MICROBIAL PROFILING, ANTIMICROBIAL & MOLECULAR CHARACTERISATION OF BACTERIAL ISOLATES CAUSING SEPSIS AMONG PRETERM NEONATES AT KITALE COUNTY HOSPITAL; KENYA

BY

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APRIL, 2021

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DEDICATION

I commit this thesis to my beloved husband Henry Mochoge Ogaro for his tireless support, my daughters Marleen, Hazzel, Elizabeth and my son Gilchrist for their great inspiration to me.

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I thank God almighty for His Grace and mercy throughout my study period.

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ABSTRACT

Globally preterm births have been on the increase and sepsis has been found as a contributor to mortality and morbidity among very low birth weight (VLBW) neonates, (<1500g) in new-born units. In this descriptive cross-sectional study, we sort to determine microbial profiling, antimicrobial and molecular characterization of common bacterial isolates causing neonatal sepsis (NS) among preterm neonates in new-born unit at Kitale County Hospital (KCH), Kenya.

Blood samples (181) were collected from eligible preterm neonates and cultured using established laboratory protocols. Well-structured questionnaires were used to get relevant information about the mothers and new-borns.

Majority 107 (59.1%) were female while 40.9% (74) were male. Their gestational age ranged from 30 to 36 completed weeks with a mean gestational age of 34.10 ± 1.743. Majority of the isolates were Gram positive 35(85.4%) with Coagulase Negative *Staphylococcus* (CoNS) 31 (88.5%) being the most predominant. Among the Gram negative 6 (14.6%), *Salmonella spp* were the predominant 3 (50%). Gram positive isolates were susceptible against Levofloxacin, Moxifloxacin, Clindamycin and Linezolid but were 100% resistant to penicillin. Gram negative isolates tested against Amoxicillin/clavulanic acid, Piperacillin/Tazobactam, Cefuroxime, Cefepime, Aztreonam, Meropenem, Ciprofloxacin and Trimethoprim/sulfamethoxazole were observed to be susceptible. Further screening of the Gram-positive bacteria for mecA markers, six (6) turned positive. Cycle thresholds for *Staphylococcus epidermidis (3)* were 28.87, 34.38 and 38.43, *Staphylococcus warneri* and *Staphylococcus hominis* were 37.34 and 32.76 respectively while *Enterococcus faecalis* was 26.76. Gram negative bacteria were screened for blaOXA48 and bla KPC genes with four turning positive. *Salmonella spp* (2) had cycle threshold of 30.61 and 30.69, *Escherichia coli* had 33.64 while *Pseudomonas aeruginosa* had 35.43.

Various risk factors and organisms were strongly associated with the development of neonatal sepsis at KCH new born unit. There exists Multi Drug Resistance (MDR) genes in bacteria circulating in new-born unit of Kitale County Hospital. Policies for management and treatment of mothers and their neonates at risk of developing septicaemia should be utilized by health care providers at KCH.

TABLE OF CONTENTS

DECLARATION ii
PLAGIARISM DECLARATION, iii
COPY RIGHT PAGE iv
DEDICATION v
ACKNOWLEDGEMENT vi
ABSTRACT, vii
TABLE OF CONTENTS viii
LIST OF FIGURES xii
LIST OF APPENDICES xiii
DEFINITION OF TERMS xiv
LIST OF ABBREVIATION AND ACRONYM, xv
CHAPTER ONE, 1
1.0: INTRODUCTION, 1
1.1 Background of the study 1
1.2 Statement of the problem 4
1.3 Justification/rationale. 5
1.4: Objectives 6
1.4.1: Broad objective 6
1.4.2 Specific objectives 7
1.5 Null hypothesis 7
1.6 Assumptions of the study, 7
1.7 Scope/delimitations of the study, 8
1.8 Limitation of the study 8
LITERATURE REVIEW 9
2.1 Introduction 9
2.2 Aetiology and risk factors for neonatal sepsis 9
2.3 Prevalence and incidence of neonatal sepsis 12
2.4 Diagnosis of neonatal sepsis 14
2.5 Management of neonatal sepsis 15
2.5.2 Antibiotics commonly used to manage NS 17
MATERIALS AND METHODS, 25
3.1 Study site 25
3.2 Research design 26
3.3 Target population 26
3.4 Eligibility criteria 26
3.4.1 Inclusion criteria 26
3.4.2 Exclusion criteria 27
3.5 Sample size 27

3.7 Specimen collection 29
3.8 Bioassays 29
3.8.1 Blood culturing 29
3.8.2 Isolation and identification of organisms 30
3.8.2.1 Gram staining 30
3.8.2.2 Biochemical Tests 31
3.9 Antimicrobial sensitivity tests – Disc diffusion test and MIC 38
3.9.1 Preparation of bacterial inoculum 38
3.9.3 Disc diffusion test 39
3.9.4 Minimum Inhibitory Concentration (MIC) 40
3.10 Screening for carbapemenase and methicillin genes from isolates responsible for neonatal sepsis by QIAamp DNA Mini Kit (250) 41
3.11 DNA fragment analysis from bacterial isolates by QIAGEN QIAxcel®-Pure 42
Excellence 42
3.11 Data analysis and presentation 43
3.12 Ethical considerations. 44
CHAPTER FOUR 45
RESULTS 45
4.1 Introduction 45
4.2 Response Rate 45
4.3 Preterm neonate demographic findings 45
4.3.1 Gender of the participants 46
4.3.2 Age of Participants 46
4.3.3 Gestational Age (in weeks) completed at delivery 47
4.3.4 Neonate body temperatures 48
4.3.5 Neonate birth weight 49
4.3.6 Age (in days) of first presentation with sepsis 50
4.3.7 Parenteral nutrition, breast feeding and foetal distress 51
4.4 Mother's medical history findings 52
4.5 Factors associated with occurrence of neonatal sepsis 53
4.5.1 Maternal factors and development of neonatal sepsis 54
4.5.2 Neonatal factors and development of neonatal sepsis 56
4.6: Isolation and profiling of common neonatal sepsis causing bacterial pathogens among preterms 58
4.6.1 Culturing 58
4.6.2 Gram staining 59
4.6.3 Biochemical tests 61

4.7 Antimicrobial characteristics of the pathogens inducing NS among the preterms 67
4.7.1 Antimicrobial susceptibility patterns of Gram-positive isolates. 69
4.7.2: Antimicrobial resistant patterns of Gram-positive isolates. 72
4.7.3 Antimicrobial susceptibility patterns of Gram-negative isolates. 74
4.7.4: Antimicrobial resistant patterns of Gram-negative isolates... 76
4.7.5 Minimum Inhibitory Concentration (MIC) of isolated bacterial causing neonatal 78
sepsis 78
4.7.6 Minimum Inhibitory Concentrations (MIC) of antimicrobial agents on Gram 78
positive isolates 78
4.7.7 Minimum Inhibitory Concentrations (MIC) of antimicrobial agents on Gram 81
negative isolates 81
4.7.8 Screening for carbapemenase: blaOXA-48, blaKPC and mecA genes from isolates responsible for neonatal sepsis 83
4.7.9: Screening for Carbapemenase producing (blaOXA-48 and bla KPC genes) in 83
Gram negative isolates 83
4.7.10: Screening for Methicillin resistance (mec A gene) in Gram positive isolates 84
4.7.11: DNA fragment analysis from bacterial isolates that had resistant genes 85
responsible for Neonatal sepsis 85
CHAPTER FIVE 87
DISCUSSION.. 87
CHAPTER SIX 95
6.0 CONCLUSION AND RECOMMENDATIONS 95
6.1: Conclusions 95
6.2 Recommendations 96
REFERENCES 97

LIST OF TABLES

Table 4. 1: Response Rate 45
Table 4. 2: Parenteral nutrition, breast feeding and foetal distress 52
Table 4. 3: Mother's past medical history (antenatal history)..................................... 53
Table 4. 4: Association between maternal factors and development of neonatal sepsis 55
Table 4. 5: Association between neonatal factors and development of neonatal sepsis 57
Table 4. 6: Blood culture and Gram staining 60
Table 4. 7: Summary of biochemical tests.. 66
Table 4. 8: Distribution of Coagulase negative Staphylococcus (CoNS)...................... 67
Table 4. 9: Antimicrobial susceptibility patterns (Average Zones of inhibition-mm) of Gram-positive bacterial isolates responsible for neonatal sepsis 71
Table 4. 10: Antimicrobial resistant patterns (Average Zones of inhibition-mm) of Gram-positive bacterial stains responsible for neonatal sepsis 73
Table 4. 11: Antimicrobial susceptibility patterns (Average Zones of inhibition-mm) of Gram-negative bacterial isolates responsible for neonatal sepsis................................ 75
Table 4. 12: Antimicrobial resistance patterns (Average Zones of inhibition-mm) of Gram-negative bacterial isolates responsible for neonatal sepsis................................ 77
Table 4. 13: Minimum Inhibitory Concentration (MIC) of Gram-positive isolates responsible for neonatal sepsis... 80
Table 4. 14: Minimum Inhibitory Concentration (MIC) of Gram-negative isolates responsible for neonatal sepsis... 82

LIST OF FIGURES

Figure 3. 1 Map of Kenya showing Trans Nzoia County and the study site 26

Figure 4. 1: Gender of preterm neonates 46
Figure 4. 2: Age of neonates in days. 47
Figure 4. 3: The mothers gestational age (weeks) completed before delivery. 48
Figure 4. 4: The neonates body temperature (°C) 49
Figure 4. 5: The Neonates body weights. 50
Figure 4. 6: Age (days) of first presentation with sepsis 51
Figure 4. 7: Blood cultures in diphasic media:.. 59
Figure 4. 9: Gram staining results: 60
Figure 4. 10: The Catalase test plates:.. 61
Figure 4. 11: Coagulase tube test:. 62
Figure 4. 12: Triple sugar iron test:... 62
Figure 4. 13: A representative for Motility, indole and ornithine decarboxylase tests: . 63
Figure 4. 14: Citrate test representatives: 64
Figure 4. 15: A representative of Disk diffusion results on Muller Hinton Agar:......... 68
Figure 4. 16: Carbapemenase (blaOXA-48 and bla KPC) genes determination in Gram negative isolates 84
Figure 4. 17: Methicillin resistance (mec A) gene determination in Gram positive isolates.. 85
Figure 4. 18: DNA fragments of bacterial isolates with resistant genes for neonatal sepsis 86

LIST OF APPENDICES

Appendix 1.0 Consent form...109

Appendix 2.0 Structured questionnaire..111

Appendix 3.0 Kisii University (office of the Registrar Research and Extension) Introductory letter..114

Appendix 4.0 Approval letter research and ethics committee (IREC)..............115

Appendix 5.0 Approval letter to carry out research by NACOSTI..................116

Appendix 6.0 Approval permit to carry out research by NACOSTI................117

Appendix 7.0 County Commissioner (Trans Nzoia) letter of no objection to carry out research..118

Appendix 8.0 County Government of Trans Nzoia (state department of early and basic education) research authorization....................................119

Appendix 9.0 County Government of Trans Nzoia (Department of Health Services) Research Authorization..120

Appendix 10.0 Request for Research Authorization (Medical Superintendent Kitale County Hospital)...121

DEFINITION OF TERMS

Neonate: 0-28 days old

Preterm neonate: A baby born before 37 compléted weeks of géstation

Neonatal period: From day 0 to 28 days of life (first four weeks postnatal)

Early-onset sepsis: Before 72 hours of life (day 0 to day 3)

Late onset sepsis: After 72 hours of life until the end of the neonatal period

LIST OF ABBREVIATION AND ACRONYM

AMR: Antimicrobial resistance
Beta: Beta lactams
Bp: Base pairs
BPs: Bacteriophages
CBA: Chocolate blood agar
CLSI: Clinical Laboratory Scientific Institute
CONS: Coagulase-Negative Streptococcus
CRE: Carbapenem resistant Enterobacteriaceae
CS: Caesarean section
DNA: Deoxyribonucleic acid
EGNN: Egyptian Neonatal Network
EOS: Early Onset Sepsis
ERC: Ethical Review Committee
ESBL: Extended Spectrum Beta Lactamase
GBS: Group B Streptococcus
GBV: Gender Based Violence
GM: Grams
ICSU: International Council for Science
IM: Intramuscular
IMCI: Integrated Management of Childhood illness
ISSC: International Social Science Council
IV: Intravenous
KCH: Kitale County Hospital
KPC: Klebsiella pneumonia
LIMS: Laboratory information management system
LOS: Late Onset Sepsis
MDGs: Millennium Development Goals
MIC: Minimum inhibitory concentration
MM: Millimetres

MOH: Ministry of Health

MRSA: Methicillin resistant *staphylococcus aureus*

NBU: New Born Unit

NCCLC: National Committee for Clinical Laboratory standards

NICU: Neonatal Intensive Care Unit

NS: Neonatal Sepsis

NTC: Non template control

PCR: Polymerase Chain Reaction

PN: Parenteral Nutrition

PROM: Prolonged Rupture of Membranes

PSBI: Possible Serious Bacterial Infection

PTB: Preterm Births

SBA: Sheep blood agar

SD: Standard Deviation

SEM: Standard error of mean

SID: Subject Identification number

SIRS: Systemic Inflammatory Response Syndrome

SOP: Standard Operating Procedure

SVD: Spontaneous virginal delivery

TSI: Triple sugar Iron

USDG: Universal Sustainable Development Goals

UTI: Urinary tract infection

VLBW: Very Low Birth Weight

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Universally around 15 million neonates are delivered annually and about 1 million succumb due to septicaemia, while others develop lifelong complications of sepsis and many experiences the ill effects of some sort of deep rooted physical, neurological, or instructive weakness, which produces significant expenses for their families and for society. About 2.5 million preterm neonates die before the first month of life and around 1 million new born deaths occur daily in developing and middle-income countries. The incidence is highest in neonates and children which accounts to 47% of total infant deaths among the under-five due to preterm related complications (Howson *et al.,* 2014; UNICEF, 2020). It is also known that neonatal stage is the most vulnerable period for a baby since it's a period of vital developmental change from uterine climate to the outer world (Getabelew *et al*., 2018).

There is also need for achieving integrated and indivisible goals that targets the social, environment and economic dimensions of sustainable development, for operationalizing the 2030 agenda (Nunes *et al*., 2016). Neonatal sepsis (NS) is defined as an infant (< 90 days) blood infection with systematic signs and symptoms of septicaemia in the first one month of life (Nathan, 2016; Masanja *et al*., 2019) and is one of the main causers of neonatal deaths. NS is classified as early and late-onset, early-onset sepsis signs appear in 72 hours of age maternal risk exposure factors. A late-onset sepsis sign appear after the fourth day of birth and is related to environment risk factors (Camacho-Gonzalez *et al*., 2013; Nathan, 2016).

Factors such as neonate age, birth asphyxia, Prolonged Rupture of Membranes (PROM), preterm births and infections have been documented to be the main causers of neonatal sepsis among the preterm neonates (UNICEF, 2020). Maternal risk factors like mode of delivery makes a neonate more susceptible to early onset sepsis, due to their potential risk for complications to the neonate and the mother resulting to infections. While neonatal risk factors such as neonatal age and resuscitation at birth, were found to contribute to occurrence of neonatal sepsis as the neonate's immune system is compromised leading to acquisition of infections. These factors were found to have a solid relationship on the advancement of neonatal sepsis (Adatara *et al*., 2019).

The prevalence of NS is overwhelming with high statistics being documented. For instance, in East Africa, prevalence of preterm neonatal sepsis from various studies was estimated to range from 4.7% to 77.9% (Berkley *et al*, 2005 and Gebrehiwot *et al*, 2012). For example, in one study carried out in Ethiopia, the prevalence of neonatal sepsis among 351 neonates admitted in NICU Arbaminch general hospital Southern Ethiopia was 78.3% (Erkihun *et al*., 2019). In Kenya neonatal mortality rate by 2019 was at 21 deaths per 1000 livebirths (UNICEF, 2020) while prevalence of sepsis was estimated to be 23.9% among all admissions in the new-born unit in a County Referral Hospital in Central Kenya (Geyt & Hauck, 2016). This is a clear indication that NS is still and remains a major problem amongst the new-born in most developing nations.

Neonatal sepsis is caused by various bacterial, viral, and fungal pathogens depending on different times and regions. Bacterial pathogens that have been commonly associated with neonatal sepsis include *Klebsiella pneumoniae,* Coagulase negative *Staphylococcus* (CoNS), *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus agalactiae* (Guo *et al*., 2019). Fungal pathogens commonly *Candida* species have also been associated with

late-onset sepsis due to fungal colonization of maternal origin due to vaginal route of delivery, while viral pathogens such as herpes simplex viruses, enteroviruses and parenchoviruses have been associated with sepsis in neonates (Singh, 2020). Among the bacterial isolates Gram positive pathogens have been documented as the most common neonatal sepsis NS causing bacteria with obstetric factors being highly related with the development of preterm neonatal sepsis. However, in some studies the prevalence of NS causing bacteria have been documented to be high with Coagulase Negative *Staphylococcus* (CoNS) being the majority from all the isolated pathogens (Sorsa, 2019).

Effective antimicrobial agents against the common pathogens causing preterm neonatal sepsis are available like Group B *Streptococcus* (GBS) and intrapartum antibiotic prophylaxis, which can improve the immune system by preventing and also treating neonatal sepsis. However, these measures are not documented in the guidelines for the management of neonatal sepsis (Esposito & Principi., 2019). Secondly early exposure to antimicrobial agents without confirmatory diagnosis, may also increase opportunistic infections, change body's microbiota, lead to antimicrobial resistance (AMR) and may also interfere with future treatment options for the infants (Wagstaff *et al.,* 2019). These factors have also contributed to prolonged treatment which ends up increasing costs of management and it can result to impairment of mental and physical development (Meenakshi *et al*., 2019). With minimal studies being done in third world countries on neonatal sepsis, this study was geared towards generation of local data at Kitale County Hospital, Kenya on microbial profiles and their antimicrobial characteristics of NS causing bacterial with an aim of such data adding value in policy formulation that can improve early management and treatment of neonatal sepsis.

1.2 Statement of the problem

Neonatal sepsis (NS) is a condition of global concern among neonates although its global burden has not been assessed. The incidence is estimated globally to be three million cases in preterm neonates and 1.2 million cases in babies although there is low data available from low-income countries to properly give an accurate estimate of the burden (Fleischmann-Struzek *et al.,* 2018). In 1st world countries 30% of neonatal mortality has been reported with highest cases among the preterm neonates compared to term neonates, while about 75% neonatal deaths have been documented to occur in 3rd world countries. The available data is from various hospital studies which may not give a good representation of the whole community, especially where mothers do not present themselves to hospitals (Herk *et al.,* 2016).

Nosocomial infections have also been known to continue being a serious problem in the neonatal care units despite advances in neonatal care, they have been found to be greatly correlated with increased financial burden, morbidity, and mortality among the preterm neonates under medical care (Ramasethu, 2017). Additionally, invasive care procedures have greatly contributed to development of neonatal sepsis particularly in very low-birth-weight neonates and this has been attributed to microbial contamination via biofilm formation which occur in devices being used resulting to Late-Onset-Sepsis (Medeiros *et al.,* 2016). Gram positive pathogens have also been found to be the most common neonatal sepsis causing bacteria, with Coagulase Negative *Staphylococcus* (CONs) being the majority of the isolated pathogens (Fleischmann-Struzek *et al*., 2018).

There is a significant number of preterm births (PTB) at Kitale County Hospital (KCH) New-Born Unit. For instance, during the month of June 2016 alone, unpublished data from KCH indicates that 205 preterms were recorded with about 135 (66%) developing

NS (New-born unit data from KCH health records department). Therefore, there is a growing concern over the high rates of sepsis developing among these preterm babies. In addition, management of neonatal sepsis mainly relies on antimicrobial agents' usage. However, with the rising incidences of antimicrobial resistance (AMR) globally it poses a great challenge on treatment/management of several illnesses including neonatal sepsis (Department of Health and Social Care, 2013; WHO, 2016). Additionally, microorganisms have been documented to use other mechanisms such biofilm formation to avoid immune response attack or even the antibiotics administered, and this has worsened their management (Rosbjerg *et al.,* 2017). Therefore, need is there to determine the common causative agents and the response to commonly used antimicrobial agents in management of NS in third world countries including Kenya who in most cases rely on data obtained from 1st world countries to make policies to govern such infections. Such data is also not documented at Kitale County Hospital (KCH) New-Born Unit (NBU) (G/eyesus *et al.,* 2017). This study therefore seeks to evaluate the potential role gestational age, birth weight and duration of parenteral nutrition may be playing in the development of neonatal sepsis among preterm neonates in KCH. The study will also identify the main NS associated pathogens, their antimicrobial profiles and responses to various antibiotics and presence of resistant genes markers among the isolates.

1.3 Justification/rationale.

There have been numerous advances in avoidance, evaluation, and management of neonatal sepsis in the previous decades. Infection control policies and practices,

including the use of central lines and intubations for invasive procedures, hand hygiene, and use of chlorhexidine can prevent development of late neonatal sepsis (Dong *et al.,* 2014). Despite these efforts, the illness and death related with septicaemia remains high among susceptible preterm neonates. For instance, Onwuanaku *et al*., (2011) did indicate that neonatal immaturity accounts for about 10% of neonatal mortality universally. It is also clear that despite the low incidences of sepsis, the associated morbidity and mortality rates are quite high (Gardner, 2009).

On the other hand, infants exposed to prolonged parenteral nutrition (PN) are at a higher risk of developing sepsis (Smith & Egger, 2008). There is need therefore to have an up-to-date local data showing the causative agents for NS in order to provide necessary information for timely intervention (Yadav *et al*., 2018).

Therefore, understanding the extent to which variable gestational age, birth weight and duration of parenteral nutrition contributes to the development of NS can reduce neonatal mortality at KCH. Knowledge on this is key on managing of preterms and will also reduce the morbidity and mortality rates among high-risk neonates who receive intensive care. Other than providing this crucial information, the present study is vital in achieving the 3rd Universal Sustainable Development Goals (USDG) focusing on preventing deaths of new-borns and under-five children by 2030 ICSU and ISSC (2015).

1.4: Objectives

1.4.1: Broad objective

To determine the microbial profiles and antimicrobial properties of microorganisms causing neonatal sepsis (NS) among preterm neonates at Kitale County Hospital (KCH) in New-born unit

1.4.2 Specific objectives

i. To investigate the factors influencing occurrence of NS (birth weight, gestational age and parenteral nutrition) among the preterm neonates at KCH New-born unit
ii. To determine the most common microbial profiles of the pathogens causing NS among the preterm neonates at KCH New-born unit
iii. To deduce the antimicrobial characteristics and presence of resistant genes markers among common pathogens inducing NS among preterm neonates at KCH New-born unit

1.5 Null hypothesis

i. Gestational age, birth weight and parenteral nutrition play no role on the occurrence, microbial profiles and antibiograms of NS among preterm neonates at KCH.
ii. There are no most common bacterial pathogens known to cause NS amongst the preterm neonates at KCH.
iii. Microorganisms causing NS are not resistant to commonly used antibiotics and have no resistant genes markers.

1.6 Assumptions of the study

The sample size used was sufficient for analysis and fit for population representation and inference.

1.7 Scope/delimitations of the study

This study was confined to Kitale county hospital new-born unit. The study focused on determination of microbial profiles and antimicrobial characteristics of neonatal sepsis among preterm neonates.

1.8 Limitation of the study

This study was conducted out within some limitations; first to premature neonates less than 36 weeks that developed signs and symptoms suggestive of neonatal sepsis. Secondly it was limited to the tools and laboratory procedures used.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Neonatal sepsis (NS) is a bacterial blood infection occurring in infants younger than 90 days old. NS can either be classified as Early-Onset Sepsis (EOS) where symptoms occur within 0-3 days of age or Late-Onset Sepsis (LOS) with signs occurring from the fourth day of life. Early-Onset Sepsis (EOS) is related to maternal risk factors (Adatara., 2019), while Late-Onset Sepsis (LOS) may be linked to environmental causes like parenteral nutrition, mechanical and ventilation (Nathan., 2016). Also, obstetric factors like preterm delivery, chorioamnionitis or endometritis, Group B streptococcal colonization and prolonged duration of rupture of membranes have been documented to be strongly involved in the occurrence of NS while intrapartum antibiotic treatment was found to importantly reduce NS (Nathan., 2019).

NS is a condition which occurs due infection of blood and is confirmed by a positive blood culture within 28 days of life. Early-onset sepsis features of sepsis present within 48-72 hours which may be due to by pathogens obtained from the mothers' genital tract. It presents with respiratory distress, pneumonia and septicaemia while is associated with high mortality. Late-onset sepsis onset features present after 72 hours, is caused by organisms from the environment and it presents with septicaemia and meningitis (Camacho-Gonzalez *et al*., 2013; Nathan., 2019).

2.2 Aetiology and risk factors for neonatal sepsis

Occurrence of NS in 3rd world countries is quite low (1/2000) compared with developing countries especially in Africa where the incidence is 42/1000 live births (Fleischmann-Struzek *et al*., 2018). Immature immune defences, environmental and maternal factors have been documented to be among the lead contributors to occurrence of NS the especially in preterm or in very low birth weight (VLBW) neonates (Srinivasan *et al*., 2015). The unbalanced immune response to infections among preterm neonates, i.e., both their non-specific and specific immune systems which are still undeveloped because of low IgG levels predisposes them a lot to the causative agents hence making them to be more susceptible to neonatal sepsis (Softić, 2017).

"Maternal factors such as gestational age, mode of delivery, prolonged rupture of membranes (PROM) and meconium-stained amniotic fluid among others variably have contributed to the risk of acquiring neonatal sepsis among preterms" (Murthy *et al.,* 2019; Nyma, 2020). However, environmental factors also known as nosocomial or community risk factors such as parenteral nutrition, and contamination from hands of care givers are crucial in occurrence of neonatal sepsis (Spearman *et al*., 2015). For instance, in one study that was carried out at Kenyatta National Hospital (KNH), Kenya to determine the relationship of improper cord hygiene and development of NS, it did report that there is a strong association between the two variables (Moraa *et al.,* 2019). Also, in another study that was done in Tanzania, resuscitation at birth and maternal age were the main factors that were documented to be associated with development of neonatal sepsis (Jabiri *et al*., 2016).

Demographic patient characteristics, colonization of the microflora in the nosocomial environment and the policy on use of antibiotics have made the distribution pattern of causative pathogens to vary across regions (Shim *et al*., 2011). NS has been

documented to be caused by various pathogens ranging from bacterial, viral to fungal. Among the most common bacterial that have been isolated from NS patients includes various microorganisms that have been associated mostly with the early-onset infection and among them they include "Group B *Streptococcus* (GBS), *Escherichia coli*, Coagulase-negative *Staphylococcus*, *Haemophilus influenzae*, *Listeria monocytogenes* amongst many" (Klinger *et al*., 2009). For late onset sepsis "Coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *E. coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, Group B *Streptococcus*, *Serratia*, *Acinetobacter,* anaerobes amongst many more have been implicated to be lead causers" (Van den Hoogen *et al*., 2010). Further studies have indicated that Gram positive bacteria associated mostly with late-onset neonatal sepsis infection, Coagulase Negative Staphylococcus (CONs) (25%), *Staphylococcus aureus* and *Escherichia coli* each (20.3%) (Sorsa, 2019).

Fungal pathogens especially *Candida spp.,* have been also reported to cause Late-onset neonatal sepsis with 12 to 18% among neonates with low birth weight (< 1500 gm). It's also documented to be the third most common cause of neonatal sepsis (Shane *et al.*, 2017; Ahmed *et al.*, 2019). Viral infections too like *Herpes Simplex virus* have been associated with Late-onset NS with a prevalence of 85% in United States while *Enteroviruses* frequency is not yet known (Modlin, 2015; Singh, 2020).

Various risk factors have been linked to NS, but among them prematurity and low birth weight has been established as the leading course in 1st and 3rd world countries (Siakwa *et al*., 2014). Therefore, it is plausible that local data needs to be generated on the most common causative agent for NS that will guide our healthcare facilities to manage this condition that is becoming a menace in our societies.

2.3 Prevalence and incidence of neonatal sepsis

Globally about 2.5 million preterm neonates die before 28 days of life and around 1 million new-born deaths occur daily in developing and developed countries. However, the occurrence may be higher in neonates and children which accounts to 47% of total infant deaths among the under five children due to preterm related complications (UNICEF, 2020). Europe and Northern America had the lowest under-five mortality rate of 54% which usually occurs during the neonatal period. Asia had a rate of 62% while Sub-Saharan Africa, Central and Southern Asia had the highest rate of 25 deaths per 1000 live births. This neonatal death occurs during the first week of life at a rate of 75% with 1 million new-born dying within 24 hours of birth (UNICEF, 2020). Neonatal infection has been found to be responsible for high numbers of new-born deaths. For instance, the number of death linked to acute bacterial infections in infants in the first days of life is 9.8 % in Sub-Saharan Africa, South and Latin America (Seale *et al.*, 2014). The rate of blood culture proven early-onset sepsis in the United States is approximately 0.3-2 per 1000 live births (Nathan, 2019). In Eastern Europe one study done at a Neonatal Intensive Care Unit (NICU) of Kosovo Hospital, out of the 1426 neonates admitted, 18.9% were diagnosed with proven sepsis. From the 107 proven cases for NS, 68 (63.6%) had Early-Onset Sepsis while 37 (34.6%) had Late-Onset Sepsis with 75 cases having Coagulase Negative Staphylococcus (Segal *et al.*, 2018). In another study done at a single tertiary unit in Greece, incidence rate of 8.6 per 1000 live births was reported following culturing of samples proven to be from neonatal sepsis cases (Kortsalioudaki, 2018). In South Asia pooled data in hospital-based reports from India, Pakistan, Nepal and Bangladesh of culture proven sepsis in community-based studies with molecular assays incorporated did approximate the rate of NS to be 12.3

per live births, while with only conventional proven positive results, the risk of sepsis was 5.5 per 1000 live births (Chaurasia *et al.*, 2019).

Africa countries have also not been left behind as they have documented high prevalence/incidence rates. For instance, in one study carried out in neonatal intensive care units in Egypt, incidence of NS was found to be 37- 50% or more of proved cases in 2015 (El-Din *et al*., 2015). Also, it has been documented that NS is responsible for 33% of neonatal mortality despite the advanced health care system in place in Ethiopia (Berhanu, 2014; UNICEF 2020). In Soweto, South Africa the rate of early on-set sepsis was reported to be 3.2/ 1000 live births, blood culture positive was 99/1231 (8.0%) (Valaphi *et al*., 2019). Another study done in west Africa in Ahmadu Bello teaching hospital in Nigeria, prevalence was reported to be 37.6% and *Escherichia coli* was the most isolated micro-organism (Olorukooba *et al*., 2020)

Despite the advancements of healthcare systems in Kenya it has not been left behind as some study that was done in Nairobi, Kenya in NBU of KNH a prevalence of 28.6% was reported with various risk factors like low birth weight, PROM, prematurity e. t. c. being attributed to the development of neonatal sepsis (Geyt & Hauck, 2016; Okube & Komen, 2020). Also, in another study done at Kisii level 5 hospital- Kenya, prevalence of neonatal sepsis was reported to be at 19.7% (95% CI 15.9-23.9) (Muturu *et al.,* 2018). While neonatal mortality rate in Kenya by 2019 was at 21 deaths per 1000 live deaths (UNICEF, 2020). Incidence of occurrence of this condition is not only influenced by economic status, but also mode of delivery, sex, and standard of neonatal care received (Shane & Stoll, 2014). However, to the best of our knowledge such studies have not been done at Kitale County Hospital, Kenya and its environs and therefore it is crucial

to identify the main NS associated pathogens and their antimicrobial profiles as this can assist in reducing the rates of infection.

2.4 Diagnosis of neonatal sepsis

Inflamation of the whole body is considered definitive of sepsis. Diagnosis is made if two of the four criteria are observed, i.e., abnormal leukocyte count or elevated temperature $\geq 38.5^0$ C or $\leq 36^0$ C; rapid heart rate or low heart rate; increased or depressed white blood cell count for a given age group, more than 10% immature neutrophils; mean respiratory rate of ≥ ±2 SD above normal age (Du Pont-Thibodeau *et al*., 2014). Laboratory diagnosis like reduced arterial blood flow, mottled skin, high oxygen flow demand, cord blood levels of prolactin or interleukin (IL) – 6 (Cottineau *et al*., 2014; Lutsar, 2014). Neonatal sepsis is detected clinically by signs and symptoms, confirmed by clinical laboratory tests, bacteriological blood culture, isolation and identification of microscopic organisms. (Marchant *et al*., 2013). Blood samples (3mls) are collected and immediately transferred in blood culture bottles which are incubated at 37^0C within the shortest time possible after removal for a maximum period of 14 days. The cultures are monitored daily for any changes (clotting, haemolysis, and turbidity) which are indications for a positive culture. Gram staining and biochemical tests are performed to identify the species of interest while antimicrobial susceptibility tests are done to determine the ideal antimicrobial agents for treatment purposes. However, new technologies in diagnosis of neonatal sepsis are nowdays available to fight against this killer disease among the preterm neonates. Molecular pathogen detection techniques (Polymerase Chain Reaction) are also available for detection of bacterial Deoxyribonucleic Acid (DNA) with the ability to detect a true positive and a true negative result within a shorter period compared to other methods (Opota *et al.,* 2015).

Gene expression profiling techniques can be used for evaluation but are not used in current neonatal setting (Sweeney *et al.,* 2017) due to financial constraints.

2.5 Management of neonatal sepsis

2.5.1 Introduction

Since neonatal sepsis is a life-threatening infection, prompt treatment reduces chances of complications and even death. Early detection of the causes and the sooner the management may reduce neonatal mortality and morbidity (Gebrehiwot *et al*., 2012; UNICEF Division of Data, 2014). Integrated Management of Childhood Illness (IMCI) recommends provision of prophylactic parenteral ampicillin and gentamicin in preterm neonates exposed to the risk factors for sepsis within 2 days (W.H.O., 2013). For infants aged < 59 days they do have their recommended guidelines that are usually followed (Basic paediatric protocol, 2016) which involves regulations for treatment of neonates within the shortest time upon admission, which should be adhered to by health facilities. This allows the facility to have the capacity for essential investigations and avail essential drugs for the care of seriously sick children as shown in table 2.1 below.

Table 2.1: The current guidance, including recommended dose and duration. (adopted from antibiotic use for sepsis in neonates and children: 2016 evidence update).

Reference	Conditions	Antibiotics	Dosing regimen
Pocket book hospital care for children, 2013	Prophylaxis in neonates with documented risk factors	IM or IV Ampicillin and Gentamicin for at least 2 days	**Gentamicin** (IM/IV): First week of life: Low-birth-weight infants: 3 mg/kg once a day; Normal birth weight: 5 mg/kg per dose once a day Weeks 2–4 of life: 7.5 mg/kg once a day **Ampicillin** (IM/IV): First week of life: 50 mg/kg every 12 h Weeks 2–4 of life: 50 mg/kg every 8 h **Benzylpenicillin (penicillin G)** (IM): First week of life: 50 000 U/kg every 12 h; Weeks 2–4 and older: 50 000 U/kg every 6 h **Procaine Benzylpenicillin**(IM)**:** 50 000 U/kg once a day **Cloxacillin** (IV)**:** First week of life: 25–50 mg/kg every 12 h; Weeks 2–4 of life: 25–50 mg/kg every 8 h
	Case definition PSBI	IM or IV Gentamicin and Benzylpenicillin or Ampicillin for at least 7–10 days	
	Greater risk of staphylococcus infection	IV Cloxacillin and Gentamicin for at least 7–10 days	
Managing possible serious bacterial infection in young infants when referral is not possible, 2015	Referral to hospital for young infants with PSBI is not possible	Option 1: IM Gentamicin once daily for 7 days and oral Amoxicillin twice daily for 7 days.	**Gentamicin**: IM 5–7.5 mg/kg (for low-birth-weight infants gentamicin 3–4 mg/kg) once daily **Amoxicillin**: Oral 50 mg/kg twice daily oral

2.5.2 Antibiotics commonly used to manage NS

2.5.2.1 Modes of action of commonly used antibiotics in management of NS

Antibiotics used in treatment of NS infections can be grouped in various categories. One of the categories include the antibiotics that do inhibit cell wall synthesis by binding penicillin proteins like the amoxicillin, Amoxicillin/Clavulanate (Augmentin). They belong to Penicillins group of antimicrobial and hinder the protein synthesis by binding to the 30S ribosomal subunit (Anderson, 2016; Hamid & Saqib, 2017). Another category of commonly used antibiotics belongs to a group that blocks protein synthesis by inhibiting the transpeptidation/translocation step of protein synthesis and they include drugs like Azithromycin, Doxycycline and Tetracycline etc which normally inhibits protein synthesis by binding to the 30S ribosomal subunit (Anderson, 2016; Hamid & Saqib, 2017). Other antibiotics commonly used belong to those that inhibit cell wall synthesis by interfering with synthesis of peptidoglycan layer and a good example of such drugs are Cephalosporins like Cephalexin (Anderson, 2016; Hamid & Saqib, 2017). Other drugs commonly used to manage NS do inhibit folate metabolism e.g. Fluoroquinolones like- Ciprofloxacin (Cipro) which interferes with DNA synthesis by inhibition of DNA gyrase and topoisomerase IV. Sulfamethoxazole/Trimethoprim also belongs to Sulphonamides class and acts directly on folate synthesis. Sulfamethoxazole hinders bacterial production of dihydrofolic acid while Trimethoprim interferes with the synthesis of tetrahydro folic acid by binding to inhibiting the required enzyme (Anderson, 2016; Hamid & Saqib, 2017). The last class of antibiotics that are commonly used in NS management do inhibit nucleic acid synthesis. Good examples of such drugs are Metronidazole of the Azoles class and interferes with DNA

synthesis by damaging DNA, Levofloxacin (Levaquin) of the Quinolones class and inhibits DNA gyrase and topoisomerase IV (Anderson, 2016; Hamid & Saqib, 2017).

Modes of action of antimicrobial agents is based on the function that is affected by the agents such as inhibition of ribosome function or cell membrane activity, inhibition of cell wall formation or nucleic acid formation and inhibition of folate metabolism (Dowling & Adley, 2017). Inhibition of ribosome function -Targets the lipopolysaccharide, inner and outer membranes and interfere with protein biosynthesis. They include aminoglycosides and tetracyclines (Aideen *et al.,* 2017; Hamid & Saqib, 2017). Inhibition of cell membrane function – they are lipophilic hence bind the lipopolysaccharide part of the outer membrane of Gram-negative pathogen. They include colistin, polymyxin B (Aideen *et al*., 2017; Hamid & Saqib, 2017). Inhibition of cell wall synthesis – they interfere with peptidoglycan synthesis by blocking steps involved making cells to be susceptible to osmotic lysis. They are bactericidal in their mode of action and include penicillin, cephalosporins, carbapenems and monobactams (Aideen *et al*., 2017; Hamid & Saqib, 2017). Inhibition of nucleic acid synthesis – They interferes with DNA replication in target cells while some work by blocking RNA polymerase activity (Aideen *et al*., 2017; Ullah & Ali, 2017). Inhibition of folate metabolism – They function by blocking the active site for bacterial biochemical reactions in metabolic pathways and interfere with folic acid synthesis in the bacteria. They include sulphonamides group of antimicrobial agents (Dowling & Adley, 2017; Ullah & Ali, 2017).

2.5.2.2 Resistance profiles of NS causing bacteria against commonly used antibiotics

As much as they have been found to offer recommendable reduction of NS, reports on emergence of drug and multi-drug (MDR) resistant bacterial strains causing neonatal sepsis are on the increase globally. This has been attributed to by easy accessibility and misuse of the non-prescribed antimicrobial agents, misuse of broad-spectrum antimicrobial agents and mutations that has been observed in microbes generally (Basavaraj *et al.,* 2013). Antimicrobial resistance has been worsened by the fact that bacteria have now started forming biofilms which are quite common also among the invasive procedures some of which are involved in management of NS infection cases (Le *et al.,* 2018). Coagulase-negative *staphylococci* (CoNS) have been documented to be the common pathogens responsible late onset sepsis in preterm neonates. They are mostly resistant to more than one antimicrobial agent and have the capability to adhere on the surface which is their main factor in disease development. A study was done in B.P Koirala Institute of health sciences University of Dharan, in Nepal on the relationship between bacteria adherence on surfaces and antimicrobial resistance in *Staphylococcus epidermidis* isolates of biofilm producers and non-biofilm producers which did report that there was “a significance association between biofilm formation and genes encoding *ica* operon, and *aap* gene (P<0.001). Therefore, presence of all genes that were involved in biofilm formation among biofilm positive strains were significantly higher than non-biofilm-producing strains” (WHO; 2018; Akbarian-Rad *et al.*, 2020).

Also, in a study done in India on antimicrobial resistance pattern among Coagulase Negative *Staphylococcus* (CONs), found out that there was resistance to methicillin with 66% and penicillin with 94% among all isolates, and were also found to be sensitive to glycopeptides. Mec A gene was found in all phenotypically methicillin-resistant isolates and in two of the phenotypically methicillin-sensitive isolates.

Detection of Mec A gene among CONs was found out to be of beneficial for reliable and early detection of methicillin resistance (Jain *et al.,* 2004; Omran & Hussein, 2019). In another study done in Southwest Iran, the findings did indicate high rates and capability of biofilm formation among *Staphylococcus epidermidis* isolates using phenotypic methods with many isolates having matrix *icaA* and *icaD* genes (Sheikh *et al.,* 2019). Sixty-five percent (65%) of *S. epidermidis* isolates produced biofilm while CoNS non-*epidermidis* were at 26% ($P < 0.001$). *S. Epidermidis* was associated antibiotic resistance due to biofilm formation (Sheikh *et al.,* 2019). In another study done in NBU in Johannésburg, South Africa, it did isolate *Klebsiella pneumoniae* and 66% of this isolate proved to be resistant to Ampicillin and Amoxicillin/Clavulanic acid (Ballot *et al*., 2019). In Kenya, a study was done in Nakuru County Referral hospital in 2017 and did reveal resistance of the isolates (*E. coli*, *S. aureus*, and *P. aeruginosa*) to the first line antibiotics (Ampicillin and Gentamycin) among others. There was a pooled resistance of the identified pathogens to all the commonly used antibiotics as follows, *E. coli*-88%, *S. aureus*-66% and *P. aeruginosa*-52% (Mumbi, 2017). Therefore, antimicrobial sensitivity and resistant testing may be of major importance in guiding rational prescription (Newton *et al.,* 2007; WHO, 2013) and need is there for it to be conducted and be part of the strategies of management of NS and other infections.

2.5.2.3 Common resistance genes associated with resistance of NS causing bacteria

NS causing bacteria have been found to produce Extended Spectrum Beta Lactamases (ESBLs) which have been found to enhance their ability to break down beta lactamase ring of the penicillin antibiotics (Thenmozhi *et al*., 2014). This ends up making the bacteria producing this enzyme to be resistant to the beta lactam ring containing antibiotics. Various studies have documented such groups of bacteria, for instance in

one study done in Asia-New Dehli, they did report a metallo-beta-lactamase (NDM-1) producing bacteria (Folgori *et al.*, 2017). Also, in another study that was carried out in the USA they did isolate *Klebsiella pneumoniae* carbapemenase that proved to be resistant against carbapenems antibiotics (Doumith *et al.,* 2017; Folgori *et al*, 2017). A study that was done in Morocco reported Oxacillinase (OXA) – 48 isolates to be resistant against carbapemenase antibiotics (Barguigua *et al.*, 2012; Doumith *et al.*, 2017). Another study that was carried out in Egypt Neonatal Intensive Care Unit (NICU), also did report *Klebsiella pneumoniae* carbapemenase that was resistant against Meropeneme, Cefoxitin, Piperacillin/Tazobactam and Tobramycin antibiotics (Hassuna *et al.*, 2020). Similarly in another study that was carried out in Sao Luis Brazil, they did isolate *Klebsiella pneumoniae* carbapemenase that proved to be resistant against carbapenems antibiotic (Ribeiro *et al.*, 2016).

NS causing bacteria have also been found to be associated with methicillin resistance due to presence of mecA gene that confers ability to bacteria to produce alternative penicillin-binding protein PBP 2A (Miragaia, 2018). This makes the bacterial mobile genetic element to confer resistance to non-β lactam antibiotics. Various studies have documented such findings for instance, a study done in India reported MecA gene in all phenotypically methicillin-resistant and in two of the phenotypically methicillin-sensitive CONs isolates that proved resistant to Erythromycin, Clindamycin and Gentamycin antibiotics (Jain *et al.,* 2004; Omran & Hussein, 2019). In another study done in Sao Paulo state, Brazil, mecA gene was reported and it was responsible for the resistance observed to oxacillin antibiotics (Taisa Trevizani Rocchetti., *et al* 2018). Another study in Saudi Arabia also did isolate mecA gene and MCC mec that proved to be resistant to penicillin and methicillin antibiotics (Albarrang *et al.,* 2020). In Nigeria, one study also did report mecA gene presence which was attributed to the resistance

observed against ceftazidime and meropenem antibiotics (Ibadin *et al.,* 2018). Such studies have also been conducted in some regions of Kenya and reported gyrA, rpoB, mecA, art (4') bl, aph (3') - 111a, ermA, sat – 4, fusA, mphC and msrA genes which were attributed to the resistance cases observed against methicillin and vancomycin antibiotics (Kyany'a *et al*., 2019). Therefore, these amongst many more studies is a clear indication that resistant genes spread is on the rise and need is there for proper policies to be put in place to assist in curbing the resistance cases that are becoming a global issue.

2.5.3 Medicinal plants usage in management of NS

Medicinal plants usage has been an age-old practise that exists even from the Precambrian period and humankind has used them for management of various ailments among them is the NS (Mensah *et al*., 2019). They are thought to be a cheaper source of medicare as they are naturally existing, and they have been proved to be better alternatives as they possess various phytochemicals with different modes of activity hence can easily clear the pathogens as they target various sites (Mahomoodally, 2013).

Various studies have documented their activities. For instance, Asian medicinal plants have been documented to be having the capacity to improve the treatment regimen for bacterial infections. For example, in a study that was done to validate antibiotic extracts from 18 medicinal plants used in Bangladesh by Khyang community against human pathogenic bacteria, it did reveal that hexane extracts from the bark of *Cinnamomum cacia* at concentration of 750/1125 (MIC/ MBC) µg/ml inhibited growth of MRSA, *Escherichia coli*, *Acinetobacter baumannii, Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*, (Hossan *et al.,* 2018). Also, in another study

that was carried out in Northern Iran in Mozandaran province on commonly used medicinal plants used by traditional healers did prove that many herbs were recommended for treatment of neonatal sepsis and neonatal jaundice (Fakhri *et al*., 2016).

Africa has not been left behind as traditional medicare is quite common in the continent. For instance, in one study done in Lagos, Nigeria it did reveal that administration of herbal medicines amongst neonates and babies who are ≤ 6 months old was inform of a polyherbal compound consisting of four to six plants and was perceived to be efficacious (Nwaiwu & Oyelade, 2016). Also, in another study done on medicinal plants commonly used around Cherangani Hills in Western Kenya found out that Asteraceae family of plant was the most dominantly used and roots were the commonly used parts of the plants. It was also found out that the commonly method of preparation was by decoction (boiling) which was used in treatment of various ailments including respiratory tract infections which proved to be effective (Mbuni *et al*., 2020). A study done in Bondo Kenya also found out that a wide range of plant extracts were used to treat common diseases amongst young children (Geissler *et al*., 2002). Therefore, this amongst many more studies is a clear indication that with proper scientific guidance and usage of the medicinal plants they can offer alternative medicare to NS.

2.5.4 Bacteriophages usage in management of NS

"Bacteriophages are viruses with ability to infect and kill bacteria devoid of any side effect on human or animal cell. They are often used alone or as a combination with antibiotics in treatment of bacterial infections" (Damigo-Clap & Delgado-Martinez, 2018). High increase of multi-drug-resistant pathogens with few effective

antimicrobials to select from has resulted to consideration of bacteriophages for management of bacterial infection. However there do have limitations on how to make formulations for clinical use and the danger of creating bacterial super bugs with high resistance through genetic material transfer. According to a study done in Russia, documentation that bacteriophage administration resulted in anti-phage antibody production with time post-oral bacteriophage treatment in infants and children was done (Pagava *et al.,* 2011; Pagava *et al.,* 2012). Another study done in a neonatal intensive care unit in Russia also did document that administration of Klebsiella bacteriophage cocktail orally for 5 days among new-borns completely decreased *Klebsiella pneumoniae* infections to zero. This clearly indicates high efficiency of bacteriophages due to their specificity and effectiveness to their targeted bacteria (Aslanov *et al.,* 2018). Also, in a similar study done in Bangladesh in 2017 on oral application of *Escherichia coli* bacteriophage among children proved to be successful in treatment of infections (Sarker *et al.,* 2016). These amongst many more studies are a clear indication that going forward with the rise in AMR phages could offer an alternative in management of bacterial infections NS being one of them.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was conducted at Kitale County Hospital (KCH) in Trans Nzoia County, (1.0219^0 N, 35.0015^0E) as shown in Figure 3.1 below. Trans Nzoia County is one of the counties in the former Rift Valley province in Kenya that covers about 2,470 km^2 and has a population of 818,757 people. It boarders Uganda on North-West, Elgeyo Marakwet County to the East, Uasin Gishu County to the South East, West Pokot County to the North, and Kakamega County to the South West. Kitale town is the head quarter and largest town in the county. The county also has 7 health centres, 28 medical clinics and about 6 nursing homes (Kenya census-2009). The number of admissions averages at 230-350 per day, this means that several patients share beds placing them, especially the neonates at risk of contracting sepsis through direct patient to patient contact. The New-Born Unit (NBU) unit at KCH has 3 rooms, one room for preterm neonates of 1.5kgs and below with 5 incubators each accommodating a maximum of 3 preterm neonates. Another room is for sick term infants and the last room is for referral neonates from outside facilities or home delivery which has 1 incubator. Average admissions in a day are 5 and sometimes it can be zero.

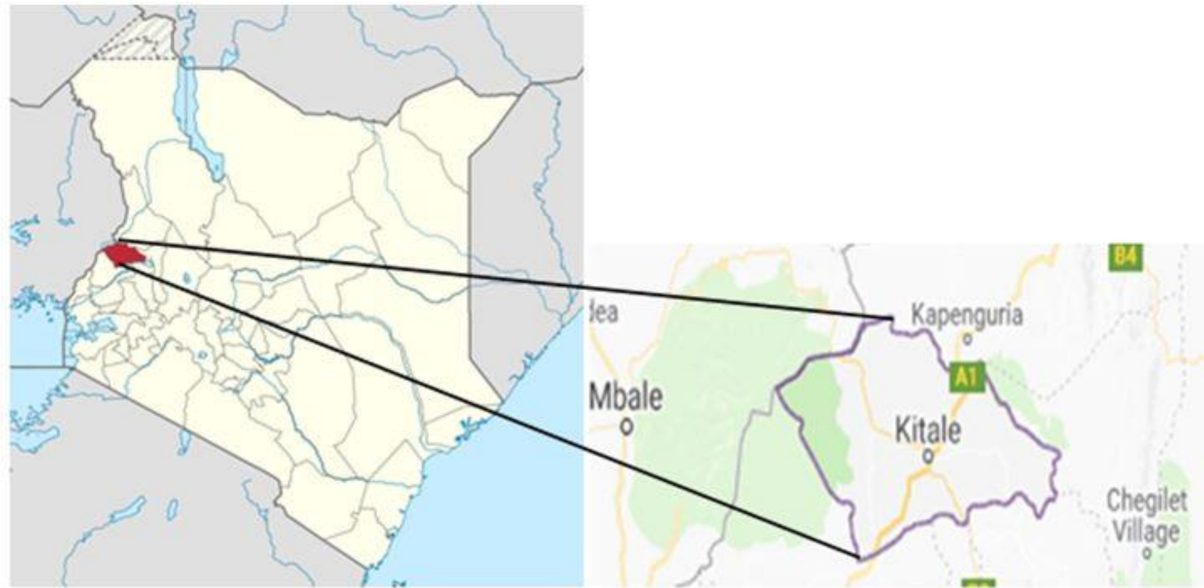

Figure 3. 1 Map of Kenya showing Trans Nzoia County and the study site
(Source: https://lands.go.ke/wp-content/uploads/2018/11/ The-map-of-Kenya.pdf).

3.2 Research design

Descriptive cross-sectional design was used, data was collected and assessed at one specific point in time.

3.3 Target population

The study targeted preterm neonates (born before 37 weeks of gestation) presenting with signs and symptoms of neonatal sepsis admitted in New Born-Unit (NBU) at KCH within the study period.

3.4 Eligibility criteria

3.4.1 Inclusion criteria

Preterm neonates of four weeks and below presenting with signs and symptoms of neonatal sepsis admitted in the new born unit at KCH during the study period were the

targets of this study. The study enrolled preterm neonates with existence of three of the four determinants (Lutsar *et al.,* 2014); e.g. chorioamnionitis, prematurity, symptoms of NS like lethargy, absence of reflexes, bradycardia, severe lung condition, difficult in breathing, low haemoglobin and seizure, temperatures of more than 38°C or less than 36°C.

3.4.2 Exclusion criteria

Preterm neonates whose parent/guardian did not give consent (Appendix 1.0) for their participation, those who were more than 37 weeks of gestation and those who had received antibiotics were also excluded from the study. If a neonate had a limb anomalies, blood was drawn from femoral artery.

3.5 Sample size

A prevalence rate of 13% bacteraemia was used to calculate the sample size as shown below in accordance to the findings of a study conducted at Kilifi District Hospital, Kenya (Berkley *et al*., 2005). Sample size was determined using Cochran's formula of 1963 as used before (Cochran, 1963).

$$n_o = \frac{z^2 pq}{e^2}$$

Where,

n_0 is the sample size

z is the critical value based on the desired confidence level (e.g. z = 1.96 for 95% confidence level).

m is the margin of error or precision of the estimate, m=0.05.

p is prevalence of the study. The prevalence of 13% was used to calculate the sample size, because there was no data showing the prevalence rate from previous studies in Trans Nzoia County.

$q = 1 - p$

e is the desired level of precision

$$n_0 = \frac{(1.96)^2 (0.13)(1 - 0.13)}{(0.05)^2}$$

$$n_0 = \frac{3.8416 (0.13 \times 0.87)}{0.0025}$$

$n_0 = 174$

This study therefore required the enrolment of a minimum of 174 preterm neonates (with a maximum age of twenty-eight (28) days) presenting with signs and symptoms of neonatal sepsis admitted in the new-born unit at KCH.

3.6 Structured questionnaires

Consenting parents of preterm neonates signed consent (appendix 1.0) on behalf of the subjects who qualified for the study. The parents also underwent face-to-face interviews and by use of well-structured and validated questionnaire (appendix 2.0). This was to capture information on the gestational age, birth weight of preterm neonate, history of maternal infection during pregnancy, breastfeeding practices, nutritional status, whether neonate is parenteral nutrition or not, for how long they were on parenteral nutrition, and whether they developed sepsis or not when on parenteral nutrition and previous antibiotic exposure.

3.7 Specimen collection

Blood specimen were collected aseptically as per methods that were used before (Ntusi, 2010; Holstege, 2011). Rubber cap of Himedia blood culture bottles (Himedia- *India*), with modifications, were prepared with alcohol swab in a circular motion, allowing alcohol to dry up. The preterm neonate was placed in a good position and the tourniquet was applied to palpate and identify appropriate vein. A venepuncture was performed and 3ml of blood sample was drawn in diphasic blood culture bottle while ensuring that bottles were not overfilled. The bottle was gently rotated to mix the blood and the broth (Ntusi *et al.*, 2010). Blood culture bottles were labelled with patient's serial number, date and time of sample collection and the request forms attached. The samples of blood cultures were sent to clinical microbiology laboratory receiving area as soon as possible between 8.00am and 5.00pm (normal hospital working hours) on Mondays to Fridays (normal working hours). Documentation in the laboratory Information Management System (LIMS) system was done with all the patient's details (in patient no, type of specimen, age, sex, residence e. t. c). For difficult venepunctures, proper assessment was done and enough time was taken to pick the right vein. Nitro-glycerine was used to increase the smaller veins. If neonate had hypovolemia, blood was drawn from larger veins.

3.8 Bioassays

3.8.1 Blood culturing

Continuously monitored blood culture systems was done at right conditions as per previously documented methods (Wayne, 2007). The diphasic blood culture bottle with blood sample was incubated at 37°C within the shortest time possible after removal for a maximum period of 14 days. The culture was then monitored daily for any changes

(clotting, haemolysis and turbidity). A positive blood culture (those that showed evidence of clotting, haemolysis or turbidity) was a critical result and was reported within the soonest time possible so as it could be used in making decisions about management of the patient concerned (Beekmann *et al*., 2003; Munson *et al*., 2003). For positive blood cultures a Gram staining and sub culturing were performed.

3.8.2 Isolation and identification of organisms

Isolated bacteria were identified based on cultural, morphological and physiological characteristics of each bacterial isolate, while colonies were described according to their colonial morphology (shapes, size, odour, haemolysis and colour). Gram staining reaction and biochemical test were performed as described in the standard Medical Microbiology laboratory manual (Hussein, 2016). Identification of Coagulase Negative Staphylococci (CONs) was done by biochemical tests followed by Vitek 2 compact microbiological analyser (bio Mérieux Inc. USA), Gram Positive identification card REF21342 for Gram Positive bacteria identification was used. All laboratory procedures were carried out according to the manufacturer’s directives.

3.8.2.1 Gram staining

The positive blood cultures (presence of bacteria in blood) were subjected to Gram staining for purposes of identifying the pathogens of interest using established protocols as used before (Behera *et al.,* 2010; Hussein, 2016). Briefly, a suspension of bacteria from a positive culture was used to make a smear on the microscope slide by using a sterile inoculating loop. The bacterial smear was heat fixed before adding 2-3 drops of Crystal Violet and left on the slide for 1 minute (Leboffe., 2014). Rinsing off the stain with water gently followed before adding the mordant (Iodine) to the smear and allowed

to stain for 1 minute (Stains File - Stain theory., 2016). Thereafter the rinsing of the mordant with water gently followed before adding a decolourizer (acid acetone) and left for 2 seconds on the smear (Welcome to Micro-bugz, 2017). Then 2-3 drops of Safranin Red Stain were added onto the smear for 1 minute to counter-stain the Gram negative Bacteria (Gram's Serendipitous Stain, 2016). The smear was then rinsed with water and air dried prior to microscopic examination (Barenfanger *et al*., 2008). For a positive Gram stain, the bacteria stained violet due to the presence of a thick layer of peptidoglycan in their cell walls which retained the crystal violet. Gram negative bacteria stained red due to the thinner peptidoglycan wall. All experiments were done in independent triplicates to enhance validity of the outcomes. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as control organisms.

3.8.2.2 Biochemical Tests

Various biochemical tests were used to identify species of interest among the isolated bacteria found to cause neonatal sepsis in preterm neonates. All experiments were done in independent triplicates to enhance validity of the outcomes. The various biochemical tests done for this study includes.

i. Catalase test

It was used to differentiate catalase-positive Micrococcal from catalase-negative *Streptococcal* species as used in previously established protocols and was done by slide drop method (Karen, 2010). Briefly, presence of enzyme catalase in bacterial isolate was evidenced by bubble formation when a small amount was introduced into hydrogen peroxide on a slide. While a negative result was evidenced by lack of bubble formation or weak bubble formation. *S. aureus* (ATCC 33592) was used as the positive control

while *E. faecalis* (ATCC 29212) was used as the negative control. Catalase positive test indicated presence of *Staphylococcus aureus* species and *Enterobacteriaceae.*

ii. Coagulase test

Coagulase tube test method was used to differentiate catalase-positive micrococcal and *Staphylococcal* species from Coagulase negative *Staphylococci* species as used before (Sagar, 2018). Briefly, a small amount of test organism was added in a tube containing 0.5 ml diluted rabbit plasma, mixed gently and incubated at 37^{0}C for 6 hours. Presence of coagulase enzyme in bacterial isolate was evidenced by a clot formation in the tube after 30 minutes interval examination as a positive result, while negative result was evidenced by no clot formation. *Staphylococcus aureus* (ATCC25923) and *Staphylococcus epidermidis* (ATCC12228) were used as control organisms. Coagulase positive test indicated presence of *Staphylococcus aureus*.

iii. Triple-Sugar Iron Agar (TSI) test

It was used to test the ability of organisms to ferment sugars and produce hydrogen sulphide and gas. This test was carried out to differentiate Gram negative isolates as per established protocols as used before (Sagar, 2019). Briefly, a well isolated bacterial colony from a pure culture was picked and stabbed through the centre of TSI medium (Himedia, India) to the bottom of the tube and streaked on the surface of the agar slant. The cap of the tube was closed loosely and incubated and at 37°C for 18 hours, the reaction medium was examined for any colour change and gas production. "Expected results were indicated as alkaline/acid (red slant/yellow butt) reaction showing presence of dextrose fermentation only, acid/acid (yellow slant/yellow butt) reaction showing presence of fermentation of dextrose, lactose and sucrose, alkaline/alkaline (red

slant/red butt) reaction showing of absence of carbohydrates fermentation, darkening of the medium (presence of hydrogen sulfide) and gas production (bubbles or cracks). Positive controls were *Shigella flexneri* ATCC 12022 with characteristic reaction of red slant/yellow butt H_2S (-), gas (-), *Protius vulgaris* ATCC 13315 with reaction of yellow slant/yellow butt H_2S (+), gas (+), *Salmonella typhimurium* ATCC 14028 with reaction of red slant/yellow butt H_2S (+), gas (+), *Escherichia coli* ATCC 25922 with reaction of yellow slant/yellow butt H_2S (-), gas (+)" (Sagar, 2019).

iv. Indole test

It was used for the identification of Enterobacteriaceae by demonstrating "the ability of certain bacteria to decompose the amino acid tryptophane to indole following already established protocols as used before (Sagar, 2019). Briefly, a well isolated bacterial colony was carefully picked and inoculated aseptically into a test tube containing 4 ml tryptophan broth (Deben diagnostic, United Kingdom). The tube was incubated at 37°C for 18 hours and 0.5 ml of Kovac's reagent was added to the broth culture. Presence of a red ring was indicative for positive result while absence of a ring formation was a negative result. *E. coli* ATCC25922 was used as positive control while *Klebsiella pneumoniae* (ATCC 13883) was used as negative control. Indole test positive test indicated presence of *Escherichia coli* species." (Sagar, 2019).

v. Methyl red test

The test was used to evaluate the ability of bacterial pathogen to "produce and maintain stable acid end products from glucose fermentation by production of a red colour following already established protocols as used before" (Sagar, 2019). *Escherichia coli* (ATCC 25922) was used as positive control while *Enterobacter*

aerogenes (ATCC13048) was used as negative control. Briefly, a pure culture of bacterial isolate was inoculated into Methyl Red Voges Proskauer (MRVP) medium (Biolife, Italy) and incubated at 37°C for 24 hours. Six drops of methyl red reagent was added to the broth culture and colour change was observed. Positive result was indicated by bright red colour/red-orange colour, while negative result was indicated by a yellow colour. Methyl red positive test indicated presence of *Escherichia coli*, *Salmonella species* and *Acinetobacter species*.

vi. Voges Proskauer test

It was used to "determine the ability of an organism to produce neutral end products (acetoin) from glucose fermentation." (Sagar, 2019). *Enterobacter aerogenes* (ATCC 13048) was used as positive control while *Escherichia coli* (ATCC25922) was used as negative control with already established protocols as used before were applied (Sagar, 2018). Briefly, an isolated colony of pure culture of bacterial isolate was inoculated into MRVP (Biolife, Italy) broth and incubated at 37^0C for 24 hours. Six drops of reagent I (alpha napthol) and two drops of reagent II (40 % KOH) were added into broth medium. Presence of pink-red colour at the surface of the media was indicative for positive result, while no colour change was indicative for negative result. Voges Proskauer test positive indicated presence of *Acinetobacter species*.

vii. Citrate test

It was used "to test an organism's ability to utilize citrate as a source of energy by growth with colour change from green to intense blue along the slant" according to already established protocols as used before (Sagar, 2019). *Klebsiella pneumoniae* ATCC 13883 and *Escherichia coli* ATCC 25922 were used as control

organisms as per methods used before Briefly, a slight inoculum of bacterial isolate was streaked back and forth on the slant of the citrate medium (Becton, Dickinson, United States of America) and incubated at 37^0C for 18 hours. Blue colour change was indicative for positive result, while green colour change was indicative for negative result. Citrate positive test indicated presence of *Pseudomonas aeruginosa*, *Salmonella species* and *Acinetobacter species*.

viii. Motility test

It was used to determine the motility of bacteria by differentiating motile bacteria from non-motile bacteria as per previously established protocols (Sagar, 2019). Briefly, an isolated colony of a bacterial isolate from a pure culture was stabbed in the medium (Deben diagnostics, United Kingdom). The tube was closed with cap loosely and incubated at 37^0C for 24 hours. Positive result is indicated by mobile organism extending from the stab line and producing turbidity or cloudiness throughout the medium, while non motile organisms grew only along the stab line. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control organisms. Mortility positive test indicated presence of *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella species*.

ix Ornithine decarboxylase test

It was used to differentiate decarboxylase producing Enterobacteriaceae from non-producing Enterobacteriaceae by measuring the organism's enzymatic ability to hydrolyse an amino acid to form an amine which results to an alkaline pH and a colour change following already established protocols as used before (Sagar, 2019). *Enterobacter aerogenes* ATCC13048 and *Klebsiella pneumoniae* ATCC 13883 were

used as control organisms. Briefly, an isolated colony of bacterial isolate from a pure culture was inoculated into ornithine decarboxylase broth tube (Deben diagnostics, United Kingdom). Four millimetre layer of sterile mineral oil was added to the tube and incubated at 37^0C for 18 hours. Positive result is indicated by alkaline (purple) colour, while negative result by acid (yellow) colour change. Ornithine decarboxylase test positive indicated presence of *Escherichia coli* and *Salmonella species*.

x. Urease test

It was used to “determine the ability of a bacterial pathogen to break down urea by the action of urease enzyme to produce ammonia and carbon dioxide” (Sagar, 2019). following established protocols as used before. Briefly, an isolated colony of a test organism was streaked on the surface of urea agar slant on urea broth (Scharlau, Spain) containing phenol red as indicator. It was incubated at 37^0C for 24 hours and positive result was indicated by deep pink colour, while negative result by yellowish-orange colour. *Proteus vulgaris* (ATCC 13315) and *Escherichia coli* ATCC 25922 were used as control organisms. Urease test positive indicated presence of *Proteus vulgaris*.

xi. Oxidase test

It was used “to evaluate the ability of bacterial pathogen to produce cytochrome oxidase enzyme by development of deep purple colour” (Sagar, 2019). following already established protocols as used before. *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC 25922 were used as control organisms. Briefly, an isolated colony of test organism from a fresh culture was picked and inoculated in 4.5 ml of nutrient broth (Himedia, India), 0.2 ml of 1% naphthol was added and then 0.3 ml of 1% of amino dimethylaniline oxalate. The mixture was shacked vigorously and observed for

colour change, expected results were development of purple colour within 2 to 3 minutes for positive results while no colour change for negative results. Oxidase test positive results indicated presence of *Pseudomonas aeruginosa*.

xii. Arabinose (1%) test

It was used to determine if a bacterium was able to ferment carbohydrate arabinose as a source of carbon by colour change from red to yellow following already established protocols as used before (Sagar, 2018). "Inoculum from a pure culture transferred aseptically into a sterile tube of phenol red arabinose broth medium (Himedia, India). The inoculated tube was incubated at 37^0C for 24 hours and results determined. Positive result was indicated by colour change from red to yellow while red colour was for negative result." (Sagar, 2019). *Escherichia coli* ATCC 25922 and *Proteus vulgaris* ATCC 13315 were used as control organisms. Arabinose (1%) test positive results indicated presence of *Enterococcus species*.

xiii. Bile esculin (40%) test

It was used to differentiate bacteria that were able to break down esculin to esculetin in presence of 4% bile seen by growth and blackening of the slant following already established protocols as used before (Sagar, 2017). *Enterococcus faecalis* (ATCC 19433) and *Escherichia coli* (ATCC25922) were used as control organisms. Briefly, two colonies from a pure culture was inoculated onto the surface of the slant of bile esculin agar medium (Becton Dickinson, United States of America) and incubated at 37^0C for 24 hours. The broth culture was then observed for growth and blackening of the medium. Positive result was indicated by growth and blackening of the agar slant

while negative result by growth and no blackening of medium. Bile esculin (40%) test positive results indicated presence of *Enterococcus species*.

Xiv. NaCl broth (6.5%) test

It was used to evaluate the ability of bacterial pathogen to survive in a salt-rich environment and was used to differentiate Enterococci from non-enterococci following already established protocols as used before (Sagar, 2020). *Enterococcus faecalis* (ATCC29212) was used as positive control while *Streptococcus bovis* (ATCC9809) as negative control. "Medium inoculated with a test pathogen and incubated at 37% for 24 hours. A positive test was indicates by colour change from purple to yellow while a negative test was denoted by no colour change." (Sagar., 2019). NaCl broth (6.5%) test positive results indicated presence of *Enterococcus species* (Sagar, 2019).

3.9 Antimicrobial sensitivity tests – Disc diffusion test and MIC

3.9.1 Preparation of bacterial inoculum

Isolated bacterial colonies (4-5) were obtained from a pure 18-hour culture, suspended into 3ml heart infusion broth and mixed well. The mixture was adjusted to a 0.5 McFarland standard using a densitometer (Biomerieux, USA) following already established protocols (Sagar., 2020).

3.9.2: Medium preparation

Mueller Hinton agar (38g) was suspended in 1000ml of distilled water, mixed well to dissolve and autoclaved at 15pa of pressure, 121^0C for 15 minutes using already established standards (Sagar., 2018). The agar medium was cooled to 50^0C, carefully

dispensed into sterile plates (20ml) on a sterile flat surface and allowed to solidify at room temperature.

3.9.3 Disc diffusion test

Susceptibility testing was done using Kirby-Bauer disk diffusion method on Muller Hinton media (Himedia, India) and incubated at 37^0C for 18 hours to evaluate the antimicrobial activity of commonly used antibiotics (Penicillin G 10 Units, Oxacillin 1µg, Gentamicin 10µg, Levofloxacin 5µg, Moxifloxacin 5µg, Clindamycin 2µg, Erythromycin 15µg, Linezolid 30µg, Tetracycline 30µg, Vancomycin 30µg, Ampicillin 10µg, Amoxicillin/ Clavulanic acid 20/10µg, Piperacillin/ Tazobactam 100/10µg, Cefazolin 30µg, Ampicillin/ Sulbactam 10/10µg, Cefuroxime 30µg, Cefepime 30µg, Cefoxitin 30µg, Aztreonam 30µg, Meropenem 10µg, Trimethoprim/ Sulfamethoxazole 1.25/23.75µg Amikacin 30µg, Gentamicin 10µg, and Ciprofloxacin 5µg) (Magaldi *et al*., 2004). Already manufactured discs impregnated with antibiotics were sourced from established suppliers (Biomerieux-France) and were used in this study. The agar plate surface was inoculated with the bacteria of interest (100µl) by spreading plate method. Then the sterile discs were placed gently and aseptically on the surface of the agar with inoculated bacteria (100µl) before the agar plates were incubated under suitable conditions depending upon the test microorganism. The zone of inhibition was read and compared with the standards set for various antibiotics and microorganisms (CLSI standards). The amount of space (measured in mm) around every antibiotic plate indicated the lethality of the antibiotic on the bacteria. Highly effective antibiotics produced a wide ring of no bacterial growth, while an ineffective antibiotic showed no change in the surrounding bacterial concentration. *S. aureus* ATCC 33592 and *E. coli* ATCC 25922 were used as standard microorganisms for quality control.

3.9.4 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) for individual antimicrobial agents were determined by Epsilometer test (E test) method which involves the dilution and diffusion of the antibiotic into the medium. Antimicrobial agent concentrations (Oxacillin, Gentamicin, Clindamycin, Erythromycin, Linezolid, Vancomycin and Tetracycline had MIC concentration range of 0.016-256 µg/ml each, while Levofloxacin and Moxifloxacin had an MIC concentration range of 0.002-32 µg/ml) were immobilized along the test strips in a continuous and exponential gradient. Drop shaped inhibition zones intersected the graded test strip at the inhibitory concentration of the antibiotics after 18 hours of incubation. The intersections of the lower parts of the ellipse shaped growth with the test strips indicated the MIC values (µg/ml). Dilutions of 0.5 MacFarland standard of individual test strains were prepared in tubes of saline, sterile cotton swabs were used to streak the prepared inoculums onto the entire surface of the agar plates (Muller Hinton) and were left for 5 minutes to allow absorption of excess moisture. The strips were applied to agar surfaces using sterile forceps while the E end of the strips were placed at the edges of the plates with the scales facing upwards. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as controls. Plates were incubated at 37^0C for 18 hours while MIC values were read at the points where ellipses intersected the scales and were interpreted as Susceptible, Intermediate and Resistant, while breakpoints values were compared with the criteria recommended by CLSI.

3.10 Screening for carbapemenase and methicillin genes from isolates responsible for neonatal sepsis by QIAamp DNA Mini Kit (250)

Bacterial Deoxyribonucleic acid (DNA) was extracted and purified using QIAamp DNA Mini Kit (250) (Qiagen, Germany) manually as used before (www.qiagen.com). This involved three steps Lysis (The cell was broken to release DNA by proteinase K enzyme (20µl), Precipitation (DNA) was separated from cellular debris by addition of ethanol (200µl)), and Purification (Rinsing of DNA with alcohol). Real time (quantitative) Polymerase Chain Reaction (PCR) technique was used for the amplification of target DNA sequences using forward and reverse primers by Qiagen Rotor-Gene Q machine (SN-R0212229, Qiagen, Germany). Non template control (NTC) was used as negative control and a known positive control were incorporated during testing procedure. Rotor-Gene Q series Software 2.1.0 was used for visualization of amplification plots tabulated on lineal scale. Gram negative isolates were screened for carbapemenase genes namely: blaOXA-48, using forward primer (5´-TTACGGCCTGGGAAGTGTTC-3´) and reverse primer (5´-AAGGGATTCTCCCAAGCTGC-3´) (Yousfi *et al*, 2016).

BlaKPC using forward primer (5´-GATACCACGTTCCGTCGTG-3´) and reverse primer (5´-GCAGGTTCCGGTTTTGTCTC-3´) as used previously (Tenover *et al.,* 2006). Gram positive isolates were screened for methicillin mecA gene using forward primer (5´-GTTGTAGTTGTCGGGTTTGG-3´) and reverse primer (5´-CCACCCAATTTGTCTGCCAGTTTCTCC-3´) as used previously (Unal *et al*., 1992).

PCR involved three steps; Denaturisation (DNA became single-stranded at a higher temperature (95^0C) for 30 seconds and the hydrogen bonds broken), Annealing (Primers bond to complementary sequences) at 65^0C for 30 seconds and Extension (Taq DNA

polymerase enhance DNA synthesis by adding nucleotides to the growing DNA strand) at 72^0C for 60 seconds. All the steps were based on temperature variations of heating and cooling which involved repeated cycles (45 cycles) where DNA became double-stranded. Tubes containing primers were reconstituted by addition of nuclease free distilled water (300μl) and were vetoed to mix the contents. Master mix (10μl) was pipetted into PCR tubes followed by 2μl of forward primer and 2μl of reverse primer. DNA templates (6μl) for the isolates were added to the tubes. Non template control (NTC) was used as negative control and a known positive control were incorporated during testing procedure. Rotor-Gene Q series Software 2.1.0 was used for visualization of amplification plots tabulated on lineal scale. Test results were interpreted as positive at the intersection between threshold line and the start of amplification curve (exponential phase) at fixed signal threshold in number of cycles. DNA that was produced in each cycle was represented by fluorophore dyes produced on amplification plot. Fluorescence was released every time a new DNA copy was synthesized; therefore, the amount of fluorescence was proportional to quantity of DNA produced.

3.11 DNA fragment analysis from bacterial isolates by QIAGEN QIAxcel®-Pure Excellence

Automated QIAxcel Advanced system® (SN-30649, Qiagen Hilden, Germany) using QIAxcel DNA high resolution kit® (Qiagen, Germany) was used for the separation of DNA for all isolates that were positive for resistant gene markers as used before (http://www.Qiagen.com). QIAxel was used for detecting and measuring the base pair (bp) size of PCR-amplified DNA fragments following the manufacturer's instructions. Gel cartridges were loaded and the buffer tray was filled, samples were loaded and the process profile was then selected. DNA is negatively charged and therefore was

attracted to the positive terminus. Nucleic acid molecules were size separated by application of a current to a gel-filled capillary, which were detected as they migrated towards the positively charged terminus. Fragments that were migrating through a gel matrix within the capillary passed excitation and detection spots and the signal was transmitted through a photomultiplier tube to the QIAxcel Screen Gel Software for data interpretation. The distance of migration was inversely correlated to the size of DNA molecules with smaller molecules travelling a longer distance through the gel compared to the larger molecules. Sizes of DNA fragments within samples were estimated by comparison to fragments of known size in a DNA ladder. Results were visualized by QIAxcel Screen Gel software®, were evaluated and interpreted according to their DNA size in base pairs (bp) and were compared with known positive and negative controls.

3.11 Data analysis and presentation

Demographic data was analysed using IBM®SPSS® (version 21). While anti-microbial susceptibility data was analysed using World Health Organization WHONET software ®, Descriptive and inferential statistics were used to analyse quantitative data such as prevalence of NS. The effect of variable parenteral nutrition, gestational age, birth weight, infant nutritional status among preterm infants was analysed using Fisher exact test, univariate and multivariate test according to variable characteristics at the significance level of $p \leq 0.05$. Data was presented using tables, spread graphs, pie charts and pictures. Soft copy data was entered into data bases in password protected files while hard copy was kept under key and lock.

3.12 Ethical considerations.

The study was conducted according to the declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice (DICH-GCP). The protocol and informed consent form (Appendix 1.0) were reviewed and approved by Research and Ethics Committee of Moi University (Appendix 4.0) prior to any protocol-related procedures that were conducted. The investigator informed IREC on the progress of the study on a regular basis per the IREC requirements. Additionally, the National Commission for Science, Technology and Innovation (NACOSTI) research approval (Appendix 5.0), clearance from the County Government of Trans Nzoia (Appendix 9.0), Ministry of education (Appendix 8.0) and the KCH hospital management (Appendix 10.0) were sort and obtained. Written informed consent was obtained from each parent/guardian prior to any protocol/-procedures being conducted. Parent/guardian were informed on the minimal potential risks such as pain, haematoma during vene puncture. Pain and bleeding was minimized by finding a suitable vein, use of a right gauge needle, applying dry cotton swab after sample collection and observation after the puncture for about 5 minutes. All blood samples were drawn by a qualified phlebotomist. Benefits such as availability of a drug that was susceptible for client management were provided. Coded numbers and initials were used to identify participant’s laboratory information to maintain confidentiality. No clinical information was released without the parent’s consent except as necessary for monitoring of the study.

CHAPTER FOUR

RESULTS

4.1 Introduction

The overall objective of this study was to determine the microbial profiles and antimicrobial properties of microorganisms causing neonatal sepsis (NS) among preterm neonates at Kitale County Hospital (KCH) new born-unit, Kenya. This chapter summarizes the findings of the study.

4.2 Response Rate

In this study a total of 181 participants who met the eligibility criteria were recruited against the proposed sample size of 174 according to Cochran's formula of 1997, yielding a response rate of 104% (Table 4.1 below).

Table 4. 1: Response Rate

Response rate	Frequency	Percentage
Recruited	181	104%
Target	174	100%

4.3 Preterm neonate demographic findings

The study established the demographic set up of the participants within the study period. The demographic information analysed included the respondents' gender and age in days.

4.3.1 Gender of the participants

The findings on neonates' gender were studied and it was revealed that majority of the neonates were females 59.1% (107) as compared to males 40.9% (74). This information was provided by the neonate's mothers/guardians and results are summarized in figure 4.1 below.

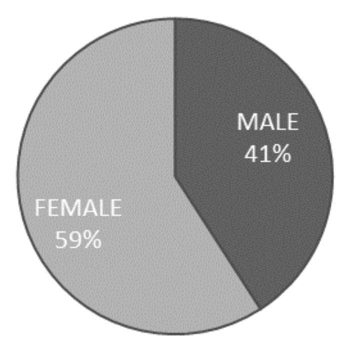

Figure 4. 1: Gender of preterm neonates

4.3.2 Age of Participants

This study also sought to establish the distribution of participant's age (in days) enrolled into the study and findings are as presented in figure 4.2 below. According to the findings of this study the age ranges among the participants ranged from zero (0) day to 10 days old neonates. One day old neonates were the most prominent at 57 (31.5%), followed by two days old 49 (27.1%) while three days old were 29 (16%). The least were eight and nine days old that had 1 (0.6%) neonate each, while the neonates' mean age was 2.48 ± 1.899 days old.

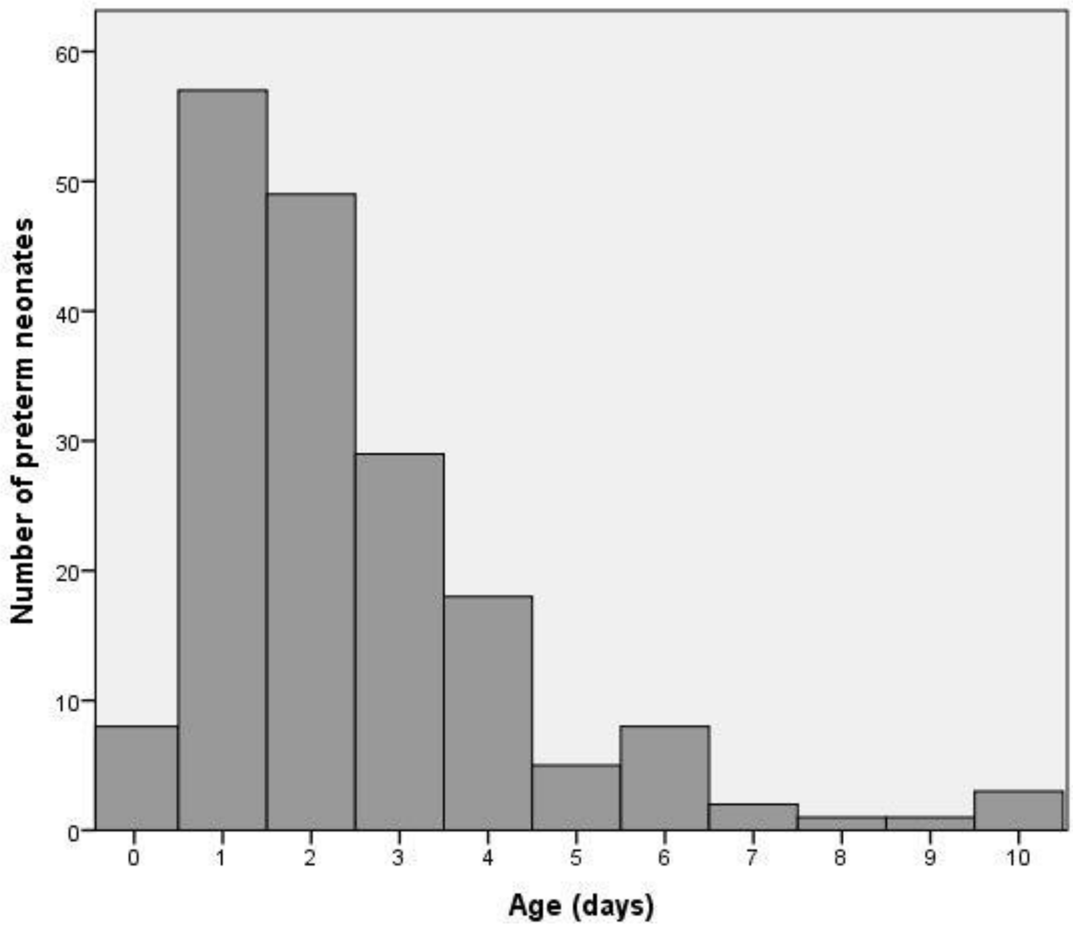

Figure 4. 2: Age of neonates in days with mean age 2.48 ± 1.899 days old (N=181).

4.3.3 Gestational Age (in weeks) completed at delivery

The study further established mothers' gestational age in weeks completed at delivery and results obtained are as presented in figure 4.3 below. The gestational age ranged from 30 to 36 completed weeks and 48 of the gravid mothers had a gestational age of 36 weeks, comprising of 48 (25.8%) followed by 35 weeks 41 (22%), 34 weeks (21%), on the other hand 30 weeks was the least 9 (4.8%) as shown in figure 4.3 below. The overall mean for the gestational age in weeks was 34.10 ± 1.743 weeks.

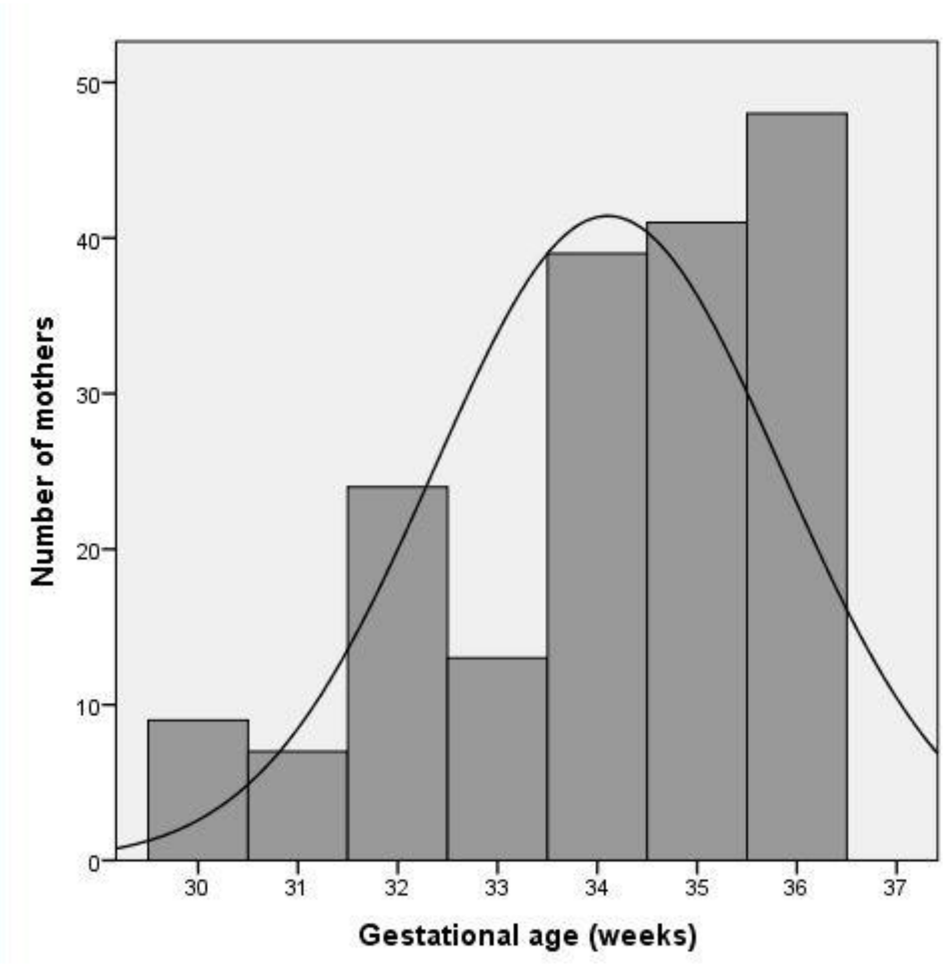

Figure 4. 3: The mothers gestational age (weeks) completed before delivery (N=181).

4.3.4 Neonate body temperatures

The neonates' temperatures were also investigated in this study and the findings did indicate that the neonates' temperature ranged between 38.0 ^{0}C and 39.6^{0}C with a median and mode of 38.70 and mean of 38.66 ± 0.3728, as illustrated in figure 4.4 below.

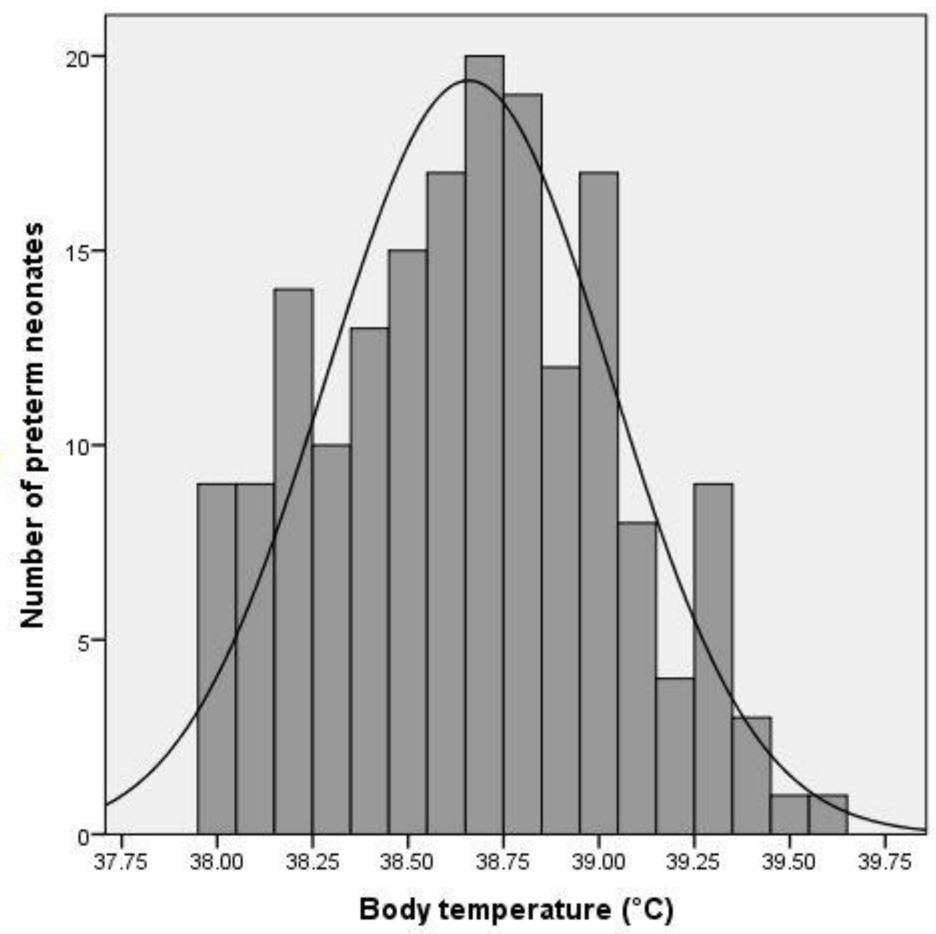

Figure 4. 4: The neonates body temperature (°C) distribution (N=181)

4.3.5 Neonate birth weight

The neonates' body weight was also checked in this study and it was measured in grams (gms) as illustrated in figure 4.5 below. Our findings did indicate that the neonates' body weight ranged from 1300 grams to 3600 grams with 28 (15.5%) having 2100 grams, followed by 24 (13.3%) with 1700 grams. On average the neonates birth weight had a mean of 2142.87 ± 482.01 grams.

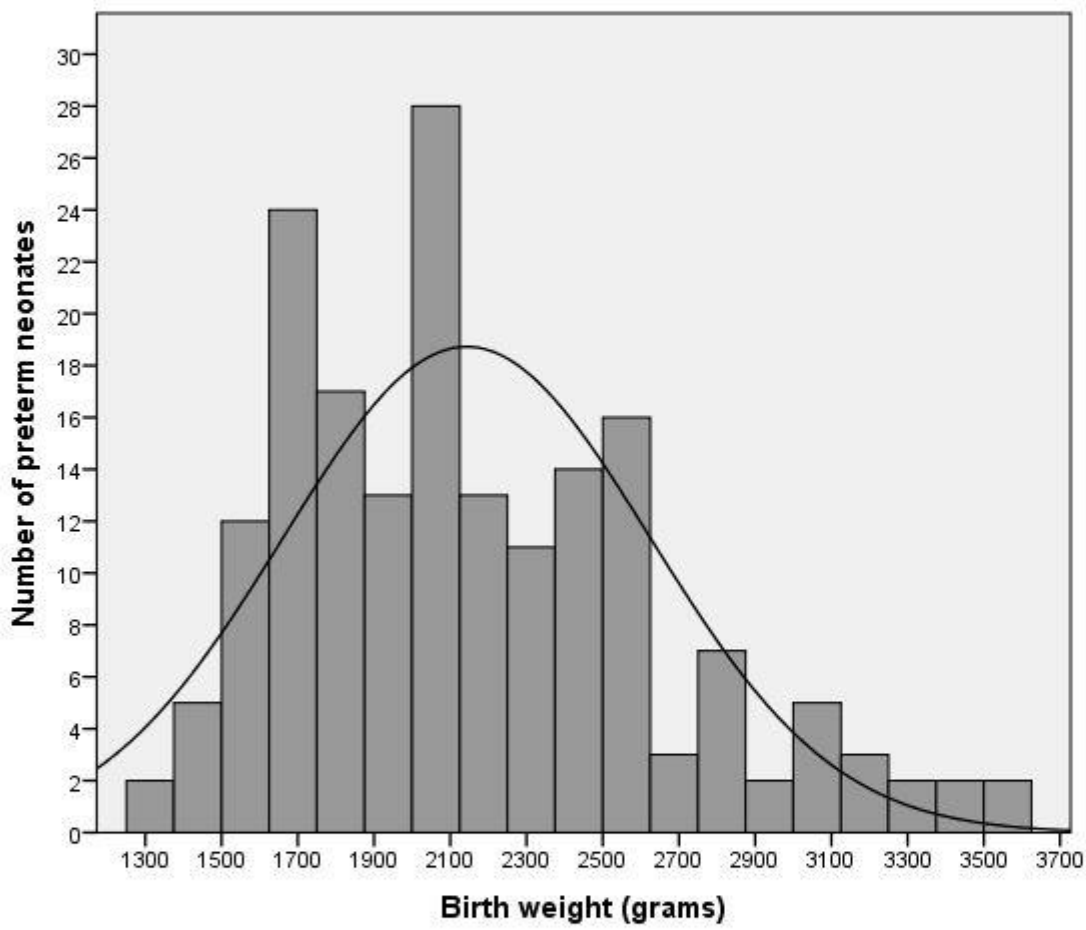

Figure 4. 5: The Neonates body weights (N=181).

4.3.6 Age (in days) of first presentation with sepsis

Amongst the neonates who had presented with sepsis, the study also did document the age in days on first presentation with sepsis, those born less than 24 hrs were designated as age zero. The findings did range from zero (< 24 hours) to ten (10) days. Most of the respondents (89%) did indicate that they developed sepsis within the first four (4) days, at one day old 57 (31.5%), followed by two day old 49 (27.1%), third day 29 (16%) and on the fourth day 18 (9.9%) amongst others as presented in Figure 4.6 below. However, the mean age in days for the neonates presenting sepsis symptoms/signs was deduced to be at 2.35 ± 1.939 days.

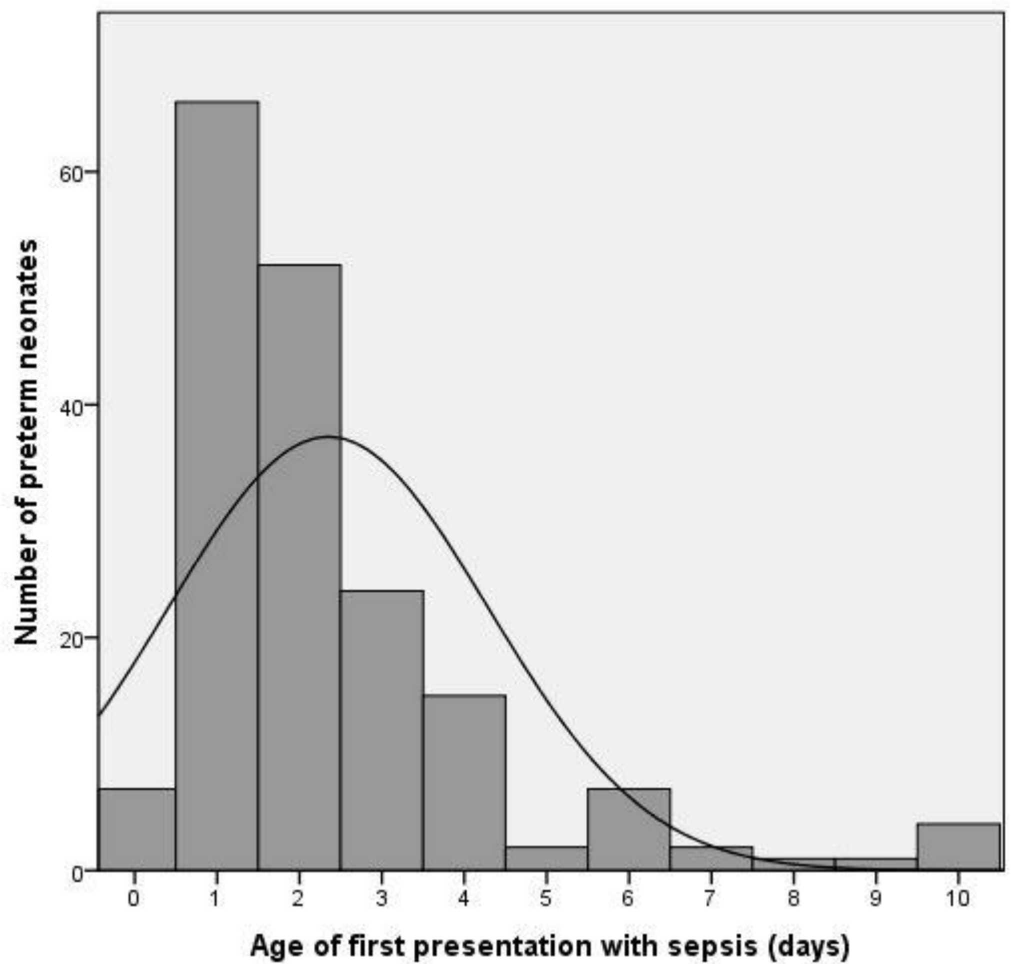

Figure 4. 6: Age (days) of first presentation with sepsis (N=181)

4.3.7 Parenteral nutrition, breast feeding and foetal distress

This study also did document findings on parenteral nutrition, breast feeding and foetal distress as illustrated in table 4.2 below. The neonates whose mothers had foetal distress were 65 (36%) while 9 (5%) were not breast feeding well. Others who didn't breast feed well were not put on parenteral nutrition and none developed sepsis, while only one had a central line 1 (0.6 %).

Table 4. 2: Parenteral nutrition, breast feeding and foetal distress

Variable	Yes (%)	No (%)
Central line prior to infection	1(0.6)	180 (99.4)
Parenteral nutrition	0 (0)	181(100)
Development of sepsis during parenteral nutrition	0 (0)	181(100)
Baby NOT breast feeding well	9 (5)	172 (95)
Foetal distress	65(36)	116 (64)

4.4 Mother's medical history findings

The study established mothers' medical history pre- and post-delivery. The information analysed included mothers' mode of delivery, history of urinary tract infection, illness in pregnancy, khan test, discharge during pregnancy, history of siblings born with neonatal sepsis, and maternal Group B *Streptococcus* (GBS) status. Study findings did indicate that majority of women had spontaneous virginal delivery (SVD) 149 (82%) compared with caesarean section mode of delivery 32 (18%).

However, it was clear that majority of the mothers 177 (97.8 %) did not have unspecified urinary tract infections. Only four (2.2 %) of the mothers had history of unspecified urinary tract infection during pregnancy period preceding delivery. The study found out the history of neonatal sepsis among previous birth from mothers/guardians and the findings are presented in table 4.3 below. Majority of the mothers 112 (61.9%) were not sure whether siblings had neonatal sepsis, 67 (37%) had knowledge of no neonatal sepsis, while 2 (1.1%) had knowledge of presence of neonatal sepsis in previous siblings.

Lastly, the mothers' medical antenatal history was also investigated with 28 (15%) of the 181 mothers indicating that they had illness during pregnancy, 2 (1.1%) tested

positive for khan test while 4 (2.2%) had discharge during pregnancy. Ten (5.5%) had elevated white blood cell counts, five (2.8%) received antibiotics during pregnancy whereas eight (4.4%) had premature rapture of membrane. The findings are presented in Table 4.3 below.

Table 4. 3: **Mother's past medical history (antenatal history)**

Variables	Frequency (N=181)	Percent (%)
a. Mode of delivery		
i. Spontaneous virginal delivery (SVD)	149	82
ii. Cesarean Section	32	18
b. Unspecified urinary tract infection	4	2.2
c. Sibling with history of neonatal sepsis		
i. Yes	2	1.1
ii. No	67	37
iii. Not sure	112	61.9
d. Any illness in pregnancy	28	15.5
e. Khan positive	2	1.1
f. Any discharge during pregnancy	4	2.2
g. Elevated maternal WBC	10	5.5
h. Maternal antibiotics given	5	2.8
i. Premature (PROM)	8	4.4

4.5 Factors associated with occurrence of neonatal sepsis

The study did also assessed the factors associated with the occurrence of neonatal sepsis among the preterm neonates. Mothers' antenatal and current medical history (mode of delivery, illness in pregnancy, khan test, discharge during pregnancy, prolonged rapture of membranes, premature rapture of membranes and urinary tract infection (UTI) in pregnancy) were taken as independent variables. Neonate factors (birth weight, central line, parenteral nutrition, foetal distress and breast feeding) were computed against dependent outcome (41 positive blood cultures) as evidence of neonatal sepsis out of the 181 blood cultures

Descriptive and inferential analysis was used to explain the nature and strength of association between independent and dependent variables. The odds ratio (OR) assessed the factors as exposure necessary for development of neonatal sepsis and were measured as OR =1 as not associated with NS, OR>1factor was associated with development of NS, while OR<1 factor was not associated with development of NS. The 95% confidence Interval (CI) was used to estimate the precision of OR. A large CI indicated lower precision of OR, whereas a smaller CI indicated a high precision of OR. Chi-square and p-value were calculated at p<0.05 and was considered statistically significant. The results are discussed in sub-sections 4.5.1 to 4.5.2

4.5.1 Maternal factors and development of neonatal sepsis

The maternal factors were studied as illustrated in table 4.4 below, the study found out that illness in pregnancy at p = 0.259, CI = 0.243-1.495 and premature rapture of membranes at p=0.358, CI=0.050-3.134 were not statistically significant with development of neonatal sepsis. Mode of delivery (SVD and C-section) had OR=1.101, $\chi 2$=0.046, CI=0.457-2.650 p=0.830 and prolonged rapture of membranes appeared to be associated with development of neonatal sepsis however did not reach statistical significance. Urinary tract infection (UTI) and maternal antibiotics taken had similar findings and had no association with development of NS, while none of the preterm neonate whose mother had Khan test positive results developed sepsis.

Table 4. 4: Association between maternal factors and development of neonatal sepsis

Factors	Neonatal sepsis (N = 41)					
	n	Percent (%)	OR	Chi-square	95% CI	P-value
a. Mode of delivery			1.101	$\chi2$ =0.046	0.457-2.650	p = 0.830*
i. SVD	34	82.9				
ii. C-Section	7	17.1				
iii. UTI in pregnancy	1	2.4	0.693	$\chi2$ = 0.112	0.079-6.045	p = 0.738
b. Any illness in pregnancy	5	12.2	0.602	$\chi2$ = 1.275	0.243-1.495	p = 0.259
i. Khan Test	0	0				
ii. Any discharge during pregnancy	1	2.4	0.924	$\chi2$ = 0.005	0.098-8.667	p = 0.945
iii. Prolonged rapture of membrane >18 hrs	2	4.9	1.108	$\chi2$ = 0.016	0.222-5.524	p =0.900*
c. Premature rapture of membrane	1	2.4	0.396	$\chi2$ = 0.844	0.050-3.134	p = 0.358
i. Maternal antibiotics taken	1	2.4	0.693	$\chi2$ = 0.112	0.079-6.045	p = 0.738

Significant at p<0.05, OR -Odds Ratio. Bold; is statistically significant (*) CI, confidence Interval

4.5.2 Neonatal factors and development of neonatal sepsis

Foetal distress was strongly linked to development of neonatal sepsis with OR=2.244. It was statistically significant at ($p = 0.018$, CI =1.143-4.407) followed by birth weight with OR=1.867 but was not statistically significant at ($p = 0.193$, CI =0.721-4.834). Low birth weight was linked to development of neonatal sepsis OR=1.867, $\chi 2$ =1.691, CI=0.721-4.834 ($p=0.194$). All the neonates were breast feeding, however those who were not breast feeding well and put on other mechanisms of feeding were more likely to develop NS, OR=1.688 and was not statistically significant at $p = 0.247$, CI = 0.691-4.123. One of the neonates had central line that was used to administer drugs. These study findings are summarised and presented in Table 4.5 below.

Table 4. 5: Association between neonatal factors and development of neonatal sepsis

Variable	Neonatal sepsis (N =41)					
	n	Percent (%)	OR	Chi-square	95% CI	P-value
a. Birth weight			1.867	$\chi2$ =1.691	0.721-4.834	p = 0.193
i. Normal	36	87.8				
ii. Low Birth weight	5	12.2				
iii. Central Line	1	2.4	1.008	$\chi2$ = 0.365	0.993-1.023	p = 0.574
iv. Breast feeding	41	100				
v. Parenteral nutrition	0	0				
b. Foetal distress	24	58.5	2.244	$\chi2$ = 5.633	1.143-4.407	p = 0.018*
c. NOT Breast feeding well	9	21.9	1.688	$\chi2$ = 1.338	0.691-4.123	p =0.247

Significant at p<0.05, OR -Odds Ratio. Bold; is statistically significant
(*) CI, confidence Interval

4.6: Isolation and profiling of common neonatal sepsis causing bacterial pathogens among preterms

4.6.1 Culturing

The research determined microbial profiles of the isolated pathogens responsible for neonatal sepsis among preterm neonates. Blood samples were collected from 181 eligible study participants, cultured in diphasic blood culture bottles, and incubated aerobically at 37^0C for up to 14 days. Presence of haemolysis, clotting and agglutination were considered positive for sub culturing as shown in figures 4.7 below. Positive blood cultures were sub cultured on sheep blood agar (SBA) and chocolate blood agar and incubated aerobically and anaerobically at 37^0C respectively for 18 hours and their growth is presented in figure 4.8 below. For positive subcultures (41), colony morphology reading was done and the morphologies observed were round, convex golden-yellow with beta haemolysis, big circular with grey moist beta haemolytic colonies. Some were small, white colonies with no haemolysis, and some appeared large metallic sheen with beta haemolysis. Culture result findings are illustrated in table 4.6 below. Sheep blood agar (5%) was used to differentiate bacteria based on their ability to haemolyse the red blood cells around their surroundings. Some bacteria 1 (2.9%) demonstrated Beta (complete) haemolysis and 3(8.6%) Gamma (no haemolysis) when cultured on sheep blood agar, while further tests were performed to identify species of interest.

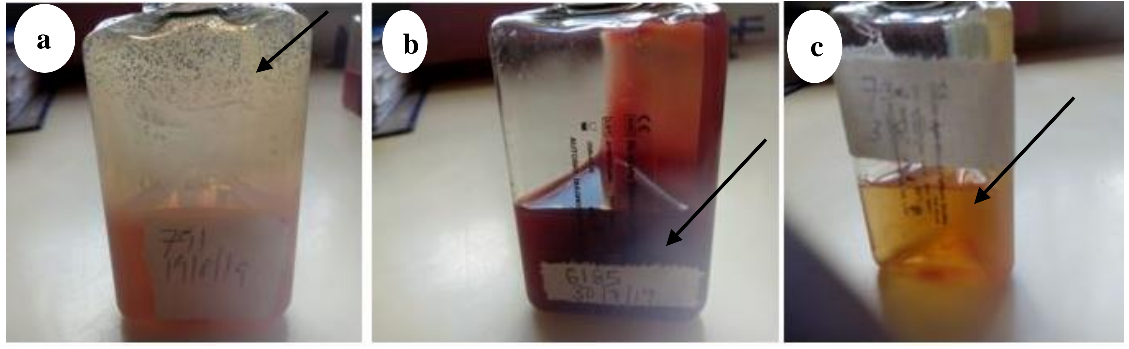

Figure 4. 7: Blood cultures in diphasic media

Plate a. Showing a positive blood culture growth (see arrow), Plate b. Showing a positive Blood culture growth with haemolysis (see arrow) and Plate c. Blood culture negative (no growth- see arrow).

The blood samples were also sub cultured on blood agar and 41 of the samples did grow on blood agar confirming the previous findings (Figure 4.7 above). They did produce various colonies with different morphologies as presented in figure 4.8 below. Among the morphologies observed were large metallic sheen-like colonies with beta haemolysis, which most likely represented *Pseudomonas aeruginosa*. Some colonies appeared big, circular, and grey in colour with beta haemolysis and likely represented *Escherichia coli*. Small colonies were white in colour with no haemolysis were observed and most likely represented *Staphylococcus epidermidis*.

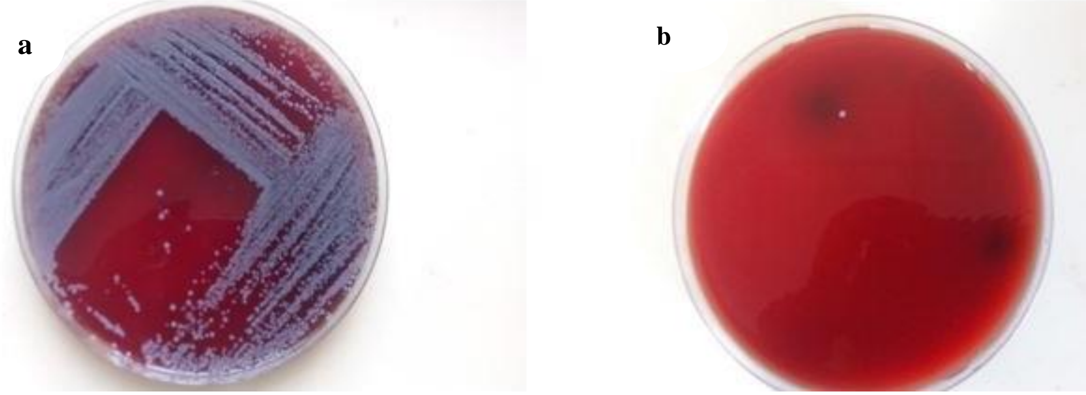

Figure 4. 8: Colony morphologies as observed from growth of bacterial sub cultures on blood agar media

Plate a. showing a positive culture growth and **plate b.** showing a negative culture growth on blood agar

4.6.2 Gram staining

Gram staining was also performed on all the positive cultures 41(100%) to identify the isolated bacteria. By use of their presentations after Gram staining, the bacteria were

separated into two groups, Gram positive cocci 35 (85.4 %) and they did present themselves as purple in colour while the Gram-negative rods 6 (14.6%) did present themselves as pink in colour as shown in figure 4.9 and Table 4.6 below

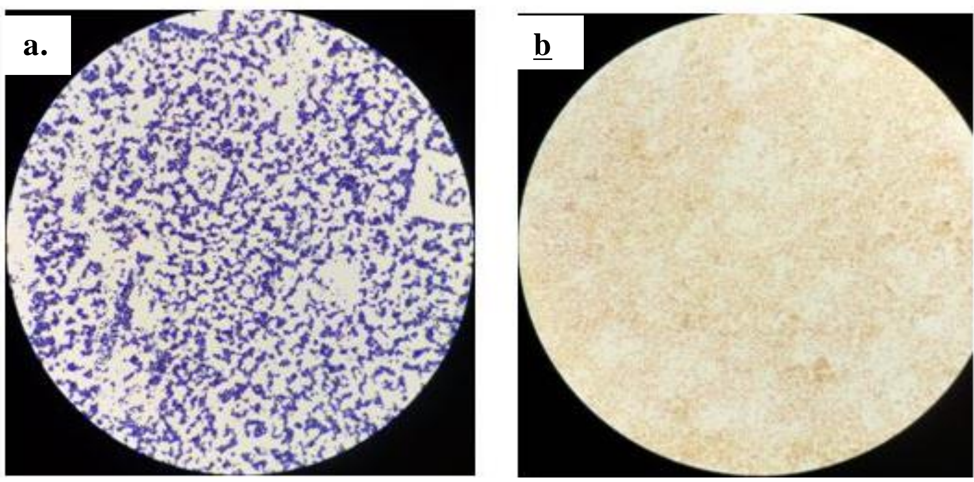

Figure 4. 9: Gram staining results

Gram positive cocci representative (plate a) and Gram negative rods representative (plate b).

The summary of these findings are presented in table 4.6 below.

Table 4. 6: Blood culture and Gram staining

Variables	Frequency (n=181)	Percent (%)
a. Blood culture	**181**	**100**
i. Blood culture positive (Hemolysis /Clotting / Agglutination)	41	22.7
ii. Subculture and purity plating	41	22.7
b. Gram staining (n=41)		
i. Gram positive	35	85.4
ii. Gram negative	6	14.6

4.6.3 Biochemical tests

Various biochemical tests were performed to identify species of interest. Microbiological quality protocols and standard operating procedures were followed in all stages (pre analytical, analytical and post analytical). Frequencies and percentages were used to demonstrate the distribution of the profiles as indicated in Table 4.7 below.

Catalase test positive findings were 1(2.9%) out of the 35 Gram positive isolates while negative findings were 34 (97.1%) as illustrated in figure 4.10 below while results are presented in table 4.7 below.

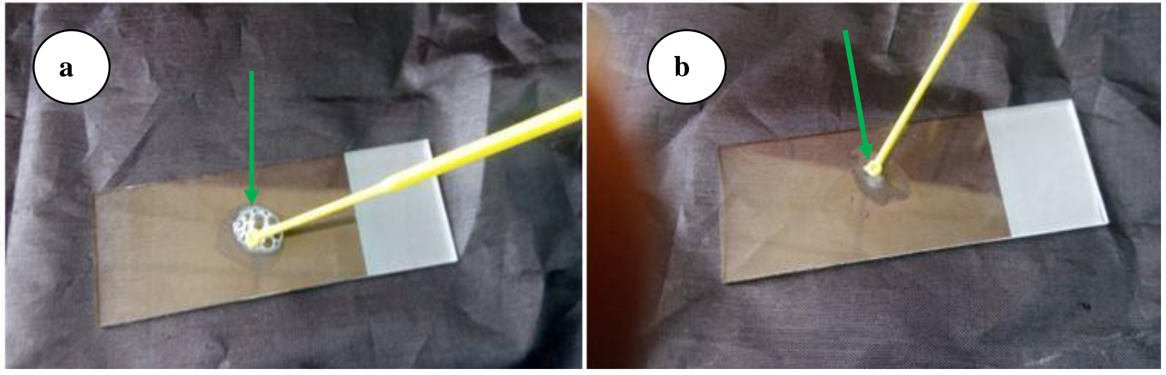

Figure 4. 10: The Catalase test plates

Plate a show a Catalase test positive result representative as shown by the green arrow while **plate b** is showing Catalase test negative representative finding as shown by the green arrow.

On the other hand, Coagulase test positive findings were 38 (92.6%) while coagulase negative findings were 3 (7.3%) as illustrated in figure 4.11 below. A summary of all test results are shown in table 4.7 below.

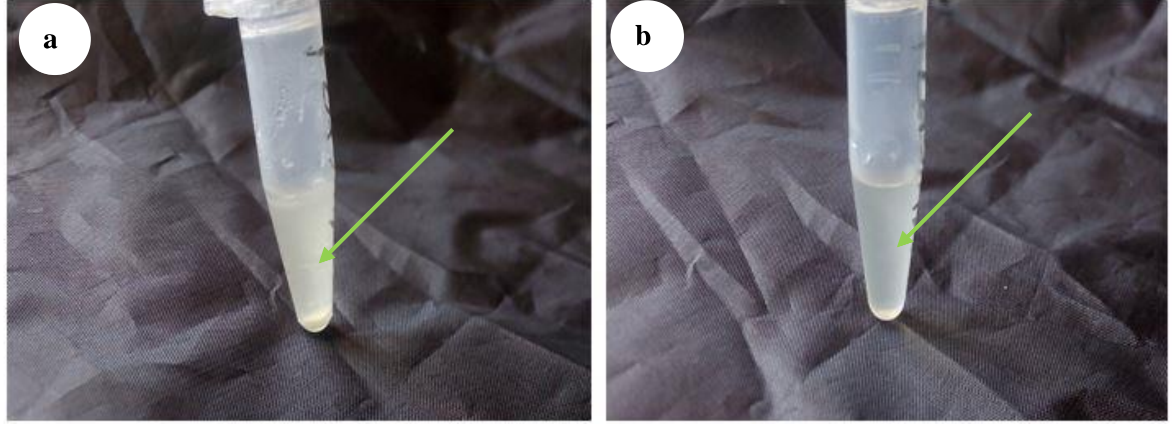

Figure 4. 11: Coagulase tube test

Plate a showing representative for coagulase positive result and plate b showing representative for coagulase negative result as shown by the green arrow.

Also, Triple Sugar Iron (TSI) test had different findings on the Gram-negative isolates (6). Among the positive findings for gas production were 4 (66.7%), while negative findings for gas production were 2 (33.2%). Positive findings for hydrogen sulphide production were 3 (50%) while for negative were 3 (50%) as shown in figure 4.12 below. A summary of result findings are shown in table 4.7 below.

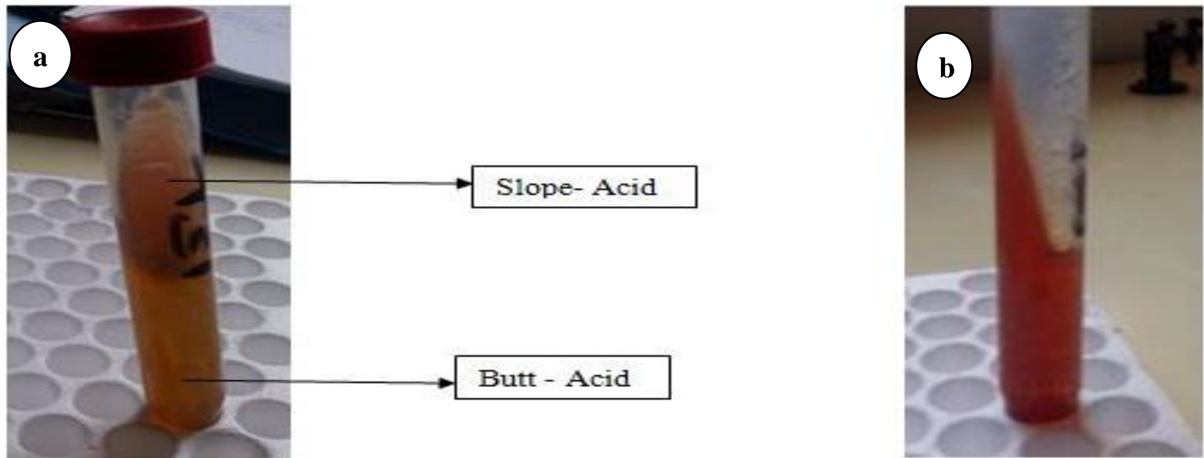

Figure 4. 12: Triple sugar iron test

Plate a showing a representative for TSI positive result while plate b is representing the TSI negative result.

Indole test was also done on the isolates and positive findings among Gram negative isolates (6) were 1 (16.6%) while negative findings were 5 (83.4%). Additionally, motility test was also performed on the Gram-negative isolates (6) with positive findings being observed in 5 (83.4%) isolates out of the 6 Gram negative isolates while negative findings were 1 (16.6%). Lastly, Ornithine decarboxylase biochemical test was also done with 4 (66.6%) positive findings being reported while negative result findings were 2 (33.4%) as illustrated in figure 4.13 below and summarised in table 4.7 below.

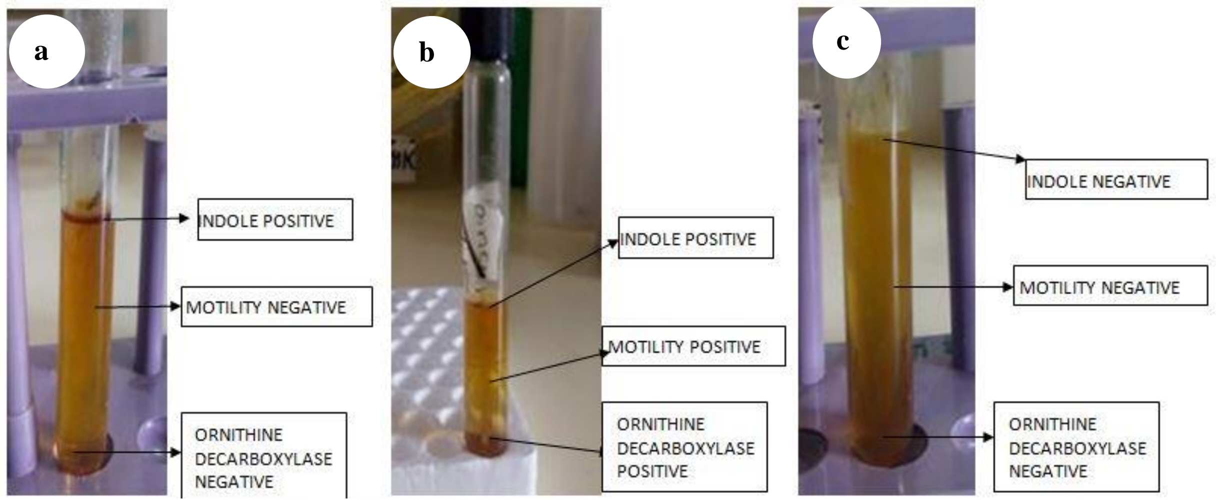

Figure 4. 13: A representative for Motility, indole and ornithine decarboxylase tests

Plate a and b showing Motility, indole and ornithine decarboxylase positive results while plate c representing Motility, indole and ornithine decarboxylase negative results.

Positive findings for citrate test among Gram negative isolates (6) were 5 (83.4%), while negative findings were 1(16.6%) as presented in figure 4.14 below. Voges Proskauer test positive findings among Gram negative isolates (6) was 1 (16.6%), while negative findings were 5 (83.4%). Urease test positive findings among Gram negative isolates (6) was 1 (16.6%) while negative findings were 5 (83.4%). Sodium chloride broth (6.5%) test among Gram positive isolates positive findings were 3 (8.6%) among

the *Enterococcus* spp. Bile esculin (40%) test among Gram positive isolates positive findings were 3 (8.6%) for *Enterococcus* spp. All test results are shown in table 4.7 below

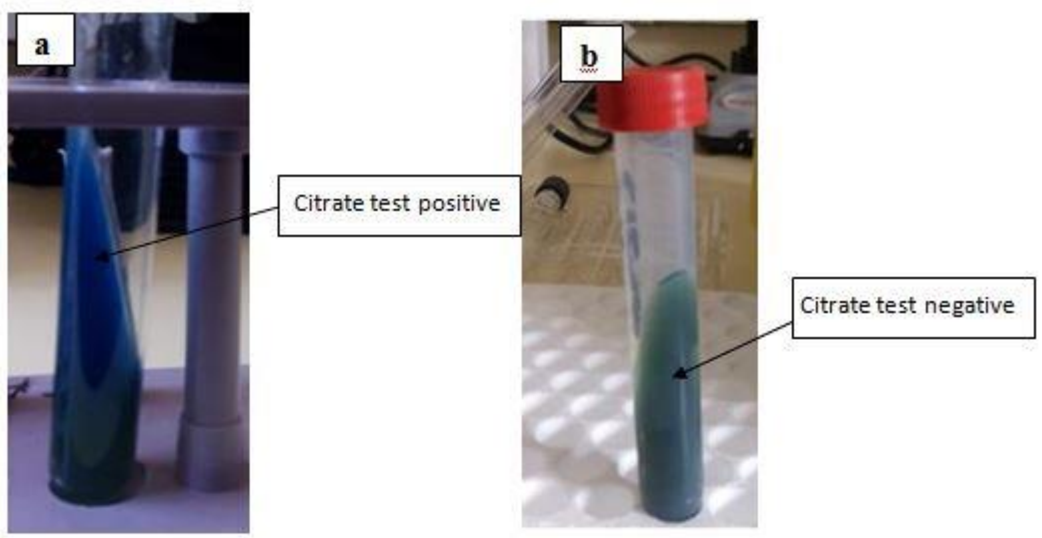

Figure 4. 14: Citrate test representatives

Plate a showing a citrate positive result while plate b is representing the citrate negative result.

In conclusion, the Gram negative 6 (14.6) % isolates that were Coagulase (-), Catalase (+), TSI (butt –Alkaline/slope Acid, Gas (-) H_2S (-), Indole (-), Methyl red (-). Voges prosker (-), Citrate (+), Urease (-), Oxidase (+), Motility test (+), and Ornithine decarboxylase (-), from these biochemical tests reactions most likely indicated presence of *Pseudomonas aeruginosa.* Those that were Coagulase (-), Catalase (+), TSI (butt –Acid/slope Acid, Gas (+) H_2S (-), Indole (+), Methyl red (+). Voges Proskauer (-), Citrate (-), Urease (-), Oxidase (-), Motility test (+), and Ornithine decarboxylase (+) are most likely indicating the presence of *Escherichia coli*. In addition, for those isolates that proved to be Coagulase (-), Catalase (+), TSI (butt –Alkaline/slope Acid, Gas (+) H_2S (+), Indole (-), Methyl red (+). Citrate (+), Urease (-), Oxidase (-), Motility (+),

and Ornithine decarboxylase (+), they most likely indicate presence of *Salmonella spp*. Lastly, among the Gram-negative isolates that proved to be Coagulase (-), Catalase (+), TSI, Indole (-), Methyl red (+). Voges Proskauer (+), Citrate (+), Urease (-), Oxidase (-), Motility test (-), and Ornithine decarboxylase (-), are most likely indicative of presence of *Acinetobacter spp* as shown summarized in Table 4.7 below.

For the Gram positive 35 (85.4%) isolates that were Coagulase (+), Catalase (+) from these biochemical tests reactions most likely indicate presence of *Staphylococcus aureus.* For the Coagulase (-), Catalase (-), Arabinose 1% (+), Bile esculin 40% (+) & Sodium chloride broth 6.5% (+) from these biochemical tests reactions most likely indicated presence of *Enterococcus spp.* While isolates that proved to be coagulase (-), and Catalase (+) from these biochemical tests reactions most likely indicated presence of Coagulase Negative *Staphylococci* (CoNS) as shown in table 4.7 below.

Table 4. 7: Summary of biochemical tests

Biochemical test		Gram staining N=41						
		Gram negative n=6 (14.6%)				Gram positive n=35 (85.4%)		
Coagulase		-	-	-	-	+	-	-
Catalase		+	+	+	+	+	-	+
TSI	Butt/ Slope	Alkaline/Acid	Acid/Acid	Alkaline/Acid	Alkaline/Alkaline	NT	NT	NT
	Gas	-	+	+	-	NT	NT	NT
	H_2S	-	-	+	-	NT	NT	NT
Indole		-	+	-	-	NT	NT	NT
Methyl red		-	+	+	+	NT	NT	NT
Voges prosker		-	-	NT	+	NT	NT	NT
Citrate		+	-	+	+	NT	NT	NT
Urease		-	-	-	-	NT	NT	NT
Oxidase		+	-	-	-	NT	NT	NT
Motility test		+	+	+	-	NT	NT	NT
Ornithine decarboxylase		-	+	+	-	NT	NT	NT
Arabinose test (1%)		NT	NT	NT	NT	NT	+	NT
Bile esculin (40%)		NT	NT	NT	NT	NT	+	NT
Sodium chloride broth (6.5%)		NT	NT	NT	NT	NT	+	NT
	Pathogen	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella spp*	*Acinetobacter spp*	*Staphylococcus aureus*	*Enterococcus spp*	*CoNS*
	Number (%)	1 (2.4%)	1 (2.4%)	3 (7.3%)	1 (2.4%)	1 (2.4%)	3 (7.3%)	31(75.6)

NT (Not done), Spp (species) CoNS (Coagulase negative staphylococcus)

Identification of Coagulase Negative *Staphylococcus* (CoNS) was done by biochemical tests (catalase test positive and coagulase test negative) and confirmed by automated Vitek 2 Compact (Bio Mérieux Inc.USA®) with highest detectable percentage probability scores per each isolate. *Staphylococcus epidermidis* were found out to be the majority 19 (61.2%) with percentage probabilities of 99% for nine isolates, 96% for five isolates, 94% for two isolates, 95%, 93% and 86% for three isolates. *Staphylococcus haemolyticus* 5 (16.1%) with percentage probability of 99% for four isolates each and 93% for one isolate. *Staphylococcus hominis* had two cases (6.5%) with probability of 95% each, *Staphylococcus lentus* had two cases (6.5%) at percentage probability of 89% and 87%, *Staphylococcus warneri* had two cases (6.5%) at percentage probability of 96% per each case while *Staphylococcus saprophyticus* had one case (3.2%) at percentage probability of 95%. The distribution of CoNS is shown and summarised in Table 4.8 below.

Table 4. 8: **Distribution of Coagulase negative *Staphylococcus* (CoNS)**

	N=31		
Coagulase negative Staphylococcus (CoNS)	**Frequency**	**Percent (%)**	**Detectable percentage Probability (Vitek 2)**
S. epidermidis	19	61.2	(86-99)
S. haemolyticus	5	16.1	(93-99)
S. saprophyticus	1	3.2	95
S. hominis	2	6.5	95
S. lentus	2	6.5	(87-89)
S. warneri	2	6.5	96

4.7 Antimicrobial characteristics of the pathogens inducing NS among the preterms

The study deduced antimicrobial characteristics of the 41 identified pathogens responsible for neonatal sepsis at Kitale County Hospital new born unit. Antimicrobial

agents were chosen based on specific organism group for routine testing and reporting as per Clinical and Laboratory Standards Institute (CLSI) guidelines. CLSI performance standards for Antimicrobial Susceptibility Testing 30th ed. CLSI supplement M100 (2020). Breakpoints of individual antimicrobial agents were used to determine the zone diameter values (mm) and results interpreted to the nearest millimetre as; Susceptible (S) where (a zone diameter at or above the susceptible break point was inhibited by the antimicrobial agent), Intermediate (I) where (a zone diameter was within the intermediate range) and Resistance (R) where (a zone diameter at or below the resistant break point was not inhibited by the antimicrobial agent). Frequencies, and percentages were used to demonstrate the characteristic of organisms. The results were discussed in sub-section 4.7.1

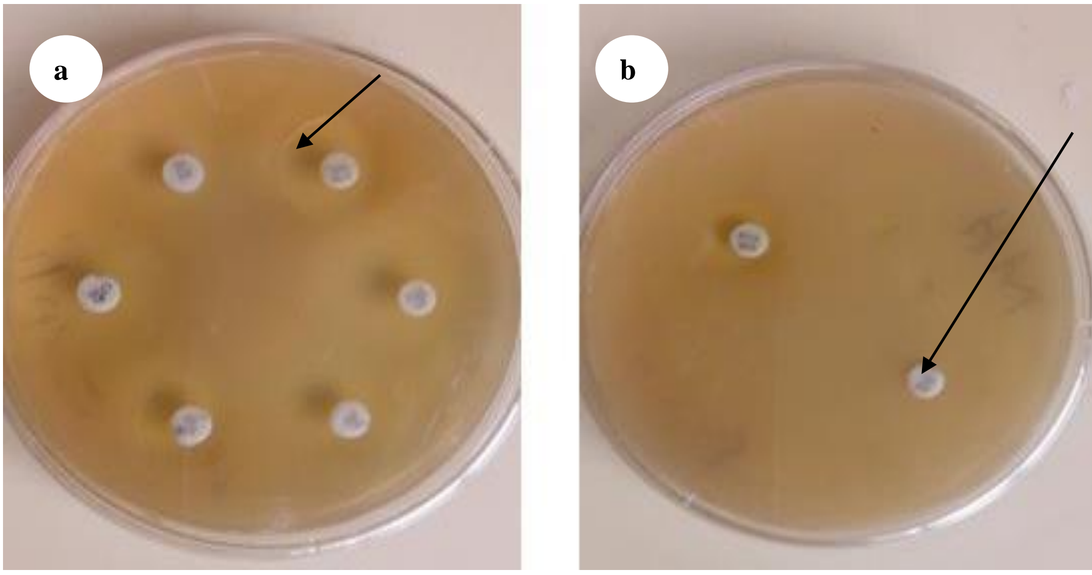

Figure 4. 15: A representative of Disk diffusion results on Muller Hinton Agar

Plate a showing susceptibility while plate b representing non susceptibility/resistance as shown with arrow.

4.7.1 Antimicrobial susceptibility patterns of Gram-positive isolates.

A total number of ten (10) antimicrobial agents were used to characterize the Gram positive (35) isolated and identified bacteria according to their zones of inhibition and break points. *Staphylococcus aureus* (1) was subjected to nine antimicrobial agents and was susceptible to eight antimicrobial agents (Oxacillin (24 ± 0.00 mm), Gentamicin (17 ± 1.00 mm), Levofloxacin (19.33 ± 0.58 mm), Moxifloxacin (24.67 ± 1.53mm), Clindamycin (21 ± 0.00 mm), Erythromycin (23.33 ± 1.16 mm), Linezolid (23.67 ± 0.58 mm), and Tetracycline (18 ± 0.00 mm).

Enterococcus spp isolates (3) were susceptible to Linezolid (21.67 ± 0.58 mm), Vancomycin (20.67 ± 0.58 mm), and Levofloxacin (19.67 ± 0.58 mm). *Staphylococcus epidermidis* (19) were susceptible to linezolid (24.32 ± 0.48 mm), other 15 (78.9%) isolates were susceptible to Clindamycin (21.6 ± 0.737 mm), 13(68.4%) were susceptible to Levofloxacin (22.54 ± 1.33 mm) and Moxifloxacin (24 ± 0.816 mm). *Staphylococcus haemolyticus* (5) were subjected to nine antimicrobials and all were susceptible to Linezolid (23 ± 0.00 mm) while two isolates (40%) were susceptible to (Levofloxacin (21.33 ± 0.816 mm), Moxifloxacin (24.5 ± 0.816 mm), Clindamycin (20.5 ± 0.548 mm). *Staphylococcus saprophyticus* (1) was subjected to nine antimicrobial agents and were susceptible to seven antimicrobials agents (Gentamicin (19.67 ± 0.577 mm), Levofloxacin (19.33 ± 0.577 mm), Moxifloxacin (24.67 ± 0.577mm), Clindamycin (22 ± 0.00 mm), Erythromycin (24 ± 0.00 mm), Linezolid (22.67 ± 0.577 mm) and Tetracycline (25 ± 0.00 mm). *Staphylococcus hominis* (2) was also subjected to nine antimicrobial agents and were susceptible to seven antimicrobials agents (Gentamicin (20.33 ± 0.516 mm), Levofloxacin (20 ± 0.00 mm), Moxifloxacin

(24.0 ± 0.00 mm), Clindamycin (20.83 ± 0.41mm), Erythromycin (24.67 ± 0.516 mm), Linezolid (24 ± 0.00 mm) and Tetracycline (24.17 ± 0.41mm). *Staphylococcus lentus* (2) were also proved to be susceptible to (Gentamicin (19.3 ± 0.58), Levofloxacin (20 ± 0.00 mm), Moxifloxacin (23.67 ± 0.58 mm), Clindamycin (20.33 ± 0.58 mm) and, Linezolid (20 ± 1.00 mm). Lastly, *Staphylococcus warneri* (2) was also subjected to nine antimicrobial agents and were susceptible to seven antimicrobial agents (Gentamicin (20 ± 1.169 mm), Levofloxacin (21.5 ± 1.378 mm), Moxifloxacin (24.33 ± 0.516 mm), Clindamycin (22.67 ± 0.516 mm), Erythromycin (24.67 ± 0.52 mm), Linezolid (23 ± 0.00 mm) and Tetracycline (25.83 ± 0.983 mm). All these findings are summarised and illustrated in table 4.9 below

Table 4. 9: Antimicrobial susceptibility patterns (Average Zones of inhibition-mm) of Gram-positive bacterial isolates responsible for neonatal sepsis

Antibiotics (Concentration used)	Break points (mm)	Microorganism (N=35)							
		Staphylococcus aureus (1)	*Enterococcus spp (3)*	*S. epidermidis (19)*	*S. haemolyticus (5)*	*S. saprophyticus (1)*	*S. hominis (2)*	*S. lentus (2)*	*S. warneri (2)*
Penicillin G (10 Units)	S ≥29	R	NT	R	R	R	R	R	R
Oxacillin (1µg)		24±0.00 mm (S)	NT	23.33±0.58mm(S)	R	R	R	R	R
Gentamicin (10µg)	13-14	17±1 mm (S)	NT	21.09±0.70 mm(S)	R	19.67 ±0.577mm (S)	20.33±0.516mm(S)	19.3±0.58 mm(S)	20±1.169 mm(S)
Levofloxacin (5µg)	16-18	19.33±0.58 mm(S)	19.67±0.58 mm(S)	22.54±1.33 mm(S)	21.33±0.816 mm(S)	19.33±0.577 mm (S)	20 ±0.00 mm (S)	20±0.00 mm (S)	21.5±1.378 mm(S)
Moxifloxacin (5µg)	21-23	24.67±1.53mm (S)	NT	24±0.816 mm(S)	24.5±0.816 mm (S)	24.67±0.577mm (S)	24±0.00 mm (S)	23.67±0.58mm(S)	24.33±0.516mm(S)
Clindamycin (2µg)	15-20	21±0 mm (S)	NT	21.6±0.737 mm(S)	20.5±0.548 mm (S)	22±0.00 mm(S)	20.83±0.41 mm (S)	20.33±0.58mm(S)	22.6±0.516 mm(S)
Erythromycin (15µg)	14-22	23.33±1.16 mm (S)	R	25.38±0.75 mm(S)	R	24±0.00 mm (S)	24.67±0.516mm(S)	R	24.67±0.516mm(S)
Linezolid (30µg)	S ≥21	23.67±0.58 mm (S)	21.67±0.58 mm(S)	24.32±0.48 mm(S)	23±0.00 mm (S)	22.67±0.577 mm (S)	24±0.00 mm (S)	23±1.00mm (S)	23±0.00 mm (S)
Vancomycin (30µg)		NT	20.67 ±0.58 mm(S)	NT	NT	NT	NT	NT	NT
Tetracycline (30µg)	15-18	18±0.00 mm (S)	R	25.25±0.96 mm(S)	R	25±0.00 mm (S)	24.17±0.41mm (S)	R	25.83±0.983mm(S)

NT (Not Tested), µg (Micrograms disk content), mm (millimeters), R (Resistant), S (Susceptible).

4.7.2: Antimicrobial resistant patterns of Gram-positive isolates.

Staphylococcus aureus (1) and *Staphylococcus saprophyticus* (1) were resistant to Penicillin G producing average zones of inhibition of 6 ± 0.577 mm and 7 ± 0.00 mm respectively. *Enterococcus spp* (3) were resistant to Erythromycin with an average zone of inhibition of 8.11 ± 1.05 mm and Tetracycline (7.78 ± 0.66 mm). *Staphylococcus epidermidis* (19) were resistant to Penicillin G (8.84 ± 2.41 mm). *Staphylococcus haemolyticus* (5) were resistant to Penicillin G (6.20 ± 1.35 mm), Oxacillin (8.2 ± 1.095 mm), Gentamicin (9.6 ± 0.548). Erythromycin (6.8 ± 0.837 mm) and tetracycline (6 ± 0.00 mm), while 3 (60%) were resistant to Levofloxacin, Moxifloxacin and Clindamycin. *Staphylococcus hominis* (2) were resistant to Penicillin G (6.5 ± 0.548 mm) and Oxacillin (8 ± 0.632 mm). *Staphylococcus lentus* (2) were also resistant to Penicillin G (6.83 ± 0.41mm), Oxacillin (7.17 ± 0.75 mm), Erythromycin (8 ± 0.632 mm) and tetracycline (6.33 ± 0.516 mm). Lastly, *Staphylococcus warneri* (2) were found also to be resistant to Penicillin G (8 ± 0.894) and Oxacillin (7.67 ± 0.52 mm). All these findings are summarised and illustrated in Table 4.10 below. They clearly demonstrate that AMR is really becoming a menace in the current society as these antibiotics may not offer any medicare against the NS condition if its caused by these pathogens.

Table 4. 10: Antimicrobial resistant patterns (Average Zones of inhibition-mm) of Gram-positive bacterial stains responsible for neonatal sepsis

Antibiotics (Concentration used)	Break points (mm)	Microorganism N=35							
		Staphylococcus aureus (1)	*Enterococcus spp (3)*	*S. epidermidis (19)*	*S. haemolyticus (5)*	*S. saprophyticus (1)*	*S. hominis (2)*	*S. lentus (2)*	*S. warneri (2)*
Penicillin G (10 Units)	S ≥29	6±0.577 mm(R)	NT	8.84±2.41 mm (R)	6.20±1.35mm (R)	7±0.00 mm(R)	6.5±0.548mm(R)	6.83±0.41 mm (R)	8±0.894mm(R)
Oxacillin (1µg)		S	NT	S	8.2±1.095 mm (R)	7.67±0.577mm(R)	8±0.632 mm(R)	7.17±0.75 mm (R)	7.67±0.52mm(R)
Gentamicin (10µg)	13-14	S	NT	S	9.6±0.548 mm (R)	S	S	S	S
Levofloxacin (5µg)	16-18	S	(S)	S	S	S	S	S	S
Moxifloxacin (5µg)	21-23	S	NT	S	S	S	S	S	S
Clindamycin (2µg)	15-20	S	NT	S	S	S	S	S	S
Erythromycin (15µg)	14-22	S	8.11±1.05 mm(R)	S	6.8±0.837 mm (R)	S	S	8±0.632 mm (R)	S
Linezolid (30µg)	S ≥21	S	S	S	S	S	S	S	S
Vancomycin (30µg)		NT	S	NT	NT	NT	NT	NT	NT
Tetracycline (30µg)	15-18	S	7.78±0.66(R)	S	6±0.00 mm (R)	S	S	6.33±0.516 mm(R)	S

NT: Not Tested (orange), R: Resistant (clear), S: Susceptible (green), µg: micrograms disk content, mm: millimeters

4.7.3 Antimicrobial susceptibility patterns of Gram-negative isolates.

A total number of fourteen (14) antimicrobial agents were used to characterize the Gram negative (6) isolated and identified bacteria according to their zones of inhibition and break points. *Pseudomonas aeruginosa* (1) was subjected to six antimicrobial agents and proved to be sensitive/susceptible to (Piperacillin/ Tazobactam (21.33 ± 0.77 mm), Cefepime (24.33 ± 0.577 mm), Meropenem (25.33 ± 0.577 mm), Amikacin (20.67 ± 1.155 mm), Gentamicin (17.67 ± 0.577 mm) and Ciprofloxacin (31.67 ± 0.577 mm). *Escherichia coli* (1) isolate also proved to be susceptible to some antimicrobial agents bioassayed (Amoxicillin/ Clavulanic acid (17.67 ± 0.577 mm), Piperacillin/ Tazobactam (20 ±0.00 mm), Cefazolin (23.33 ± 0.577 mm), Cefuroxime (23.67 ± 0.577 mm), Cefepime (24 ± 0.00 mm), Cefoxitin (18.33 ± 1.15 mm), Aztreonam (24.33 ± 0.577 mm), Meropenem (25 ± 0.00), Amikacin (21 ± 1.00 mm), Gentamicin (18 ± 0.00 mm), Ciprofloxacin (32 ± 0.00 mm) and Trimethoprim/ Sulfamethoxazole (16.67 ± 0.577). *Salmonella* spp (3) were susceptible to Ampicillin (16.67 ± 1.00 mm), Amoxicillin/ Clavulanic acid (19.56 ± 0.527 mm), Ampicillin/ Sulbactam (15.44 ± 0.527 mm), Piperacillin/ Tazobactam (21.67 ± 0.50 mm), Cefuroxime (23 ± 1.00 mm), Cefepime (25.67 ± 0.866 mm), Aztreonam (23.44 ± 0.527 mm), Meropenem (24.44 ± 0.527 mm), Ciprofloxacin (31.56 ± 0.27 mm) and Trimethoprim/ Sulfamethoxazole (15.67 ± 0.5 mm). *Acinetobacter* spp (1) was susceptible to Ampicillin (17.67 ± 0.577 mm), Amoxicillin/ Clavulanic acid (20.67 ± 0.577 mm), Ampicillin/ Sulbactam (16 ± 0.00 mm), Piperacillin/ Tazobactam (21.67 ± 0.577 mm), Cefepime (25 ± 0.00 mm), Meropenem (26.33 ± 0.577 mm), Ciprofloxacin (33.33 ± 0.577 mm) and Trimethoprim/ Sulfamethoxazole (16 ± 1.00 mm) as summarised and illustrated in table 4.11 below.

Table 4. 11: Antimicrobial susceptibility patterns (Average Zones of inhibition-mm) of Gram-negative bacterial isolates responsible for neonatal sepsis

Antibiotics (Concentration used)	Break points (mm)	Microorganism N=6			
		Pseudomonas aeruginosa (1)	*Escherichia coli (1)*	*Salmonella spp (3)*	*Acinetobacter spp (1)*
Ampicillin (10µg)	14-16	NT	R	16.67±1.00 mm(S)	17.67±0.577mm (S)
Amoxicillin/ Clavulanic acid (20/10µg)	14-17	NT	17.67±0.577mm(S)	19.56±0.527 mm(S)	20.67 ±0.577 mm (S)
Ampicillin/Sulbactam (10/10µg)	12-14	NT	R	15.44±0.527 mm (S)	16 ±0.00 mm (S)
Piperacillin/ Tazobactam (100/10µg)	18-20	21.33±0.577 mm (S)	20±0.00 mm (S)	21.67±0.5 mm (S)	21.67 ±0.577 mm (S)
Cefazolin (30µg)	20-22	R	23.33±0.577 mm (S)	R	NT
Cefuroxime (30µg)	15-22	NT	23.67±0.577 mm (S)	23±1.00 mm (S)	NT
Cefepime (30µg)	19-24	24.33±0.577 mm (S)	24±0.00 mm (S)	25.67±0.866 mm (S)	25±0.00 mm (S)
Cefoxitin (30µg)	15-17	NT	18.33±1.15 mm (S)	R	NT
Aztreonam (30µg)	18-20	NT	24.33±0.577 mm (S)	23.44±0.527 mm (S)	NT
Meropenem (10µg)	20-22	25.33±0.577 mm (S)	25±0.00 mm (S)	24.44±0.527 mm (S)	26.33±0.577 mm (S)
Amikacin (30µg)	15-16	20.67±1.155 mm (S)	21±1.00 mm (S)	R	R
Gentamicin (10µg)	13-14	17.67±0.577 mm (S)	18±0.00 mm (S)	R	R
Ciprofloxacin (5µg)	21-30	31.67±0.577 mm (S)	32 ±0.00 mm (S)	31.56±0.27 mm (S)	33.33±0.577 mm (S)
Trimethoprim/ Sulfamethoxazole (1.25/23.75µg)	11-15	NT	16.67±0.577 mm (S)	15.67±0.50 mm (S)	16±1.00 mm (S)

Spp (species), NT (Not Tested), µg (Micrograms disk content), mm (millimeters) R (Resistant), S (Susceptible).

4.7.4: Antimicrobial resistant patterns of Gram-negative isolates.

Among the Gram-negative bacteria isolates that were found to be resistant to some commonly used antibiotics is *Pseudomonas aeruginosa* (1). It was found to be resistant to Cefazolin (9 ± 0.00 mm). *Escherichia coli* (1) was also found to be resistant against Ampicillin (6.33 ± 0.577 mm) and Ampicillin/ Sulbactam (6.67 ± 0.577 mm). *Salmonella* spp (3) strains also proved to be resistant to Cefazolin (7.78 ± 0.441mm), Cefoxitin (9.89 ± 0.333 mm), Amikacin (7.89 ± 0.333 mm) and Gentamicin (7.33 ± 0.50 mm), while two (66.7%) were resistant to Cefuroxime. *Acinetobacter* spp (1) was resistant to Amikacin (7 ± 0.00 mm) and Gentamicin (7.67 ± 0.577 mm). These findings are summarized and presented in table 4.12 below and they clearly demonstrate that resistance is arising to commonly used antibiotics against not only NS but also to other bacterial infections.

Table 4. 12: Antimicrobial resistance patterns (Average Zones of inhibition-mm) of Gram-negative bacterial isolates responsible for neonatal sepsis

		Microorganism N=6			
Antibiotics (Concentration used)	**Break points (mm)**	*Pseudomonas aeruginosa (1)*	*Escherichia coli (1)*	*Salmonella spp (3)*	*Acinetobacter spp (1)*
Ampicillin (10µg)	14-16	NT	6.33±0.577mm(R)	(S)	(S)
Amoxicillin/ Clavulanic acid (20/10µg)	14-17	NT	(S)	(S)	(S)
Ampicillin/Sulbactam (10/10µg)	12-14	NT	6.67±0.577mm(R)	(S)	(S)
Piperacillin/ Tazobactam (100/10µg)	18-20	(S)	(S)	(S)	(S)
Cefazolin (30µg)	20-22	9 ± 0.00 mm(R)	(S)	7.78±0.44mm (R)	NT
Cefuroxime (30µg)	15-22	NT	(S)	(S)	NT
Cefepime (30µg)	19-24	(S)	(S)	(S)	(S)
Cefoxitin (30µg)	15-17	NT	(S)	9.89±0.33mm(R)	NT
Aztreonam (30µg)	18-20	NT	(S)	(S)	NT
Meropenem (10µg)	20-22	(S)	(S)	(S)	(S)
Amikacin (30µg)	15-16	(S)	(S)	7.89±0.33mm (R)	7±0.00mm (R)
Gentamicin (10µg)	13-14	(S)	(S)	7.33±0.50 mm (R)	7.67±0.57mm(R)
Ciprofloxacin (5µg)	21-30	(S)	(S)	(S)	(S)
Trimethoprim/ Sulfamethoxazole (1.25/23.75µg)	11-15	NT	(S)	(S)	(S)

Spp (species), NT (Not Tested), µg (Micrograms disk content), mm (millimeters)

4.7.5 Minimum Inhibitory Concentration (MIC) of isolated bacterial causing neonatal sepsis

Minimum inhibitory concentration of antimicrobial agents against the isolated susceptible bacterial strains on Kirby-Bauer disk diffusion method was determined by use Epsilometer test (E Test) based on Clinical Laboratory Standards Institute (CLSI) guidelines. CLSI Performance standards for Antimicrobial Susceptibility Testing 30th ed. CLSI supplement M100. (2020), Minimum inhibitory concentration (MIC) values in µg/ml were read at the point where edge of the ellipses intersected the strips. E test values (µg/ml) were computed to mean and Standard Error of Mean (SEM) and the findings are as presented in sub-sections 4.7.6-4.7.7

4.7.6 Minimum Inhibitory Concentrations (MIC) of antimicrobial agents on Gram positive isolates

Nine antimicrobial agents (Oxacillin, Gentamicin, Levofloxacin, Moxifloxacin, Clindamycin, Erythromycin, Linezolid, Vancomycin and Tetracycline.) were used to determine MIC values of the isolated Gram-positive isolates responsible for neonatal sepsis. *Staphylococcus aureus* (1) had an average MIC of 0.25 ± 0.00 µg/ml for Moxifloxacin, Clindamycin and Erythromycin. Also, an average MIC of 0.5 ± 0.00 µg/ml for Oxacillin and Gentamicin were reported from this study. Additionally, against this isolate an average MIC of 1 ± 0.00 µg/ml for Linezolid and Tetracycline were reported while Levofloxacin had an average MIC of 0.12 ± 0.00 µg/ml.

For *Enterococcus spp* (3) the average MICs of 3.5 ± 1.146 µg/ml for Levofloxacin, 1 ± 0.00 µg/ml for Linezolid, and 1.67 ± 1.333 µg/ml for Vancomycin were also documented. *Staphylococcus epidermidis* (19) bacterial isolate also had an average MICs 3.4 ± 0.324 µg/ml for Oxacillin, 3.83 ± 0.903 µg/ml for Gentamicin, 2.79 ±

0.831 µg/ml for Levofloxacin, 0.722 ± 0.174 µg/ml for Moxifloxacin, 0.25 ± 0.00 µg/ml for Clindamycin, 4.986 ± 0.916 µg/ml for Erythromycin, 1 ± 0.00 µg/ml for Linezolid, and 5.833 ± 0.749 µg/ml for Tetracycline., Similarly, *Staphylococcus haemolyticus* (5) isolates had an average MICs 2.726 ± 1.309 µg/ml for Levofloxacin, 0.958 ± 0.350 µg/ml for Moxifloxacin, of 0.291 ± 0.416 µg/ml for Clindamycin, and 1 ± 0.00 µg/ml for Linezolid. *Staphylococcus saprophyticus* (1) isolate also had varying average MICs across the antibiotics bio assayed. For instance, it did produce an average MIC of 2 ± 0.00 µg/ml for Oxacillin and Tetracycline, 0.5 ± 0.00 µg/ml for Gentamicin, Levofloxacin, and erythromycin, 0.25 ± 0.00 µg/ml for Moxifloxacin and Clindamycin, and 1 ± 0.00 µg/ml for Linezolid. *Staphylococcus hominis (*2) also did produce varying average MICs against the antibiotics bio assayed: -0.5 ± 0.00 µg/ml for Gentamicin, Levofloxacin and Erythromycin, 0.25 ± 0.00 µg/ml for Moxifloxacin and Clindamycin, and 1 ± 0.00 µg/ml for Linezolid. Similar findings were recorded with *Staphylococcus lentus* (2) that had an average MIC of 4.25 ± 1.677 µg/ml for Gentamicin, 4.06 ±1.76 µg/ml for Levofloxacin, 1.125 ± 0.39 µg/ml for Moxifloxacin, 0.37 ± 0.055 µg/ml for Clindamycin, 8 ± 0.00 µg/ml for Erythromycin, 1 ± 0.00 µg/ml for Linezolid. Lastly *Staphylococcus warneri* (2) isolate also produced varying average MICs against the antibiotics bioassayed: - 0.25 ± 0.00 µg/ml for Levofloxacin, Moxifloxacin, Clindamycin and Erythromycin, 0.375 ± 0.06 µg/ml for Gentamicin, 1 ± 0.00 µg/ml for Linezolid, 0.5 ± 0.00 µg/ml for Tetracycline. This varying average MICs also clearly demonstrates that the isolates do react differently to various antibiotics and these findings are summarised and presented in Table 4.13 below.

Table 4. 13: Minimum inhibitory concentration (MIC) of Gram-positive isolates responsible for neonatal sepsis

		Organism N=35							
		Staphylococcus aureus (1)	*Enterococcus spp* (3)	*S. epidermidis* (19)	*S. hemolyticus* (5)	*S. saprophyticus* (1)	*S. hominis* (2)	*S. lentus* (2)	*S. warneri* (2)
Antibiotics	**Concentration range (µg/ml)**	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)	MIC (µg/ml)
Penicillin G		R	NT	R	R	R	R	R	R
Oxacillin	0.016-256	0.5±0	NT	3.4 ±0.324	R	2±0	R	R	R
Gentamicin	0.016-256	0.5±0	NT	3.83 ±0.903	R	0.5±0	0.5±0	4.25±1.677	0.375±0.06
Levofloxacin	0.002-32	0.12±0	3.5±1.146	2.79 ±0.831	2.726 ±1.309	0.5±0	0.5±0	4.06±1.76	0.25±0
Moxifloxacin	0.002-32	0.25±0	NT	0.722 ±0.174	0.958 ±0.350	0.25±0	0.25±0	1.125±0.39	0.25±0
Clindamycin	0.016-256	0.25±0	NT	0.25 ±0	0.291 ±0.416	0.25±0	0.25±0	0.37±0.055	0.25±0
Erythromycin	0.016-256	0.25±0	R	4.986 ±0.916	R	0.5±0	0.5±0	8±0	0.25±0
Linezolid	0.016-256	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0
Vancomycin	0.016-256	NT	1.67 ±0.333	NT	NT	NT	NT	NT	NT
Tetracycline	0.016-256	1±0	R	5.833 ±0.749	R	2±0	R	R	0.5±0
MIC Range		0.12-1	0.25-8	0.12-8	0.12-8	0.25-2	0.12-2	0.12-8	0.25-1

Spp (species), NT (Not Tested), R (resistant), µg/ml (Micrograms/ milliliter)

4.7.7 Minimum Inhibitory Concentrations (MIC) of antimicrobial agents on Gram negative isolates

Fourteen (14) antimicrobial agents were used to determine MIC values of the isolated Gram-negative isolates responsible for neonatal sepsis. *Pseudomonas aeruginosa* (1) had varying average MICs against various antibiotics used: - 0.25 ± 0.00 µg/ml for Meropenem and Ciprofloxacin, 4 ± 0.00 µg/ml for Piperacillin/ Tazobactam 1 ± 0.00 µg/ml for Cefepime and Gentamicin, 2 ± 0.00 µg/ml for Amikacin. *Escherichia coli* (1) similar findings were obtained with varying average MICs: - 8 ± 0.00 µg/ml for Amoxicillin / Clavulanic acid and Trimethoprim/ sulfamethoxazole, 4 ± 0.00 µg/ml for Piperacillin/ Tazobactam, Cefazolin, Cefuroxime and Cefoxitin, 1± 0.00 µg/ml for Cefepime, Aztreonam and Gentamicin, 0.25 ± 0.00 µg/ml for Meropenem and ciprofloxacin, and 2 ± 0.00 µg/ml for Amikacin. *Salmonella* spp (3) had also varying average MICs against the antibiotics screened: - 2 ± 0.00 µg/ml for Ampicillin, Amoxicillin/ Clavulanic acid and Ampicillin Sulbactam, 4 ± 0.00 µg/ml for Piperacillin/Tazobactam and Cefuroxime, 1 ± 0.00 µg/ml for Cefepime and Aztreonam, 0.25 ± 0.00 µg/ml for Meropenem and Ciprofloxacin, and 8 ± 0.00 µg/ml for Trimethoprim/ sulfamethoxazole. *Acinetobacter spp* (1) also had similar findings on the average MICs against commonly used antibiotics used to manage this isolate:- 2 ± 0.00 µg/ml for Ampicillin, Amoxicillin/ Clavulanic acid and Ampicillin / Sulbactam, 4 ± 0.00 µg/ml for Piperacillin/ Tazobactam and Trimethoprim/ Sulfamethoxazole, 1 ± 0.00 µg/ml for Cefepime, and 0.25 ± 0.00 µg/ml for Meropenem and Ciprofloxacin. All these findings are summarised and presented as shown in Table 4.14 below. From our findings it's clear that different isolates react differently to various antibiotics as some need small doses to inhibit their growth while other need higher doses.

Table 4. 14: Minimum inhibitory concentration (MIC) of Gram-negative isolates responsible for neonatal sepsis

		Organism N=6			
		Pseudomonas aeruginosa (1)	*Escherichia coli* (1)	*Salmonella spp* (3)	*Acinetobacter spp* (1)
Antibiotics	**Concentration range (µg/ml)**	**MIC (µg/ml)**	**MIC (µg/ml)**	**MIC (µg/ml)**	**MIC (µg/ml)**
Ampicillin	0.016-256	NT	R	2±0.00	2±0.00
Amoxicillin/ Clavulanic acid	0.016-256	NT	8±0.00	2±0.00	2±2.0
Ampicillin/ Sulbactam	0.016-256	NT	R	2±0.00	2±0.00
Piperacillin/ Tazobactam	0.016-256	4±0.00	4±0.00	4±000	4±0.00
Cefazolin	0.016-256	R	4±0.00	R	NT
Cefuroxime	0.016-256	NT	4±0.00	4±0.00	NT
Cefepime	0.016-256	1±0.00	1±0.00	1±0.00	1±0.00
Cefoxitin	0.016-256	NT	4±0.00	R	NT
Aztreonam	0.016-256	NT	1±0.00	1±0.00	NT
Meropenem	0.002-32	0.25±0.00	0.25±0.00	0.25±0.00	0.25±0.00
Amikacin	0.016-256	2±0.00	2±0.00	R	R
Gentamicin	0.016-256	1±0.00	1±0.00	R	R
Ciprofloxacin	0.002-32	0.25±0.00	0.25±0.00	0.25±0.00	0.25±0.00
Trimethoprim/ Sulfamethoxazole	0.002-32	NT	8±0.00	8±0.00	4±0.00
MIC Range		0.25-4.00	0.25-8.00	0.25-8.00	0.25-4.00

Spp (species), NT (Not Tested), R (resistant), µg/ml (Micrograms/ milliliter).

4.7.8 Screening for carbapemenase: blaOXA-48, blaKPC and mecA genes from isolates responsible for neonatal sepsis

The study screened for the presence of resistant gene markers (carbapemenase: blaOXA-48 and blaKPC) from the six Gram negative isolates and mecA gene from the 35 Gram positive isolates. Test results were interpreted as positive at the intersection between threshold line and the start of amplification curve (exponential phase) at fixed signal threshold in number of cycles. These findings are presented in sub-sections 4.7.9

4.7.9: Screening for Carbapemenase producing (blaOXA-48 and bla KPC genes) in Gram negative isolates

Deoxyribonucleic acid (DNA) from Gram negative bacteria were screened for the plasmid-encoded blaOXA-48 and bla KPC genes that confer resistance to bacteria against carbapenem class of antibiotics considered to be the drugs of last resort. Six (6) DNA templates were screened and four (4) turned positive for the markers giving 66.7%. *Salmonella ssp.* 2 (50%) had cycle threshold of 30.61 and 30.69 while *Escherichia coli* one (1) (25%) at 33.64 and *Pseudomonas aeruginosa* one (1) 25% at 33.43 as illustrated in figure 4.7 below.

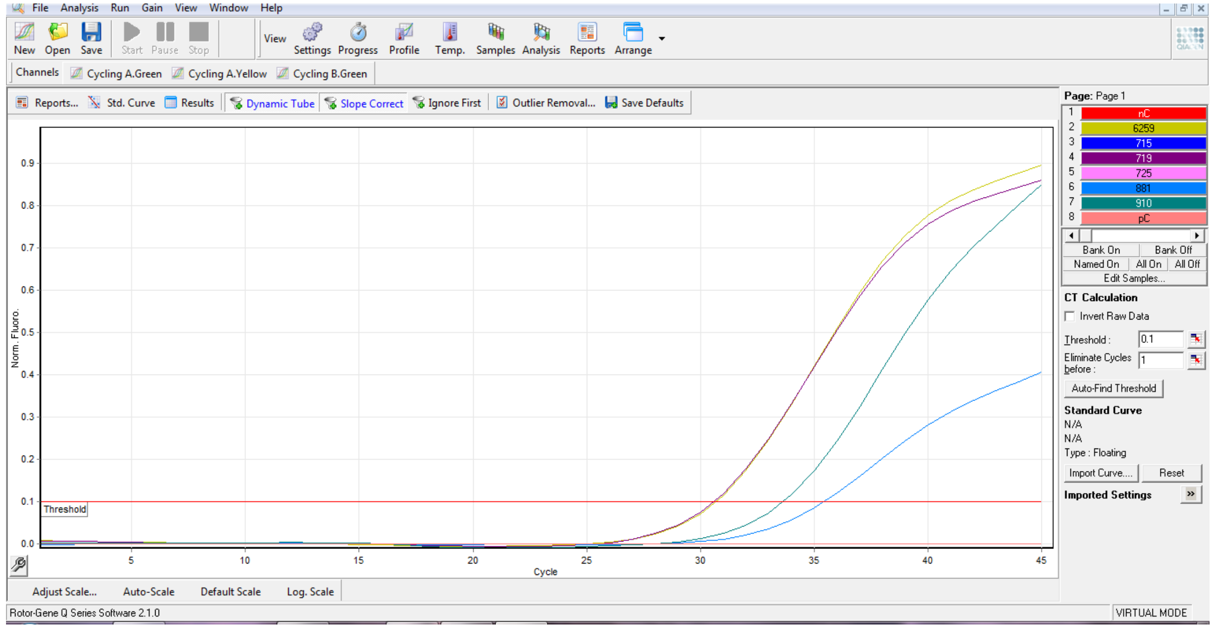

Figure 4. 16: Carbapemenase (blaOXA-48 and bla KPC) genes determination in Gram negative isolates

Y axis (represents visualization of fluorescence produced). X axis (represents visualization of number of cycle threshold). While lines above the threshold line are (positive results with different cut off values). The different coloured lines represent (the isolates) Purple 30.61 (Salmonella spp), Jungle green 30.69 (Salmonella spp), Light blue 33.43 (Pseudomonas aeruginosa), Cornflower blue 33.64 (Escherichia coli)

4.7.10: Screening for Methicillin resistance (mec A gene) in Gram positive isolates

Deoxyribonucleic acid (DNA) from Gram positive bacteria were screened for mecA gene that confers resistance to bacteria against selected antimicrobial agents like Penicillin, Oxacillin Gentamicin, Levofloxacin, Moxifloxacin, Clindamycin, Erythromycin, Linezolid, Vancomycin and Tetracycline. Thirty-five (35) DNA templates were screened, six (6) turned positive for the markers giving 17.1%. For *Staphylococcus epidermidis* three (50%) isolates had cycle threshold of 28.87, 34.38 and 38.43. Also, one of *Staphylococcus warneri* isolate (16.7%) and one of the *Staphylococcus hominis* isolate (16.7%) had 37.34 and 32.76 respectively, while one *Enterococcus spp* isolate (16.7%) had 26.76 as illustrated in figure 4.8 below.

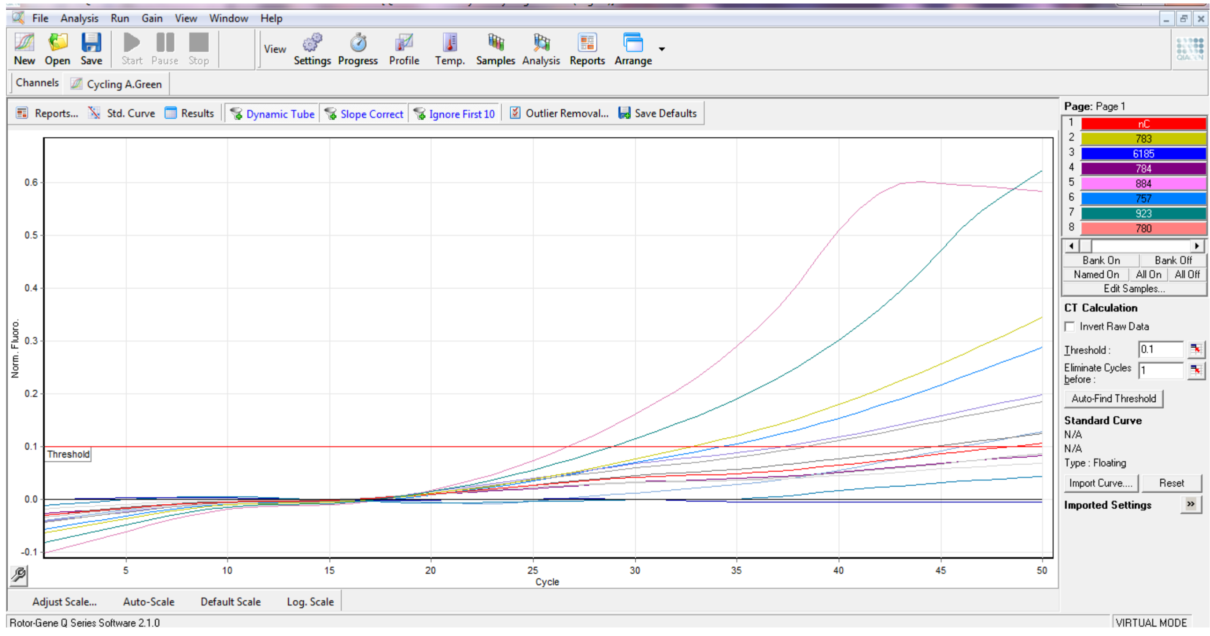

Figure 4. 17: Methicillin resistance (mec A) gene determination in Gram positive isolates

Y axis (represents visualization of fluorescence produced). X axis (represents visualization of number of cycle threshold). While lines above the threshold line are (positive results with different cut off values). The different coloured lines represent (the isolates) Pink 26.76 (Enterococcus spp.), Jungle green 28.87 (Staphylococcus epidermidis), Lime green 32.76 (Staphylococcus hominis), Cornflower blue 34.38 (Staphylococcus epidermidis), purple 37.34 (Staphylococcus warneri), and Grey 38.43 (Staphylococcus epidermidis).

4.7.11: DNA fragment analysis from bacterial isolates that had resistant genes responsible for Neonatal sepsis

Deoxyribonucleic acid (DNA) fragments of the ten (10) bacterial isolates with resistant gene markers were separated based on their size and charge and visualized as base pair bands (bp) compared to the DNA ladder by use of Automated QIAxcel Advanced system® using QX DNA high resolution kit® and the results were visualized by QIAxcel Screen Gel software®. Out of the ten (10) DNA fragments, four (4) turned positive A4 *(Salmonella spp)* at 148 bp, A6 *(Escherichia coli)* at 148 bp, A7 *(Staphylococcus epidermidis)* at 148 bp A11 *(Staphylococcus hominis*) at 296 bp and six (6) turned

negative A3 (*Salmonella spp*), A5 (*Pseudomonas aeruginosa*), A8 (*staphylococcus warneri*) A9 (*Enterococcus spp*) A10 (*Staphylococcus epidermidis*) A12 (*Staphylococcus epidermidis*), while A1 was a known negative control and A2 was a known positive control. as illustrated on figure 4.9 below. Therefore, it can be concluded that bla OXA-48 and bla KPC genes were present in *Salmonella* and *Escherichia coli* isolates, while mecA gene was present in *Staphylococcus epidermidis* and *Staphylococcus hominis* isolates.

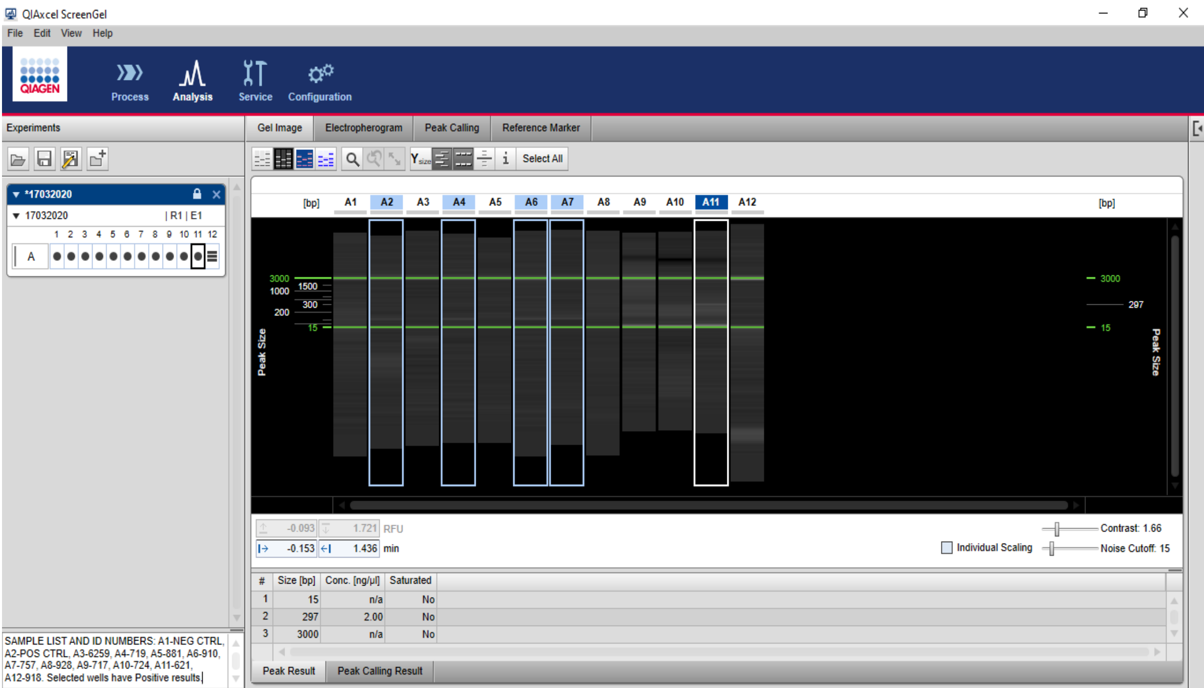

Figure 4. 18: DNA fragments of bacterial isolates with resistant genes for neonatal sepsis

bp (base pairs). A1-A12 (Wells), A1 (Negative control), A2 (Positive control), (Positive wells A2, A4, A6, A7 and A11, A4 (Salmonella spp), A6 (Escherichia coli), A7 (Staphylococcus epidermidis) A11 (Staphylococcus hominis). Negative results A3 (Salmonella spp), A5 (Pseudomonas aeruginosa), A8 (staphylococcus warneri), A9 (Enterococcus spp), A10 (Staphylococcus epidermidis), A12(Staphylococcus epidermidis)

CHAPTER FIVE

DISCUSSION

This study did recruit 181 preterm neonates against the sample size of 174 as the respondents did seem to be interested with the study. This is a positive indicator that if data obtained from such studies can be disseminated to the local populations and healthcare departments it can add value to management of various infections including NS. From the preliminary data obtained in this study, female neonates were 107 (59.1%) while 74 (40.9%) were male and ranged from (<24 hours zero (0) to 10 days old. One day to three days old 135 (75%) were the majority, this may be due to early onset sepsis which is caused by vertical transmission (Adatara., 2019). These study findings are in consistent with findings reported in a study done by Mandot & Gandhi, (2017) which found out that neonates are mostly infected with NS during the first three days of childbirth. Our findings on gender also did concur with other studies done before which indicated that female neonates were more infected compared to male neonates. This finding contrasts a study by Adatara *et al*., (2019) which found out female neonates being less likely to develop neonatal sepsis than male neonates.

Maternal and neonatal factors were assessed against the development of neonatal sepsis among preterm neonates. Findings of this study revealed that antenatal maternal health is crucial and is protective to the unborn. The study found out that illness during pregnancy and premature rupture of membranes were not associated to neonatal sepsis, these findings are different with findings that were documented from a similar study that was done at the centre of neonatology Podgorica Montenegro in the university clinical centre, institute for children diseases by Lekić *et al.,* (2019). Other factors like maternal syphilis-Khan test positive (2), urinary tract infections (28) and vaginal

discharge (4) did not influence occurrence of neonatal sepsis. However, it should be noted that they can result to development of NS as evidenced by studies that have been done before (Murthy *et al.,* 2019; Nyma, 2020). These findings are different from findings of a study done in Northwest Ethiopia that showed virginal discharge as one of the factors related to neonatal sepsis (Yismaw *et al.,* 2019). Maternal antibiotic exposure during pregnancy was prophylactic, this finding is similar to the findings of a study that was carried out in Shenzhen China women and children hospital which did document that prenatal antibiotic exposure reduced the chances of neonates developing sepsis (Zhou *et al.,* 2020). This could also be reason as to why the various UTIs and syphilis did not have any direct association with NS in our study.

Childbirth and labour is a natural process that occurs through spontaneous virginal delivery (SVD) or C-section when foetus is term (Agnche *et al.,* 2020). The study findings indicated that C-section as a mode of choice and had a high likelihood of preterm neonate developing NS with the OR=1.101. Preterm neonates delivered by mothers who had prolonged rapture of membranes (PROM) greater than >18 hours had higher odds and association to development of neonatal sepsis and could be attributed to chorioamnionitis (infection of membranes that surround the foetus). Such a finding has been reported by a study that was carried out in North India and it did demonstrate that there was a relationship between chorioamnionitis and prolonged rapture of membranes (Gupta, 2019).

On neonatal factors, the study found out that foetal distress, one of the uncommon complications of labour where foetus oxygen levels were severely low was strongly linked to development of neonatal sepsis with OR=2.244. Foetal distress can result to respiratory failure and cardiopulmonary arrest which can lead to death of a preterm

neonate, therefore early management can prevent such deaths (Reuter *et al.,* 2014). These findings are similar to those documented in a study which opined that neonatal sepsis in preterm neonates was associated with pneumonia like respiratory distress (Ghafoor *et al.,* 2020). Low birth weight in some neonates may be an indicator of ill health and require prompt attention and clinical management. This study found out that a preterm neonate with low birth weight was the most single important factor associated with development of neonatal sepsis with OR=1.867. This concurs with a study done in South Africa which showed that low birth weight was an important risk factor compared with other factors that were studied (Schrag *et al.,* 2012).

Breast feeding on the other hand is important for optimal growth and development with good health for neonates as it reduces the risk of NS (Mugadza *et al*., 2017). From our findings, majority of the respondents had no issues pertaining breast feeding, however those that were not breast feeding well and were put on other mechanisms of feeding were more likely to develop the infection with OR=1.688. Breast feeding has been documented to enhance protection of neonates against neonatal septicaemia, by providing defence factors (large amounts of secretory IgA antibodies a probable reason that our findings can be attributed to (Gopalakrishna & Hand, 2016; Mugadza *et al*., 2017). These findings however do not concur to those obtained in a study that was done in Zimbabwe where early and correct breast-feeding initiation was associated with development of neonatal sepsis (Mugadza *et al*., 2017). This finding was attributed to increased risk of NS with early initiation of breast feeding.

From the current study, isolated bacteria that were found to be responsible for neonatal septicaemia were predominantly Gram positive 35(85.4%) followed by Gram negative 6(14.6%). Such a finding in most cases differs from site to site because of different

niche characteristics and cleanliness. For instance, a study done by Ghafoor *et al.,* (2020) did report that majority of isolates were Gram negative a finding that clearly differs from ours. From this study, Coagulase Negative *Staphylococcus* (CoNS) were found to be the majority isolates 31 (88.5%) causing NS which concurs to a study in Egypt by El-Din *et al*., 2015. CoNS are important as they are ubiquitously present on human skin and are a leading cause of nosocomial blood infection, as they have been attributed to their ability to form biofilms hence persisting in environments and their ability to resist several antimicrobial agents like Methicillin and Oxacillin (Seng *et al.,* 2017). These findings are comparable with those obtained in a study done by Grace & Obaro, (2019) a systematic review of eight randomly selected journals from different countries (Nigeria, France, Kenya, Zambia, India, Sweden, China). which did document that majority of the isolates were *Staphylococcus epidermidis* and other isolates of clinical significance were *Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus hominis* and *Staphylococcus saprophyticus.* From our findings, Gram positive isolates were more resistant especially the CoNS, this may be due to presence of virulence genes like the *icaA, icaB, icaC* and *icaD* that have been documented previously to be responsible for biofilm formation which is associated with antibiotic resistance (Al-Haqan *et al.,* 2020). Antimicrobial resistance is a global concern mostly in 3rd world countries where high cases have been documented (75%). This study also found out presence of resistant genes (carbapenemes; bla oxa- 48, bla KPC and methicillin mecA gene) among bacteria that were isolated from preterm neonates. This clearly indicates presence of antimicrobial resistance among isolated bacteria against Beta-lactam antibiotics which also adds the numbers to the resistance incidences statistics globally. It further possesses a serious challenge on treatment and management

of several illnesses including neonatal sepsis not only to the study site but also regionally and globally (Department of Health and social care, 2013; WHO, 2016).

On isolation and characterisation of the bacterial pathogens known to cause NS, *Staphylococcus aureus* was isolated in the current study. *Staphylococcus aureus* an opportunistic pathogen that is known to cause a wide range of infections due to its enormous virulence traits like ability to coagulate plasma (Crosby *et al*., 2016). Our findings also did document its presence among the isolated pathogens a finding that is consistent to the findings obtained in a South Asia study that documented it as a major course of death among preterm neonates and was described as a huge burden (Chaurasia *et al.,* 2019). *Enterococcus ssp* was also isolated from our study and it is considered as a harmless commensal of little clinical significance. However, when associated with hospital acquired infection and multidrug resistance patterns especially with Vancomycin, it becomes a very significant pathogen of concern. This is comparable to study findings of Subramanya *et al.,* (2019) among preterm neonates admitted in intensive unit that did indicate that *Enterococcus ssp* has the ability to form biofilms that contributes to their virulence and resistance to antibiotics. Gram negative bacteria are a group of Enterobacteriaceae responsible for late onset sepsis, these pathogens are also responsible for multi drug resistance. From our study findings, *Salmonella spp*, *Enterococcus ssp*, *Pseudomonas aeruginosa*, *Acinetobacter ssp, Escherichia coli* were isolated. These study findings are similar with those in a study by (Ghafoor *et al.,* 2020) who did report similar pathogens isolated from blood samples obtained from preterm neonates suspected of neonatal sepsis.

The isolated pathogens from the blood samples obtained from the neonates were further characterized on their antimicrobial properties. From our findings it was clear that

Oxazolidinones (linezolid) was the most effective antibiotic class against all Gram-positive isolates with least MIC of 1 ± 0.00 µg/ml. The activity of this antibiotic is based on its ability to inhibit bacterial protein synthesis and it has been documented to be more effective against methicillin resistant *Staphylococcus spp* (Anderson, 2016; Hamid & Saqib, 2017). Penicillin (penicillin G and Oxacillin) belong to the same class of antibiotics and had the highest resistance. This scenario may be attributed to the narrow spectrum of activity of the antibiotic and the resistance against the antibiotic developed over years by the bacteria. *Staphylococcus aureus* had the highest susceptibility pattern across all microbial agents tested among Gram positive isolates. It had the least variation of resistance pattern with MIC range of 0.12-1 µg/ml, this is similar to results of a study done by (Lyngdoh *et al.,* 2017) where *Staphylococcus aureus* had the highest susceptibility pattern with MIC range of 0.12-1µg/ml. On the other hand, *Staphylococcus epidermidis* was the only isolate that had the highest variation of resistance pattern across all the antimicrobial agents with MIC range of 0.12-8 µg/ml. This clearly demonstrates that proper intervention measures need to be put in place to manage any condition that could be caused by this isolate. Similar findings have also been reported elsewhere where MIC range of 0.12-8 µg/ml was documented (Sheikh *et al.,* 2019). A clear indication of the trouble this pathogen can cause just in case its causes a pandemic more so amongst the neonates.

On the other hand, *Escherichia coli* had the highest susceptibility pattern with an MIC range of 0.25-8 µg/ml across all antimicrobial agents tested among the Gram-negative isolates. This is a very positive finding as this pathogen has been associated with various outbreaks and resistances globally (Maguire, 2006). Additionally, it is a pathogen that can easily be isolated from most environments (Maguire, 2006). Also, the *Salmonella spp* had the highest resistant pattern across all the microbial agents tested

with MIC range of 0.25-8 µg/ml. This is comparable with similar results in a study done by Sheikh *et al.,* (2019) who did report MIC of *Salmonella typhi* to ciprofloxacin to be 8 µg/ml which was upward trend among 20% of the strains (Gautam, 2002).

Beta lactam inhibitors (Amoxicillin/ clavulanic acid and piperacillin/Tazobactam), 4th Generation cephalosporins, (Monobactams, carbapenems, quinolones) were the most effective against Gram negative isolates and this could be attributed to their ability to inhibit the formation of bacterial cell wall and DNA synthesis (Aideen *et al*., 2017). Aminoglycosides (Gentamicin and Amikacin) were the least effective antibiotics against *Pseudomonas aeruginosa* and *Escherichia coli* as they did show MIC of 1± 0.00 and 2 ± 0.00 respectively. This scenario could be attributed to the fact that there is a high exposure levels and misuse of this drug in the study population as it is easily obtained over the counter prescription (Sheikh *et al.,* 2019).

Severe or high-risk bacterial infections are treated by the most effective available antimicrobial agents, however bacteria have evolved and developed mechanism to defeat antimicrobial agents by developing sophisticated mechanisms like production of enzymes from specific genes that neutralize drugs. Methicillin resistant (mecA-gene) and carbapenamase (bla OXA 48 and bla KPC) genes are few of the known genes that produce enzymes that deactivate antibiotics (Barguigua *et al.,* 2012). Among the Gram-negative pathogens, Extended Spectrum Beta lactamases (ESBLs) are enzymes which have the ability to inactivate broad spectrum cephalosporins, aztreonam, penicillins and comprises of TEM, SHV, OXA and CTX-M derivatives (Rahman *et al.,* 2018). SHV enzymes are encoded by self-transmissible plasmids and have a hydrolysing activity to carbapenems and monobactams CTX-M enzymes are plasmid based encoded cefotaximases with an extended activity to cefotaxime antibiotic. TEM enzymes are plasmid mediated resulting from mutations by amino acid substitution around the active

site. OXA enzymes have a high hydrolytic activity against cloxacillin and oxacillin antimicrobial agents (Liakopoulos et al., 2016). Gram positive bacteria produce different enzymes that have different mechanisms of resistance to antimicrobial agents. Penicillin binding-proteins, DNA-dependent RNA polymerase and type II topoisomerases work by targeting antimicrobial drugs. Some work by modifying cellular targets of antimicrobial agents like the phosphoethanolamine, others work as antimicrobial drug-modifying enzymes like the Transferases and the Hydrolases, while some work as the antimicrobial drug-metabolizing enzymes like the Pyrazinamidase enzymes (Brodolin., 2019).

In our study, Methicillin resistant (mecA-gene) was found in six isolates, three *Staphylococcus epidermidis* with a cycle threshold of 28.87, 34.38 and 38.43, *Staphylococcus warneri* with a cycle threshold of 37.34, *Staphylococcus horminis* with cycle threshold of 32.76 and *Enterococcus spp* with cycle threshold of 26.76. These findings are similar to those documented in a study done in Brazil where mecA gene was detected among Coagulase Negative Staphylococcus (CoNS) Rocchetti *et al.,* (2018). Bla Oxa 48 and Bla KPC genes were isolated in four isolates, two *Salmonella spp* with a cycle threshold of 30.61, *Escherichia coli* spp with cycle threshold of 33.64 and *Pseudomonas aeruginosa* with cycle threshold of 35.43. Bla Oxa 48 gene was also isolated in *Klebsiella pneumoniae* isolates that were carbapenem resistant at a tertiary care centre in Western Turkey (Ece *et al.,* 2018). Another study done in Huashan hospital, China did report that Bla KPC gene was dominant in clinical *Klebsiella pneumoniae* isolates (Shen *et al*., 2016). From this study findings, it clearly indicates that there exist resistant genes among the isolates found in preterm neonates at Kitale county Hospital New Born Unit (NBU).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1: Conclusions

Based on the findings of this study, a larger proportion of the neonates were one day old 57 (31.5%) with a gestation age of ≤36 completed weeks, mean weight of 1700 grams and presented with signs of sepsis on the first day after delivery. A higher proportion of deliveries were spontaneous virginal delivery with a high frequency of prolonged rapture of membranes.

With regard to maternal factors associated with development of neonatal sepsis; mode of delivery and prolonged rapture of membranes and neonatal factors; foetal distress, body weight, poor breast feeding were risk factors strongly associated with the development of neonatal sepsis at KCH new born unit.

On microbial profiles, Gram positive isolates constituted the majority of microbials responsible for neonatal sepsis among the preterm neonates with Coagulase negative *Staphylococcus* (CoNS) being the predominant isolates.

On Antimicrobial characteristics, the study found out that there was significant resistance to extended spectrum ß lactam antibiotics with varying patterns to cephalosporins as demonstrated by the presence of resistance gene markers (carbapemenase: blaOXA-48 and bla KPC for Gram negative isolates and mec A for Gram positive isolates).

On microbial profiles and antibacterial properties of microorganisms causing neonatal sepsis, the study concludes that there exists Multi-Drug Resistance (MDR) genes in bacterial isolates circulating in Kitale County Hospital new born unit.

6.2 Recommendations

- Management at KCH should develop and implement Hospital based specific Policy, guidelines and Standard Operating Procedures (SOPS) on Management and treatment of preterm neonatal sepsis with multidrug resistant microbials.
- The Infection Prevention and Control (IPC) at KCH should adopt Standard Operating Procedures (SOP) for management of mothers and neonates at risk of developing sepsis
- Clinicians, pharmacists and microbiology laboratory staff at KCH should work together to develop antibiogram for management and treatment of preterm neonatal sepsis and revise hospital formulary where necessary.
- KCH new born unit should commission further study to identify major risk factors and should include investigations on environment, tools and equipment used during delivery, training/ competency of staffs and development of neonatal sepsis.
- Despite many neonates having signs and symptoms of neonatal sepsis, their blood culture results turned negative for bacteria, therefore health ministries in counties within western region should consider further studies of a wider scale that will include several hospitals at different levels needs to determine the presence of other potential aetiological agents (viral or fungal).
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APPENDICES

APPENDIX 1.0

CONSENT FORM

Title of study: Determinants, microbial profiles and antimicrobial characteristics of neonatal sepsis among preterms in Kitale County Hospital (KCH).

Introduction

My name is Everlyne Pauline Kweyu. I am a MSc Microbiology student at Kisii University currently at the level of generating data for my thesis. My research interest is on Neonatal sepsis, which have been found to be a major contributor to morbidity and mortality among preterm infants. Studies have been conducted in Kenya, there is a high rate of neonatal deaths, and the proportion is rising. A number of different bacteria, including *Escherichia coli* (*E. coli*), *Listeria*, and certain strains of *streptococcus*, may cause NS. This situation is compounded by rising antimicrobial resistance through mechanisms such as mutations and increased enzyme production, which is a worldwide problem and the changing pattern of antibiotic use and changes in lifestyles. The local pathogen profiles and antimicrobial data necessary for rational treatment are not available in Kenya. I am therefore, conducting this study to evaluate the potential role of gestational age, birth weight and duration of parenteral nutrition may play in the development of neonatal sepsis among preterm infants, and to identify the main NS associated pathogens and establish their antimicrobial characteristics. This will aid in the management of sepsis in your baby.

Description of study: If you agree to be in this study, you will allow us to collect a blood sample from your infant. The sample will be taken to the laboratory for culture, which will take a period of more than a week for the results to come out.

Risks of the study: Blood will be drawn from the preterm neonate and there will be some minimal risks such as pain and little discomfort, the mother will be encouraged to breastfeed thereafter. There will be some bleeding which will be controlled through safe phlebotomy. There is no monetary benefit for your participation in this study. The benefit, which may reasonably be expected to result from this study, includes your contributions to

efforts of understanding the burden of NS and how to manage this infection locally. If we find that your child has NS through blood culture, we will advise your health provider for proper management. Your decision whether or not to participate in this study will not affect your current treatment you are receiving from the hospital in any way.

Confidentiality: The records of this study will be kept strictly confidential. Research records will be kept in a locked file and all electronic information will be coded and secured using a password protected file.

Right to withdraw: The decision to participate in this study is entirely voluntarily. You may refuse to take part in the study at any time. You have the right not to answer any single question, as well as to withdraw completely from the interview at any point during the process.

Right to ask questions: You have the right to ask questions about this research study and to have those questions answered by me before, during or after the research. Feel free to contact me, Everlyne P Kweyu, email epkweyu@gmail.com or by telephone: 0728 985675. If you have any other concerns about your rights as a research participant that have not been answered by the investigators, you may contact the secretary, Kisii University Review Committee, P.O. Box 408-40200 Kisii, Kenya; Email address: info@kisiiuniversity.ac.ke

Consent: My signature below indicates that I have accepted my infant as a research participant for the study, and I have read and understood the information provided above.

Subject's signature/thump-print _______________ Date _________________

Investigator's name _________________________ Signature __________

Date __________________

APPENDIX 2.0

STUCTURED QUESTIONNAIRE

A. NEONATE DEMOGRAPHIC DATA

I. SID ____________________________

II. Age in days_____________________

III. Gender __________________________

IV. Date of birth: _____________________

V. Time of birth: _____________________

B. MOTHER'S MEDICAL HISTORY

I. Gestational age at birth: ____________ weeks completed

II. What type of delivery did you have? (Tick where applicable)

- Vaginal delivery □
- Cesarean section □

III. Maternal GBS status: (Please tick where applicable)

Positive □

Negative □

Unknown □

IV. Gestational age tested ________________

V. Did you have any maternal urinary tract infection during pregnancy? Yes □ No □

If yes, specify GBS bacteriuria ______________ Other, specify: _____________

C. FAMILY HISTORY

I. Any previous sibling with neonatal sepsis? Yes □ No □ Not sure □

If yes, specify type _________________________

D. NEONATE EXAMINATION ON ADMISSION

I. BP? ___________________________

II. HB level? ______________________

III. Any fever? ______________________

E. NEONATES CLINICAL DATA

I. APGAR score at: 1 minute _____ 5 minutes _____ 10 minutes _____

II. Birth weight: _____ grams □ Normal □ Not normal

III. Height: _____ cm □ Normal □ Not normal

IV. Head circumference: _____ cm □ Normal □ Not normal

V. Date of presentation: _____ /_____ /_____

VI. Age of 1st presentation with sepsis: _____ hours (if<24hrs) or _____days

VII. If readmitted, date of readmission: _____ /_____ /_____

VIII. Weight on readmission: _____ grams

IX. Did the infant have a central line (peripherally inserted central catheter line, umbilical line, central venous line) prior to infection: Yes □ No □ Unknown □

X. What type of feeding do you give to your baby? (Please tick where applicable)

Breast □

Formula □

XI. Is the preterm neonate on parenteral nutrition? Yes □ No □

XII. For how long has your baby been on parenteral nutrition?

____

XIII. Did your baby develop sepsis when on parenteral nutrition? Yes □ No □

F. MOTHER'S PAST MEDICAL HISTORY (ANTENATAL HISTORY)

I. Did you have any illness in pregnancy? Yes □ No □

II. What were your KHAN results: Reactive □ Non-reactive □

III. Did you have any discharge during pregnancy? Yes □ No □

IV. Prolonged rupture of membranes (PROM>18 hrs prior to delivery) Yes □ No □

V. If yes, specify duration of ROM: ______ hours

VI. Premature PROM: Yes □ No □ If yes, specify gestational age at rupture ______ weeks

VII. Elevated maternal white blood count (WBC >10,000; after onset of labour) Yes □ No □

VIII. Maternal antibiotics (GBS prophylaxis given) Yes □ No □ If yes, specify: Type of antibiotic ______________________ Duration ___________

IX. Did you have foetal distress? Yes □ No □

G. NEONATE FEEDING

I. Is the baby breastfeeding well? Yes □ No □

II. Is it on parenteral nutrition? Yes □ No □

III. Is the baby on antibiotic treatment? Yes □ No □

IV. How long has the baby been on parenteral nutrition?

________Days and ______Weeks and ______Months

APPENDIX 3.0

KISII UNIVERSITY (OFFICE OF THE REGISTRAR RESEARCH AND EXTENSION) INTRODUCTORY LETTER

KISII UNIVERSITY

Telephone: +254 773452323
Facsimile: +254 020 2491131
Email: research@kisiiuniversity.ac.ke

P O BOX 408 – 40200
KISII
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OFFICE OF THE REGISTRAR RESEARCH AND EXTENSION

KSU/R&E/ 03/5/vol.1/183 **DATE:** 9th August, 2018

The Head, Research Coordination
National Council for Science, Technology and Innovation (NACOSTI)
Utalii House, 8th Floor, Uhuru Highway
P. O. Box 30623 – 00100
NAIROBI - KENYA.

Dear Sir/Madam,

RE: EVERLYNE PAULINE KWEYU REG.NO: MHS13/40035/15

The above mentioned is a student of Kisii University currently pursuing Master Degree of Biomedical Science (Microbiology Option) in the School of Health Sciences. The topic of the research is, ***"Microbial Profiles and Antimicrobial characteristics of Neonatal Sepsis among Preterm Infants at Kitale County Hospital, Kenya".***

We are kindly requesting for assistance in acquiring a research permit to enable the student carry out the research.

Thank you.

KISII UNIVERSITY
09 AUG 2018
REGISTRAR RESEARCH & EXTENSION

Prof. Anakalo Shitandi, PhD
Registrar, Research and Extension
Cc: DVC (ASA)
Registrar (AA)
Director SPGS

AS/sm

KISII UNIVERSITY IS ISO 9001:2008 CERTIFIED

APPENDIX 4.0

APPROVAL LETTER FORM RESEARCH AND ETHICS COMMITTEE (IREC)

MU/MTRH-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET

Reference: IREC/2018/228
Approval Number: 0003174

6th December, 2018

Everlyne Pauline Kweyu,
Kisii University,
School of Health Sciences,
P.O. Box 408-40200,
KISII-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
06 DEC 2018
APPROVED
P.O. Box 4606-30100 ELDORET

Dear Ms. Kweyu,

RE: FORMAL APPROVAL

The MU/MTRH- Institutional Research and Ethics Committee has reviewed your research proposal titled: -

"Microbial Profiles and Antimicrobial Characteristics of Neonatal Sepsis among Preterm Neonates at Kitale County Hospital, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 3174*** on 6th December, 2018. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; hence will expire on 5th December, 2019. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date. You will be required to submit progress report(s) on application for continuation, at the end of the study and any other times as may be recommended by the Committee.

Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. You will also be required to seek further clearance from any other regulatory body/authority that may be appropriate and applicable to the conduct of this study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc	CEO - MTRH	Dean - SOP	Dean - SOM
	Principal - CHS	Dean - SON	Dean - SOD

APPENDIX 5.0

APPROVAL LETTER TO CARRY OUT RESEARCH BY NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/80813/27724**

Date: **29th January, 2019**

Everlyne Pauline Kweyu
Kisii University
P.O. Box 408-40200
KISII

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Microbial profiles and antimicrobial characteristics of neonatal sepsis among preterm neonates at Kitale County Hospital, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Trans Nzoia County** for the period ending **29th January, 2020.**

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

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

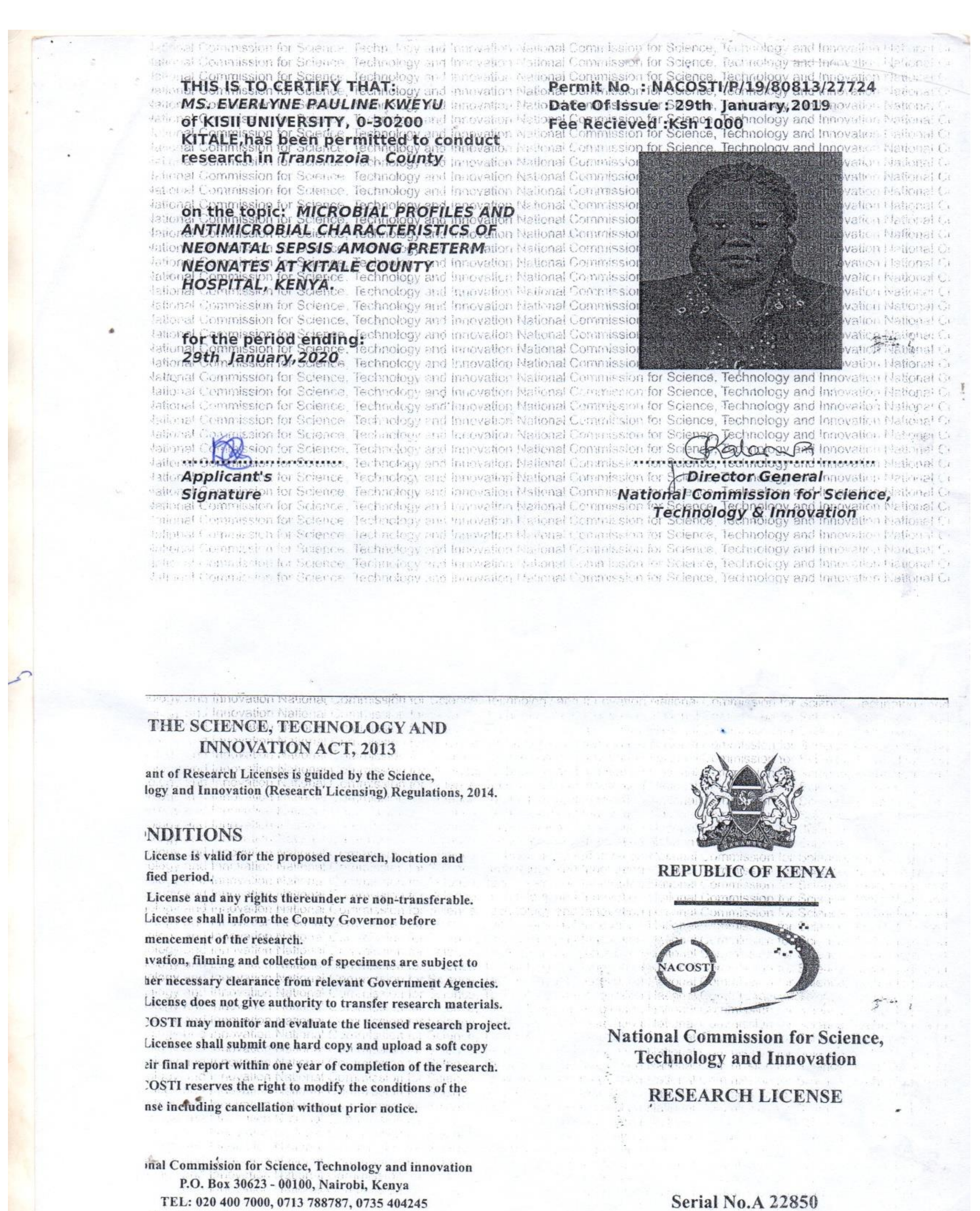
Copy to:

The County Commissioner
Trans Nzoia County.

The County Director of Education
Trans Nzoia County.

APPENDIX 6.0

APPROVAL PERMIT TO CARRY OUT RESEACH BY NACOSTI



THIS IS TO CERTIFY THAT:
MS. EVERLYNE PAULINE KWEYU
of KISII UNIVERSITY, 0-30200
KITALE,has been permitted to conduct
research in Transnzoia County

on the topic: MICROBIAL PROFILES AND ANTIMICROBIAL CHARACTERISTICS OF NEONATAL SEPSIS AMONG PRETERM NEONATES AT KITALE COUNTY HOSPITAL, KENYA.

for the period ending:
29th January,2020

Permit No. : NACOSTI/P/19/80813/27724
Date Of Issue : 29th January,2019
Fee Recieved :Ksh 1000

Applicant's Signature

Director General
National Commission for Science, Technology & Innovation

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

ant of Research Licenses is guided by the Science, logy and Innovation (Research Licensing) Regulations, 2014.

NDITIONS

License is valid for the proposed research, location and fied period.
License and any rights thereunder are non-transferable.
Licensee shall inform the County Governor before mencement of the research.
ıvation, filming and collection of specimens are subject to her necessary clearance from relevant Government Agencies.
License does not give authority to transfer research materials.
COSTI may monitor and evaluate the licensed research project.
Licensee shall submit one hard copy and upload a soft copy eir final report within one year of completion of the research.
COSTI reserves the right to modify the conditions of the nse including cancellation without prior notice.

nal Commission for Science, Technology and innovation
P.O. Box 30623 - 00100, Nairobi, Kenya
TEL: 020 400 7000, 0713 788787, 0735 404245
Email: dg@nacosti.go.ke, registry@nacosti.go.ke
Website: www.nacosti.go.ke

REPUBLIC OF KENYA

NACOSTI

National Commission for Science, Technology and Innovation

RESEARCH LICENSE

Serial No.A 22850

CONDITIONS: see back page

APPENDIX 7.0

COUNTY COOMISSIONER (TRANS NZOIA) LETTER OF NO OBJECTION TO CARRY OUT RESEARCH

THE PRESIDENCY

Telephone: 054 – 30020
Fax No: 054 – 30030

MINISTRY OF INTERIOR
AND
COORDINATION OF
NATIONAL GOVERNMENT

COUNTY COMMISSIONER'S OFFICE
TRANS NZOIA COUNTY
P.O BOX 11 - 30200
KITALE

E-mail: cctransnzoiacounty@yahoo.com
When replying please quote

TNZC/CONF/ED.12/2/VOL.II/107

27th March, 2019

TO WHOM IT MAY CONCERN

RESEARCH AUTHORIZATION

This is to inform you that **Everlyne Pauline Kweyu** of **Kisii University** been authorized by National Commission for Science, Technology and Innovation to carry out research on **"Microbial profiles and antimicrobial characteristics of neonatal sepsis among preterm neonates at Kitale County Hospital, Kenya"** in **Trans Nzoia County,** for a period ending **29th January, 2020**

Please accord her the necessary assistance she may require.

COUNTY COMMISSIONER
TRANS-NZOIA COUNTY
P. O. Box 11 - 30200 KITALE

NANCY KURGAT
FOR: COUNTY COMMISSIONER
TRANS NZOIA COUNTY

APPENDIX 8.0

COUNTY GOVERNMENR OF TRANS NZOIA (State Department of Early and Basic Education) RESEARCH AUTHORIZATION

REPUBLIC OF KENYA
Ministry of Education
State Department of Early Learning and Basic Education

Telegrams: ....
Telephone: Kitale 054-31653 – 30200
Fax: 054-31109
Email: transnzoiacde@gmail.com
When replying please quote:

Ref. No. TNZ/CNT/CDE/R.GEN/1/VOL.II/14

County Director of Education
Trans Nzoia
P.O. Box 2024 – 30200
KITALE.

Date: **27th March, 2019**

TO WHOM IT MAY CONCERN

RE: RESEARCH AUTHORIZATION – EVERLYNE PAULINE KWEYU

This office acknowledges receipt of a letter on the above subject Ref: **NACOSTI/P/19/80813/27724** dated **29th January, 2019**

Everlyne Pauline Kweyu a student at Kisii University is authorized to carry out research on **"Microbial Profiles and Antimicrobial characteristics of Neonatal Sepsis among Preterm Infants at Kitale County Hospital in Trans-Nzoia County - Kenya** for a period ending **29th January, 2020**

The purpose of the letter is to request you to accord her the necessary assistance.

pp.

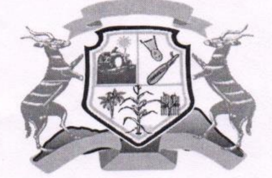
DR. S. W. MAINA (PhD)
COUNTY DIRECTOR OF EDUCATION
TRANS-NZOIA COUNTY.

APPENDIX 9.0

COUNTY GOVERNMENT OF TRANS NZOIA (DEPARTMENT OF HEALTH SERVICES) RESEARCH AUTHORIZATION

REPUBLIC OF KENYA

TEL: 054-30301;
054-30302
Fax;
EMail: barazoo2000@yahoo.co.uk



Office Of The COHCS
P.O BOX 4211-30200
KITALE

COUNTY GOVERNMENT OF TRANS NZOIA
DEPARTMENT OF HEALTH SERVICES

When replying please quote
Our Ref: CGTN /HS/COHCS/2018
Your Ref:

Date: Wednesday, April 17, 2019

TO WHOM IT MAY CONCERN

RE: RESEARCH AUTHORIZATION – EVERLYNE PAULINE KWEYU P/NO.1997093010.

The above named officer has authority to carry out research on **Microbial Profiles and Antimicrobial Characteristics of Neonatal Sepsis among Preterm Neonates at Kitale County Hospital in Trans Nzoia** County for a period ending 29th January 2020.

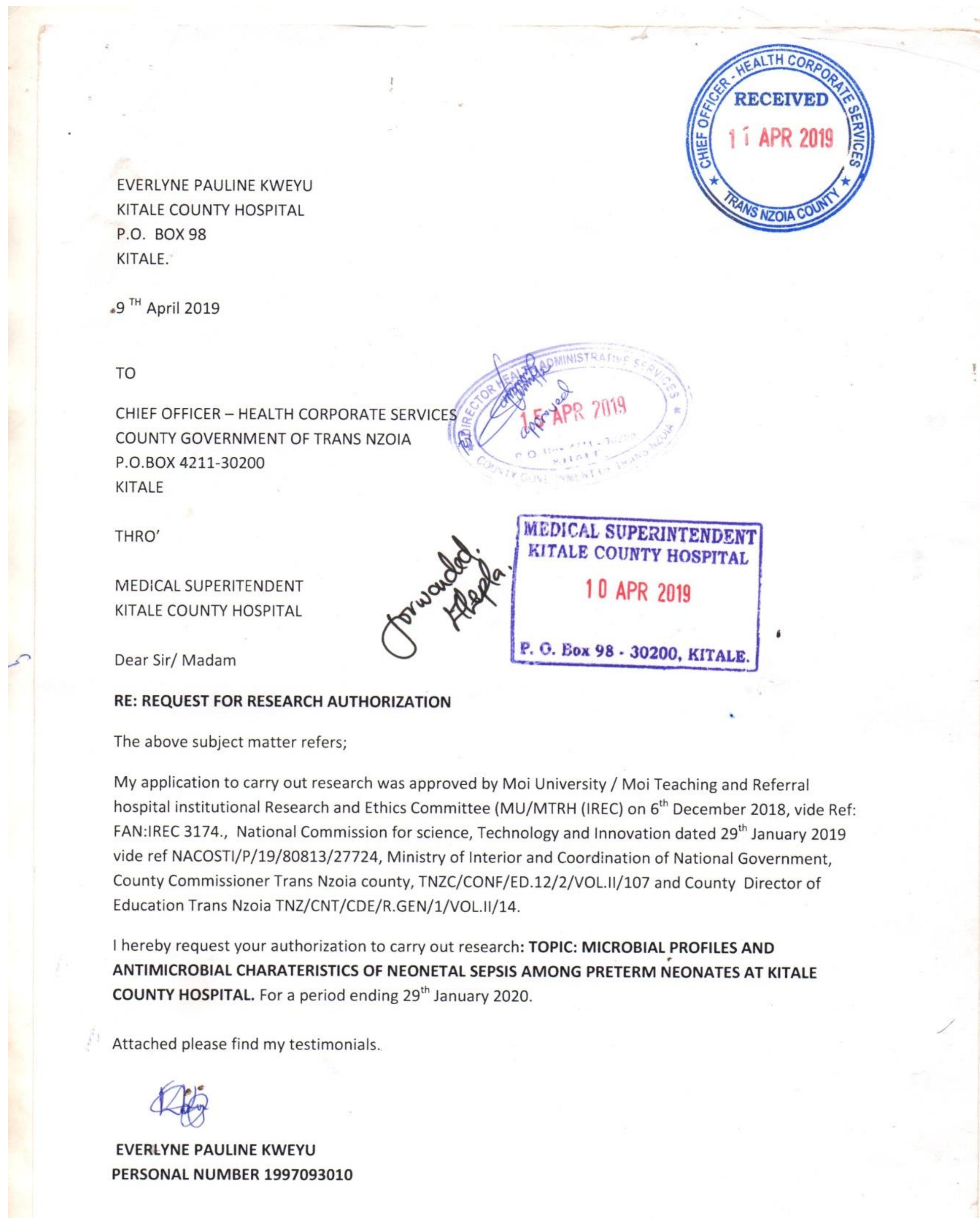
Please accord her the necessary assistance she may require.

DIRECTOR HEALTH ADMINISTRATIVE SERVICES
17 APR 2019
P.O. Box 4211 - 30200
COUNTY GOVERNMENT OF TRANS NZOIA

Dr Sammy Masibo
FOR: CHIEF OFFICER – HEALTH CORPORATE SERVICES

APPENDIX 10.0

REQUEST FOR RESEARCH AUTHORIZATION (Medical superintendent Kitale County Hospital)



EVERLYNE PAULINE KWEYU
KITALE COUNTY HOSPITAL
P.O. BOX 98
KITALE.

9 TH April 2019

TO

CHIEF OFFICER – HEALTH CORPORATE SERVICES
COUNTY GOVERNMENT OF TRANS NZOIA
P.O.BOX 4211-30200
KITALE

THRO'

MEDICAL SUPERITENDENT
KITALE COUNTY HOSPITAL

Dear Sir/ Madam

RE: REQUEST FOR RESEARCH AUTHORIZATION

The above subject matter refers;

My application to carry out research was approved by Moi University / Moi Teaching and Referral hospital institutional Research and Ethics Committee (MU/MTRH (IREC) on 6th December 2018, vide Ref: FAN:IREC 3174., National Commission for science, Technology and Innovation dated 29th January 2019 vide ref NACOSTI/P/19/80813/27724, Ministry of Interior and Coordination of National Government, County Commissioner Trans Nzoia county, TNZC/CONF/ED.12/2/VOL.II/107 and County Director of Education Trans Nzoia TNZ/CNT/CDE/R.GEN/1/VOL.II/14.

I hereby request your authorization to carry out research: **TOPIC: MICROBIAL PROFILES AND ANTIMICROBIAL CHARATERISTICS OF NEONETAL SEPSIS AMONG PRETERM NEONATES AT KITALE COUNTY HOSPITAL.** For a period ending 29th January 2020.

Attached please find my testimonials.

EVERLYNE PAULINE KWEYU
PERSONAL NUMBER 1997093010

RECEIVED 11 APR 2019 — CHIEF OFFICER - HEALTH CORPORATE SERVICES ★ TRANS NZOIA COUNTY ★

MEDICAL SUPERINTENDENT
KITALE COUNTY HOSPITAL
10 APR 2019
P. O. Box 98 - 30200, KITALE.

15 APR 2019