ANTIMICROBIAL SENSITIVITY AND TREATMENT OUTCOMES OF NEONATAL SEPSIS AT PUMWANI MATERNITY HOSPITAL

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DECLARATION OF ORIGINALITY FORM

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DEDICATION

I dedicate this work to my dear husband, Gideon and our children Leo and Lulu.

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ABBREVIATIONS

ADRs	Adverse drug reactions
AMC	Amoxicillin/Clavulanic acid
APGAR score	Appearance, Pulse, Grimace, Activity, and Respiration
BD	Twice daily
BP	Blood pressure
CoNS	Coagulase Negative *Staphylococcus aureus*
EOS	Early onset neonatal sepsis
ESBL	Extended spectrum beta-lactamases
GBS	Group B *Streptococcus*
GNB	Gram negative bacteria
GPB	Gram Positive bacteria
INJ	Injection
LBW	Low birth weight
LOS	Late onset neonatal sepsis
MRSA	Methicillin Resistant *Staphylococcus aureus*
MDG 4	Millennium Development Goal Four
NW	Normal Weight
OD	Once daily
PROM	Premature rupture of membranes
SDs	Standard Deviations
SIRS	Systemic inflammatory response syndrome
SXT	Sulphamethoxazole/trimethoprim
VLBW	Very low birth weight

UK	United Kingdom
UoN	University of Nairobi
WHO	World Health Organization

DEFINATION OF TERMS

Neonates:	0-28 days old
Neonatal period:	From day 0 to 28 days of life (first four weeks postnatal)
Early onset sepsis:	Within the first 72 hours of life (Day 0 to Day 3)
Late onset sepsis:	After the first 72 hours of life until the end of the neonatal period

ABSTRACT

BACKGROUND: Neonatal sepsis is one of the most common causes of mortality and morbidity among infants in the developing countries. The spectrum and antimicrobial sensitivity patterns of bacteria responsible for neonatal sepsis could vary in different hospitals and regions. A periodic survey of the etiological agents and their susceptibility pattern is indeed necessary for the timely detection of the changing trend of antibiotic resistance. This will guide initial empirical choice of antimicrobial therapy in absence of culture and sensitivity results. This research focused on the sensitivity patterns of bacteria responsible for neonatal sepsis to antimicrobial agents used to treat neonatal sepsis.

OBJECTIVE: To identify bacteria in blood cultures of neonates with clinically suspected septicemia, their susceptibility to commonly used antimicrobial agents and the treatment outcomes of neonatal sepsis.

STUDY METHODOLOGY: A longitudinal design was used and the target population was all neonates born in the hospital or admitted to Pumwani Maternity Hospital with suspected sepsis. A sample size of 150 neonates with suspected sepsis was reached using consecutive sampling. Data was collected using a questionnaire and blood cultures were analyzed at Pediatric Department Laboratory, School of Medicine at the University of Nairobi. The statistical analysis was done using a software SPSS version 21.0. Data was expressed as mean +/- Standard Deviation, and comparison of proportions was performed using Exact Fisher's test and Chi-square. The significance level was set at $p = \leq 0.05$. A bivariable analysis was done for all the variables used for the comparison of the neonates included in the study in relation to the outcomes. The key outcomes considered were length of inpatient treatment, culture positivity, and death.

RESULTS: Out of 150 blood specimens cultured, the prevalence of confirmed bacterial sepsis was 32% (48/150). Gram positive pathogens were predominant with isolates of *Staphylococcus aureus* and *Streptococcus viridans* accounting for 70% of the total isolates. Gram negative bacteria comprised of 18% of the total isolates with *E.coli* and *Klebsiella* spp being the only isolates. *Staphylococcus aureus* was the main pathogen in early onset sepsis while in late onset sepsis, 2 isolates were obtained that included *Staphylococcus aureus* and *Streptococci*

pneumoniae. All the isolates were absolutely sensitive to meropenem. *Staphylococcus aureus* showed a high resistance to piperacillin (40%) and amoxicillin clavulanic acid (57%). All Gram positive isolates showed high sensitivity (above 80%) to gentamicin, ceftriaxone, ofloxacin and amikacin, except for Coagulase negative *Staphylococcus aureus* that showed 100% resistance to ofloxacin. The Gram negative pathogens exhibited a high resistance to ampicillin and some resistance to amikacin but a good sensitivity to gentamicin both at above 80%.The most common regimen prescribed is benzylpenicillin and gentamicin.

Bacterial sepsis was higher in neonates with hyperthermia (p=0.003), vomiting (p=0.034) and respiratory distress. Male sex (p=0.018) and premature rupture of membranes (p=0.049) were predictors of positive blood culture. The overall death rate was 3.3% (5/150).

CONCLUSIONS: *Staphylococcus aureus* predominates the etiology of neonatal septicaemia followed by *E.coli*. There was high resistance to ampicillin and amoxicillin clavulanic acid. Routine antimicrobial surveillance should be done to identify the trend of the causative agents along with their susceptibility patterns so as to guide the choice of antibiotics for empirical treatment of neonatal sepsis.

CHAPTER ONE: INTRODUCTION

1.1 Background

Neonatal sepsis is broadly defined as a systemic inflammatory response occurring in the first four weeks of life as a result of a suspected or proven infection (1). Neonatal sepsis has also been defined as a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life (2). It may be classified according to time of onset of disease: early onset and late onset. Early onset neonatal sepsis (EOS) occurs within the first 72 hours of life and late onset neonatal sepsis (LOS) if it occurred beyond 72 hours of life until the end of the neonatal period. Few studies differ on the definition of EOS and LOS. This is mainly on their duration of onset. EOS has been defined to range from 48 hours to 6 days and LOS from 72 hours to 28 days after delivery (3,4).

Neonatal sepsis remains a leading cause of mortality and morbidity among infants in the developing countries. According to the World Health Organization (WHO) estimates there are about 5 million neonatal deaths a year globally, 98% of which occur in developing countries (5,6), accounting for about 26-34% of total deaths each year (3,7,8). Prevention of neonatal sepsis and decision making on a rational treatment plan using the antibiotics, still remains an important clinical problem internationally (9–12). Despite substantial progress, the world has not achieved Millennium Development Goal (MDG) 4 that aimed to reduce child mortality by two thirds by 2015. Preventable diseases are substantially associated with under-five deaths (13). The incidence of neonatal sepsis varies from 1-4/1000 live births in developed countries, to 10-50/1000 live birth in developing countries(14). The reported neonatal sepsis in South America and Caribbean is 3.5 to 8.9 per 1000 live births (3). By comparison, the reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live births in Asia(15), and from 6 to 22.9 per 1000 live births in Sub-Saharan Africa (16).

The bacteremia rate in Kenya is 5.46 cases per 1000 live births (17). High mortality rates have been reported in Saudi Arabia (18) and Mexico (19), while declining mortality rates were detected in USA (20) and Nigeria (21). The neonatal mortality rate in Kenya is 31 per 1000 live births (22). Overall, 8% to 80% of all neonatal deaths in developing countries are attributable to

infections and as many as 42% of deaths occur in the first week of life (8). In the developed countries where the neonatal infection ranges from 1-5 per 1000 live births (14,23,24).

Clinical presentation of neonatal sepsis varies and there are no specific signs and symptoms (7). In a study on predictors of neonatal sepsis, Kayange *et al*., reported inability to feed as a common and early symptom, as well as lethargy, convulsion, chest wall indrawing, jaundice and umbilical redness (25). Tripathi *et al*., reported manifestations that included: poor perfusion, bradycardia or tachycardia, respiratory distress, hypoglycemia or hyperglycemia, poor cry and hypothermia or fever. Blood culture is the gold standard for diagnosis of septicemia, while lumbar puncture, urine culture, radiology, acute phase reactants, cell surface markers, cytokines, granulocyte colony stimulating factor, molecular genetics and proteomics can also be used. Management is mainly supportive therapy and antimicrobial treatment (26).

The spectrum of organisms responsible for neonatal sepsis in developing countries differs from those in developed countries (3). Also the spectrum of organisms associated with EOS differs from those implicated in LOS sepsis (27). In developing countries Gram negative organisms are common, mainly *Klebsiella* spp*, Escherichia coli, Pseudomonas* and *Salmonella* spp (2,11). Gram positive organisms implicated include *Staphylococcus aureus* (3,28).

Antimicrobial drug resistance is a growing threat due to emergence of microorganisms that are resistant to the currently used medicines (29). Antimicrobial sensitivity and resistance testing is important as a guide for rational prescribing. The gold standard for assessing antimicrobial susceptibility is determination of the minimum inhibitory concentration (30). It is been reported that between 4.4% and 10.5% of all infants born in the United States (130,000-400,000) receive systemic antibiotics (31–33). Comparatively there is no available documented information on the percentage of newborns that received antibiotics in the developing countries. However controversy exists with respect to newborns that are asymptomatic though high risk and to those newborns whose mothers received intrapartum antibiotics (34–37). Intrapartum antibiotic treatment may partly suppress bacterial growth leading to false-negative culture results (38). According to Berkley *et al*., antimicrobial susceptibilities ranged from 31% when using benzyl penicillin alone to 97% with a combination of ampicillin and gentamicin (17). Studies have also shown that resistance of Gram negative organisms to empiric first-line antibiotics remains high(25,39,40).

In management of neonatal sepsis in resource poor settings diagnosis and empirical treatment of neonatal sepsis is based on the existing guidelines. However the etiology of neonatal sepsis and antimicrobial sensitivity may vary significantly from time to time and geographically which may affect the choice and efficacy of empirical management (25,41,42).

The diagnostic criterion of neonatal sepsis varies from country to country. Systemic Inflammatory Response Syndrome (SIRS) is considered a definitive of sepsis and diagnosis is made if two of the following four criteria is observed, one of them being abnormal leukocyte count or elevated temperature. Temperatures of more than 38.5 $^{\circ}$C or less than 36 $^{\circ}$C; tachycardia or bradycardia; leukocyte count elevated or depressed for age or more than 10% immature neutrophils; and mean respiratory rate of more than 2 Standard Deviations (SDs) above normal for age (1). Cottineau *et al*.,and Lutsar *et al*., listed some predictive clinical and laboratory criteria: impaired peripheral perfusion, increased oxygen requirement, mottled skin, cord blood levels of prolactonin or interleukin (IL)-6 or both (43,44). Blood culture is the gold standard for diagnosis of septicemia and blood should be drawn before starting antibiotics (26).

WHO recommends that serious bacterial infection or sepsis should be managed by administration of oxygen by nasal catheter in cyanosed infants, extensive fluid management and antimicrobials in combinations of penicillin or ampicillin and gentamicin. This regimen cover most likely causative bacteria but have poor coverage of both *Salmonella* and increasingly penicillin-resistant *Staphylococcus aureus*. WHO also recommends hospitalization for suspected cases of sepsis and ten or more days of parenteral therapy with penicillin/ampicillin and gentamycin for neonates with serious bacterial infections or sepsis. A change of antibiotics is recommended if the condition is not improving in 2-3 days after initiation of therapy(45,46).

The complications associated with neonatal sepsis include: necrotizing enterocolitis, meningitis, vision impairment, impaired head growth, functional disability in terms of difficulties in standing, locomotion eye-hand co-ordination or limb movement disorders that have long-term consequences for the neonates and the family. They predispose an infant to increased risk of future neurological impairment (47,48).

1.2 Problem statement

There is an estimated five million neonatal deaths per year globally, 95% percent occurring in developing countries. Infection is pointed out to be one of the leading causes (3,7,49). Neonatal sepsis is increasingly identifiable as a public health issue worldwide. The prevalence of neonatal sepsis is low in the developed world. The reported neonatal mortality rate is 2-4 per 1000 live births. In contrast neonatal mortality is at 34 per 1000 live births in developing countries. Neonatal mortality is 34 per 1000 live births in Asia, 42 per 1000 live births in Africa and 17 per 1000 live births in Latin America and the Caribbean(3,50,51).

Neonatal sepsis can cause life threatening complications including meningitis and long term neurodevelopment delays and damage. Consequently this leads to increased mortality and morbidity among young infants.

The pathogens implicated in neonatal sepsis in developed countries differ from those implicated in developing countries (3,25,52). Variations are also seen within hospitals of the same region (17,25,53), which necessitates periodic surveillance for data on the implicated organisms that also keep changing periodically. Unfortunately surveillance is not readily done in sub-Saharan Africa. Currently, as evidenced by the scarcity of data regarding etiology, antimicrobial sensitivity patterns, insufficient knowledge about appropriate antibiotic choice, has led to development of guidelines based on data from the developed countries. This has posed a challenge in adequate management of neonatal sepsis in this region (28). The situation is further compounded by the increasing trend of antibiotic resistance to commonly used first line treatment and this too warrants availability of current data locally (29,54). This will ultimately hamper the ability to successfully treat neonatal sepsis in our region. Strict antibiotic policy and up-to-date guidelines will greatly impact management of neonatal sepsis today and the future.

1.3 Justification

Historical reviews have demonstrated that the predominant organisms responsible for neonatal sepsis have changed with time (6). Community-based studies to estimate infection rates and infection specific mortality are limited especially in the developing world (8). In Kenya, such studies are very limited in quality and quantity (17). This justifies need for periodic surveys on susceptibility patterns of implicated bacteria and the need to evaluate the treatment outcomes in each case.

Neonatal sepsis is a life threatening emergency that requires accuracy in choice of empiric therapy to save lives. In view of the above, this study aims to determine if the current empiric treatment is adequate and to survey the etiological agents and their susceptibility patterns. Information obtained will guide steps in management of neonatal sepsis. This study will help inform timely and accurate empiric decisions in absence of cultures that may not be feasible in some situations, thereby reducing the risk of under treatment or over treatment of infections, both of which are associated with emergence and increasing of resistance to antibiotics (29).

The current data also will be necessary in policy decisions and development of treatment guidelines that can help to mitigate neonatal mortality.

1.4 Research questions

1. What are the bacteria implicated in clinically suspected sepsis?
2. What are the antimicrobial susceptibility patterns of the isolated bacteria?
3. What are the treatment outcomes in neonatal sepsis?
4. Which drugs are used to treat neonatal sepsis?

1.5 Objectives

1.5.1 Main Objective

To determine the etiology, antimicrobial susceptibility and treatment outcomes of neonatal sepsis at Pumwani Maternity Hospital, Nairobi.

1.5.2 Specific objectives

To:

1. Isolate and identify the bacteria that cause neonatal sepsis.
2. Determine the antimicrobial susceptibility patterns of the isolated bacteria.
3. Establish the antibiotic regimens used to treat bacterial neonatal sepsis.
4. Evaluate the treatment outcomes of neonatal sepsis.

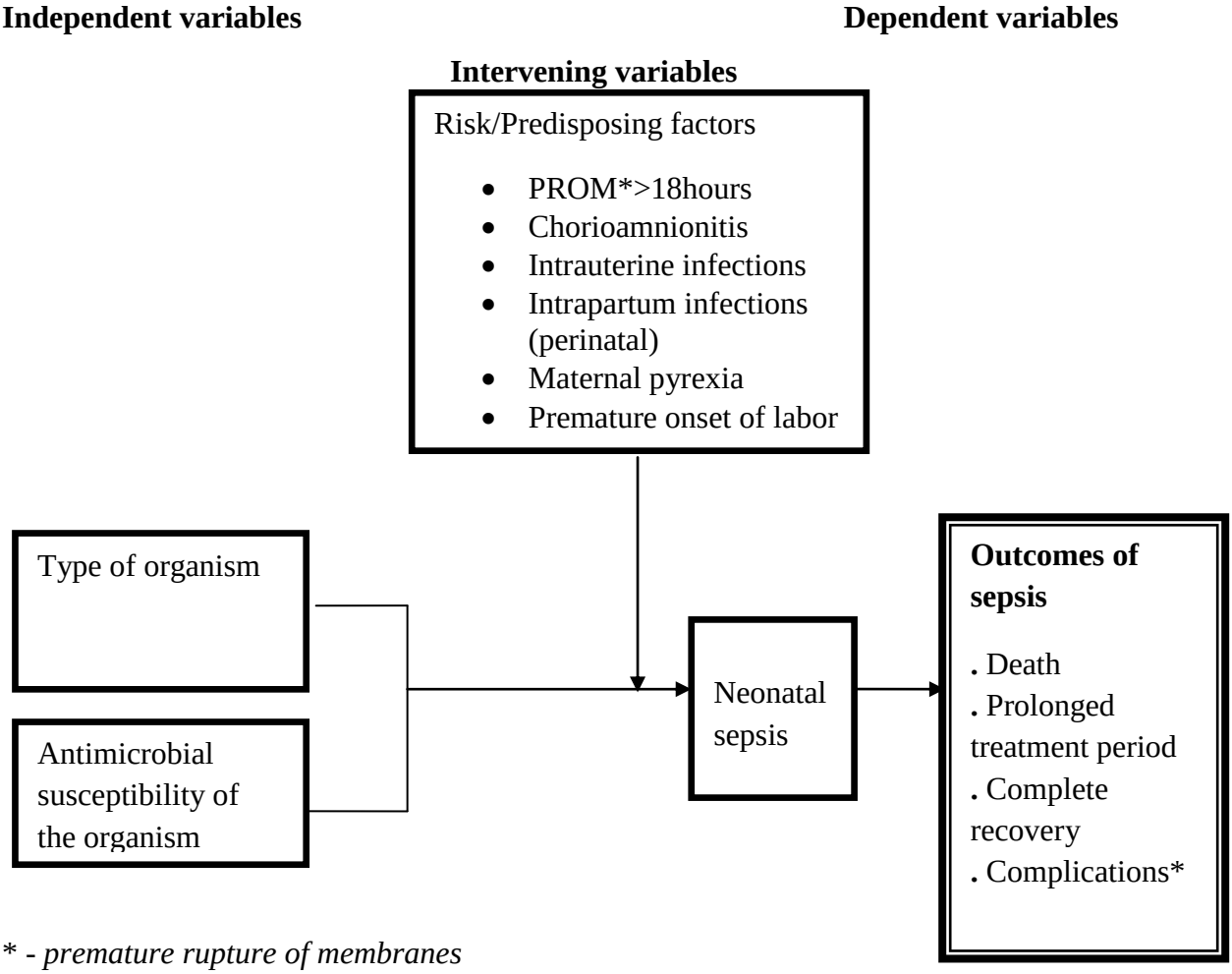
1.6 Significance of the study

Neonatal sepsis is one of the most common causes of morbidity and mortality among neonates in the developing countries (5,6). It is well known that the spectrum of organisms that cause neonatal sepsis very in regions and even within the same setting. Antibiotic resistance is a problem worldwide, as evidenced by emergence of microorganisms that are resistant to commonly used antimicrobial agents. The information obtained from study will help in identifying the pathogens implicated in neonatal sepsis and their susceptibility patterns in this setting. This will guide in empirical treatment and also provide up to date information for appropriate management of neonatal sepsis. This will ultimately contribute towards achievement of Kenya Millennium Development Goal 4.

1.7 Conceptual/Theoretical framework

This is the relationship between variables: Independent, dependent and intervening variables.

Figure 1: Conceptual Frame work



* - *premature rupture of membranes*

* -*including meningitis, necrotizing enterocolitis*

functional disability and neurological impairment

Neonatal sepsis is mainly caused by bacterial pathogens. The risk factors associated with its occurrence are; intrauterine infections during transcervical chorionic villus sampling and amniocentesis, intrapartum infections (perinatal infections), postnatal infections, obstetric complications such as PROM>18hours, premature onset of labor and, maternal pyrexia among others.

The type of organism that causes neonatal sepsis determines the outcome of the disease. Bacteria differ in their virulence and those that are most invasive cause serious infections that carry high mortality and morbidity. Organisms also differ in their susceptibility to antimicrobial drugs and use various mechanisms to resist being killed. Therefore the type of drug used will be effective or not depending on the sensitivity of the causative agents. The choice of regimen thus depends on the severity of the disease, implicated pathogen, the sensitivity pattern.

The treatment outcome of neonatal sepsis includes: death, prolonged stay in the hospital, disability and complete recovery. The outcome depends on the severity of the infection and effectiveness of the medications used as well as the duration before seeking treatment.

CHAPTER TWO: LITERATURE REVIEW

2.1 Etiology and risk factors for neonatal sepsis

Due to underdeveloped skin barriers and immature or compromised immune systems, neonates are more susceptible to infections (55).The burden of disease attributed to neonatal infection varies by geographic region and maternal and neonatal risk factors. Early onset neonatal sepsis is associated with vertical transmission during labor or birth. The other important factors that predispose neonates to early onset sepsis is prematurity (56–59), low birth weight (57,59), premature rupture of membranes (PROM) longer than 18 hrs before birth, Group B *Streptococcus* infection during pregnancy, immunologic immaturity and chorioamnionitis (60). Late onset neonatal sepsis is usually considered to originate from the care giving environment either at the community or hospital setting (41).

The prevalence reported from different hospitals varies. Mugalu *et al.,* reported confirmed septicemia in 37% of cases (61). This is similar with what was reported by Owa *et al.,*(62) 35% and 33% by Mondal et al., (63), which was in contrast to the findings of Haque *et al*., (64) of Saudi Arabia, (15%) Ako-Nai *et al*., of Nigeria (55%) (65), Shitaye *et al*., of Ethiopia 44.7% (59) and (60%) by Aurangzeb *et al*., (66). The differences could be due to sample size and selection of patients.

The predictors of positive blood culture mainly comprises of perinatal risk factors and clinical characteristics. Perinatal factors like PROM and meconium stained liquor were strongly associated with a positive blood culture in both LOS and EOS (25). In this study the inability to feed, lethargy convulsions, hypothermia, chest indrawing, umbilical redness, jaundice and cyanosis were the clinical characteristics that were associated with a positive culture. Similar findings were reported in a study done in Uganda (61). In a study done by Soman *et al*., an increased risk for being male and low birth weight is reported. They also found a strong association between an APGAR score of 6 or less at 5 minutes with neonatal sepsis. A routine evaluation in neonates born in areas with high incidence rates of early neonatal sepsis, has been recommended (67).

Organisms causing neonatal sepsis vary from place to place and also keep changing periodically in the same area. Consequently the implicated pathogens in neonatal sepsis in developing countries differ from those in developed countries (3). In developed countries, group B *Streptococcus* (GBS) is the organism mainly implicated in neonatal sepsis (68), followed by *E. coli* (69). Pathogens implicated in EOS are GBS and *E. coli* where as in LOS, *Staph. aureus*, *Enterococcus species* and GBS are implicated (4). Concurring with this findings are studies , in USA and Australia where GBS and *E. coli* were the predominant organisms in EOS and Coagulase Negative *Staphylococci* (CoNS) followed by *Staph. aureus* in LOS (52,70). In another study done in the USA Group B *Streptococcus* was the predominant organism, followed by *E. coli* and *Staphylococcus* species. The study also reported a decreasing trend in occurrence of Group B *Streptococcus* and *E. coli* cases over seventy years between 1928-2003 (69). In the United kingdom GBS was the most frequent pathogen isolated followed by CoNS, non pyogenic *Streptococci* and *E.coli* (71).

In the developing world, *Staphylococcus aureus, Klebsiella species* and *E.coli* are most common pathogens causing EOS, whereas incase LOS, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* are implicated (72,73). The causative agents in LOS and EOS are similar especially in hospitals in developing countries (74).

A study done at a private hospital in Kenya found Gram-positive organisms to be the predominant pathogens in both early and late onset sepsis. Common isolates were *Staphylococcus epidermidis* and *Staphylococcus aureus.* EOS was mainly caused by *Staphylococcus aureus, Klebsiella* spp*, Staphylococcus epidermidis* and LOS was caused by *Staphylococcus aureus, Streptococcus* spp*, Enterococcus* spp *and Staphylococcus epidermidis* (53).

In another study done in Kenya, Gram positive causative organisms included *Streptococcus pneumoniae*, *Staphylococcus aureus*, Group A *Streptococcus* and Group B *Streptococcus*. The main Gram negative causative isolates were *E.coli*, *Klebsiella* spp, *Acinetobacter* spp, *Hemophilus influenza*e and *Pseudomonas* spp among others (17).

A WHO multicentre study in developing countries that included Ethiopia, Gambia, Papua New Guinea and Philipines reported *Streptococcus Pneumoniae, Staphylococcus aureus* and Group A *Streptococcus* as predominant organism and that Group B *Streptococcus* was uncommon (72). This differs with a study by English *et al*., that reported Group B *Streptococcus* to be the most common isolate in EOS (75). Differences in findings could be due to the different locations of the studies or difference in age of neonates recruited.

Other studies on causative organisms have been summarized in the table below.

Table 1: Causative bacterial pathogens in neonatal sepsis

Country/Author	**EOS/LOS (where specified)**	**Most common isolates**
Malawi (Gray *et al*)(76)	EOS	*Staphylococcus agalactiae*
Kenya (English *et al)*(75)		*Staphylococcus agalactiae*
Tanzania (Mhada *et al*)(42)		*Staphylococcus aureus, Klebsiella* spp *and Escherichia coli*
Ethiopia (Shitaye *et al*)(59)		*Klebsiella* spp, *Staphylococcus aureus*
Nigeria (Owa *et al*)(62)		*Staphylococcus aureus*
Nigeria (Ako-Nai *et al*)(65)		*Staphylococcus aureus*
Saudi Arabia(64)		*Staphylococcus aureus*
Zimbabwe(77)		*Staphylococcus aureus*
Uganda (Mugalu *et al*)(61)		*Staphylococcus aureus, Escherichia coli,* GBS
Nigeria(78)		*Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Enterobacter, Pseudomonas*
Tanzania (Kayange *et al*)(25)	EOS LOS	*Klebsiella* spp, *Escherichia coli*, GBS *Escherichia coli, Staphylococcus aureus*
Tanzania (Nelson *et al*)(79)		*Klebsiella* spp, *Escherichia coli*
India (Sharma *et al*)(80)		*Staphylococcus aureus, Klebsiella pneumoniae*

Nepal (Shaw *et al*)(81)		*Escherichia coli*, CoNS, *Pseudomonas*, *Acinetobacter*, *Enterobacter* spp
Sharma *et al*(82)	EOS LOS	*Staphylococcus aureus* *Staphylococcus aureus*, *Escherichia coli*
Mondal *et al*(63)		CoNS, *Klebsiella* spp, *Acinetobacter* spp
Bhat *et al*(83)		GNB comprised of 90% of the isolates. *Pseudomonas* spp, *Klebsiella* spp, *Acinetobacter* ,*Escherichia coli*, *Citrobacter*, *Staphylococcus aureus* and *Enterobacter* spp
Pakistan(66)	EOS	*Escherichia coli*, *Pseudomonas* spp, *Klebsiella* spp, *Staphylococcus aureus*
Vergnano *et al*(3)	EOS LOS	*Enterococcus*, *Listeria monocytogenes* *Pseudomonas* spp, *Salmonella* and *Serratia* spp

2.2 Antimicrobial susceptibility of agents that cause Neonatal Sepsis

The spectrum of bacteria and their susceptibility patterns may vary depending on prevailing conditions especially antimicrobial drug use. Antibiotic resistance has become a global problem. There are several reports of multi resistant bacteria causing neonatal sepsis, and the trend shows increasing resistance to commonly used antibiotics in developing countries (3,84).Vergnano *et al*., in a review in developing countries reported that most Gram negative bacteria are now resistant to ampicillin and cloxacillin, and many are becoming resistant to gentamicin (3). Similarly this was also observed by Aurangzeb *et al*., where they reported that Gram negative bacteria showed a high degree of resistance to commonly used antibiotics (66). In other studies it was also reported that there is an emerging reduction in sensitivity to third generation cephalosporins and quinolones (85,86).

The following are susceptibility patterns reported in other studies;

Table 2: **Antimicrobial susceptibility of agents that cause neonatal sepsis**

Study/Country	**Pathogens isolated**	**Antimicrobial Susceptibility**	
		Sensitive	**Resistant**
Jerusalem(87)	GPB*	vancomycin	
	All GPB except *Staphylococci*	Ampicillin	
	GNB*		Showed an increased resistance to commonly used antibiotics
Pakistan(66)	GNB		High degree resistance to ampicillin, amoxicillin, ceftazidime, cefotaxime, Low resistance to tobramicin, amikacin, imipenem, ofloxacin and ciprofloxacin
Sharma *et al* (80)	GPB and GNB	cefotaxime, amikacin, cefepime, meropenem, vancomycin	
	GPB except *Streptococcal* spp		Displayed resistance to most penicillins and ciprofloxacin
	Staphylococcus aureus		Absolute resistance to benzyl penicillin

Bhat *et al*(83)	GNB	amikacin followed by aminoglycosides, ciprofloxacin and cefotaxime	
	GPB	erythromycin, ciprofloxacin and aminoglycosides	
	GNB and GPB	amikacin and cefotaxime	
Kohli-kochhar *et al*(53)	*Staphylococcus aureus*		Ampicillin
	Klebsiella pneumoniae		Gentamicin
Monjur *et al*(40)	*Pseudomonas*, *E.coli*, *Klebsiella pneumoniae*		ampicillin and gentamicin
Sharma *et al*(82)	*Staphylococcus aureus*		about 57% found to be methicillin resistant
	Gram negative rods		Amikacin
	GNB and GPB	imipenem, piperacillin/tazobactam	third generation cephalosporins and ciprofloxacin
Gwayali *et al*(39)	GPB	aminoglycosides and quinolones	cephalosporins and penicillin
	GNB	third generation cephalosporins, other aminoglycoside	Ampicillin

Kayange *et al*(25)	*Klebsiella pneumoniae, E.coli*		ampicillin and gentamicin
	Most GNB	ciprofloxacin and meropenem	third generation cephalosporins and aminoglycosides
India(88)	*Escherichia coli, Klebsiella pneumoniae*	ceftriaxone, cefotaxime	ampicillin, chloramphenicol, cotrimoxazole, tetracycline
	CoNS*	gentamicin	

*GPB: Gram positive bacteria

*GNB: Gram negative bacteria

*CoNS: Coagulase negative bacteria

2.3 Treatment regimens and outcomes in management of neonatal sepsis

Neonatal sepsis is a life threatening emergency and prompt treatment reduces chances of complications associated with sepsis and even death. Therefore in suspected sepsis, empirical antibiotic therapy should be initiated in high–risk neonates, after blood for cultures have been obtained (89). Early and prompt initiation of empirical antimicrobial treatment, is based in the presence of risk-factors in EOS (90,91) and clinical presentation of LOS (92); this has shown to reduce mortality in neonates.

The initial choice of antibiotics for empirical treatment is dependent on knowledge of probable causative agents, their susceptibility patterns, perinatal history, and any maternal symptoms and cultures (93). There are randomized controlled studies(RCTs) (94),(95),(96) , that compared appropriate regimens for empirical treatment in suspected neonatal sepsis. They all failed to show that one regimen was superior to the other. In the study by Snell *et al*., (94), ceftazidime was compared to gentamicin plus benzyl penicillin, where they concluded that ceftazidime was as good as penicillin and gentamicin.

A study done by Miall-Allen *et al*., (95) they compared ticarcillin plus clavulanic acid versus piperacillin with or without gentamicin and concluded that there was no difference in mortality or treatment failure. Also Metsvaht *et al*., (96) in their study found no difference in treatment failure or mortality rate in the comparison between ampicillin plus gentamicin versus penicillin plus gentamicin. Based on the common antibiotic sensitivities of the predominant organisms in EOS, recommended empirical treatment in the developed countries includes ampicillin and an aminoglycoside (2). This may however not be the case for the developing countries where *Staph. aureus* and *Klebsiella* spp showed high resistance to ampicillin, ceftriaxone, macrolides, cotrimoxazole and gentamicin (28). They also reported that the available studies from the developed world focused on community-acquired infections, and that the segregation of EOS and LOS was not available. The empirical treatment for LOS should cover both GNP and GPM (89).

In the developed countries where CoNS is the predominant isolate and where resistance to gentamicin and penicillin is common ,vancomycin has been recommended to be used in empiric treatment (50). In another study done in the developed countries, it was suggested that the attempts to reduce the incidence of CoNS infection using antibiotics was not necessary since many of those infections are relatively benign (52). The acceptable approach would be to start with cloxacillin and gentamicin in a stable neonate (89). Zaidi *et al*., in their review reported a higher percentage of Gram negative bacteria and an increased resistance among these organisms. They also reported that most of these organisms are not covered by the empiric regimen of ampicillin and gentamicin (97).

Empirical antifungal treatment should be considered in infants exposed to broad- spectrum cephalosporins or carbepenem, those that have a central vascular access and gestational age less than 28 weeks (98). The antibiotics used for empirical treatment should be reevaluated in reference to the results of cultures and susceptibility tests (99). The duration of treatment depends on the severity of the disease and intravenous antibiotics are usually prescribed for 21 days for cases of neonatal meningitis and for 10 to 14 days in other severe neonatal infections (100). However when to stop the antibiotics is still not clear (101).

Cordero and Ayers *et al*., reported that it would be safe to discontinue empiric antibiotics when blood cultures are negative and asymptomatic in very low birth weight neonates (102). Prolonged durations of initial empirical antibiotic treatment have been associated with an increased risk of necrotizing enterocolitis, neonatal candidiasis, alteration of the gut flora and even death in neonates (103–106). Hence it is important to restrict the duration of initial antibiotic treatment to less than 3 days where blood cultures are negative and neonates asymptomatic (89). Supportive care of dysfunctional organs is the mainstay of therapy which includes mechanical ventilation, fluids, vasopressors or inotropes, and blood transfusion (1).

Anti-microbial agents are the cornerstone therapy for sepsis. The choice of regimen for management of neonatal sepsis varies widely in both developed and developing countries. The choice of antimicrobial agents is generally based on the local policy for most countries. The duration of therapy is not clear since it also varies from region to region, where the practice is at the discretion of the treating physician based on clinical symptoms and blood culture results (25,44,89).

In some countries there are no clear guidelines for management of neonatal sepsis, especially for distinguishing between LOS and EOS, where the organisms implicated differ. In the UK there is no clear guideline in place for management of EOS and LOS. This has led to variations in patterns of practice among British practitioners. Group B *Streptococcus* is the most common organism implicated in EOS in the UK and the most common choice of antibiotics is a combination of benzyl penicillin and intravenous gentamicin (107). Lutsar *et al*., in a prospective study done in Europe found 18 different empiric antibiotics regimens used for management of LOS. The empiric regimens mostly comprised of ampicillin, a third generation cephalosporin or meropenem plus aminoglycoside or vancomycin (44). Similarly Du Pont-Thibodeau *et al*., found variations in management of LOS that involved 18 different regimens that included benzyl penicillin, ampicillin, a third generation cephalosporin, or meropenem plus aminoglycoside or vancomycin. EOS was mainly treated with benzyl penicillin and gentamycin (1). In the developed countries it has been noted that CoNS was resistant to methicillin (27). It was recommended that a broad spectrum antibiotic including vancomycin be used for treatment, but not for prophylaxis.

A study done in Serbia concluded that the most adequate initial treatment for neonatal sepsis was cefotaxime plus amikacin (100). Also from this study it was recommended that the most adequate treatment for nosocomical sepsis is carbepenem, whereas Bibi *et al.*,in a study done at an urban hospital in Bangladesh, concluded that ampicillin plus gentamicin are still useful in initial treatment of sepsis but preferred for post neonatal sepsis probably because the study population was mostly post neonatal age group (108).Yurdakök *et al*., recommended a combination of third generation cephalosporin and gentamicin as the appropriate initial therapy for neonatal sepsis in places where there is increased aminoglycoside resistance (99). A study done in Tanzania used the WHO integrated management of childhood illness protocol that recommends ampicillin, cloxacillin, and gentamicin as first line therapy(42). Similarly a study done at a tertiary hospital, used ampicillin and gentamicin as first-line therapy (25).

Ampicillin plus gentamicin has been recommended for management of suspected community acquired neonatal sepsis where resistant strains are unlikely. Also recommended was that cefotaxime be added if meningitis is present. In case of a hospital acquired neonatal sepsis, treatment with cefotaxime plus aminoglycoside as initial therapy was recommended. Piperacillin-tazobactam or methicillin or vancomycin was also recommended for use in cases of high resistant strains (26).

Sivanandan *et al*., in their study concluded that a combination of ampicillin and gentamicin was the appropriate choice of empirical treatment in EOS, where GBS and *E. coli* are predominant organisms in the developed countries. Also recommended in the study is that empiric antibiotic treatment for the developing countries should be individualized for each hospital and regions (89).

In a study that considered antibiotic regimens for suspected late onset sepsis, the Primary outcomes considered were: Mortality prior to discharge from the hospital, development of septic shock. Secondary outcomes considered were complications to antibiotics and complications of sepsis like meningitis (27). In another study the outcome assignment was based on culture results or clinical factors like results of physical examinations or chest roentgenograms. They classified

them as definite infection, probable infection and possible infection. In the study deaths and discharges were reported those with infection. The rehospitalized patients in this study were due to jaundice and/or feeding difficulties, ruled out sepsis with negative cultures and miscellaneous diagnoses (109).

Primary outcomes considered in the study done at Karachi-Pakistan were: treatment failure within 7 days of start of therapy, death and/or worsening of condition in any day. Secondary outcome considered was ADRs due to treatment drugs. Also concluded in this study was that the simplest safe and effective antimicrobial in young infants was not known, although a 7 day treatment with gentamicin and procaine penicillin injection appear to be safe and effective (45).
In Kenya the treatment guidelines options provided by the Ministry of Health for management of neonatal sepsis are penicillin or cloxacillin plus gentamycin as first line therapy. Second line therapy includes ceftazidime, ceftriaxone with amikacin or according to antimicrobial sensitivity of isolated organism (110).

CHAPTER THREE: METHODOLOGY

3.1 Research design

The design was a longitudinal study. Neonates in the study were observed prospectively till they were discharged to determine the treatment options used and their outcomes. The causative agents for neonatal sepsis were identified in the blood of the neonates with suspected sepsis.

3.2 Study area

The study was conducted at Pumwani Maternity Hospital, Nairobi City, Kenya. Pumwani maternity hospital is the largest public maternity hospital in East and Central Africa catering for both low and middle income earners. It is run by the County Government of Nairobi. It has one labor ward, one ante-natal ward and five post-natal wards. The hospital has an average of 70 deliveries per day translating to 30,000 deliveries per year. It was chosen because it serves both low income and middle income population and has a laboratory facility that was used for the processing of the samples

3.3 Target population

The target population were neonates (aged 0-28 days of life) born in the hospital or outside admitted to the hospital with suspected sepsis during the study period.

3.4 Eligibility criteria

3.4.1 Inclusion criteria

- Neonates of 0-28 days of life with clinically suspected sepsis admitted during the study period (March 2015 to August 2015).
- Parents/Guardians who gave written consent.
- Infants who had not received antibiotics on admission.

3.4.2 Exclusion criteria

Neonates excluded from the study were those with the following characteristics:

- Preterm less than 35 weeks gestational age
- Congenital malformations
- Severe birth asphyxia

- Low birth weight less than 2000 grams
- Meconium aspiration syndrome
- Mothers / Guardians who refuse to consent
- Neonates exposed to antibiotics prior to recruitment.

3.5 Sampling

3.5.1 Sample size determination

According to a study done by Berkley *et al*., (14) in a rural hospital in Kenya at Kilifi, the prevalence of bacteremia among the neonates was 15%.

Sample size was calculated using the Fischer's formula (111).

$$n = \frac{(Z\alpha/2)^2 * P(1-P)}{d^2}$$

Where:

n=sample size

Z= Standard normal deviate at 95% confidence interval.

P= Proportion of target population with neonatal sepsis

q= 1-p

d= expected margin of error

Z=1.96, p=0.15, q=1-0.15=0.85. d=0.05

Thus;

$$N=\frac{1.96^2*0.15(0.85)}{0.05^2}$$

$$=195.92$$

$$=196$$

The target sample size was 196 neonates with suspected sepsis.

Due to the constraints encountered during the study, a sample of 150 was achieved.

3.5.2 Sampling technique

Samples were drawn by use of consecutive sampling method. All the neonates born in the hospital and those admitted to the hospital with suspected sepsis during the study period (March 2015 to August 2015), that meet the inclusion and exclusion criteria, were recruited. A sample size of 150 neonates with suspected sepsis was attained.

3.6 Data collection

The parent/guardian was presented with the consent form, and the purpose, procedures, risks and benefits of the study were explicitly explained. The parents/guardians were informed that it is voluntary and that they could opt out of the study at any stage. Parents/guardians were then asked to sign the consent form (Appendix 3). A copy of the consent form was then left with the guardian. Patient's particulars were filled in a data collection form after consent had been obtained. This was done by the investigators or the research assistants who were trained to take part in the research. Parents and neonates demographic and clinical data was collected using a data collection form in (Appendix 1).

3.6.1 Sample collection, handling and transport

Diagnosis of sepsis was made by the attending paediatrician. After the consent was obtained from the parent/guardian, 1-3 ml of blood was obtained from the neonate by the pediatrician for culture using aseptic precautions: The skin at the venepuncture site was meticulously disinfected using a bactericidal disinfectant that comprised of tincture of iodine, polyvidone-iodine 10%, alcohol 70%. The disinfectant was allowed to dry before blood was drawn by a pediatrician. The specimen obtained was immediately inoculated into BACTEC Peds Plus^{TM}/F culture vials (enriched with Soybean-Casein digest broth with CO2). These vials used for aerobic blood cultures and they are manufactured by Becton, Dickson and company, United States. The Culture bottles with the specimen were then incubated at the Pumwani Hospital laboratory at 37 °C until transportation to the Department of Pediatrics laboratory of the University of Nairobi, School of Medicine. This transfer of the samples was done in temperature-maintained insulated cool boxes and did not take more than one hour. At the University laboratory, bacterial growth isolates were identified using standard microbiologic procedures outlined in Appendix 2 and their susceptibility pattern to commonly used antimicrobial agents was determined using the Kirby

and Bauer Disc Diffusion sensitivity test (112,113). Sensitivity testing was done for the following antibiotics that are used to treat neonatal sepsis at Pumwani Maternity Hospital: benzyl penicillin, gentamycin, ampicillin, ceftriaxone, ceftazidime, amikacin, vancomycin, flucloxacillin, meropenem, and amoxicillin-clavulanic acid. Categorization of antimicrobial susceptibility was done as resistant, intermediate or sensitive. The culture report obtained was recorded in a form (Appendix 1).

3.6.2 Treatment outcomes

Patients file records were reviewed to capture information on the treatment regimen, change of regimen and treatment outcomes: complications, discharges, length of stay in the hospital, or death. This was the Data Collection Form in Appendix 1.

3.7 Quality assurance and Data Management

All aspects of quality assurance were adhered to accordingly. All personnel and data collectors involved in the study were screened to determine whether they had proper qualification for the research. A data base on qualifications and experience was continuously maintained. All study personnel were trained on study objectives and relevant procedures. The data collectors were trained. The data collection tool was pre-tested on 10 subjects at the Kenyatta National Hospital to determine whether it was adequate and whether any modifications needed to be done. The same questions were put to a number of respondents to see if they give the required response.

Quality Assurance was ensured in all aspects, from the specimen collection done under aseptic conditions, to the handling and laboratory analysis. This was enhanced by having the procedures performed by qualified personnel and using appropriate biochemical tests and sensitivity discs. The specimens were analyzed at the University of Nairobi, Department of Pediatrics Laboratory, School of Medicine that has the capacity to carry out culture and sensitivity analysis. The principal investigator conducted audits at predetermined intervals and supervised other personnel to ensure the maintenance of quality. The Data obtained was kept under lock and key with access by only one individual, which ensured security. Data was backed up every day and was password Protected. External validity was ensured through appropriate non-biased sampling and adequate sample size.

3.8 Data analysis

Data was analyzed using SPSS version 21.0 software. All variables were subjected to descriptive and inferential statistics. This included mean, range, percentile and proportions. Comparison of proportions was performed using Exact Fisher's test and Chi-square. The data was presented in form of tables and figures as shown in chapter 4. For any comparisons a P-value of ≤ 0.05 was considered statistically significant.

3.9 Ethical considerations

Permission was sought from the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON-ERC) and Pumwani Maternity Hospital. Each of the respondents was given information on the rationale of the study and its objectives. They were then allowed to voluntarily opt to participate. They were asked to sign a consent form (Appendix 3) and a copy was given to them to keep. Confidentiality of the respondents as well as all records evaluated were observed. No names were recorded during the study. All information obtained was treated with confidentiality and only the chief investigator had access. After the completion of this study the information obtained was kept in a secured storage site for up to two years before destruction.

Risks involved like contamination was mitigated by ensuring the procedure was carried out by a qualified clinical practitioner and that the procedure was done under aseptic conditions to prevent development of any infections. The neonates were also monitored to ensure no complications arose when drawing blood from the baby. The neonates benefited from the findings in the study, since the laboratory results that were obtained guided treatment while they were in the hospital.

3.10 Dissemination Plan

The dissemination of information began with a brief presentation about the study and the study objectives to the Pumwani Hospital research committee. During the study period the investigator made available the culture and sensitivity results of the patient from the UoN, School of Medicine Pediatric department laboratory to the primary care giver that aided in management of the patients at Pumwani Maternity Hospital.

After completion of this study the findings on implicated organisms in sepsis and their susceptibility patterns were communicated to Pumwani Hospital in form of an end-of-study seminar. A copy of the dissertation was also be given to the Hospital.

The final write up was submitted to the Medical Library at UoN and another copy to the Department of Pharmaceutics and Pharmacy practice, which can be accessed by students and faculty members at the University of Nairobi.

A manuscript was prepared and published in a peer-reviewed, open-access biomedical journal, ensuring that the study findings can be accessed worldwide through the internet**.**

CHAPTER FOUR: RESULTS

4.1 Study population

Neonates admitted for suspected cases of sepsis were investigated for bacterial infection between March and August 2015. The age and sex distribution of the neonates are presented in Table 3.There were 78 (53%) males and 70 (47%) females resulting in a male to female ratio of 1.1:0.9. The mean age of the neonates at diagnosis was one day (SD of 3days). The number of neonates that presented with early-onset sepsis (EOS) were 138 (96.5%) neonates, while the rest 5 (3.5%) presented with late-onset sepsis (LOS) Figure 2. Among the neonates with EOS, 73 (52.9%) were male and 65 (47.1%) were female. Among the neonates with LOS, 2 (40%) were male and 3(60%) were female.

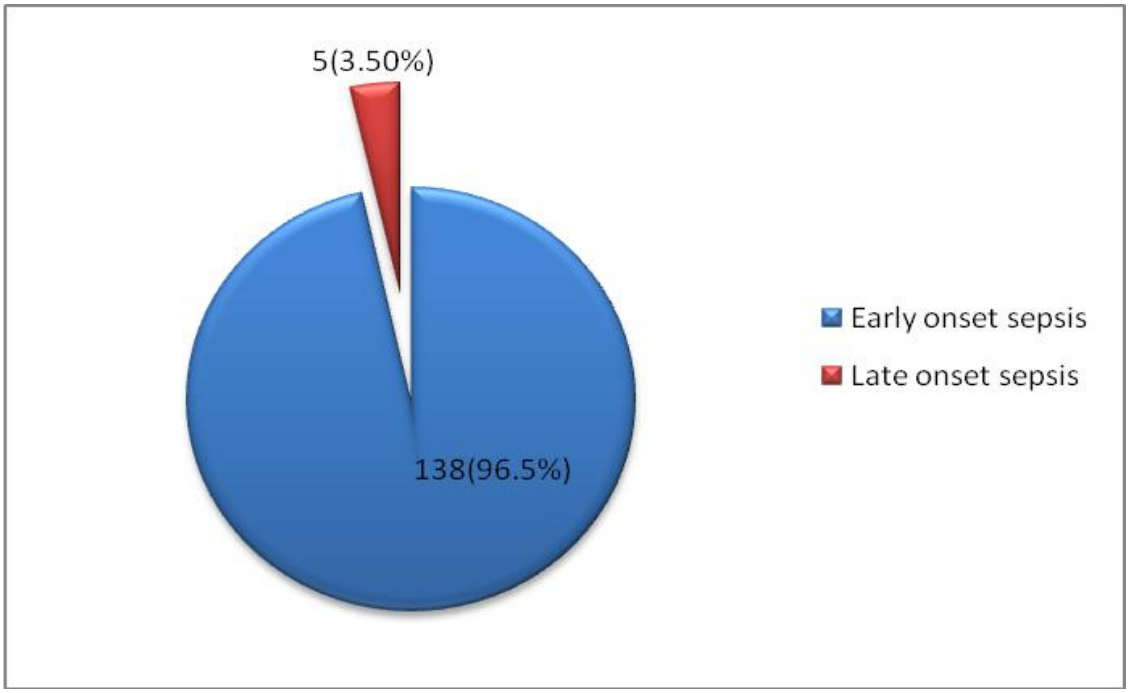
Table 3: Age and sex distribution of neonates with suspected neonatal sepsis

	Male n (%)	**Female n (%)**	**Total n (%)**
0-3 days (EOS)	73(97.3)	65(95.6)	138(96.7)
4-28 days(LOS)	2(2.7)	3(4.4)	5(3.3)
Total	75(100)	68(100)	143(100)

EOS: Early-onset sepsis

LOS: Late-onset sepsis

Figure 2: EOS and LOS Bacterial isolates prevalence



4.2 Maternal and neonatal social-demographic data

Most (n=126, 86%) of the maternal respondents had either a primary or secondary level of education. The minority had tertiary or no formal education at all (Table 4). Of the 150 neonates, 10 (6.8 %) were preterm. Majority of the neonates were born at term (89.7%). A few neonates were underweight (<2.5kg) and there were no very low birth weight (<1.5kg) neonates in this study where majority of the neonates had a normal weight.

Table 4: Maternal and neonatal socio-demographic data of the participants

Characteristic	n (%)
Maternal educational background	
Illiterate	3 (2.1)
Primary	32(21.9)
Secondary	94(64.4)
College and above	17(11.6)
Gestation age	
<37 weeks	10(6.8)
37 - 42 weeks	132(89.8)
42 weeks and above	5(3.4)
Birth Weight	
<1.5 kgs(VLBW)	0(0)
1.5 - <2.5 kgs(LBW)	10(6.7)
2.5 - <4.0 kgs(NW)	132(88.6)
>4 kgs(overweight)	7(4.7)
Place of delivery	
Hospital/Health center	148(98.7)
Home	2(1.3)
Apgar score at the 1st minute	
<5	11(8.7)
>5	115(91.3)
Apgar score at the 5th minute	
<5	9(7)
>5	118(93)

The mean birth weight and gestation age of the study population were 3.12kgs with a SD of 0.45kgs and 39weeks with a SD of 2 weeks, respectively. Most of the neonates were either born at the hospital or a health centre. Only two children were born at home. Majority of births (n=81,55.9%) were via Spontaneous Vaginal Delivery (SVD) mode of delivery, while those who were born through caesarian section were 64 (44.1%). In the first and fifth minute after birth, 11/126 (8.7%) and 9/127 (7%) of the neonates had an Apgar score less than 5. This information is summarized in Table 4.

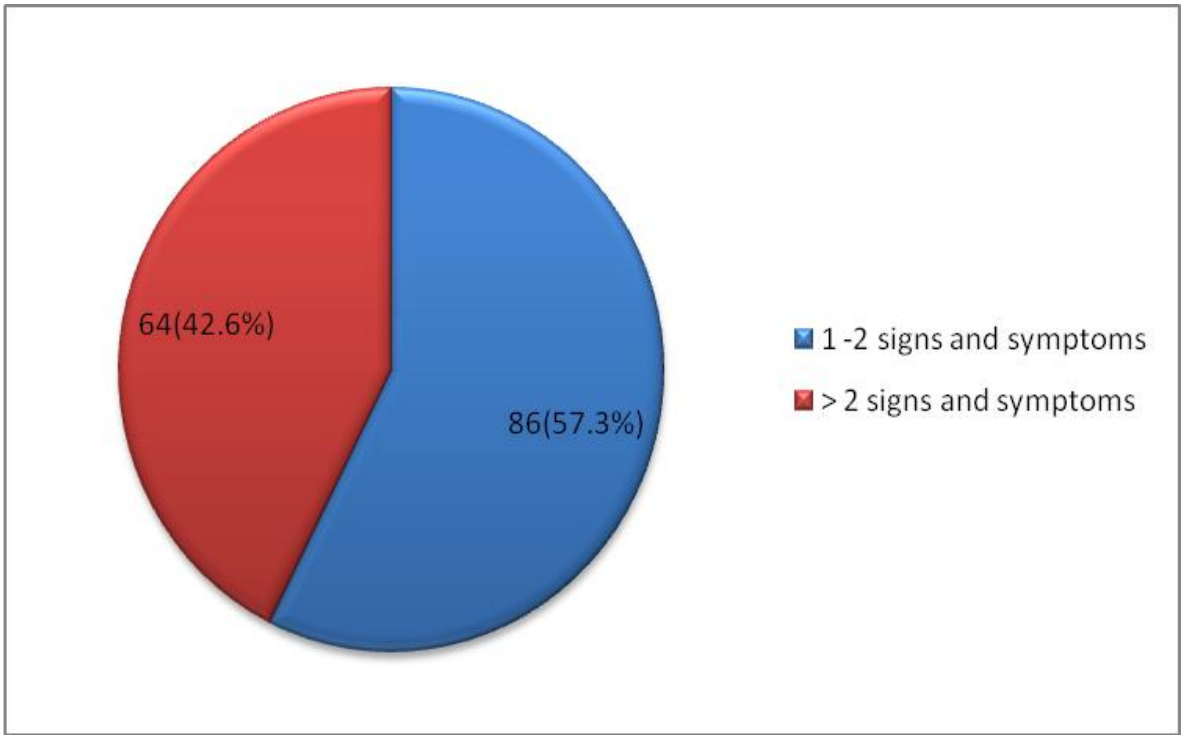
4.3 Signs and symptoms of neonates with suspected sepsis in early onset and late onset sepsis

The signs and symptoms that the neonates with EOS and LOS, presented with are summarized in Table 5 and Figure 3. The number of patients that had one or two signs was 86 (57.3%) while those with more than two signs and symptoms were 64 (42.6%). The most common features in EOS were jaundice 59 (42.4%) followed by irritability, respiratory distress, failure to feed and hyperthermia. In LOS hyperthermia and irritability were most prevalent features followed by jaundice and failure to feed. The features that cut across EOS and LOS were irritability, jaundice, failure to feed and hyperthermia. The other signs and symptoms observed were lethargy, vomiting, convulsions, abdominal distension, poor skin color, and hypothermia.

Table 5: Signs and symptoms of neonates with suspected sepsis according to age

Signs and symptoms	Age at diagnosis				Total	
	0-3(EOS)		4 - 28 days(LOS)			
	Frequency (n)	Percent (%)	Frequency (n)	Percent (%)	Frequency (n)	Percent (%)
Irritability	58	41.7	3	60	61	42.4
Jaundice	59	42.4	2	40	61	42.4
Respiratory distress	53	38.1	0	0	53	36.8
Failure in feeding	38	27.3	2	40	40	27.8
Hyperthermia	36	25.9	3	60	39	27.1
Dehydrated	15	10.8	0	0	15	10.4
Septic Rash	12	8.6	1	20	13	9.0
Caput	11	7.9	0	0	11	7.6
Vomiting	9	6.5	0	0	9	6.3
Lethargic	8	5.8	0	0	8	5.6
Poor Skin Colour	6	4.3	0	0	6	4.2
Convulsions	6	4.3	0	0	6	4.2
Hypothermia	4	2.9	0	0	4	2.8
Abdominal distension	4	2.9	0	0	4	2.8
Chest indrawing	4	2.9	0	0	4	2.8
Tachypnoea	4	2.9	0	0	4	2.8
Grunting	4	2.9	0	0	4	2.8
Cyanosis	2	1.4	0	0	2	1.4

Figure 3: Proportions represented by number of signs and symptoms in neonates with suspected sepsis

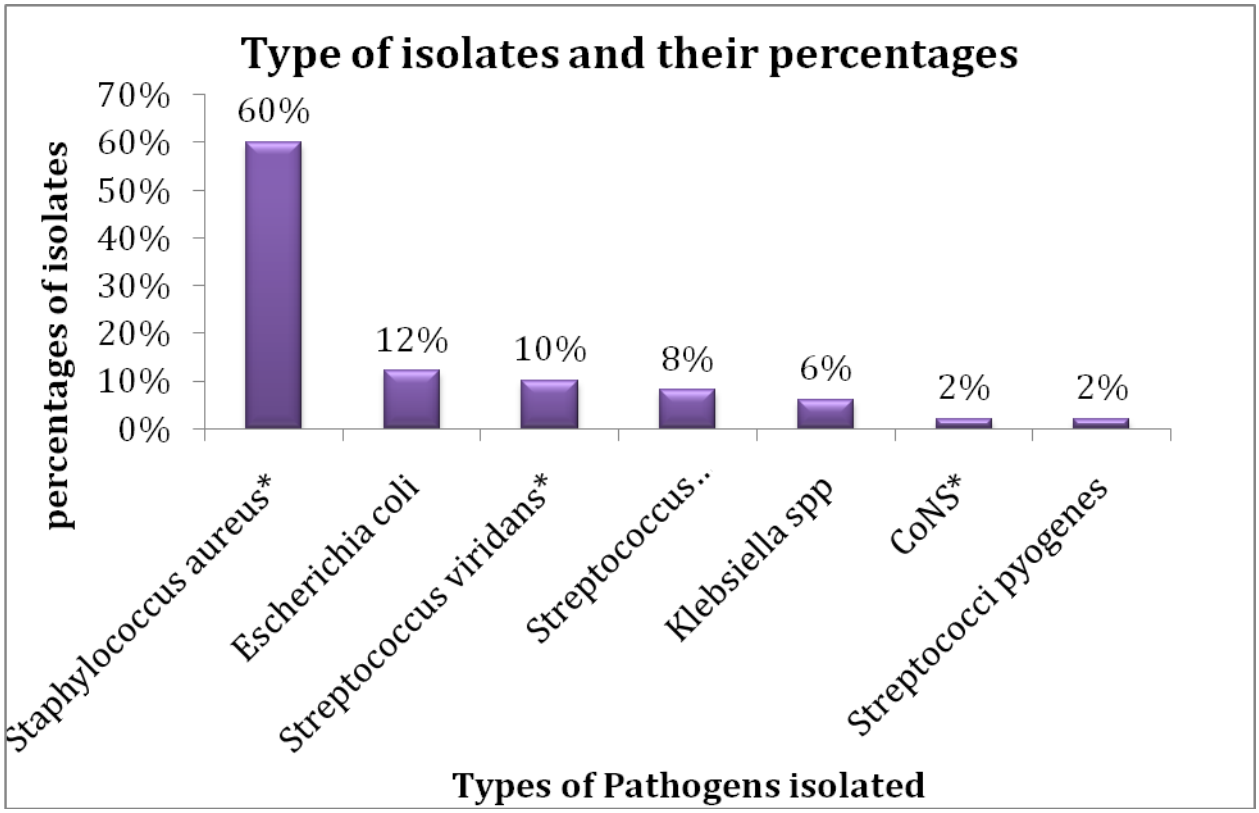


4.4: Etiological agents of suspected sepsis

4.4.1 The prevalence of bacteria causing neonatal sepsis

The prevalence of bacteria that cause neonatal sepsis is summarized in Figure 4. Out of the 150 neonates with suspected sepsis, 48 (32%) had a positive blood culture for bacterial sepsis. Of these, 46 (95.8%) had single organism while 2 (4.2%) had a combination of two pathogens isolated. These comprised of a combination of *Staphylococcus aureus* and *Streptococcus viridans* as well as *Staphylococcus aureus* and *Streptococcus pyogenes*. *Staphylococcus aureus* was the most common isolate (n=30, 60%), followed by *Escherichia coli* (n=6, 12%), *Streptococcus viridans* (n=5, 10%), *Streptococcus pneumoniae* (n=4, 8%), *Klebsiella* spp (n=3, 6%), *Streptococcus pyogenes* and Coagulase negative *Staphylococcus aureus* (CoNS) each had a prevalence of 2% (n=1). Gram negative and Gram positive bacteria accounted for 9/50 (18%) and 41/50 (82%) of the isolates respectively (Figure 5).

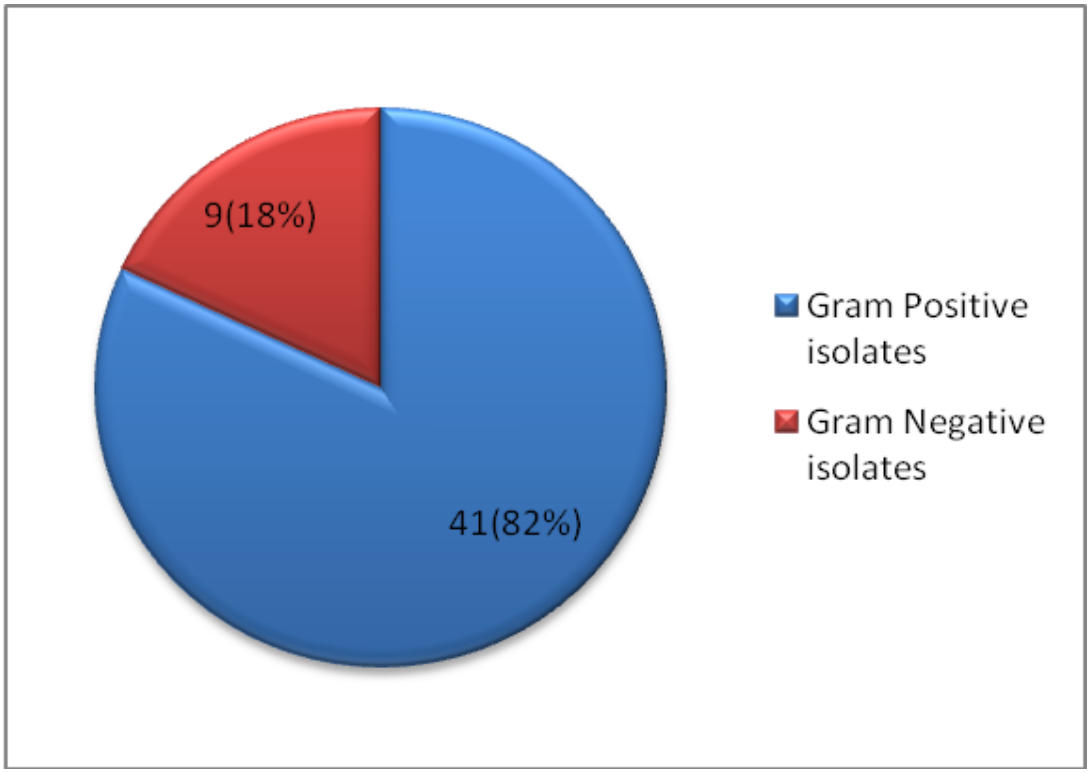
Figure 4: The prevalence of bacteria causing neonatal sepsis



*Two patients had a growth of two organisms (that comprised of *Staphylococci aureus* plus *Streptococci viridans* and *Staphylococci aureus* plus *Streptococci pyogenes*)

*CoNS: Coagulase negative *Staphylococcus aureus*

Figure 5: Prevalence of gram negative and gram positive bacteria



4.5.2: Early and late onset sepsis

Among the150 neonates admitted with cases of suspected EOS, 46 (30.6%) had sepsis confirmed by a positive blood culture. *Staphylococcus aureus* was the predominant pathogen (29 (60.4%)), followed by *Escherichia coli* and *Streptococcus viridans* .The other isolates are shown in Table 6. In LOS there were 2 (4%) culture positive isolates, namely; *Staphylococcus aureus* and *Streptococcus pneumoniae* (Table 6). There was no statistical significance seen in the different types of sepsis.

Table 6: Bacteria isolated from neonates with suspected sepsis

Pathogen	Age		
	<3days(EOS) n (%)	>3-28days(LOS) n (%)	p value
Gram positive			
*Staphylococcus aureus**	29 (60.4)	1 (50)	0.800
Streptococcus viridans	5 (10.4)	0 (0)	0.808
Streptococci pneumoniae	3 (6.25)	1 (50)	0.160
Coagulase Negative Staphylococcus aureus	1 (2.08)	0 (0)	0.960
Streptococcus Pyogenes	1 (2.08)	0 (0)	0.960
Gram negative			
Escherichia coli	6 (12.5)	0 (0)	0.772
Klebsiella spp	3 (6.25)	0 (0)	0.882
Total	48(96%)	2(4%)	

*For two neonates two organisms were isolated.

4.5 Antimicrobial susceptibility patterns

Antimicrobial sensitivity analysis was carried out and the bacteria were classified into three categories: susceptible, intermediate and resistant. Susceptible means the bacteria cannot grow if the drug is present and indicates an effective antibiotic. Resistant means the organisms grow even in the presence of drug indicating an ineffective antibiotic. Intermediate means a higher dose of the antibiotic is needed to prevent growth of the pathogens.

4.5.1 Antimicrobial susceptibility of *Staphylococcus aureus and* Coagulase Negative *Staphylococcus aureus*

The antimicrobial sensitivity for *Staphylococcus aureus* is summarized in Table 7. The highest sensitivity was seen with meropenem at 30 (100%) followed by ofloxacin, gentamicin and ceftriaxone. There were intermediate sensitivity seen with benzylpenicillin, ceftriaxone and ofloxacin. Piperacillin showed the highest resistance with 12 (40%) of the isolates demonstrating resistance to the drug. Benzyl penicillin resistance was observed in 5 (17%) of the isolates. There were intermediate sensitivity in the following antimicrobials: benzyl penicillin, ceftriaxone, ofloxacin and ciprofloxacin.

There was one isolate of CoNS that was resistant to ofloxacin and totally susceptible to penicillins, third generation cephalosporins. There were no intermediates seen with this organism (Table 7).

Generally there is a good sensitivity to the aminoglycosides, and third generation cephalosporins. There is also a good sensitivity to the later generations of fluroquinolones and quinolones (but this class is contraindicated in children). There was least sensitivity to penicillins even piperacillin with this organisms.

Table 7: Antimicrobial susceptibility of Gram positive isolates

ANTIBIOTIC	**BACTERIA**				
	***Staphylococcus aureus* (n=30**	***Streptococcus viridans* (n=5)**	***Streptococcus pneumoniae* (n=4)**	**CoNS* (n=1)**	***Streptococcus Pyogenes* (n=1)**
Ampicillin	11 (37%) **	4 (80%)	3 (75%)	1 (100%)	1 (100%)
Benzyl penicillin	12 (40%)	4 (80%)	3 (75%)	1 (100%)	1 (100%)
Piperacillin	18 (60%)	5 (100%)	4 (100%)	1 (100%)	1 (100%)
Amoxicillin Clavulanic Acid	13 (43%)	5 (100%)	3 (75%)	1 (100%)	1 (100%)
Gentamicin	27 (90%)	5 (100%)	4 (100%)	1 (100%)	1 (100%)
Amikacin	29 (97%)	5 (100%)	4 (100%)	1 (100%)	1 (100%)
Ceftriaxone	26 (87%)	4 (80%)	4 (100%)	1 (100%)	1 (100%)
Ceftazidime	10 (33.3%)	3 (60%)	2 (50%)	1 (100%)	1 (100%)
Flucloxacillin	13 (43%)	3 (60%)	1 (25%)	1 (100%)	-
Ofloxacin	29 (97%)	5 (100%)	3 (75%)	0 (0%)	1 (100%)
Ciprofloxacin	-	4 (80%)	-	1 (100%)	1 (100%)
Meropenem	30 (100%)	5 (100%)	4 (100%)	1 (100%)	1 (100%)

*CoNS: Coagulase negative *Staphylococcus aureus*

(-) Represents those that were not tested at all

4.5.2 Antimicrobial Susceptibility of *Streptococcus* species

The Streptococcus species isolated were: *Streptococcus viridans, Streptococcus pneumoniae,* and *Streptococcus pyogenes* (Table 7). *Streptococcus viridans* showed a good sensitivity to penicillins, aminoglycosides, third generation cephalosporins and meropenem. They showed some resistance to fluroquinolones and quinolones but not ofloxacin. Intermediate sensitivity was observed with ceftriaxone and flucloxacillin.

Streptococcus pneumoniae was resistant to flucloxacillin and ciprofloxacin. There was a good sensitivity to penicillins, aminoglycosides, third generation cephalosporins and carbepenems. Intermediate sensitivity was observed in flucloxacillin and ofloxacin. There was one isolate of *Streptococcus pyogenes* that was absolutely sensitive to all antibiotics tested.

4.5.3 Antimicrobial Susceptibility of Gram negative isolates

Escherichia coli and *Klebsiella* spp were the gram negative isolates obtained. Their antimicrobial sensitivities are presented in Table 8. There was absolute sensitivity to meropem in these organisms. *Klebsiella* spp showed a good sensitivity 100% to piperacillin and amoxicillin clavulanic acid. The highest resistance to ampicillin 67% was observed and also some resistance to, fluroquinolones, benzyl penicillin and amikacin 33%. Generally least resistance to aminoglycosides especially with gentamicin and third generation cephalosporins observed.

Escherichia coli showed the highest resistance with to ampicillin 50%, followed by penicillins. Some resistance to aminoglycosides and third generation cephalosporins was also observed. There was also one intermediate sensitivity observed in flucloxacillin. They showed a good sensitivity above 80% to ofloxacin, amikacin and gentamicin

Table 8: Antimicrobial sensitivity of Gram negative bacteria

ANTIBIOTIC	BACTERIA	
	***Escherichia coli* (n=6)**	***Klebsiella* spp (n=3)**
Ampicillin	1 (17%)	0 (0%) (**resistance 67%)**
Benzyl penicillin	4 (67%)	2 (67%)
Piperacillin	4 (67%)	3 (100%)
Amoxicillin Clavulanic Acid	4 (67%)	3 (100%)
Gentamicin	5 (83%)	3 (100%)
Amikacin	5 (83%)	2 (67%)
Ceftriaxone	4 (67%)	3 (100%)
Ceftazidime	3 (50%)	1 (33%)
Meropenem	6 (100%)	3 (100%)
Flucloxacillin	3 (50%)	1 (33%)
Ofloxacin	6 (100%)	2 (67%)
Ciprofloxacin	-	1 (33%)

(-) Represents those that were not tested

4.6: Antibiotic prescription patterns in the newborn unit

The number of antibiotics used per neonate, antibiotics prescribed and the various regimens used to treat neonatal sepsis are summarized in Table 9. All neonates with suspected sepsis were treated with antibiotics. The most commonly prescribed regimen was benzylpenicillin and gentamicin at 123 (82%). Two neonates received 4 or 5 different types of antibiotics at different periods in their treatment. Only one patient was on the ceftriaxone and amikacin regimen.

Table 9: Antibiotics prescribed and regimens used in neonatal sepsis

Antibiotics prescribed and regimens used to treat sepsis	Frequency (n)	Percent (%)
Number of antibiotics prescribed per patient		
1	5	3.3
2	130	86.7
3	12	8.0
4	1	0.7
5	1	0.7
None	1	0.7
Type of antibiotics prescribed		
Benzyl Penicillin	128	85.3
Gentamycin	128	85.3
Amikacin	1	0.7
Flucloxacillin	7	4.7
Ceftriaxone	6	4.0
Ceftazidime	3	2.0
Amoxclav*	2	1.3
Metronidazole	3	2.0
Antibiotic regimens prescribed		
Benzylpenicillin plus Gentamycin	123	82.0
Flucloxacillin plus Gentamycin	6	4.0
Ceftriaxone	6	4.0
Ceftriaxone plus Gentamycin	5	3.3
Ceftriaxone plus Amikacin	1	0.7
Ceftazidime	3	2.0
Amoxicillin Clavulanic Acid	2	1.3
Others	4	2.7

* Amoxicillin clavulanic acid

4.7: Association between signs and symptoms of neonatal sepsis and culture proven sepsis

Neonatal risk factors associated with sepsis are outlined in Tables 10 and 16 in Appendix 5. Hyperthermia and vomiting were the only features that were associated with positive blood culture (p=<0.05). The other signs and symptoms were difficulty in breathing, irritability, lethargy, hypothermia, cyanosis, diarrhea, jaundice, abdominal distention, septic rash and chest indrawing, all of which were not statistically significant. In the signs and symptoms (1-2 signs and >2 signs) there was no association seen, where an increase in the number of signs and symptoms presenting during admission did not predict the culture growth in suspected sepsis. Also there was no association observed between number of signs and symptoms, and duration of inpatient treatment.

Table 10: Number of signs and symptoms in relation to culture growth

		Grouped number of signs and symptoms			
		1-2 signs/symptoms	**>2 signs/symptoms**	**OR[95% CI of OR**	**P value**
Culture growth					
No growth	**n (%)**	60 (69.8)	42 (65.6)	1.21 [0.61 – 2.41]	0.591
Growth	**n (%)**	26 (30.2)	22 (34.4)		
Length of stay					
<7 days	**n (%)**	65 (78.3)	48 (80)	0.90 [0.40 – 2.05]	0.807
7 days and above	**n (%)**	18(21.7)	12 (20)		

4.8 Risk factors associated with blood culture proven sepsis

4.8.1 Association between Neonatal risk factors with culture proven sepsis

The analysis of possible neonatal risk factors associated with blood culture proven sepsis is shown in Table 11. There was an association observed between the sex of the neonate and the possibility of having culture proven sepsis (OR: 2.3, 95% CI: 1.1-4.8, p=0.018). Male neonates were more likely to have culture proven sepsis. There was no statistical significance observed with the neonate's gestation age, birth weight, mode of delivery, APGAR scores, history of maternal fever and chorioamnionitis among others.

Table 11: Neonatal risk factors associated with culture proven neonatal sepsis

Variables		Growth					
		Growth		No growth			
		n	Percent	n	Percent	OR [95% CI of OR]	P value
Neonate Gender	Male	32	41.0	46	59.0	2.3 [1.1 – 4.8]	**0.018**
	Female	16	22.9	54	77.1		
Grouped gestation age	<37 weeks	2	20.0	8	80.0	-	0.544
	37 - 42 weeks	45	34.1	87	65.9		
	42 weeks and above	1	20.0	4	80.0		
Mode of delivery	SVD (Normal)	26	32.1	55	67.9	1.0 [0.5 – 2.1]	0.913
	Caeserian section	20	31.3	44	68.8		
Birth weight	<1.5 kgs	0	.0	0	.0	-	0.670
	1.5 - <2.5 kgs	2	20.0	8	80.0		
	2.5 - <4.0 kgs	44	33.3	88	66.7		
	4 kgs and above	2	28.6	5	71.4		
APGAR at 1 min	<5	3	27.3	8	72.7	0.7 [0.2 – 2.8]	0.616
	5 and above	40	34.8	75	65.2		
APGAR at 5 min	<5	1	11.1	8	88.9	0.2 [0.02 – 1.9]	0.135
	5 and above	42	35.6	76	64.4		

4.8.2 Maternal risk factors associated with blood culture positivity

The analysis of possible maternal risk factors associated with blood culture sepsis is shown in Table 12. Neonates born to mothers with a history of premature rapture of membranes (OR: 3.3, 95% CI: 0.1-1.1, p=0.049) are at a higher risk of developing culture proven neonatal sepsis. The other perinatal risk factors in this study included; history of antenatal care, maternal fever and chorioamnionitis.

Table 12: Maternal risk factors associated with culture proven neonatal sepsis

		Growth					
		Growth		No growth			
		n	Percent	n	Percent	OR [95% CI of OR]	P value
History of antenatal care	No	6	27.3	16	72.7	0.7 [0.3 – 2.1	0.607
	Yes	42	32.8	86	67.2		
History of Maternal fever	No	44	31.9	94	68.1	0.9 [0.3 – 3.3]	0.918
	Yes	4	33.3	8	66.7		
History of Chorioamnionitis	No	44	33.6	87	66.4	1.9 [0.6 – 6.1]	0.274
	Yes	4	21.1	15	78.9		
History of premature rupture of membranes	No	42	30.0	98	70.0	3.3 [0.1 – 1.1]	**0.049**
	Yes	6	60.0	4	40.0		

4.9 Pathogens isolated in relation to the inpatient treatment duration

The associations between isolated pathogens from the neonates with suspected sepsis and relation to duration of inpatient treatment are summarized in Table 13.

Table 13: Pathogens isolated in relation with duration of in treatment

Organisms		length of stay			
		<7 days	7 days and above		
		n (%)	n (%)	OR [95% CI of OR]	P value
Streptococci pneumoniae	No	110 (79.1)	29 (20.9)	1.01 [0.59 – 1.87]	0.841
	Yes	3 (75)	1 (25)		
Streptococci viridans	No	109 (79)	29 (21)	0.94 [0.10 – 8.73]	0.956
	Yes	4 (80)	1 (20)		
Coagulase Negative *Staphylococci*	No	112 (78.9)	30 (21.1)	-	0.605
	Yes	1 (100)	0 (0)		
Staphylococci aureus	No	90 (76.9)	27 (23.1)	0.43 [0.12 – 1.56]	0.191
	Yes	23 (88.5)	3 (11.5)		
Escherichia coli	No	108 (79.4)	28 (20.6)	1.54 [0.11 - 1.86]	0.613
	Yes	5 (71.4)	2 (28.6)		
Klebsiella spp	No	112 (80)	28 (20)	8.02 [0.21 -1.36]	**0.049**
	Yes	1 (33.3)	2 (66.7)		
Streptococci pyogenes	No	112 (78.9)	30 (21.1)	-	0.605
	Yes	1 (100)	0 (0)		

The patients from whom the *Klebsiella* spp was isolated, had an increased inpatient treatment period (OR: 8.02, 95% CI: 0.21 -1.36, p=0.049). The rest of the isolates showed no statistical significance in relation to the inpatient treatment duration.

4.10 Relation of antibiotic use and duration of treatment and mortality

The inpatient treatment period for the neonates with suspected sepsis ranged from 0 to 14 days, where the majority of the neonates 120 (80%), were treated in less than seven days. In this study 5 (3.3%) patients developed complications, 5 (3.3%) neonates succumbed to death and the remaining 140 (93.3%) got well and were discharged.

4.10.1 Antibiotic treatment regimen in relation to duration of inpatient treatment

The association between antibiotic regimen and duration of stay in inpatient treatment is presented in Table 14. There was an association observed between some regimens used in treatment of neonatal sepsis in relation with the duration of inpatient treatment. The regimens that comprised of ceftriaxone plus gentamycin (OR: 6.17 95% CI:0.98 – 38.75,p=0.029), ceftriaxone plus amikacin (p=0.051), ceftazidime (OR: 8.0 95% CI: 0.70 – 91.4: p=0.049), amoxicillin clavulanic acid (p=0.006) were seen to reduce the length of the inpatient treatment period compared to other regimens.

Table 14 : Antibiotic treatment regimen in relation to duration of inpatient treatment

Regimens prescribed		Grouped length of stay			
		<7 days n (%)	7 days and above n (%)	OR[95% CI of OR]	P value
Benzyl and Gentamycin	No	22 (88)	3 (12)	2.1 [0.65 – 9.7]	0.225
	Yes	91 (77.1)	27 (22.9)		
Flucloxacillin and Gentamycin	No	108 (78.8)	29 (21.2)	0.7 [0.08 – 6.63]	0.791
	Yes	5 (83.3)	1 (16.7)		
Ceftriaxone	No	110 (80.3)	27 (19.7)	4.07 [0.78 –21.31]	0.074
	Yes	3 (50)	3 (50)		
Ceftriaxone and Gentamycin	No	111 (80.4)	27 (19.6)	**6.17 [0.98 – 38.75]**	**0.029**
	Yes	2 (40)	3 (60)		
Ceftriaxone and Amikacin	No	113 (79.6)	29 (20.4)	**-**	**0.051**
	Yes	0 (0)	1 (100)		
Ceftazidime	No	112 (80)	28 (20)	**8.0 [0.70 – 91.4]**	**0.049**
	Yes	1 (33.3)	2 (66.7)		
Amoxicillin Clavulanic Acid	No	113 (80.1)	28 (19.9)	**-**	**0.006**
	Yes	0 (0)	2 (100)		

4.11 Multivariate analysis

4.11.1 Independent predictors for growth

The Independent predictors of growth in neonatal sepsis are presented in Table 15. Gender of the neonates (male) is strongly associated (p=0.007) with neonatal sepsis independent of other factors. Among the signs and symptoms, Hyperthermia is also significantly associated (p=0.002) with neonatal sepsis independent of other factors. The maternal risk factor: a history of premature rupture of membranes could be associated with neonatal sepsis independent of other factors.

Table 15: The independent predictors of growth in neonatal sepsis

Variable	Coefficient	S.E. of the coefficient	P value	OR	95% CI for OR	
					Lower	Upper
Gender of the neonate(male)	1.060	0.396	**0.007**	2.888	1.329	6.273
Hyperthermia	-1.284	0.413	**0.002**	.277	0.123	0.622
History of premature rupture of membranes	-1.322	0.694	.057	.266	0.068	1.038

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The majority of the neonates in this study were one day old and the proportions of both sexes were almost equal. Most of the neonates had a normal birth weight and were delivered via Spontenous Vaginal Delivery. Almost all the neonates in the study were born within the hospital. Babies born at the hospital and discharged are not admitted back at the facility in case they fall sick. They are seen at the outpatient clinic and then referred elsewhere for admission.

The clinical presentation of neonatal sepsis can be with or without focal signs of neonatal sepsis. Often the early signs are non specific, such as irritability, failure to feed, difficulty in breathing, lethargy, poor skin color (50). Clinical features of sepsis in the this study are similar to those reported in Tanzania (25) and Uganda (61) which included inability to feed, lethargy, convulsions, hypothermia, hyperthermia, jaundice, skin rash, cyanosis and chest in drawing. Umbilical redness and umbilical pus discharge, hypoglycemia and hyperglycemia were reported in these studies but were not observed in the present study. The most common signs and symptoms were jaundice, inability to feed and respiratory distress, which is comparable to the findings in Tanzania and India (25,26). Early onset sepsis (EOS) was characterized by jaundice, irritability, hyperthermia and failure to feed but in late onset sepsis (LOS), hyperthermia and irritability were observed. There was no statistical significance in the association between the number of signs and symptoms with culture positivity and increase of the length say in the hospital. Clinical assessment using a combination of signs and symptoms may be a useful guide in the provisional diagnosis of neonatal sepsis.

The outcome of treatment of neonates with infections is strongly related to appropriate diagnosis and management. Diagnosis is however a challenge in cases where the signs and symptoms are not specific or not available. As a consequence, deciding whether to treat or not, balancing optimal patient care with varied aspects of antibiotic resistance and possible adverse effects is a challenge. In line with this recognition of risk factors of neonatal sepsis are very relevant in settings where there is resource constraints and as a contribution to diagnostics. This knowledge will also help in coming up with strategies to mitigate sources of infection in the hospital to eventually minimize morbidity and mortality related to sepsis. In this study male neonates were

at risk of developing culture proven neonatal sepsis, an observation made in a similar study (69). Premature rapture of membranes (PROM) was the only maternal risk factor that was associated with positive culture in the both LOS and EOS. Neonates born to mothers with PROM>18hrs were likely to develop sepsis as also (25,61).

The prevalence of the neonates with culture proven sepsis was 32% which was comparable to the rates reported in Uganda, Nigeria and India (61–63). In contrast lower yield (15%) were reported in Saudi Arabia (64) and higher ones in Ethiopia, Nigeria and Pakistani (59,65,66). EOS contributed 96.% of the cases similar with findings from India ,(15). In contrast studies done in Kenya, Gambia, Papua New Guinea, Philippines and Ethiopia by WHO reported LOS was more common (72,85). The difference in the definition of could be the explanation of the differences seen in the percentages and also because Pumwani hospital has a policy of not admitting neonates once they are discharged home unless they are emergencies. Therefore the information on LOS was a challenge to capture since most of the neonates are referred on re-admission.

Blood culture remains the gold standard in diagnosis of neonatal sepsis, where the results take 48-72hrs.This necessitates the use of antibiotics empirically (35,88). For rational prescribing knowledge of the causative agents and their sensitivity is important. In this study out of 150 neonates with suspected sepsis, 48 (32%) had a positive blood culture. Of these, only two neonates had more than one isolate. The frequency of isolation of Gram positive bacteria (GPB) and Gram negative bacteria (GNB) was 96% and 4% respectively. This is comparable with other studies (19,54,63,67,91) that showed that GPB was responsible for most cases in the isolates. In contrast other studies reported the predominance of the GNB (3,53,57,80) but in another study there was almost equal prevalence observed (91).

Staphylococcus aureus was the predominant pathogen isolated in this study that accounted for 60.4% this is comparable with the study done by Mugalu *et al*., (61). This was followed by *Escherichia coli*, *Streptococcus viridans, Streptococci pneumoniae, Klebsiella* spp, *Streptococcus pyogenes,* and Coagulase negative *Staphylococcus aureus*. *Staphylococcus aureus* was the predominant isolate of the GPB whereas *E.coli* was the most common GNB isolate. This finding is consistent with other studies (34,78).

In EOS GPB accounted for 81% of the cases and 19% accounted for the GNB. The predominant organism in EOS was *Staphylococcus aureus* followed by *E. coli.* This is comparable with studies that isolated *Staph aureus* as the predominant organism (55,59,74,). It is also comparable to the studies that isolated *E coli* as the second most common isolate (66,83).In contrast some other studies (17,25,63,66,75,76) showed varied isolates.

In LOS there were only two isolates of which both were Gram positive that comprised of *Staphylococcus aureus* and *Streptococcus pneumoniae.* This is comparable with studies that isolated *Staphylococcus aureus* among other organism in the LOS (3,25,53,59) which contrasted the observations made in our study. There was a study that reported the predominance of CoNS in LOS (78); however in the current study there was no isolate of CoNS in LOS, which comprised of only 2% of isolates in EOS. In general there are studies that showed different and varied organisms in their isolates, which were not part of the spectrum of isolates in the present investigation. This included Group B *Streptococci*, Group A *Streptococci*, *Hemophilus influenzae*, *Pseudomonas* spp, *Acinetobacter* spp, *Enterobacter* spp and *Citrobacter* spp. *Staphylococcus agalactiae* was also seen to be the most common isolate in some studies (75,76) which was not the case in the present investigation.

Streptococci viridans was the third most common isolate in this study but the there was no comparative seen in literature. The spectrum of isolates is generally similar with those of the developing countries (34,54,56). A difference was observed in the type of bacteria that is predominant., Gram positive bacteria comprised of the majority of the isolates unlike in many studies in the developing countries where the Gram negative bacteria were most common isolates (3,25,63,66). The isolates in early and late onset sepsis though are seen to be the same in the developing countries is not the case in the developed countries where they are distinct (28,89). The spectrum of bacteria and their susceptibility patterns may vary depending on the prevailing conditions especially antimicrobial use. Antibiotic resistance has become a global problem, evidenced by several reports show a trend of increasing resistance to commonly used antibiotics in the developing world (3,28,80). *Staphylococcus aureus* Gram showed highest sensitivity to meropenem (100%), gentamicin, ceftriaxone and amikacin which was similar with the findings in other studies (25,39,40,53,80,83). Amikacin showed the best sensitivity among the aminoglycosides which is consistent with other studies(83). In contrast some studies (39,40)

reported a high resistance to 3rd generation cephalosporins especially ceftriaxone which was not the case in the current study. Some resistance was seen with piperacillin (40%), ampicillin (10%), benzylpenicillin (17%), and amoxicillin clavulanic acid showed the highest resistance at (57%). This concurred with other finding in various studies (59,82) some studies have reported Methicillin resistant *Staphylococcus aureus* (MRSA) show resistance to amikacin (40). This was not covered in the current study.

The other Gram positive organisms isolated were; *Streptococcus viridans, Streptococci pneumoniae, Streptococcus pyogenes,* and Coagulase negative *Staphylococcus aureus*. They generally showed an absolute sensitivity to meropenem. This was consistent with the other findings (40,66). *Streptococcus pneumoniae* also showed an absolute sensitivity to piperacillin, gentamicin, ceftriaxone and amikacin which concurs with the study done by Sharma *et al*., (80). The highest resistance was seen with flucloxacillin which concurs with other studies (15,80). Only one isolate of CoNS was obtained that showed absolute resistance to ofloxacin but susceptible to ampicillin, benzylpenicillin, gentamicin, ceftriaxone, flucloxacillin, ciprofloxacin and amikacin. This was in contrast with a previous study that showed 96% resistance to ampicillin and penicillin with 72% sensitivity to gentamicin (87).

Escherichia coli showed a high resistance to ampicillin, mild resistance to ceftriaxone, piperacillin, and amoxicillin clavulanic acid. This is comparable with the findings in various studies (25,40,66,88). A good sensitivity was observed in meropenem, ofloxacin, gentamicin, and amikacin. This is similar with what's reported in other studies (25,66,83).

Klebsiella pneumoniae was isolated in EOS only, and it exhibited an absolute sensitivity to piperacillin, gentamicin, ceftriaxone, meropenem and amoxicillin clavulanic acid as observed in similar studies (66,80). A high resistance was observed with ampicillin, some resistance was also seen towards benzylpenicillin, flucloxacillin, ofloxacin and amikacin. This is comparable to some studies that observed a high resistance especially to ampicillin (39,40). In contrast no resistance was observed against 3rd generation cephalosporins and gentamicin as reported in some studies (15,25,80).

Our results for the sensitivity patterns generally demonstrated that meropenem was the ultimate antibiotic of choice in case of treatment failure with the other regimens. The isolates from the

blood culture from both Gram negative and Gram positive showed a low resistance to ciprofloxacin, amikacin, gentamicin, benzylpenicillin and gentamicin. Some resistance was observed with flucloxacillin in GNB and GPB, Ofloxacin also showed some resistance towards GPB. There was observed a high resistance by GPB towards amoxicillin clavulanic acid. Similarly a high resistance pattern was observed toward ampicillin by the GNB and not necessarily form cephalosporin or gentamicin as observed in other studies. It was also observed a statistical significance of some regimen used to treat sepsis that led to a reduction in the duration of inpatient treatment regimen that included; ceftriaxone plus gentamicin, ceftriaxone plus amikacin, ceftazidime and amoxicillin clavulanic acid. It is thus preferable to use regimens that comprise of combined antibiotics of a ceftriaxone with an aminoglycoside like amikacin or gentamicin.

In this study there was no comparison done to determine the superiority of one regimen to the other. Snelling *et al*., (94) compared ceftazidime to gentamicin plus benzylpenicillin and found none of the regimens superior to the other.

5.2 Conclusion

Staphylococcus aureus and *Escherichia coli* are the most common pathogens implicated in neonatal sepsis. Premature rapture of membranes >18hrs and male gender were the risk factors strongly associated with culture proven neonatal sepsis. All the organisms showed absolute sensitivity to meropenem. GPB exhibited a high resistance to amoxicillin/clavulanic acid and GNB showed substantial resistance to ampicillin. The regimens that showed a statistical significance were ceftriaxone plus amikacin and ceftriaxone and gentamicin.

5.3 Limitations to this study

The anticipated sample size of 196 neonates with suspected sepsis, which could have given us precedence sensitivity and precision, was not attained due to unavoidable administration issues at the study site during the study period. The short study period was inadequate to assess for periodic and seasonal variations in frequency of causative organisms and a recent intrapartum antibiotics use could have reduced the possibility of detecting causative organisms and may have influenced the types and antimicrobial susceptibility of isolated bacteria. The Laboratory did not have the capacity to analyze anaerobic bacteria and only a single blood culture was performed from each neonate for isolation and identification. Due to stock outs of some of the

antimicrobials for sensitivity testing it was not possible to subject all the isolated organisms to the antibiotics included in the study.

5.4 Recommendations

5.4.1 Recommendations for policy and practice

A routine bacterial surveillance of prevalent organisms and the study of the sensitivity patterns of the pathogens responsible for neonatal sepsis to be made an essential component of neonatal care. This information from many parts of the country will be important in policy making on antimicrobial use not only locally but also internationally.

A part from a blood sample for investigation, other clinically relevant samples should be investigated since the neonates can have other coexisting infections.

5.4.2 Recommendations for research

A research on the role of anaerobic bacteria and fungi in neonatal sepsis should be carried out as well as studies on simple and sustainable interventions to help reduce the burden of neonatal sepsis.

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APPENDICES

Appendix 1: Questionnaire

*Please fill **ALL** sections in by interviewing the patient's caregiver.*

Questionnaire Serial No.			Date(dd/mm/yy)	[ ]/[ ]/[ ]
Data Collectors Code	[1] [2] [3] [4] [5] [6] [7] [8]			
1.0Patient's Biodata				
1.1Personal details **A)Neonate**				
I)Demographic Data/General data				
1.1.1Gender	[0]Male		[1]Female	
1.1.2 Date of birth(dd/mm/yy)	[ ] Don't know		[ ]/[ ]/[ ]	
1.1.3 Birth Weight	.....kg	1.1.4 Gestational age	.....wks .....days	
1.1.5 Date of admission	[ ]/[ ]/[ ]			
1.1.6 Date of Discharge	[ ]/[ ]/[ ]			
1.1.7 Ward of Admission	[1]..... [2]..... [3].....			
II) clinical data				

Signs and symptoms of sepsis.	**Yes[1]**	**No[2]**	**Signs and symptoms of sepsis**	**Yes[1]**	**No[2]**
1.Irritability	.....	.....	7.Cyanosis	.....	.....
2.Respiratory distress	.....	.....	8.Diarrhoea	.....	.....
3.Lethargic	.....	.....	9.Vomiting	.....	

4.Failure in feeding	.....	.....	10.Jaundice	.....	.....
5.Hyperthermia	.....	.....	11.Abdominal distension	.....	.....
6. Hypothermia	.....	.....	12.APGAR score at **1**and **5** minute	.....	.....
			Other (specify).....	.....	.....

B)Mother/Guardian

I.SOCIAL -DEMOGRAPHIC DATA

1.1.8 Educational background. [1] illiterate [2]primary school [3] Secondary school [4] college and above

II.OBSTETRIC DATA	1.1.9 History of antenatal care [1] Yes [2]No
	1.1.10 History of Maternal fever [1] Yes [2] No
	1.1.11 History of Chorioamnionitis [1] Yes [2] No
	1.1.12 History of premature rupture of membranes [1]Yes [2] No If yes how long (in hours).....
	1.1.13 History of **previous medication** [1] Yes [2] No If yes specify what medication..... What was the treatment.....?
	1.1.14 Medication in Pregnancy [1]Yes [2] No If yes specify what medication..... ..... In which trimester: First [1] Second [2] Third [3]
	1.1.15 Mode of delivery [1] SVD (normal) [2] caesarean section [3]other..... .....
	1.1.16 Place of delivery [1] Hospital /Health centre [2]

	Home							
2.0 Treatment prescribed in the ward. Indicate appropriately :[0] Not prescribed [1] Prescribed								
2.1 Number of antibiotics used	A)[1] B) [2] C)[3] D)[4] **More specify.....**							
2.2 Antimicrobial agent used	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
2.2.1 Benzyl penicillin inj								
2.2.2 Gentamycin								
2.2.3 Amikacin								
2.2.4 Flucloxacillin								
2.2.5 Ceftriaxone								
2.2.6 Ceftazidime								
2.2.7 Vancomycin								
2.2.8 Piperacillin /tazobactum								
2.2.9 Ampicillin								
2.2.10 Meropenem								
2.2.11 Amoxicillin-clavulanic acid.								
Other Antibiotic used (specify) a)								
b)								
c)								
3.0 Treatment outcomes								
3.1 What was the outcome of the	[1]Patient		[2]Patient		[3]Patient		[4]Patient	

inpatient treatment of neonatal sepsis	Discharged.	Died.	developed complications.	absconded.
3.2 Complications developed	[1]Yes [2] No **Specify if any.....**			
3.3 Patient developed Adverse drug reactions [1] Yes [2] No				
3.4 Indicate days of inpatient treatment	1. [1-3] 2. [4-7] 3.[7-14] 4.[above 14 days]			

4.0 Identified micro-organisms		
4.1 Gram positive bacteria		**(Indicate total number of types of organisms isolated.....)**
Bacteria		Isolate(tick where applicable)
1.	*Streptococci pneumoniae*	
2.	*Streptococci viridans*	
3.	Coagulase Negative Staphylococci	
4.	Group B streptococci	
5.	*Staphylococci aureus*	
6.		
7.		
4.2 Gram negative bacteria		
Bacteria		Isolate(tick where applicable)

1.	*Pseudomonas* spp	
2.	*Escherichia coli*	
3.	*Klebsiella* spp	
4.	*Enterobacter* spp	
5.	*Citrobacter*	
6.	*Acinetobacter*	
7.		

5.0 Antibiotic Sensitivity

Bacteria.....			
Antibiotic	Resistant	Intermediate	Sensitive
Ampicillin			
Benzylpenicillin			
Gentamycin			
Ceftriaxone			
Meropenem			
Ceftazidime			
Vancomycin			
Flucloxacillin			
Amoxicillin-clavulanic acid			
Amikacin			
Erythromycin			

Bacteria.....			
Antibiotic	Resistant	Intermediate	Sensitive
Ampicillin			
Benzyl penicillin			
Gentamycin			
Ceftriaxone			
Meropenem			
Ceftazidime			
Vancomycin			
Flucloxacillin			
Amoxicillin-clavulanic acid			
Amikacin			
Erythromycin			

Appendix 2: Laboratory procedure

I) Identification tests

Gram stain

This test was used to differentiate bacteria based on whether they retain or lose the 'primary stain' (crystal violet) after mordanting with iodine, treatment with alcohol and counter staining with safranin. The following steps were followed.

Procedure:

1) Prepare a smear of bacteria (from a culture not more than 24 hours old) on a slide. Air dry. Fix the smear by passing over the pilot flame. Stain the smear with 3 to 5 drops of crystal violet solution for 1 minute. Wash with water for a few seconds.
2) Apply 3 to 5 drops of Gram's iodine solution and let sit for 1minute.Wash slide in water.
3) Decolorize with 3 to 5 drops of alcohol-acetone until free color has been washed off (approximately 5-15 seconds).Wash slide with water and blot dry.
4) Counter stain smear for 10 seconds with 3 to 5 drops of safranin. Wash slide and blot dry.
5) To view a stained cell, use oil immersion objective and set the slide condenser to the blank side (empty hole for brightened microscopy).

Interpretation

Gram positive retained crystal violet (appear as dark blue, purple or violet).

Gram negative lost the crystal violet are subsequently stained by the 'counter stain' (safranin) appear as red.

Indole test

This was used to differentiate Enterobacteriaceae and other genera. It also aided in species differentiation: *Klebsiella* species and *Citrobacter* species. Most strains of enterobacteria break down the amino acid tryptophan with the release of indole.

Method

Using a sterile straight wire, 5ml of sterile medium was inoculated with test organism.

An indole paper strip was placed in the neck of the tube and stopper put. Incubation was done at 35-37^{o}C overnight.

Indole production was exhibited by reddening of the lower part of the strip.

Motility

The spreading of turbidity throughout the medium was a positive proof.

Catalase test

This test was used to differentiate the bacteria that produce the enzyme catalase such as *Staphylococci* from non-catalase producing bacteria such as *Streptococci.*

Method

i. 2-3ml of hydrogen peroxide solution was be poured into a test tube.

ii. Using a wooden stick or a glass rod several colonies of the test organism were removed and immersed in the hydrogen peroxide solution.

iii. Active bubbling indicates a positive catalase test.

Optochin susceptibility test

The optochin susceptibility test is performed with a 6-mm, 5-microGram optochin disk, and is used to differentiate between *Streptococcus pneumoniae* and *Viridans Streptococci. Optochin*-susceptible strains can be identified as *Streptococci pneumonia.*

Method

i. Touch the suspect-hemolytic colony with a sterile bacteriological loop and streak for isolation onto a blood agar plate in a straight line. Several strains can be tested on the same plate at once, streaked in parallel lines and properly labeled.

ii. Asceptically place an optochin or "P" disk with a diameter of 6 mm (and containing5 microGram of ethylhydrocupreine) on the streak of inoculums, near the end where the wire loop was first placed. Because the inoculums are streaked in a straight line, three to four colonies may be tested on the same plate.

iii. Incubate the plates in a carbon dioxide incubator or candle-jar at 35^0C for 18-24 hours.

iv. Read, record and interpret the results.

Interpretation

Hemolytic strains with a zone of inhibition of growth greater than 14mm in diameter are pneumococci.

Hemolytic strains with no zones of inhibition are viridians streptococci.

Coagulase test

This test was used to identify *Staphylococcus aureus* which produces coagulase. Both tube test and slide test was be employed.

Method

Slide test (detects bound coagulase)

i. A drop of distilled water was placed on each end of a slide or on two separate slides.

ii. A colony of the test organism was be emulsified in each of the drops to make two thick suspensions.

iii. A loop full (not more than) was added to one of the suspensions and mixed gently.

iv. Clumping of the organisms occurred within 10 seconds if the organism *Staphylococcus aureus*.

v. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Tube test (detects free coagulase)

i. Plasma was be diluted in the ratio of 1:10.

ii. Three small test tubes were to be availed and labeled; test organism, positive control and negative control.

iii. 0.5ml of the diluted plasma was pipetted into each tube.

iv. Five drops (about 0.1ml) of the test organism was added into the labeled positive and 5drops of the *Staphylococcus aureus* culture was added to the tube labeled positive and 5 drops of sterile broth in the tube labeled negative.

v. The tubes was incubated at 35-37C after mixing gently. Clotting was occur after an hour, if no clotting occurs after one hour examination was be repeated after every 30minutes for up to 6hours.

vi. Clotting is indicative of *Staphylococcus aureus*.

Oxidase test

This test was used to identify *Pseudomonas*.

Method

1) Apiece of filter paper is placed in a Petri dish and soaked with 2-3 drops of freshly prepared oxidase reagents.
2) Using a piece of stick or glass rod, a colony of the test organism was then be smeared on the filter paper.
3) Development of blue- purple color within a few seconds indicates positive oxidase test.

Voges-proskeur (v-p) test.

This test was used to identify *Klebsiella* spp.

Method

i. 2ml of sterile glucose phosphate peptone water was inoculated with the test organism and incubated at 35-37^{o}C for 48hours.

ii. A small amount of creatinine was added and mixed well.

iii. 3ml of sodium hydroxide was added and mixed well.

iv. The bottle cap was be removed and left for one hour at room temperature.

v. Development of pink color was indicative of *Klebsiella pneumoniae*.

Urease test.

This test was used to identify *Proteus* spp.

Method.

i. A straight wire was used to inoculate a tube of MIU with a colony of the test organism.

ii. An indole paper strip was placed in the neck of the tube above the medium. The tube was stoppered and incubated at 35-37^{o}C overnight.

iii. Production of urease was change the color of the paper strip to pink.

Bacitracin test

This test was used to identify *Streptococcus pyogenes*.

Method

i. Bacitracin disk was placed on a culture plate inoculated with the organism and incubated at 35-37^{o}C overnight.

ii. A zone of inhibition around the disc was indicative of *Streptococcus pyogenes*.

II) Antimicrobial sensitivity testing- Disc diffusion method

Antimicrobial susceptibility testing is a standard method that is used to measure the effectiveness of antibiotics and other chemotherapeutic agents on pathogenic microorganism. In many cases, it is an essential tool in prescribing appropriate treatment.

Method

A disc of blotting paper is impregnated with known volume and appropriate concentration of an antimicrobial.

The disc is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism.

The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism.

Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc.

To ensure reproducibility and comparability of results, the modified Kirby-Bauer diffusion technique was be used.

Modified Kirby-Bauer susceptibility testing technique

A sterile medium was prepared according to the manufacturer's instructions. The PH of the medium was be set at 7.2-7.4.

The media is poured into a 90mm sterile Petri dish to a depth of 4mm (about 25ml per plate). This is done on a level surface so that the depth of the medium is uniform. NB If the media is too thin the inhibition zone was be falsely large and if too thick the zones was be falsely small.

Each new batch of agar was controlled using *E. faecalis* (ATCC 29212 or 33186) and co-trimoxazole disc. The zone of inhibition should be 20mm or more in diameter.

The plates was be stored at 2-8^{o}C in sealed plastic bags. For use the plates was be dried with their lids slightly raised in 35-37^{o}C incubator for about 30minutes.

About one hour before use, the working stock of the discs was to be allowed to warm to room temperature, protected from direct sunlight.

Method

1) Using a sterile wire loop, touch 3-5 well isolated colonies of similar appearance to the test organism and emulsify in 3-4ml of sterile physiological saline or nutrient broth.

2) In a good light match the turbidity of the suspension to the turbidity of the standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper.

3) Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by rotating and pressing the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o}C to ensure even distribution.

4) With the Petri dish lid in place, allow 3-5 minutes (no longer than 15minutes) for the surface of the agar to dry.

5) Using sterile forceps, needle mounted in a holder, or multidisc dispenser, place appropriate antimicrobial discs, evenly distributed on the inoculated plate. The discs should be 15mm from the edge of the plate and no closer than about 25mm from disc to disc. No more than eight discs was be applied on each Petri dish. Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved in one place.

6) Within 30minutes of applying the discs, invert the plate and incubate it aerobically at 35^{o}C for 16-18 hours.

7) After overnight incubation, examine the control and the test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the

diameter of each zone of inhibition in mm. the endpoint of inhibition is where growth starts.

Interpretation of zone sizes

Using the interpretative chart, the zones of each antimicrobial was interpreted reporting each organism as Resistant, Intermediate susceptible, Susceptible.

Appendix 3A: Patient consent form

To be read and explained in a language that the respondent understands.

Study title: Antimicrobial sensitivity and treatment outcomes of neonatal sepsis at Pumwani maternity hospital.

Objective of study: To determine the profile of the pathogenic bacteria in blood cultures of neonates with clinically suspected septicemia, and their susceptibility patterns to commonly used antibiotics.

Institution: Department of Pharmaceutics and Pharmacy Practice, School of Pharmacy; University of Nairobi. P.O BOX 19676-00202 Nairobi.

Investigator: Dr Norah Maore

Supervisors: Dr Peter Karimi: Department of Pharmaceutics and Pharmacy Practice, Dr Eric. Guantai: Department of Pharmacognosy and pharmacology.

Ethical approval: The study has been approved by the Ethics and Research Committee of Kenyatta National Hospital/University of Nairobi P.O BOX 20723-00100, Nairobi.Tel.no. 2726300/2716450.Ext 44102.Attached is a copy of the same.

My name is Dr. Norah Maore, a student of clinical pharmacy at the University of Nairobi. I am carrying out a study on the way microorganisms respond to the antibiotics used in treatment of diseases that they cause in this hospital (Pumwani Hospital).This study involves identification of microorganism that cause disease and how they respond to different medicines used in treatment of diseases. Normally the organisms involved are different and change overtime. So this makes it necessary keep surveillance of the sensitivity patterns. I was also follow up to see how the baby responds to the treatment till you leave the hospital.

Procedure to be followed: With your permission, one blood sample approximately 1-3ml was drawn from the baby by the doctor. This blood was be put in bottles that contain culture material

and taken to the laboratory at the university. Then after a few days we will identify the bacteria and test with antibiotics that treat sepsis.

Risks: Care was being taken to ensure the procedure is carried out by a qualified clinical practitioner and was be done under aseptic conditions to prevent development of any infections. Your baby was be monitored to ensure that no complications arise from the drawing of blood baby and any problem that may occur was be treated before discharge from the hospital.

Benefits/Cost/Reimbursement: The results from this study will guide in treatment choice for other sick babies in this hospital. The results from the test will also be brought to your doctor to use in better management of the baby while in the hospital. I am not going to charge you any amount for the test we was do. I was also not give any monetary benefit for participating in the study.

Assurance of confidentiality: No names were recorded during the study. All information obtained will be treated with confidentiality and only the investigator was have access to it during the entire study period. After the study is complete all identifiable information obtained was be destroyed.

I request your permission in order to be able to carry out the tests. Your baby's privacy was be respected and confidentiality maintained as I was not include any names in the study. In case of any questions you can contact me on the address below. You are free to withdraw your baby from the study any time you feel you do not want to continue. The study has been approved by the KNH/UON Ethics and Research Committee.

Please sign below if you agree that your baby to participates in the study.

Contacts: In case of any enquiries please contact me (the investigator) on:

Norah Maore Tel no. 0726-033-901

Patient consent

I have read the above consent form and understood it. The nature of the study has been explained to me by Dr. Norah Maore. I voluntarily agree to participate in the study and to respond to questions asked. I give consent for one blood sample to be drawn from my baby.

Signature of parent/guardian……

Name of participant…….

Date…….

Researcher/Research assistant (staff consenting)

Name.....

Signature.....

Date.....

Kiambatacho 3B: Cheti cha waraka wa idhini

Isomewe na ielezewe mhusika kwa lugha aielewayo zaidi.

Cheo utafiti : Tathmini ya kisababichacho,unyeti na matokeo ya matibabu ya sepsis kwa watoto wa umri chini ya siku ishirini na nane baada ya kuzaliwa Pumwani maternity hospital.

Taasisi: Idara ya Pharmaceutics and Pharmacy Practice, Shule ya pharmacy; Chuo kikuu cha Nairobi.P.O BOX 19676-00202 Nairobi.

Mchunguzi: Dr. Norah Maore

Wasimamizi: Dr P.N Karimi: Idara ya Pharmaceutics and Pharmacy Practice, Dr E. Guantai: Idara ya Pharmacognosy and pharmacology.

Utafiti huu umepitishwa na kamati ya Maadili ya Hospitali kuu ya Kenyatta/Chuo kikuu cha Nairobi(KNH/UON).P.O BOX 20723-00100,Nairobi.Nambari ya simu 2726300/2716450.Ext 44102 na kamati ya hospitali ya Pumwani maternity.Nakala hiyo imeambatishwa hapa.

Jina langu ni daktari Norah Maore,niko masomoni katika chuo kikuu cha Nairobi.Ninafanya utafiti kuhusu kisababichacho,unyeti na matokeo ya matibabu ya ugonjwa kwa watoto wa umri chini ya mwezi mmoja wa kuzaliwa.Lengo kuu ni kujua kisabishacho ugonjwa huu, Pumwani na dawa ambazozinatumika kutibu kama za fanya hivyo vikamilivu.Somo hili litasaidia kwa uamuzi wa dawa zitakazo tumika kutibu ugonjwa huu.

Utaratibu utakao fwatwa ni:kwa idhini yako damu kiasi cha milimita moja kitatolewa kwa mkono wa mototo safari moja.Hii itatumika kwa vipimo vya damu kwenye maabara ya chuo kikuu cha Nairobi, kuthamini kisababichacho ugonjwa huu motto alionayo.

Kushiriki ni kwa hiari: Kushiriki katika utafiti huu ni kwa hiari.Kama unakubali kusaidia utafiti huu na baadaye kubadili uamuzi wako kinyume na ushirika,uko huru kutenda hivyo.Hautabaguliwa kwa namna yoyote kama utakataa kushiriki,au kujiondoa baadaaye.uamuzi wako utaheshimiwa.

Usiri: Habari zitatumika kwa madhumuni ya utafiti tu,kuhakikisha usiri, hatuta fichua kutambulika kwako kwa kutumia jina lako au la mtoto popote katika utafiti huu.Maneno ya jumla tu ndio yatatumika kuonyesha ushirika ulivyo kuwa.

Hatari za utafiti: Hatua za kina zita chukuliwa kuhamasisha kuwa utaratibu utakao fanywa, utafanywa na Daktari aliye hitimu kufanya kazi hiyo na utaratibu wa mikakati kufwatwa kwa njia ya usafi wa hali ya juu kuzuia kusambaza uchafu na magojwa.

Faida/gharama za utafiti: Ingawa hakuna faida ya moja kwa moja kwako wewe,habari hii itatumika kuboresha matibabu kwa watoto wengine.Repoti ya kipimo hiki italetwa kwa daktari na itasaidia kwa matibabu ya mototo wako. Mtoto ataangaliwa kwa kina kuhakikisha kuwa matatizo yoyote yatakayompata yatatibiwa kabla ya kuruhusiwa kwenda nyumbani.

Naomba idhini yako nifanye vipimo.Faragha ya mtoto itazingatiwa,hatutatumia majina kutambulisha mtoto au wewe.Ukiwa na swala lolote au tashwishi kuhusu utafiti huu ,wasiliana na Dkt.Norah Maore kwa anawani **Sanduku la posta 15634 -00503Mbagathi. Nambari ya simu 0726033901.**

Idhini ya mgonjwa kuhusika

Nimesoma cheti hiki na nimeelewa.Dkt.Norah amenieleza kuhusu utafiti huu.Nimejitolea kuhusika katika utafiti kwa hiari na kujibu mawsali nitakayo ulizwa.Nimepeana idhini ya mtoto kutolewa damu kiasi ya sampuli moja.

Sahihi ya mzazi/mlezi.....

Jina la mhusika.....

Tarehe.....

Mchunguzi/mchunguzi msaidizi(idhini ya mchunguzi mhusika)

Jina la mchunguzi.....

Sahihi.....

Tarehe.....

Appendix 4: Budget

ITEM	QUANTITY	UNIT PRICE	TOTAL (KSH)
SUPPLIES			
Biro Pens	6	20.00	120.00
Pencils	2	12.00	24.00
Box file	2	150.00	300.00
Spring files	2	120.00	240.00
Pencils sharpener	1	45.00	45.00
White out pen	1	85.00	85.00
Folder	2	120.00	240.00
Staple	1	245.00	245.00
Paper Punch	1	550.00	550.00
Staple Remover	1	235.00	235.00
Note book	2	85.00	170.00
TOTAL SUPPLIES			**2,254.00**
OTHERS			
Printing	30	10.00	300.00
Photocopying	4000	3.00	12,000.00
Final dissertation booklet	8	500.00	4,000.00
Ethic committee Approval	1	2,000.00	2,000.00
A poster	4	2,500.00	10,000.00
TOTAL OTHER			**28,300.00**

Transport	**1**	**10,000.00**	**10,000.00**
Communication	**1**	**5,000.00**	**5,000.00**
Research Assistant	**1**	**30,000.00**	**30,000.00**
Data Statistician	**1**	**10,000.00**	**10,000.00**
Laboratory services	**216.00**	**1,500.00**	**324,000.00**
TOTAL PERSONNEL			**349,000.00**
TOTAL EXPENSES			**409,554.00**

Appendix 5: Results Tables

Table 16 : Neonatal risk factors associated with culture proven neonatal sepsis

		Culture growth				
		Growth		No Growth		
		Frequency (n)	Percent (%)	Frequency (n)	Percent (%)	P value
Irritability	No	29	33.7	57	66.3	0.600
	Yes	19	29.7	45	70.3	
Respiratory distress	No	35	37.6	58	62.4	0.059
	Yes	13	22.8	44	77.2	
Lethargic	No	47	33.1	95	66.9	0.224
	Yes	1	12.5	7	87.5	
Failure in feeding	No	33	30.6	75	69.4	0.543
	Yes	15	35.7	27	64.3	
Hyperthermia	No	28	25.2	83	74.8	0.003
	Yes	20	51.3	19	48.7	
Hypothermia	No	47	32.2	99	67.8	0.761
	Yes	1	25.0	3	75.0	
Cyanosis	No	48	32.4	100	67.6	0.329
	Yes	0	.0	2	100.0	
Diarrhoea	No	48	32.2	101	67.8	0.491
	Yes	0	.0	1	100.0	
Vomiting	No	48	34.0	93	66.0	0.034
	Yes	0	.0	9	100.0	
Jaundice	No	25	28.1	64	71.9	0.215
	Yes	23	37.7	38	62.3	
Abdominal distension	No	47	32.2	99	67.8	0.761
	Yes	1	25.0	3	75.0	
Caput	No	43	30.9	96	69.1	0.320
	Yes	5	45.5	6	54.5	
Dehydrated	No	42	31.1	93	68.9	0.484
	Yes	6	40.0	9	60.0	
Septic Rash	No	41	30.1	95	69.9	0.129
	Yes	7	50.0	7	50.0	
Cold Extremities	No	48	32.2	101	67.8	0.491
	Yes	0	.0	1	100.0	
Chest Indrawing	No	47	32.2	99	67.8	0.761

	Yes	1	25.0	3	75.0	
Tachypnea	No	47	32.2	99	67.8	0.761
	Yes	1	25.0	3	75.0	
Grunting	No	48	32.9	98	67.1	0.164
	Yes	0	.0	4	100.0	
Poor Skin Color	No	46	31.9	98	68.1	0.943
	Yes	2	33.3	4	66.7	
Hypotonic	No	48	32.2	101	67.8	0.491
	Yes	0	.0	1	100.0	
Pedal Edema	No	48	32.2	101	67.8	0.491
	Yes	0	.0	1	100.0	
Crackles	No	48	32.2	101	67.8	0.491
	Yes	0	.0	1	100.0	
Stridor	No	48	32.2	101	67.8	0.491
	Yes	0	.0	1	100.0	
Ectodermal	No	47	31.5	102	68.5	0.144
Displasia	Yes	1	100.0	0	.0	
Convulsions	No	45	31.3	99	68.8	0.335
	Yes	3	50.0	3	50.0	
Other Signs	No	47	32.2	99	67.8	0.761
	Yes	1	25.0	3	75.0	

Appendix 6: Ethical approval letters

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/105

6th March, 2015

Dr. Norah K. Maore
U56/69094/2013
Department of Pharmaceutics and Pharmacy Practice
School of Pharmacy
University of Nairobi

Dear Dr. Maore

Research Proposal: Antimicrobial sensitivity and treatment outcomes of neonatal sepsis at Pumwani Maternaity Hospital (P731/12/2014)

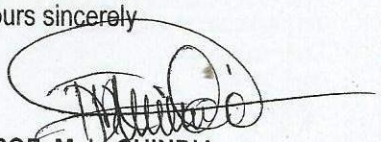
This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 6th March 2015 to 5th March 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

Yours sincerely



PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
The Dean, School of Pharmacy, UoN
The Chair, Dept. of Pharmaceutics and Pharmacy Practice, UoN
Supervisor: Dr. Peter Karimi, Dr. E.M. Guantai

NAIROBI CITY COUNTY

Telephone: 020 344194
Web.www.nairobi.go.ke

City Hall
P. O. Box 30075 - 00100
Nairobi
Kenya

COUNTY HEALTH SERVICES:
PUMWANI MATERNITY HOSPITAL

PMH/DMOH/75/0193/2014

23RD MARCH 2014

TO:
Dr. Norah K. Maore
U56/69094/2013
Department of Pharmaceutics and Pharmacy Practice
School of Pharmacy
University of Nairobi.

RE: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the research entitled "**Antimicrobial Sensitivity and Treatment Outcomes of Neonatal Sepsis at Pumwani Maternity Hospital (P731/12/2014)**" has been approved.

You are expected to pay Kshs. 6000/- only.

You are hereby allowed to collect data. We look forward to receiving a summary of the research findings upon completion of the study.

Yours sincerely,

PUMWANI MATERNITY HOSPITAL
P. O. Box 42849-00100, NAIROBI.
TEL. NRB. 6763291-4/ 6762965

DR. L.O. KUMBA
MEDICAL SUPERINTENDENT