carried out in Nakuru County Referral Hospital. The hospital is located within Nakuru town, 2 kilometers north of town center. Nakuru town is located in the Rift Valley along the great north road, on geographical coordinates of 0° 17' S and 36° 41' E, 156 kilometers from Nairobi city, the capital of Kenya (Appendix IV). The hospital was preferred as a study area due to availability of the study population and laboratory facilities required for the research. The hospital also serves as a referral hospital for six District hospitals (Molo, Njoro, Olenguruone, Ol-Kalau, Gilgil and Naivasha) and the municipal health centers. This provides the hospital with a large population of patients from diverse backgrounds.

3.2 Study design

The study was done on all the newborns in NCRH who were clinically diagnosed with neonatal sepsis and were admitted in the general pediatric ward and those with normal health that were discharged through the same ward after delivery over a period of four months. The neonates were grouped based on whether they had sepsis or normal health. Blood samples for cytokine assay and bacterial culture were obtained aseptically from peripheral veins of patients (2 ml each) by a qualified phlebotomist. Analysis for cytokines was done in Kenya Medical Research Institute (KEMRI) Laboratory in Kisumu while bacteria culture and sensitivity was done in the laboratory within the hospital. Factors associated with neonatal sepsis were obtained from the patient’s parent or guardian using a questionnaire (Appendix III).
3.3 Inclusion and exclusion criteria

3.3.1 Inclusion criteria
All neonates admitted in the general pediatric ward of Nakuru County Referral Hospital with positive clinical diagnosis for sepsis and those born with normal health and aged between one and ninety days were included in this study.

3.3.2 Exclusion criteria
Neonates within the age of 0 to 90 days but were admitted in the neonatal intensive care unit (NICU) and neonates whose parents or guardians declined to consent were excluded from this study.

3.4 Sample size determination
The sample size was determined by the formula published by the research division of the National Education Association in the article “Small sample techniques” (NEA Research bulletin, 1998);

\[ S = \frac{X^2 NP (1-p)}{d^2 (N-1) + X^2 P (1-P)} \]

Where; \( S = \) sample required.
\( X^2 = \) the table value of Chi-Square for 1 degree of freedom at the desired confidence level (3.841).

\( N = \) Population size. This study involved 104 neonates.
P= the population proportion (assumed to be 0.5 since it would provide a maximum sample size).

d= the degree of accuracy expressed as a proportion (0.05).

The relationship between sample size and total population indicates that, as the population increases, the sample size increases at diminishing rate and remains relatively constant. The sample size for this study was 79 neonates with clinical diagnosis of neonatal sepsis.

### 3.5 Bacterial culture

Two milliliters of blood obtained was inoculated into broth culture media (Brain heart Infusion broth-BH I) in the ratio of blood to broth culture of 1:10. The preparation was incubated for 5-7 days at 37\(^0\)C and was checked daily for evidence of bacterial growth (Forbes et al., 2002). Bacterial cultures that showed growth were sub-cultured in blood agar from sheep, then incubation for further growth was done up to 48 hours at a temperature of 37\(^0\)C. The grown bacteria was identified by colony morphology, gram stain and standard biochemical tests (AMREF, 2008).

### 3.6 Antibiotic sensitivity testing

Antibiotic susceptibility for the identified bacteria was determined by disc diffusion method according to clinical and laboratory standard institute guidelines (CLSI, 2002). Antibiotic kit comprised of Erythromycin, Cefotaxime, Gentamycin, Ampicillin, Ciprofloxacin, Chloramphenical, Clindamycin, Streptomycin, Penicillin, Doxycycline and sulfurtrimethaxazole. Small wafers containing antibiotics were placed into a plate
upon which bacteria were growing. Bacteria sensitivity to the antibiotic was denoted by a zone of inhibition seen and measured around the wafer indicating that the bacteria is susceptible or resistant to the antibiotic (AMREF, 2008). According to the kit, a radius less than 6mm was considered resistant.

3.7 Cytokine measurement

The 2 ml of blood sample collected was immersed into ice immediately before processing. Plasma was separated by centrifugation (1900 rpm for 5 minutes) at 4°C and stored in 1ml vials at -80°C until analysed. Interleukin-1β, IL-6, TNF-α, IL-10 and IL-8 levels were estimated using a high sensitive sandwich ELISA kit (BD OptEIA™, BD Biosciences-Pharmigen, USA) according to the manufacturer’s instructions. Briefly, microtitre plates (Nunc Immuno™ plate Maxisorp™ surface, Denmark) were coated with capture antibody specific for each cytokine (IL-1β, IL-6, IL-8, IL-10, and TNF-α) after dilution in 1:250 phosphate buffered saline (PBS, SIGMA, P4417-100TAB St. Louis MO, USA) at the rate of 100 µl per well. The plates were then sealed with parafilm (PECHINEY plastic packaging-Chicago) and incubated overnight at 4°C.

Wells were aspirated and washed three times with 100 µl well wash buffer; PBS+ 0.05% TWEEN 20 (SIGMA, P7949-500 ml- USA). Plates were blocked with 200 µl well assay diluents; PBS+ 20% fetal bovine serum-FBS (Gibco, 16000-044-500 ml, USA) then incubated at room temperature (RT) for one hour. Wells were aspirated and washed 3 times. Standard dilutions were then prepared as per manufacturer’s
instructions where concentration for all cytokines was 250 pg/ml. Multi-channel Pipette
was used to pace 100 µl of standard, control and sample to appropriate wells.
Following incubation of plates for 2 hours at RT wells were aspirated and washed 5
times. Detector antibody was prepared as per instruction manual. For TNF-α was 1:500,
IL-6 and IL-8 1:250 while for IL-1β and IL-10 it was 1:1000 dilutions in assay diluent.
Each detector antibody was added to appropriate wells at the rate of 100 µl per well.
Plate for IL-1β was sealed and incubated for one hour at RT. Enzyme reagent
(Streptavidin-horseradish peroxidase conjugate; SA-HRP) was then diluted at 1: 250 in
diluted detection antibody for TNF-α, IL-8, IL-10 and IL-6. The plates were sealed and
incubated for 1 hour at RT. The wells of IL-1β plate were aspirated and washed 5 times.

Enzyme reagent for IL-1β was diluted in assay diluents at 1: 250 then 100 µl was added
to appropriate wells, the plate sealed and incubated for 30 minutes at RT. Wells of all
the plates were aspirated and washed 7 times. After the final wash, 100µl of substrate
solution (3,3',5,5'-Tetramethylbenzidine-TMB, SIGMA, T0440-USA) was added to each
well. The plates were incubated without seals in dark for 30 minutes. The reaction was
stopped using 50 µl per well of 1M phosphoric acid, then the absorbance was measured
at 450 nm using a micro-plate reader (BIO TECH, CA –USA). Cytokine concentrations
were determined by reference to standard curves construction.

3.8 Data analysis

Data were analysed using the Statistical Programme for Social Sciences (SPSS)
software version 22. Continuous variables such as age (term or preterm) and weight
(low birth weight or normal birth weight) were compared using paired t-test while categorical variables (Gender, Gestational age and mode of delivery) were compared using Chi-square analysis. Analysis of variance (ANOVA) was used to determine the mean levels of individual cytokines and Spearman Rank correlation was used to establish the relationship between: pro-inflammatory and anti-inflammatory cytokine responses in neonatal sepsis, bacterial pathogen and sepsis type and relationship between gestational age and type of pathogen. Data were presented using percentages, tables and graphs. A $P$ value $\leq 0.05$ was considered statistically significant.

### 3.9 Ethical consideration

The informed consent of each patient’s parent or guardian was obtained without coercion (Appendix I). The dignity, integrity and privacy of each patient was treated as important and respected at all times. Research permit was obtained from the National Commission for Science, Technology and Innovation (Appendix II), Graduate school of Kenyatta University and an approval from the Research and Ethics Committee of Nakuru County Referral Hospital.
CHAPTER 4: RESULTS

4.1 Characteristics of the study participants

The study enrolled 104 newborn infants admitted in the general pediatric ward of Nakuru County Referral Hospital (NCRH). Among the neonates, 79 were clinically diagnosed with neonatal sepsis infection while 25 had normal health hence acted as the control group in the study. The sick group had 39 (49.4%) females and 40 (50.6%) males while the healthy group (control) had 15 (60%) females and 10 (40%) males (P > 0.05). The mean age of the control group was 11.36 ± 21.22 days while that of the sick group was 21.98 ± 26.47 days (P = 0.008).

The mean birth weight in kilograms (Kg) for the control and the sick group was 3.04 ± 0.56 and 2.73 ± 0.52 respectively, indicating that the sick children had significantly lower birth weights when compared with the control group (P < 0.05). Based on gestational age, the control group had 23 (92%) term neonates and 2 (18%) preterm neonates, while the sick group had 60 (75.9%) term neonates and 19 (24.6%) pre-term neonates, indicating a higher sepsis prevalence in term neonates (P < 0.05). On the mode of delivery, in the control group 22 (88%) were through normal or vaginal delivery (VD) and 3 (12%) through caesarian delivery (CS), while the sick group had 70 (88.6%) through VD and 9 (11.4%) through CS. This means that there was no significant difference between the mode of delivery and sepsis (P > 0.05). Of all the sick neonates, 43 (54.4%) had early onset sepsis (EOS) while 36 (45.6%) had late onset sepsis (LOS) (Table 4.1). There was a significant relationship between the age and birth weight of the neonates with neonatal sepsis infection (P < 0.05).
Table 4.1: Demographic characteristics of the study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>Sick with sepsis</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (N)</td>
<td>25</td>
<td>79</td>
<td>____</td>
</tr>
<tr>
<td>Gender n (%)</td>
<td>F-15(60)</td>
<td>F-39(49.4)</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>M-10(40)</td>
<td>M-40(50.6)</td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>11.36±21.22</td>
<td>21.98 ±26.47</td>
<td>0.008</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.04±0.56</td>
<td>2.73 ±0.52</td>
<td>0.020</td>
</tr>
<tr>
<td>Gestational age n (%)</td>
<td>Term- 23(92.0)</td>
<td>60(75.9)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Pre-term 2(18.0)</td>
<td>19(24.6)</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery n (%)</td>
<td>VD-22(88.0)</td>
<td>70(88.6)</td>
<td>0.934</td>
</tr>
<tr>
<td></td>
<td>CS-3(12.0)</td>
<td>9(11.4)</td>
<td></td>
</tr>
<tr>
<td>Early-onset sepsis n (%)</td>
<td>____</td>
<td>43(54.4)</td>
<td>____</td>
</tr>
<tr>
<td>Late onset sepsis n (%)</td>
<td>____</td>
<td>36(45.6)</td>
<td>____</td>
</tr>
</tbody>
</table>

Key: N-Study population; n- Sample size

4.2 Bacterial pathogens causing neonatal sepsis

Out of 35 samples that were cultured 18 were positive while the rest showed was no growth. Three types of bacteria pathogens were isolated from the positive blood cultures which included: *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas*
*Pseudomonas aeruginosa*. The *E. coli* was the most commonly isolated pathogen causing 44.4% of neonatal sepsis cases occurring in both early and late onset sepsis. *Staphylococcus aureus* caused 38.9% of culture proven sepsis and dominated in late onset sepsis while *Pseudomonas aeruginosa* caused the least cases of neonatal sepsis (16.7%) all of which were pre-term. There was no association between early onset sepsis and late onset sepsis with the type of bacteria isolated (P > 0.05).

Among the isolated pathogens, Gram negative bacteria were *E. coli* and *P. aeruginosa* while Gram positive bacteria were *S. aureus* species. Among the neonates with culture proven sepsis, 33.3% had EOS while 66.7% had LOS, 16.7% were pre-term while 83.3% were term neonates (Table 4.2). A significant association was established when the age of neonates at birth was compared with bacteria pathogens causing sepsis where *P. aeruginosa* caused sepsis only in preterm neonates (r= 0.6, P < 0.05). However, there was no significant relationship between birth weight of the neonates and type of pathogen causing sepsis in this study (P > 0.05).
Table 4.2: Total microbial isolates based on sepsis diagnosis and the bacterial group

<table>
<thead>
<tr>
<th>Group and species</th>
<th>Early onset neonatal sepsis (N=6)</th>
<th>Late onset neonatal sepsis (N=12)</th>
<th>Total (N=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>(50%)</td>
<td>(41.7%)</td>
<td>(44.4%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>(16.7%)</td>
<td>(50%)</td>
<td>(38.9%)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Non-fermenting bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>(33.3%)</td>
<td>(8.3%)</td>
<td>(16.7%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

4.3 Antimicrobial susceptibility

Following susceptibility test results, sensitivity to Chloramphenicol was 87.5% by E. coli, 85.7% by S. aureus and 100% by P. aeruginosa. Ciprofloxacine showed a sensitivity of 100% by S. aureus and P. aeruginosa while that of E. coli was 75%. Cefotaxime had a sensitivity of 100% by S. aureus and P. aeruginosa but none by E. coli. There was 100% sensitivity by P. aeruginosa, 71.4% by S. aureus and none by E. coli. There was 100% sensitivity by P. aeruginosa, 71.4% by S. aureus and none by E. coli. There was 100% sensitivity by P. aeruginosa, 71.4% by S. aureus and none by E. coli. There was 100% sensitivity by P. aeruginosa, 71.4% by S. aureus and none by E. coli. There was 100% sensitivity by P. aeruginosa, 71.4% by S. aureus and none by E. coli. There was 100% sensitivity by P. aeruginosa, 71.4% by S. aureus and none by E. coli.
0% by other pathogens. *Pseudomonas aeruginosa* showed 66.7% sensitivity to ampicillin while other pathogens were not susceptible to it. All the pathogens (*E. coli*, *S. aureus* and *P. aeruginosa*) were not sensitive to Gentamycin, Penicillin, Streptomycin and Doxycycline (Table 4.3).

**Table 4.3: Bacteria sensitivity profile to the antibiotics mainly used in the hospital**

<table>
<thead>
<tr>
<th>Bacteria (no)</th>
<th>Antimicrobial susceptibility, no (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHL</td>
</tr>
<tr>
<td><em>E. coli</em> (8)</td>
<td>7 (87.5)</td>
</tr>
<tr>
<td><em>S. aureus</em> (7)</td>
<td>6 (85.7)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (3)</td>
<td>3 (100)</td>
</tr>
</tbody>
</table>

**Key:** CHL– Chloramphenicol; CLM-Clindamycin; STM- Sulfurtrimethaxazole

AMP–Ampicillin; ERY–Erythromycin; DOX–doxycycline; CIP-Ciprofloxacine

STR-Streptomycin; CTX–cefotaxime; GM-Gentamycin; P-Penicillin

**4.4 Antimicrobial resistance**

The results obtained from culture sensitivity test showed that, the three pathogen isolates (*E. coli*, *S. aureus* and *P. aeruginosa*) were resistant to the first line antibiotics (Ampicillin and Gentamycin) among others. One of the *E. coli* strains was resistant to all the antibiotics used. Resistance to Chloramphenicol by *E. coli* was 12.5%, *S. aureus* 14.3% but no resistance by *P. aeruginosa*. There was 25% resistance to Ciprofloxacine
by *E. coli* while other pathogens showed no resistance. Clindamycin showed 50% resistance by *E. coli* and 100% resistance by other pathogens. Erythromycin registered 100% resistance by *E. coli*, 85.7% by *S. aureus* and 33.3% resistance shown by *P. aeruginosa*. Resistance to Sulfurtrimethaxazole was 100% by *E. coli*, 28.6% by *S. aureus* but no resistance by *P. aeruginosa*. There was 100% resistance to Ampicillin by *E. coli* and *S. aureus* but 33.3% by *P. aeruginosa*. *Pseudomonas aeruginosa* and *S. aureus* showed no resistance to cefotaxime while *E. coli* showed 100% resistance to this antibiotic. There was 100% resistance to Gentamycin, Streptomycin, Penicillin and Doxycycline by all the pathogens (*E. coli, S. aureus and P. aeruginosa*) (Figure 4.1). From the results of the present study, pooled resistance of the identified pathogens to all the antibiotics commonly used was as follows; *E. coli* (88.5%), *S. aureus* (66%) and *P. aeruginosa* (52%).
Out of 104 neonates, 69 were enrolled for the cytokine measurement at random. The 25 of the neonates were controls while 44 neonates were sick. Of the sick neonates, 29 had EOS while 15 had LOS. The IL-10 mean levels (pg/ml) for the sick was 29.75 ± 16.6 while for the control group the levels were 23.27 ± 9.7. Mean IL-6 levels for the sick were 27.32 ± 25.3 while that of control group was 21.06 ± 11.4 and IL-1β mean levels for the sick group was 2.17 ± 1.67 while that of control group was 1.90 ± 1.19. The
mean levels of IL-8 in the sick group was 57.86 ± 117.16 while that of control group was 20.60 ± 22.49. Mean level for TNF-α in the sick group was 10.75 ± 6.58 while that of the control group was 6.83 ± 3.85 pg/ml.

There was no significant difference in the mean levels of all the cytokines among the sick and the control group (P > 0.05). However, there was a significant relationship between the pro-inflammatory cytokine levels of TNF-α when correlated with anti-inflammatory cytokine levels of IL-10, (r = 0.561, p < 0.05). There was no significant relationship between the distribution of cytokines (IL-6, IL-8, IL-1β and TNF-α) with age, gender, birth weight or gestational period (P > 0.05). However, there was a significant relationship between IL-10 levels with gender (P = 0.031). This is where the sick male neonates had higher mean levels of IL-10 (34.34 pg/ml) compared to the sick female neonates (25.26 pg/ml).
Table 4.5: Mean cytokine (IL-10, IL-6, IL-1β, IL-8 and TNF-α) levels in the sick and control groups of neonates

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Participants</th>
<th>Population (N)</th>
<th>Mean levels (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>Sick</td>
<td>44</td>
<td>29.75 ± 16.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>23.27 ± 9.7</td>
</tr>
<tr>
<td>IL-6</td>
<td>Sick</td>
<td>44</td>
<td>27.32 ± 25.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>21.06 ± 11.4</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Sick</td>
<td>44</td>
<td>2.17 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>1.90 ± 1.19</td>
</tr>
<tr>
<td>IL-8</td>
<td>Sick</td>
<td>44</td>
<td>57.86 ± 117.16</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>20.60 ± 22.49</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Sick</td>
<td>44</td>
<td>10.75 ± 6.58</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>6.83 ± 3.85</td>
</tr>
</tbody>
</table>

4.6 Factors associated with neonatal sepsis

The present study revealed factors which could be associated with neonatal sepsis in Nakuru county referral hospital through a questionnaire (Appendix III). These factors include; neonatal age, birth weight, gender, mode of delivery and health status of the mother during delivery. In this study, there was no significant relationship between gender and neonatal sepsis. Although the number of male patients in the present study was slightly higher than that of females, both males (50.6%) and females (49.4%) were
evenly distributed in the sick group (P >0.05). This study found a significant relationship between neonatal age and sepsis where late onset sepsis was more common than early onset sepsis (P = 0.008). Birth weight of neonate also had a significant relationship with sepsis infection where sick neonates had significantly lower birth weight when compared to the healthy group (P = 0.02). In this study, 88.6% of septic cases were through vaginal (normal) delivery while 11.4% were through caesarian delivery (Table 4.1). This study found that, all mothers who had complications (prolonged labor, pre-term rapture of membranes, obstructed labor and chorioamnionitis) during delivery, disease like malaria in pregnancy and iron deficiency leading to anemia in pregnancy gave birth to neonates who demonstrated early onset sepsis.
CHAPTER 5: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Bacterial pathogens causing neonatal sepsis

The findings that neonatal sepsis caused by Gram negative bacteria (E. coli and P. aeruginosa) accounted for a higher percentage compared to Gram positive bacteria associated sepsis (S. aureus) in the present study were in concordance with results from other studies done in developing countries (Sundaram et al., 2009) which showed Gram negative bacteria as a major cause of neonatal sepsis. This may be due to contamination of the skin of neonates with bacteria from the environment and also from the people working in the neonatal units, together with the various procedural examinations which are invasive on the neonates such as heel prick blood sampling, endotracheal sunction and intravenous cannula insertion as reported elsewhere (Seyyed et al., 2011).

The establishment that E. coli related sepsis was the highest when compared to sepsis due to other isolated pathogens in the present study compares with results from a recent study done in South African referral hospital where E. coli was the major pathogen causing neonatal sepsis (Dramowski et al., 2015). However, the present results are different from those of an earlier study done in developing countries in Sub-Saharan Africa (Zakariya et al., 2011) that showed Klebsiella pneumonia as the most commonly isolated bacteria pathogen. This may be due to variation of bacterial pathogens causing neonatal sepsis in different regions even within the same country.
The higher rate of late onset sepsis (55.4%) compared to that of early onset sepsis (44.6%) in the present study are findings that differ from the report of a recent study done in low income countries mostly in Indian sub-continent and Sub-Saharan Africa that showed higher prevalence of early onset sepsis in neonatal intensive care unit (NICU) (Huynh et al., 2015). It is likely that more cases of early onset sepsis are in NICU compared to the general pediatric ward.

The significant association between gestational age of neonates and the pathogen type in the present study, where *Pseudomonas aeruginosa* was predominantly causing sepsis in preterm neonates may be an indication that, this pathogen is also responsible for the preterm deliveries. These nosocomial infections may be favoured by prematurity, invasive procedures and immature immune system of the neonates. Furthermore, immune system of neonates and infants is not fully developed (Marchant et al., 2013). The occurrence of culture proven sepsis in the present research (51.4%) could be under reporting because a general blood culture kit was used instead of pediatric specific kit for blood culture which could be more sensitive even in low bacteremia infection, sepsis due to anaerobic viral or fungal pathogens and may be due to improper diagnosis due to the common symptoms exhibited in sepsis and in other infectious diseases like malaria.

### 5.1.2 Antimicrobial susceptibility and resistance

The resistance of the isolated pathogens (*E. coli, S. aureus* and *P. aeruginosa*) to more than three types of antibiotics in the present study are findings that compare with results from similar studies done in low income countries in Sub-Saharan Africa (Huynh et al.,
2015), South Africa (Dramowski et al., 2015) and in India (Vinodkumal et al., 2008; Vinodkumal et al., 2008; Viswanathan et al., 2011) that demonstrated pathogen resistance to most of the antibiotics that were used. Antimicrobial ineffectiveness in treatment of biotic infections is a worldwide challenge that has led to failed attempt to eradicate most of sepsis causing pathogens. Furthermore, the present study noted that all the isolated pathogens were resistant to Amphicilin and Gentamycin and yet they are the most recommended antimicrobials for the management of neonatal sepsis by the World Health Organization (WHO). This calls for studies that will test for extended spectrum beta-lactamase (ESBL) producers, which is related to the exposure to broad-spectrum antibiotics such as Cefotaxime due to induction of chromosomal beta lactamases (Bagla et al., 2013).

It was worrying for the present study to establish that the bacterial pathogens causing the highest cases of neonatal sepsis are the most resistant to the antibiotics used in the hospital. A previous study reported that in cases of neonatal sepsis where antibiotics of choice include third generation Cephalosporins, it is possible to reduce the rate of resistance to different antimicrobials by limiting the use of Cefotaxime (Bagla et al., 2013). It is unfortunate that the hospital in the present study lacks Amikacin and Cephalosporins and yet these are the antibiotics recommended for treatment of neonatal sepsis. This is because Amikacin has low rate of resistance and it does not induce chromosomal beta-lactamases and should therefore be used instead of Cephalosporins as empirical management of neonatal sepsis. Treatment of neonatal sepsis should be
modified based on the antibiogram results as recommended in a previous study (Zakariya et al., 2011).

The few non-fermenting bacteria (*P. aeruginosa*) isolates in both early and late onset sepsis reported in the present study that were also sensitive to most of the antibiotics used, are findings similar to the results of a recent study done at a South African referral hospital (Dramowski et al., 2015) that demonstrated *P. aeruginosa* as one of the least pathogens causing neonatal sepsis (caused 4% of cases). As every hospital unit may have different patterns of antimicrobial resistance, reliable data should be analyzed and compared at each unit before making decision in terms of management, an observation reported by Juan et al., (2015).

### 5.1.3 Circulating cytokines

The elevated mean levels of IL-6 in neonates with sepsis compared to the control group of neonates are findings that compare with results from an earlier study that was carried out in Iran on the role of IL-6 in predicting neonatal sepsis (Lobat et al., 2011), which demonstrated significant increase in IL-6 levels in septic neonates compared to the healthy neonates. The higher mean levels of IL-8 in sepsis cases compared to the control group in the present study are comparable to findings of another study (Boskabadi et al., 2010) that reported IL-8 concentration being 3 times higher in mortal cases compared to the surviving cases of neonates. Interleukin-8 has been demonstrated to be efficient in the diagnosis of definite infection where an elevation in IL-8 levels has
been associated with positive blood cultures (Edgar et al., 1994). The current study confirms these findings.

The elevated mean levels of TNF-α in neonates with sepsis compared to the sepsis-free control group in the present study supports the results from previous studies that were done in China which demonstrated elevated serum levels of TNF-alpha on day one of neonatal sepsis (Martin et al., 2001). Tumor-necrosis factor- alpha (TNF-α) is recognized as a primary mediator in the pathophysiology of sepsis and septic shock and it has also been considered as an early marker of neonatal sepsis infection (Kurt et al., 2007).

Higher levels of IL-1β in neonates with sepsis reported in the present study compared to the control group are findings that support the results from previous studies that demonstrated an elevated level of IL-1β in plasma of septic neonates when compared to both healthy and clinically suspected group that was not confirmed as having sepsis (Berner et al., 1998; Kurt et al., 2007). The elevated levels of IL-1β in the present study may imply that it is one of the key mediators in the pathogenesis of sepsis.

The higher mean levels of IL-10 in neonates with sepsis compared to the control group in the present study are findings that compare with results of an earlier study (Boskabadi et al., 2011), which demonstrated elevated levels of IL-10 and IL-6 in neonates with sepsis. Quantitative measurement of IL-10 levels has been associated with predicting the severity of neonatal sepsis infection. The role of IL-10 in neonatal
sepsis is to inhibit macrophage activation and secretion of cytokines which allow the bacteria to thrive in the body of the sick newborn, hence elevated levels of IL-10 concedes with neonatal sepsis.

The significant association between IL-10 levels and gender in the present study may be an implication of severity of sepsis in male newborn patients compared to female newborn patients, or due to the variation of the genetic background of the patients as previously established elsewhere (Deasy and Read, 2010). The present study established a positive correlation between the mean levels of TNF-α and that of IL-10. This may be an indication of the role of IL-10 in sepsis which include down regulation of the inflammatory effects of pro-inflammatory cytokines through its inhibitory roles.

The findings of the present study indicating higher mean levels of all the measured cytokines (IL-6, IL-8, IL-1β, TNF-α and IL-10) in patient neonates compared to the control group of neonates are comparable with findings from similar studies done in Saudi Arabia (Gamal et al., 2015) and in Iran (Hassan et al., 2013) that showed higher levels of these cytokines especially the levels of IL-1β, IL-8 and TNF-α in the sick neonates compared to the control group of neonates. However, the results from the present study did not have a significant difference in cytokine levels between septic and non-septic neonates. This may be due to differences in severity of sepsis infection because previous studies are reported to have been carried out in the neonatal intensive care units (NICUs), while the present study was done in the general pediatric ward where the disease was not as severe. This shows that the level of cytokine elevation may
depend on severity of sepsis or bacteremia infection. The findings of the present study establishes that the cytokines IL-1β, IL-8 and TNF-α can be used as valuable markers of severity of sepsis.

It was interesting to establish that some neonates in the control group showed elevated cytokine (IL-6, IL-8, and IL-10) levels, especially within the first day of birth, but normalized by the third day. This may be due to the effects of delivery factors such as traumatic experience during the process of delivery, but further studies are also required to give more explanations for such cytokine elevation in normal or disease free neonates.

5.1.4 Factors associated with neonatal sepsis

The comparable number of cases of sepsis between male and female neonates may have led to lack of significant association between gender and neonatal sepsis. However the present results are similar to those obtained from a similar study in Iran (Seyyed et al., 2011) that showed a higher probability of sepsis in males (67%) when compared to that of females (33%). Earlier studies established a higher susceptibility of male sex to sepsis although the main cause of these differences is not well established but it may be linked to the immunological uniqueness of each gender (Liorens, 2004). The results obtained in the present study might have lacked statistical significance due to the small sample size involved.
The significant relationship between age and weight of the neonates with sepsis in the present study may be because there were more cases of early onset sepsis (Bacterial blood stream infection within the first 7 days of life), among pre-term neonates compared to late onset sepsis (Bacterial blood stream infection after the first week of life). Furthermore, most of neonates with sepsis had low birth weight (< 2.5 Kg) compared to the control group in which majority of neonates had ≥ 3.0 Kg, findings that are similar to those reported from recent studies done in low income countries (Huynh et al., 2015) that demonstrated low birth weight and pre-maturity as risk factors in neonatal sepsis. The prevalence of neonatal sepsis is inversely correlated with gestational age and birth weight hence making the newborns that are born prematurely highly susceptible to infections. This may be due immaturity of their immune system since the nonspecific innate immune mechanism and the adaptive immune system develops gradually. The adaptive immune response is immature at birth due to the limited exposure to antigens during gestation thus leaving the neonates almost entirely dependent on the innate immune component and the passively acquired antibodies from the mother to compact infections.

It was interesting to establish that among the neonates with sepsis, 88.6% were born normally and 11.4% of neonates were born through CS. It is likely that neonates born normally are more exposed to maternal body fluids and body contact. Normally, CS has less body contact between the neonate and the mother. Given these conditions, it may be right to conclude that maternal-neonatal contact may be the source of these infections. Furthermore, the present results indicate that, the more the maternal-neonatal
contact, the more the neonatal sepsis cases. However the present study established that there was no significant relationship between the mode of delivery and sepsis infection in newborns.

5.1.5 Testing of hypotheses

Neonatal sepsis was established to be a very common infection in NCRH by the present study and therefore the null hypothesis is rejected. The bacteria pathogens associated with neonatal sepsis in NCRH were found to be resistant to most of the commonly used antibiotics hence the null hypothesis is accepted. Cytokines that were tested were all elevated in neonatal sepsis infection and some of them like IL-1β, TNF-α and IL-8 could be used as variable markers in neonatal sepsis severity hence the null hypothesis is rejected. The present study established some of the factors associated with neonatal sepsis in NCRH to include; health status of the mother at delivery, birth weight and gestational age of the neonate hence the null hypothesis is rejected.

5.2 Conclusions

(i) Bacterial pathogens causing neonatal sepsis in NCRH were; *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

(ii) The bacteria pathogens isolated in NCRH were not sensitive to most of the antibiotics used including the first line antibiotics (Ampicilin and Gentamycin).

(iii) Mean levels of measured cytokines (IL-1β, IL-6, IL-8, TNF-α and IL-10) were all elevated although the IL-1β, TNF-α and IL-8 recorded higher levels in the sick neonates compared to the control group.
(iv) Factors associated with neonatal sepsis in NCRH included; mother’s condition at delivery (sick or normal), birth weight of the neonates (low or normal) where low birth weights are favored and gestational age of the neonate (term or pre-term) where pre-term are more susceptible to infection.

5.3 Recommendations

(i) There is need for studies to be carried out yearly on pathogen prevalence and trends of resistance, so that only suitable antibiotics are used in treatment.

(ii) The cytokines; IL-1β, IL-8 and TNF-α can be used as variable markers for early diagnosis of neonatal sepsis and predicting the outcome of the infection.

(iii) Cohort studies seeking to compare cytokine levels in general pediatric ward patients and NICU patients are necessary.

(iv) There is need for studies that will associate specific cytokines with specific bacterial pathogens causing neonatal sepsis.
REFERENCES


Clinical and Laboratory Standards Institute (2002). Performance standards for antimicrobial susceptibility testing. 12th informational supplement. Pennsylvania USA.


Kurt, A. N., Aygun, A. D. and Godekmerdan, A. (2007). Serum IL-1beta, IL-6, IL-8 and TNF-alpha levels in early diagnosis and management of neonatal sepsis. Mediators of Inflammation, article 31397.


APPENDIX I: Research consent on neonatal sepsis

Introduction
Neonatal sepsis has been a leading cause of neonatal morbidity and mortality. This is due to quantitative and qualitative insufficiencies of innate immunity of neonates. A research aiming at providing new direction in management and treatment of neonatal sepsis is required.

Objectives
To determine bacteria pathogen causing neonatal sepsis, their drug sensitivity, associated cytokines and other factors involved in neonatal sepsis.

Requirements
Neonates admitted in Nakuru County Referral Hospital will be considered for research. A blood sample (2 ml) will be drawn from each patient before treatment for laboratory analysis.

Benefits to Patients and Future Newborns
- Review of antibiotic regime used in treatment of neonatal sepsis in the hospital hence quick recovery from infection and reduced period of hospitalization
- Improved management procedures in neonates by clinicians and pediatrics
- Specific diagnosis and treatment of neonatal sepsis
- Future development of Anti-Sepsis vaccine for expectant women and newborns

Parent/Guardian
I freely and willingly agree to allow blood sample collection from my baby for the purpose of neonatal sepsis research.

Name of Parent /Guardian...............................................................

ID. No. .................................................................

Patient’s No ...............................................................

Date ..........................................................................

Signature...........................................................................

Thank you
APPENDIX II: Research permits

(a) Research authorization

NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

Ref. No. NACOSTI/P/16/91201/11781
Date: 25th August, 2016

Mumbi S. Njagi
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on
“Epidemiology of neonatal sepsis and the associated cytokines in Nakuru
County Referral Hospital,” I am pleased to inform you that you have been
authorized to undertake research in Nakuru County for the period ending

You are advised to report to the County Commissioner, the County
Director of Education and the County Director of Health Services,
Nakuru County before embarking on the research project.

On completion of the research, you are expected to submit two hard copies
and one soft copy in pdf of the research report/thesis to our office.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.
(b) Approval of research proposal

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

Internal Memo

FROM: Dean, Graduate School
TO: Ms. Mumbi S. Njagi
C/o Zoological Sciences Dept.
KENYATTA UNIVERSITY

DATE: 30th September, 2014
REF: 156/CE/22001/10

SUBJECT: APPROVAL OF RESEARCH PROPOSAL.

This is to inform you that the Graduate School Board at its meeting of 29th September, 2014 approved your M.Sc. Research Proposal Entitled "Epidemiology of Neonatal Sepsis and the Associated Cytokines in Nakuru County Referral Hospital".

You may now proceed with your Data collection, subject to clearance with the Principal Secretary, Higher Education, Science and Technology.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

JOSEPHINE KENDI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Zoological Sciences Dept.

Supervisors:

1. Dr. Michael Gicheru
   C/o Zoological Sciences Dept.
   KENYATTA UNIVERSITY

2. Dr. Joshua Mutiso
   C/o Zoological Sciences Dept.
   KENYATTA UNIVERSITY

JK/cao
(c) Research approval from Rift Valley Provincial General Hospital

MINISTRY OF MEDICAL SERVICES

RIIVOL I/08

Date 6/5/2014

To: Mumbi S. Nyagi

Box 16418

Nakuru

Dear Mumbi Nyagi

RE: APPROVAL TO UNDERTAKE RESEARCH AT THE
RIFT VALLEY PROVINCIAL GENERAL HOSPITAL

Reference is made to your letter dated 30/4/2014 seeking
approval to conduct a research on "A study of neonatal sepsis,
Prevalence, aetiology and associated cytokines in Nakuru Provincial Hospital"

Permission has been granted/Not granted for the research. It is hoped that you will
adhere to the ethics and standards that relate to research at our institution.

Thank you.

Yours sincerely,

[Signature]

MEDICAL SUPERINTENDENT

CHAIRPERSON
RESEARCH AND ETHICS COMMITTEE
APPENDIX III: Questionnaire on neonatal sepsis infection

(Tick Appropriately)

1. Patient’s number______________________________________________________________

2. Sex of the patient    Male [ ]    Female [ ]

3. Current age of patient. Days_________ Weeks ________ Months _____________

4. Gestational age.      Term [ ]    Preterm [ ]

5. Health status.        Normal [ ]    Neonatal sepsis [ ]

   Other conditions (specify) ___________________________________________________

6. Nutrition.            Breast fed [ ]    Not breast fed [ ]

7. Place of delivery.    Hospital [ ]    Home [ ]

8. Mode of delivery.     Caesarian [ ]    Normal birth [ ]

9. Health status of the mother.    HIV (+VE) [ ]    HIV (-VE) [ ]

   Other condition (specify) ___________________________________________________
APPENDIX IV: Geographical location of Nakuru County Referral Hospital