Aetiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis at the University Teaching Hospital in Lusaka, Zambia.

By

Mwila Patrick Kabwe

"A Dissertation submitted to the University of Zambia in partial fulfilment of the requirements of the degree of Masters of Medical Microbiology"

The University of Zambia

Lusaka

Declaration

This Dissertation represents Mwila Patrick Kabwe's own work and has not been previously submitted for a degree, diploma or other qualification at this or any other University.

Candidate Name: **Mwila Patrick Kabwe**

Signature:.....

Date:.....

Certificate of approval

This dissertation of **Mwila Patrick Kabwe** has been approved in partial fulfilment of the requirements for the degree of Master of Medical Microbiology by the University of Zambia.

……	…… ……	……. ……
Supervisor	Signature	Month/Date/Year
……	…… ……	……. ……
Examiner 1	Signature	Month/Date/Year
……	…… ……	…….. ……..
Examiner 2	Signature	Month/Date/Year
……	…… ..……	…….. ……..
Examiner 3	Signature	Month/Date/Year

Abstract

Although neonatal sepsis occurs in approximately 15% of neonatal admissions in Sub-Saharan Africa, there is minimal data on its causes and antimicrobial resistance (AMR) that might guide policy and practice. *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus,* Group B streptococcus (GBS) and other gram negative bacteria are the most common causes. These organisms especially the Enterobacteriaceae have AMR rates higher than 50%, for both first (Ampicillin and Gentamycin) and second line (Cephalosporins) drugs. Data on prevalence, aetiology, AMR patterns and risk factors for neonatal sepsis at the University Teaching Hospital (UTH) in Zambia has not been collected systematically making it difficult to utilize such information on patient management. Thus, this cross sectional study conducted in the Neonatal Intensive care Unit at UTH was aimed at providing baseline data that can be used to formulate guidelines for neonatal sepsis management. Among 313 neonates with clinically suspected sepsis, the prevalence of culture-confirmed sepsis was 33% with early onset sepsis (EOS) accounting for 85% of the cases. *K. pneumoniae* was the most prevalent pathogen accounting for 75% of the cases, followed by *S. aureus* and *E. coli* at 6% and 5%, respectively. During the study, it was observed that an increase in prevalence of neonatal sepsis due to *K. pneumonia* correlated with a high mortality rate, implying a potential outbreak. For WHO-recommended first line and second line therapy, AMR ranged from 96-99% and 94-97%, respectively. Bacterial culture diagnosis had minimal impact on treatment and outcome as only 25% of the neonates had received results for culture and drug sensitivity before discharge/death. Increasing neonatal age in days, and parity were associated with a significant increase in the odds of culture-confirmed neonatal sepsis, whereas irritability and pallor were negatively associated. Maternal human immunodeficiency virus infection was also associated with a significant reduction in the odds of culture-confirmed neonatal sepsis. These data, therefore, indicate a high burden of neonatal sepsis that needs immediate intervention. This could be done through implementation of simple and scalable infection control strategies that can reduce the frequency and rate of transmission of these infections.

Dedication

This dissertation is dedicated to my wonderful daughter Isabella Chansa Kabwe. I also dedicate this work to my parents Joyce and Rodwell Kabwe, My wife Elizabeth and my Brothers and Sister.

Acknowledgements

Firstly thanks and Praises goes to the Lord Almighty, Jesus Christ for He has been and always will be Faithfull in all ups and downs. In no particular order, I would like to thank the following for helping in my successful completion of this work. I would like to acknowledge the efforts and contributions of my supervisors Dr Chileshe Lukwesa-Musyani and Dr Matthew Bates who gave me valuable support regardless of their limited and precious time. Other professional contributors to this work, Professor Alimmudin Zumla, Dr Davidson H Hamer and Dr Yusuf Ahmed, who offered their expert opinion in the design and implementation of this work. I would like to further thank the participants' parents and the department of paediatrics and Child Health at the University Teaching Hospital in Lusaka for allowing me carry out this study. Many thanks also goes to 'Kids-Here-and-There' for funding the study through UNZA-UCLMS research and training programme. UNZA-UCLMS staffs are appreciated for their support and availing me the opportunity to be the lead researcher and carry out the study and not forgetting the advice and support from my fellow students and family. The Lord God Almighty be with you.

Table of Contents **Page**

List of Tables **Page**

List of Figures, Maps and illustrations **Page**

List of Abbreviations and Acronyms

µL	Microlitre
AMR	Anti-microbial Resistance
CLSI	Clinical and Laboratory Standards Institute
DST	Drug Susceptibility Testing
EOS	Early Onset Sepsis
ESBL	Extended Spectrum Beta-Lactamase
GBS	Group B *Streptococcus*
HAI	Hospital Acquired Infection
HEU	HIV Exposed but uninfected
HIV	Human Immunodeficiency Virus
IQR	Interquartile Range
LIA	Lysine Iron Agar
LOS	Late Onset Sepsis
MDR	Multi-Drug Resistant
MSAF	Meconium Stained Amniotic Fluid
MTCT	Mother-to-Child transmission
NICU	Neonatal Intensive care Unit
PMTCT	Prevention of Mother-to-Child transmission
SERCH	Septicaemia Etiological Research in Child Health
SIM	Sulphur Indole Motility
SIRS	Systemic Inflammatory Response Syndrome
SOP	Standard Operating Procedure
SVD	Spontaneous Vaginal Delivery
TSI	Tripple Sugar iron
UNZA	University Of Zambia
UNZA-UCLMS	University of Zambia- University College London Medical School

UTH	University Teaching Hospital
WHO	World Health Organisation

List of Appendices **Page**

Chapter 1: Introduction

1.1 Background

Neonatal sepsis, broadly defined as a systemic inflammatory response syndrome (SIRS) occurring in new borns up to 29 days of birth (Dellinger *et al*, 2008), is a significant cause of morbidity and mortality globally (Nagata *et al*, 2002; Auriti *et al*, 2003; Couto *et al*, 2007). The gold standard for neonatal sepsis diagnosis is blood culture. Pathogen isolation from venous blood within the first 72 hours of life has been referred to as early onset neonatal sepsis (EOS) while that occurring after 72 hours of life has been considered to be late onset sepsis (LOS) (Ballot *et al*, 2012). Whilst EOS has been strongly associated with maternally acquired neonatal sepsis, LOS has been associated with Hospital and/or community acquired infections. Group B Streptococci (GBS) is the most common aetiological agent of EOS and is usually acquired during child birth. In contrast, LOS is usually caused by coagulase-negative *Staphylococcus aureus, Klebsiella pneumonia, and Escherichia coli* which are mostly from contaminated intravenous devices, birthing surfaces and normal skin flora. (Zaidi *et al*, 2009). The causative agents may vary by country or region making it fundamental to have a current knowledge of the epidemiology of sepsis to facilitate early treatment that may contribute to the reduction of mortalities in neonates (Kumar *et al*, 2008; Cailes *et al*, 2015).

Alarming rates to the 1st line and 2nd line therapy have been reported in hospital based studies in developing countries (Bates *et al*, 2014). *K. pneumonia* was the most prevalent pathogen with the highest rates of AMR ranging from 86% to 51% for ampicillin and cefotaxime, respectively. *E. coli* was the second most resistant pathogen with rates of AMR ranging from 82% to 46% for ampicillin and cefotaxime, respectively. For *S.*

aureus resistance rates were reported at 64% and 38% for co-trimoxazole and methicillin, respectively (Bates *et al*, 2014).

Identification of risk factors is key in designing effective interventions to reduce the burden of neonatal sepsis (Cailes *et al*, 2015). In developing countries with limited resources, targeted infection control by disinfection in high-risk areas such as birthing theatres and labour ward nurseries could significantly reduce infection and consequently decrease neonatal mortality (Philip *et al*, 2006). Very low birth weight has been significantly associated with the risk of EOS as well as many maternal factors such as meconium stained amniotic fluid, pre-gestational diabetes mellitus, first birth, multiple gestation and duration of labour (Stephanie *et al*, 2012).

At University Teaching Hospital (UTH), neonatal sepsis has not been systematically studied. High mortalities in the NICU have led some medical personnel to suspect that sepsis could be among the major causes of these mortalities. Despite these suspicions, the prevalence, aetiology, AMR and risk factors of neonatal sepsis at UTH remain unknown.

Clinical and laboratory diagnosis of neonatal EOS is mostly hampered by inconsistent clinical symptoms and low blood volume, respectively (Van der Zwet *et al*, 2005). The bacteriology laboratory supporting NICU at UTH applies blood culture as the gold standard for the investigation of suspected neonatal sepsis (MOH-Microbiology level III SOP). This is undertaken with an automated BACTEC FX200 with positive cultures flagging between 24 to 48 hours. A further growth period of 24 hours is required on solid media and another 24 hours for phenotypic characterisation and Drug Susceptibility Testing (DST). Empirical treatment according to WHO recommendations is utilised

although it is not implemented perfectly due to equipment and staff challenges. For example the decision to switch from first to second-line therapy is often consultant led and is independent of blood culture results which are usually unavailable. Normally, patients are started on two broad spectrum antibiotics (Gentamicin and Ampicillin) and third generation cephalosporins are used as second line drugs. Ciprofloxacin is used as third line drug.

The objective of this study was to determine the prevalence, aetiology, drug resistance of culture confirmed neonatal sepsis through a cross sectional observational study of suspected neonatal sepsis cases admitted to the NICU at UTH. The study also aimed to identify maternal and neonatal factors that might influence the odds of sepsis. This was a hospital based study and EOS was divided into very early (within 72hours of life) and early (i.e. up to 7days) while LOS was defined as sepsis acquired after 7 days of life.

1.2 Statement of the Problem

Sepsis is a major cause of morbidity and mortality in neonatal admissions in UTH. Out of a monthly admission rate of approximately 300 neonates, approximately 190 (63%) are suspected cases with a case mortality of approximately 50% (UNZA-UCLMS, 2014, unpublished data). Most of these preventable mortalities are a result of blood stream bacterial infections that may be transmitted vertically and/or horizontally from a single or multiple sources. Treatment challenges due to lack of local guidelines for treatment of blood stream infections in NICU and also management guidelines for those neonates especially at risk are also unavailable. The lack of systematically collected laboratory data has made it difficult to define the epidemiology of sepsis at the institution that can be used to guide therapy.

1.3 Justification of the Study

Due to the many challenges faced in the diagnosis, treatment and management of sepsis coupled to the lack of prevalence and aetiology data in many African settings, knowing the epidemiology of sepsis is of paramount importance. Identifying the organisms responsible and their AMR patterns and risk factors associated with development of sepsis are key to alleviating this problem (Reddy *et al*, 2010). The identification of micro-organism responsible for causing blood stream infections in severely ill neonates admitted to NICU at UTH is cardinal in understanding the spread and prevention strategies that may be employed. Their identification can further lead to defining the Antibio-gram upon which treatment guidelines and antimicrobial usage guidelines may be formulated. This provides clinicians with an option to properly treat a neonate with an antibiotic likely to work in his/her own ward before laboratory results are available (Sigaúque *et al*, 2009). The epidemiology and risk factor analysis can provide for community interventions targeted at reducing morbidity and mortality in neonates in the country as well as across the sub-Saharan region.

1.4 Literature Review

1.4.1 The Epidemiology of Neonatal Sepsis

Clinically Neonatal sepsis has been divided into EOS and LOS (Cailes *et al*, 2015). However, controversies occur as to where the cut-offs exist between these two categories. Some studies have described EOS as sepsis acquired perinatally and occurs before 72 hours while LOS has been associated with hospital acquired sepsis and known to occur after 72 hours until the end of neonatal age (Cailes *et al*, 2015; Phillip *et al*, 2006). Other studies have indicated that EOS is sepsis that occurs within 7 days of life associated with perinatally acquired sepsis and LOS occur after 7 days and is associated with either Hospital or Community acquired sepsis (Zaidi *et al*, 2009; Bateman and Seed, 2010).

Worldwide, epidemiology is not well described due to the lack of a consensus definition of sepsis (Artero *et al*, 2012). The few studies that have been carried out indicate that sepsis is a worldwide problem with epidemiological estimates suggesting that there were 1.7 million cases of neonatal sepsis globally in 2010, with 600 thousand cases (Seale *et al*, 2013) and 140 thousand deaths (Liu *et al*, 2012) in sub-Saharan Africa. While the distribution and burden of sepsis have been difficult to estimate due to the lack of data on prevalence and aetiology in Africa, and broadly in developing countries.

Neonatal sepsis studies from Africa published between 2010 and 2015 do not distinguish clearly between community-acquired or hospital-acquired neonatal sepsis, and/ or between vertically or horizontally acquired infections. *Klebsiella* species were commonly identified in all but one study, accounting for median prevalence of 32%. *Staphylococcus aureus* (24%) and coagulase-negative *Staphylococci* (12%) were the second and third most prevalent organisms (Shitaye *et al*, 2010; Landre-Peigne *et al*, 2011; Kohli-Kochlar *et al*,

2011; Ogunlesi *et al*, 2011; West and Peterside, 2012; Ballot *et al*, 2012; Mhada *et al*, 2012; Acquah *et al*, 2013; Kiwanuka *et al*, 2013; Hamer *et al*, 2014; Mkony *et al*, 2014; Tann *et al*, 2014). The findings from the above studies were similar to those reported in a review of 6 studies of hospital-acquired neonatal sepsis published between 1990 and 2004. In that review, *Klebsiella species* were the major pathogens found in 28.2%, followed by *Staphylococcus aureu*s at 14.3%, *Escherichia coli* at 9.9%, other gram negatives grouped together stood at 8.8% and GBS was the least prevalent pathogen at 8.5% (Zaidi *et al*, 2005). In 2009, Zaidi *et al* reviewed 20 published studies and showed that *Klebsiella* spp. with a prevalence of 26.4% was the most common cause of very early onset sepsis that occurred within 3 days. *S. aureus* and *E.coli* followed each accounting for 17.3% and 12.6% respectively. Similar prevalence was seen up to 7 days after which *S. aureus* (13.7%), *Salmonella spp*. (13.3%), *Streptococcus pneumoniae* (12.3%), and GBS (11.5%) were most prevalent. The distribution of pathogens, however, is different depending on the region (Zaidi *et al*, 2009)

These pathogens have led to high mortality rates especially in the African region where strategies for prevention of neonatal sepsis have remained poor. Scanty data is available on the risk factors associated with neonatal sepsis in this region while other studies have only looked at factors that increase the risk of neonatal mortality as a whole and not that attributed to sepsis (Schrag *et al*, 2012; Chiabi *et al*, 2014). A South African cohort study found that preterm birth and low birth weight that have been described as well-established risk factors, were strongly associated with increased risk of sepsis and perinatal death, and that Meconium Stained Amniotic Fluid (MSAF) and primiparity were each associated with approximately twice the risk (Schrag *et al*, 2012). Other factors such as induced maternal hypertension and intrapartum antibiotic prophylaxis were not associated with

reduced risk of neonatal sepsis (Schrag *et al*, 2012). However, in this study not all cases of sepsis were culture confirmed and so their definition of sepsis could also capture non-infectious syndromes such as meconium aspiration syndrome. Apart from these limitations, the study did not mention of any associations of Human Immunodeficiency Virus (HIV) (either maternal or neonatal) with sepsis.

In the World Health Organisation (WHO) Africa region the importance of neonatal infections and their contribution to under 5-years mortality is of concern (Liu *et al*, 2012). Apart from the impact of sepsis to morbidity and mortality in neonates, and associated health system costs, there are also long-term developmental effects such as growth retardation and delay in the development of neuro-cognitive functions (Mwaniki *et al*, 2012). Improved understanding of the underlying causes and risk factors for neonatal sepsis is required to better inform on patient management and prevention guidelines.

1.4.2 Methods for Sepsis Diagnosis

Blood culture has for long been the gold standard for the diagnosis of sepsis, pathogen identification and DST. However, its diagnostic value has been limited by several factors including: small volumes of blood sampled from paediatric patients with consequent false-negative results as well as the use of antibiotics prior to testing will reduce chances of pathogen isolation. Ultimately, blood culture has a long time-to-result that fails to impact on treatment and save life (Calitri *et al*, 2014). This involves the culture of the microorganism in enrichment broth, isolation of the pathogen on agar, identification through biochemical properties, and susceptibility testing. Blood cultures can only identify viable microorganisms and is known to detect as low as 10 microorganisms per millilitre of peripheral blood. However, the bacteraemia is dependent on the course of the disease

and hence blood culture will not yield positive results all the time. Because blood cultures have been in use for more than 100 years, they have been embedded well in clinical practice to guide therapy. Semi-automated culture systems have enormously impacted on the process flow of diagnosis of sepsis thereby improving pathogen isolation, a pre-requisite to phenotypic DST that should influence commencement of appropriate therapy (Skvarc *et al*, 2014). These automated systems such as the BACTEC FX200 system, detect microbial growth by analysis of CO_2 release using fluorescent or calorimetric sensors (Liesenfeld *et al*, 2014). Among the major limitations, are that, culture cannot detect pathogens that cannot grow or poorly grows in blood cultures such as *Legionella* spp., *Bartonella* spp., and *Aspergillus* spp, while others like *Mycobacterium tuberculosis* requires specialized media for their identification and isolation. Further, antimicrobials may inhibit growth of relevant pathogens and the diagnosis requires a long time for the results to be available which is too late to guide therapy. (Skvarc *et al*, 2013)

Molecular methods have been growing in general acceptance for use in sepsis diagnosis and have tremendously reduced time-to-result hence having a potential to impact on patient care while detecting both viable and non-viable bacteria (Grif *et al*, 2012). Some assays such as SepsiTest (Molzym) and Septifast™ (LCSF) test (Roche Molecular Systems, Switzerland) have been developed and commercialized. These can detect pathogens directly from whole blood, however, they can detect a limited number of pathogens and resistance markers, and in cases where blood has been stored at 4^oC yields false negatives. The sensitivity overall range from 53-71% and specificity of 50-63% (Fernández-Cruz *et al*, 2013; Skvarc *et al*, 2013) which is generally on a low side. Other molecular tests are available that have combined polymerase chain reaction (PCR) targeting conserved regions of bacterial and fungal genomes but their value for money

have not been evaluated (Grif *et al*, 2012; Orszag *et al*, 2014). Other techniques such as Next generation sequencing still require a culture step before molecular analysis and require specialized bioinformatics software and expert Bioinformaticians to analyse the huge sets of data generated. These limitations have therefore, made it difficult to integrate these methods in clinical practice (Wyres *et al*, 2014)

1.4.3 Antimicrobial Resistance (AMR)

WHO recently declared AMR a global health security threat due to very high AMR rates globally, and highlighted the large gaps in surveillance (WHO, 2014). With respect to neonates, available data demonstrate the widespread resistance to first (Gentamicin and Ampicillin) and second line (third generation cephalosporins) therapy, for both community-acquired (Thaver *et al*, 2009; Aiken *et al*, 2011; Downie *et al*, 2013) and hospital-acquired (Zaidi *et al*, 2005; Bates *et al*, 2014) neonatal sepsis. The primary cause of resistance is plasmid-driven spread of extended spectrum beta-lactamase (ESBLs), which are common in Africa and confer resistance to all first and second-line antibiotics (Storberg, 2014). A study at UTH investigating the molecular characteristics of the *Klebsiella pneumoniae* isolated in the Hospital laboratory showed a majority of them to be ESBL producers (Mumbula *et al*, 2015). There is also a growing concern of resistance to carbapenems in Africa (Manenzhe *et al*, 2014). Many of these problems faced in the sepsis infection control and AMR may be speculated to be as a result misuse of antibiotics and lack of or inadequacies in infection control practices directed at basic cleaning and disinfection (Bates *et al*, 2014).

1.5 Research Questions

1.5.1 What are the major pathogens causing septicaemia in neonates admitted in NICU at UTH?

1.5.2 What are the Antimicrobial resistance patterns of the organisms implicated in causing sepsis in NICU?

1.5.3 What factors are linked to an increased risk of sepsis in these neonates?

1.6 Objectives

1.6.1 General Objective

To Determine the Aetiology, Antibiotic resistance and risk factors for neonatal sepsis at UTH in Lusaka, Zambia.

1.6.2 Specific Objectives

1.6.2.1 To identify the bacteriological aetiologies of neonatal sepsis in NICU at UTH in Lusaka, Zambia.

1.6.2.2 To determine the AMR patterns of the pathogens causing sepsis in neonates admitted in the NICU at UTH in Lusaka, Zambia.

1.6.2.3 To identify demographical and clinical characteristics (Risk factors) associated with an increased risk of neonatal sepsis in NICU at UTH in Lusaka, Zambia.

Chapter 2: Methodology

2.1 Study Design

This was a prospective observational study whose purpose was to evaluate the aetiology of septicaemia in neonatal admissions in NICU at UTH, as well as to assess the scale of antibiotic resistance and identify the factors associated with an increased risk of sepsis.

2.2 Study site

The study was undertaken at UTH, Lusaka, Zambia. This is a national referral centre with an average of 281 paediatric admissions per week with a bed capacity of more than 2000. Children are referred to UTH mainly from the local clinics in and around Lusaka, but some cases also from other towns across the country. HIV prevalence is 13.1%. Septicaemia is seen or suspected clinically on admission in 7.4% of admissions (20.9 cases of sepsis per week) with Mortality rates are as high as 30% (UNZA-UCLMS, 2014. unpublished data).

2.3 Study Population

The study population included neonates suspected of having sepsis and admitted in NICU at UTH in Lusaka, Zambia. As part of monitoring efforts, The University of Zambia-University College London Medical School Research and Training Programme (UNZA-UCLMS) have been maintaining an admissions register in which vital clinical information including sex, birth weight, maternal HIV status, and outcome have been collected since June 2013 on NICU. Key descriptives of the recruited neonates were compared with this register, to evaluate recruitment bias. The hospital employs WHO-recommendation Option B+ for prevention of Mother-To-Child Transmission (MTCT), so all neonates born to HIV-infected mothers are on daily nevirapine until 6 weeks of age and their mothers are on Anti-retroviral Therapy (ART) for life (WHO, 2014). The term ‘HIV-exposed’ has been used to refer to all

neonates born to HIV-infected mothers. HIV Reverse Transcriptase PCR which can detect HIV viral particles in HIV infected neonates is not routinely undertaken at birth at UTH, hence, it is difficult to distinguish neonates that are HEU from those infected.

2.3.1 Inclusion Criteria

Inclusion criteria for neonates was 'suspected sepsis', as defined by elevated temperature (More than 37.5ºC) OR leukocyte count of more than4-10x10^3 cells/mm^3 OR Tachypnea defined by a respiratory rate of more than30 breaths/minute OR heart rate of more than 80 beats/minute and indicated for blood culture by the attending clinician. This definition was based on the local UTH, NICU protocol which captured the maximal number of cases.

All neonates had a thorough clinical evaluation and had blood taken for microbiological analysis.

2.3.2 Exclusion Criteria

Neonates who met the inclusion criteria but whose parents or guardians did not consent were not included in the study just like those who did not have any parent/guardian or were an unavailable at the time of recruitment.

2.3.3 Sample size

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

Where n = sample size

Z = Z statistic for level of confidence

P = expected prevalence or proportion, and

d = precision

Considering the lack of baseline data in Africa and Zambia particularly,a pilot study in NICU was carried out before initiation of this study that estimated prevalence of neonatal sepsis to be approximately 20% giving a minimum sample size of 246 participants. In this study, a total of 342 neonates had their parents/guardians approached during the study period and 321 consented. Clinical data and other recruitment data could not be collected before discharge/death for 8 neonates, resulting in the final sample of 313 participants.

2.4 Septicaemia Aetiology, Detection and Identification

2.4.1 Blood collection, Culture and Processing

A volume of 1.5mL of blood was drawn from each participating neonate as indicated by the attending physician for blood culture analysis. Whole blood was inoculated into the Peads-plus culture bottles and processed using the BACTEC FX200 system, (Becton Dickinson, NJ, USA). A blood culture that flagged either positive or negative in the BACTEC FX200 system was removed and time-to-positivity recorded as time from placing the culture bottle into the BACTEC FX200 system up to the time it flagged. From the positive blood culture, gram stain was performed using a disposable 10 microliter (µL) loop. Another disposable 10µL loop was used to sub-culture from the positive blood culture on to 15mL of solid agar media on a petri dish. This media included Blood agar, Chocolate agar and MacConkey agar and was incubated at 37^{o}C for 18-24 hours. Cultures with mixed growths i.e. more than one type of bacteria as observed from the gram stain and/or solid media growth colonies were regarded as a potentially contaminated culture and attempts to collect another blood specimen from the patient were made.

2.4.2 Bacterial Isolation and Identification

In order to identify the bacterial species causing sepsis in a positive blood culture, pure colonies from the solid media were analysed through biochemical property testing. The biochemical testing was done using conventional (traditional) tests that included Tripple Sugar iron (TSI), Lysine Iron Agar (LIA), and Sulphur Indole Motility (SIM). Others included production of citrate and/or catalase i.e. reduction of hydrogen peroxidase as well the coagulase test using plasma (see Appendix A). These tests were done depending on the gram stain and the Clinical Laboratory Standards Institute (CLSI) algorithm of the organism isolated.

2.5 Bacterial Antimicrobial Susceptibility Testing

The antibiotic sensitivity testing was done by Bauer-Kirby disc diffusion method using Oxoid discs (Thermo Fisher, MA, U.S.A) and Mueller Hinton agar, in accordance with CLSI guidelines. The antibiotics included, penicillins, aminoglycosides, quinolones, chloramphenicol, macrolides, sulphonamide, third generation cephalosporins, carbapenems and glycopeptides. Beta-lactamase production on all *K. pneumoniae* and *E. coli* isolates was done using the Epsilometer (E-test) (AB Biodisk, Solna, Sweden) together with disc diffusion testing using Clavulanic acid and a cephalosporin.

2.6 Identification of Risk factors associated with Sepsis

Risk factors linked to sepsis were identified by univariate analysis and multivariate analysis by using binary logistic regression of the clinical data collected together with positivity of a blood culture. Demographics data was collected in a structured questionnaire addressed to the mother/guardian through an interview while clinical presentations were recorded from the patient file as recorded by the attending clinician.

2.7 Data Analysis

For comparisons of descriptive data between the study group and the broader admitted neonatal population, and by maternal HIV status, categorical and continuous descriptive variables were compared by chi square and Mann Whitney U tests respectively. Binary logistic regression was used to perform univariate and multivariate analysis of how a range of variables might affect the odds of culture confirmed neonatal sepsis. Data analysis was done in SPSS version 21.

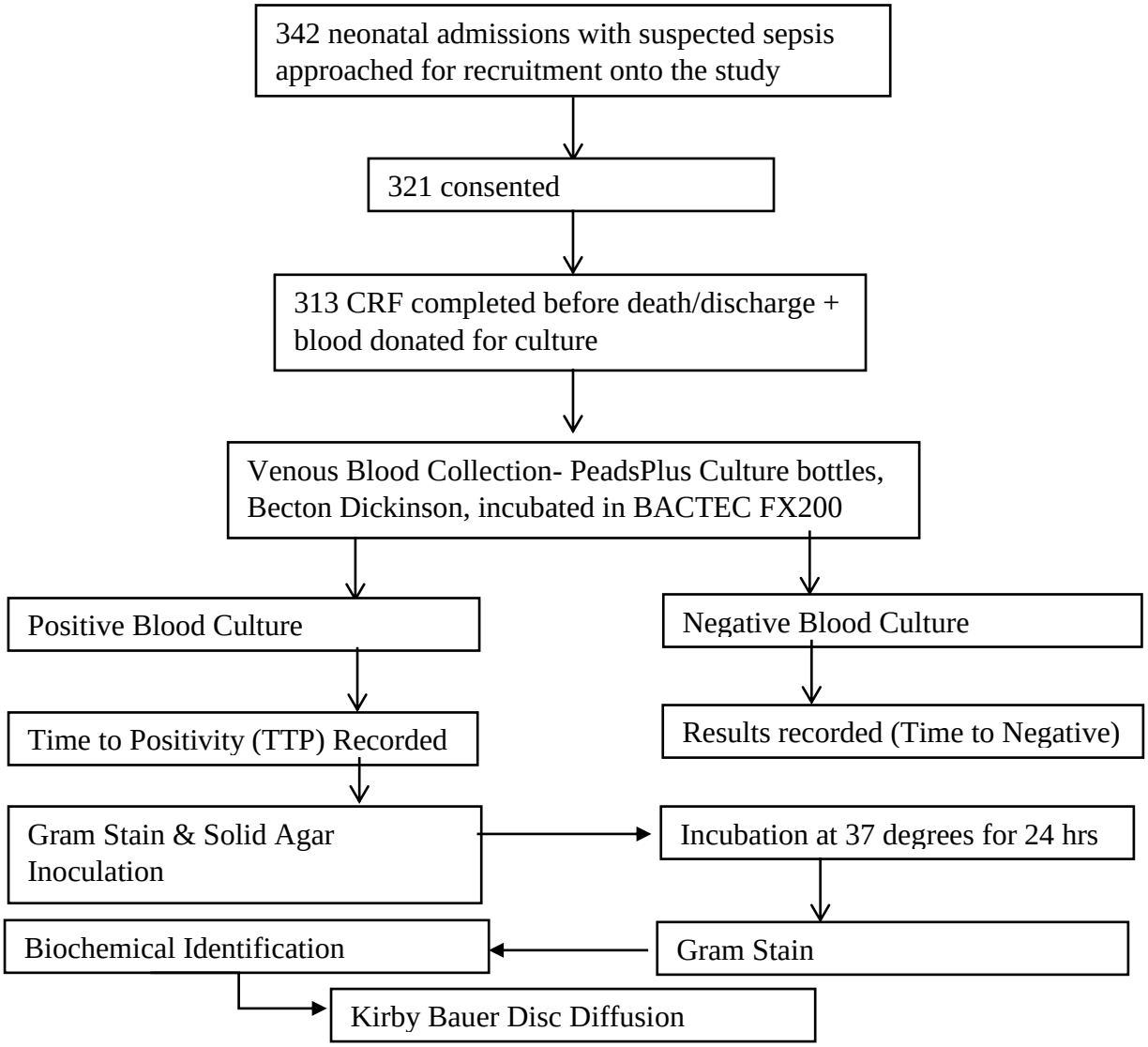
2.8 Ethical Considerations

Ethical Clearance was sort from the University of Zambia Biomedical Research Ethic committee (UNZABREC) of the School of Medicine (Appendix D). The study posed no ethical issues to the participants as no extra samples were collected from them apart from those indicated by the attending physician as part of routine standard of care. To ensure patient confidentiality, study identification numbers beginning with ‘SP’ were used in the study.

Chapter 3: Results

3.1 Patient Recruitment and Descriptives

Mothers/guardians of 342 admitted neonates with suspected neonatal sepsis were approached from October 2013 to May 2014. Of 342 mothers/guardians who were approached, 321 consented to take part in the study. However, recruitment data for 8 participants could not be collected before their discharge/death and were not included in the analysis. This resulted in a final sample size of 313 participants (Figure 1 below). Figure 3.1 below illustrates the flow of patient recruitment and data collection.



CRF= Clinical Recruitment Form

Figure 3.1: Recruitment and sample processing flow diagram

Sex was fairly representative with 170 (54.3%) of the neonates being male. A total of 246 (77%) neonates were admitted on the day they were born. The median age for neonates who participated in this study was 2 days old (IQR 1-5 days) and 62 (20%) neonates were born to HIV-infected mothers. Compared with a total neonatal inpatient population during the study period, sex, maternal HIV exposure and mortality were broadly representative. Recruited neonates had a higher mean birth weight as shown in table 3.1.1 below:

Table 3.1.1: Comparison of key descriptive variables between the study group and admitted neonatal population from NICU at UTH

Variable	**Study (n= 313)**	**Population (n = 2471)**	**P-Value**
Male sex (%)	170 (54.3%)	1248 (50.3%)	0.209
Mean weight in kg (se)	2.5 (0.048)	2.2 (0.02)	< 0.001
Premature (%)	138 (44.1%)	1336 (54.2%)	< 0.001
Maternal HIV infection (%)	60 (19.7%)	413 (17.3%)	0.303
Mortality (%)	134 (43.2%)	1067 (43.2%)	1.000

IQR = Interquartile Range
se = standard error (+/-)

The results of the study recruitment showed that within the study population, neonates born to HIV infected mothers were significantly older (p<0.029) than those born from HIV uninfected mothers. The mean age of HIV exposed neonates was 3 days with an Interquartile range of 2-5 days compared to a mean age of 2 (IQR 1-5 days) in HIV unexposed neonates. However, HIV exposed neonates had a lower mean birth weight of 2.1kg (IQR1.6-2.8) compared to 2.7kg (IQR1.8-3.2), p=0.003 in HIV unexposed neonates as shown in table 2 below. With respect to their clinical presentation, the table 3.1.2 below also shows that HIV-

exposed neonates were significantly more likely to present with cyanosis, distension, hepatomegaly or splenomegaly.

Table 3.1.2: Demographic and clinical characteristics of neonates admitted to NICU at UTH stratified by maternal HIV status

Variable	All neonates	Neonates born to HIV uninfected mothers	Neonates born to HIV infected mothers	p-value
Male Sex	54.3% (170/313)	54.3% (133/245)	58.3% (35/60)	0.572
Median Age in Days (IQR)	2 (1-5)	2 (1-5)	3 (2-5)	0.029
Aged <= 3 days	62% (194/313)	83% (161/194	17% (33/194)	0.276
Aged 4-7 days	27% (84/313)	76% (64/84)	24% (20/84)	
Aged >7 days	11% (35/313)	74% (26/35)	26% (9/35)	
Mean Weight in Kg (SE)	2.6 (2.2)	2.6 (0.05)	2.2 (0.1)	0.003
Hospital Birth	78.6% (246/313)	78% (191/245)	80% (48/60)	0.731
Delivery type				
Spontaneous Vaginal Delivery	78.6% (246/313)	76.7% (188/245)	85.0% (51/60)	0.205
Caesarean Section	18.2% (57/313)	19.6% (48/245)	15.0% (9/60)	
Instrument	3.2% (10/313)	3.7% (9/245)	0.0% (0/60)	
Clinical presentation*				
Fever	62.3% (195/313)	62% (152/245)	61.7% (37/60)	0.957
Cyanosis	13.1% (41/312)	11.5% (28/245)	21.7% (13/60)	0.038
Distension	10.2% (32/313)	8.6% (21/245)	18.3% (11/60)	0.027
Hepatomegally	5.4% (17/313)	4.1% (10/245)	11.7% (7/60)	0.022
Splenomegally	3.8% (12/313)	2% (5/245)	11.7% (7/60)	0.001
Mortality	43.2% (134/310)	43% (104/242)	41.7% (25/60)	0.854

* The prevalence of the following did not differ by maternal HIV status: Hypothermia 8.0% (25/313); Convulsions 30.4% (95/313); Poor feeding 47.3% (148/313); Vomiting 1.9% (6/313); Difficulty breathing 62.3% (195/313); Tachypnoea 71.9% (225/313); Chest recession 31.0% (97/313); Nasal Flaring 31.0% (97/313); Pallor 11.2% (35/313); Jaundice 11.5% (36/313); irritability 42.5% (133/313); Lethargy 26.2% (82/313); Bulging fontanel 7.3% (23/313); Umbilical discharge 2.6% (8/313); Eye infection 4.5% (14/313). Also vital signs: Temperature (^{o}C) 38.0 (36.4-38.5); Respiratory Rate (breaths/min) 52 (48-60); Pulse (pulses/min) 150 (140-160) and haematology: WCC (cells/ml) 15.4 (12.0-19.6); Hb (g/litre) 14.5 (13.4-16.1); Platelet (1000 plates/ml) 152 (110-210) and RBS (g/litre) 4.3 (3.2-6.1)

The median age of HIV infected mothers was 3 years older than HIV un-infected mothers (27 years (IQR23-30) vs 24 years (IQR19-29.5), p=0.008 and correspondingly, median parity

was also significantly higher for HIV-infected mothers. Maternal education did not show any associations as shown in table 3.1.3 below:

Table 3.1.3: Demographic characteristics of mothers to Neonates admitted to NICU at UTH, stratified by HIV status

Variables	All Mothers	HIV infected	HIV uninfected	p-value
Median Age in years (IQR)	25 (20-29.5)	24 (19-29.5)	27 (23-30)	0.008
Mother's Education*				
None	6.2% (17/273)	6% (12/211)	7% (4/54)	0.750
Primary	32.2% (88/273)	32% (67/211)	37% (20/54)	
Secondary	54.6% (149/273)	56% (118/211)	48% (26/54)	
Tertiary	7% (19/273)	7% (14/211)	7% (4/54)	
Married	83.3% (254/305)	82% (196/239)	88% (52/59)	0.259
Parity	2 (1-3)	2 (1-3)	3 (1.3-4)	0.012
Complications**	44.7% (140/313)	47.3% (116/245)	38.3% (23/60)	0.209

*Maternal education was collected for 267 cases

** The prevalence of the following complications in pregnancy did not differ by maternal HIV status: Abdominal pain 29.4% (92/313); Pain when passing urine 8.9% (28/313); Pain when having sexual intercourse 2.2% (7/313); PVspot 12.8% (40/313); Vaginal discharge 3.5% (11/313); Rash 1% (3/313); Fever 4.8% (15/313).

3.2 Prevalence and Aetiology of Neonatal Sepsis

Microorganisms were isolated from blood of 36.1% of neonates of which 10 were probable contaminants (6 Diptheroids, 3 mixed growth and 1 Clostridium). The prevalence of culture confirmed sepsis was hence 32.9% of all neonates in table 3.2 below. *Klebsiella* species, predominantly *K. pneumoniae,* were highly prevalent accounting for 74.8% of all cases. Among the remaining 26 cases, *S. aureus* was isolated in 5.8% cases, coagulase-negative *Staphylococci* in 6.8 % cases, *E. coli* in 4.9% cases and *Candida* spp. in 4.9% cases were most prevalent as shown in table 4. Culture confirmed sepsis was significantly more prevalent in neonates age 4-7 days at 53.6%, $p<0.001$ or those aged more than 7 days at 42.9%, $p=0.006$ compared to those who were 3 days or less who had culture confirmed sepsis

in 22.2% as shown in the table 3.2 below. Hence EOS was prevalent in 85.4% cases. Of the 200 culture negative participants, 68.5% were discharged and 31.5% died. Among the 10 from which the probable contaminants were isolated, 70% were discharged and 30% died while among the culture confirmed cases LOS accounted for 33.3% deaths and 66.7% discharges and EOS among neonates aged <=3 days, had 30.2% of cases discharged and 69.8% cases died while those aged 4-7 days recorded 26.7% discharges and 73.3% deaths.

Table 3.2: Predominant Pathogens Isolated from venous blood of neonates admitted to NICU at UTH

		Prevalence within different age groups			**Mortality**
	Overall Prevalence	Aged <= 3 days (194)	Aged 4-7 days (84)	Aged <7 days (35)	
Culture confirmed Sepsis	33% (103/313)	22% (43/194) [b,c]	54% (45/84) [b]	43% (15/35) [c]	
Established pathogens					
Klebsiella species[a]	25% (77/313)	13% (25/194)[d,e]	49% (41/84)[d]	31% (11/35)[e]	69% (53/77)
S. aureus	2% (6/313)	2% (4/194)	1% (1/84)	3% (1/35)	50% (3/6)
E. coli	2% (5/313)	2% (3/194)	2% (2/84)	0% (0/35)	100% (5/5)
Probable pathogens					
Coagulase-negative *Staphylococci*	2% (7/313)	3% (6/194)	0% (0/84)	3% (1/35)	29% (2/7)
Candida species	2% (6/313)	2% (4/194)	1% (1/84)	0% (0/35)	60% (3/5)
Gram negative Diplococcus	<1% (1/313)	0% (0/194)	0% (0/84)	3% (1/35)	0% (0/1)
Bacillus species	<1% (1/313)	0% (0/194)	0% (0/84)	3% (1/35)	100% (1/1)
Acinetobacter	<1% (1/313)	0.5% (1/194)	0% (0/84)	0% (0/35)	100% (1/1)
Probable Contaminants*	3% (10/313)				30% (3/10)
Mortality Among cases	66% (68/103)	70% (30/43)	73% (33/45)	33% (5/15)	

[a]*Klebsiella pneumoniae* (76), *Klebsiella oxytoca* (1)
[b,d] Pearson chi square p < 0.001
[c] Pearson chi square p = 0.006
[e] Pearson chi square p = 0.009
* Gram stain showing more than one organism

There was a rapid increase in the prevalence of *Klebsiella* infections from December 2013 to March 2014 was linked with a similar increase in mortality, both within the study population and within the neonatal unit as a whole. Figure 3.2 below shows a graph of the increase in the

incidence of *Klebsiella* infections and mortality in the study population and other neonates admitted to NICU prior to and after the study period.

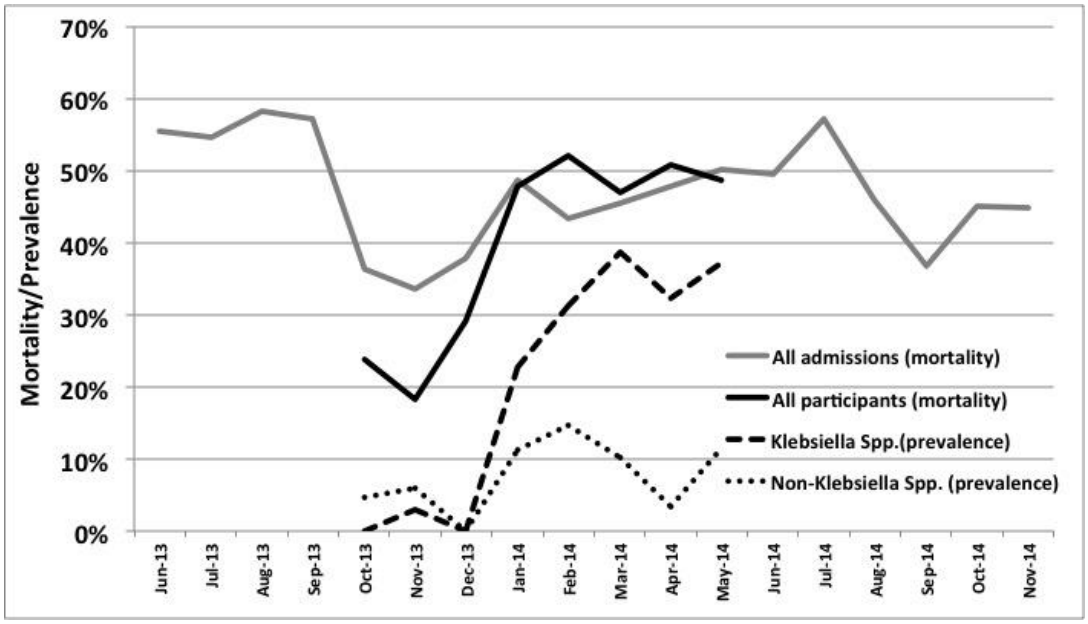


Figure 3.2: Prevalence of *Klebsiella* spp. and non-*Klebsiella* spp. among neonates in NICU at UTH, by month

3.3 *Klebsiella pneumoniae* Outbreak

During the study period an apparent outbreak of *Klebsiella* infections among neonates admitted with suspected sepsis, with incidence increasing from 0 cases per 100 admissions in December 2013, to 39 cases per 100 admissions in March 2014 was documented. During this same period, the mortality rate among study participants increased from 29% to 47%, with an increase in mortality also documented within all NICU admissions during the same period as shown in the Figure 3.2 above. The figure 3.3 below shows that EOS in neonates aged 3 days or less appear to contract sepsis the day they were born while those aged 4-7 days appears to have contracted sepsis after admission to the ward. The figure 3.3 below also shows that 14 (93.3%) LOS cases were admitted for more than 2 days.

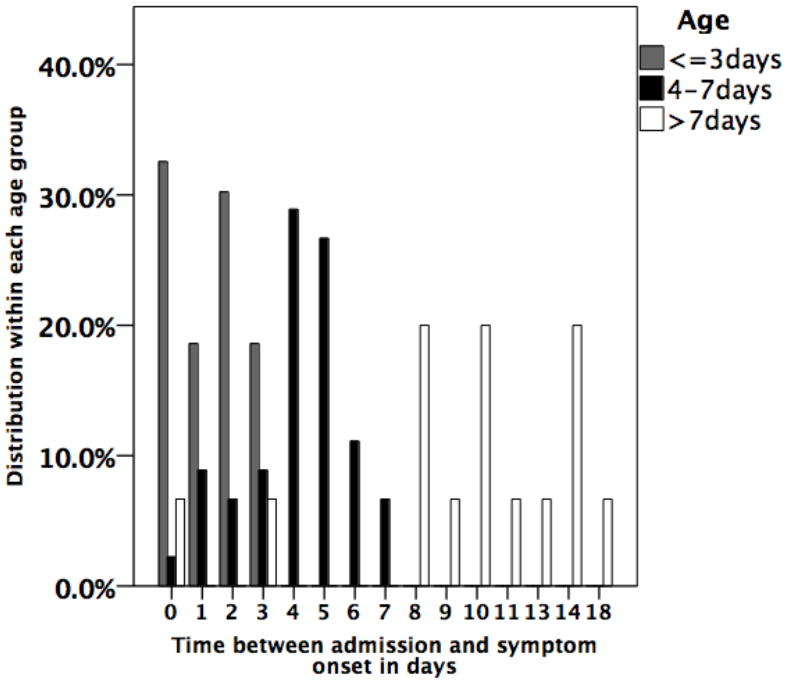


Figure 3.3: Days from admission to onset of symptoms for culture confirmed EOS and LOS cases in NICU at UTH

3.4 Impact of Culture Diagnosis on Mortality

The mortality rate among all neonates was 43% (134/311) as shown in table 3.1.1, while amongst those with culture confirmed neonatal sepsis, the mortality rate was 66% (68/103). Further, neonates with *Klebsiella* infections the mortality rate was 69% (53/77) as shown in table 3.2 above. In accordance with routine practice at the hospital, both species and DST data are reported together. Complete records of outcome, duration of admission and reporting time for culture result for 85/103 (82.5%) of culture positive cases were successfully collected. The median reporting time was 7 days (IQR 5-9 days), but the median times to discharged or death were 6 (IQR3-8) and 3 (IQR1-6) days respectively as shown in table 3.4 below. A total of 62 (73%) cases did not receive a culture result prior to death or discharge.

Table 3.4: Time-to-result of blood cultures and neonatal outcome in NICU at UTH.

	Median time (days)	Interquartile range
Admission to Discharge	6	3-8
Admission to Death	3	1-6
Time to Report (culture)	7	5-9

3.5 Anti-Microbial Susceptibility

Phenotypic DST was undertaken using a panel of antibiotics that are used locally and in line with CLSI guidelines, demonstrating near universal resistance (range 92%-100% resistant) to Penicillin/Ampicillin, Gentamicin, Cotrimoxazole, and Cephalosporins for gram-negative rods (*Klebsiella* spp. and *E. coli*) as shown in table 3.5.1 below. There were lower rates antibiotic resistance observed for *S. aureus*. Only one *K. pneumoniae* isolate that was also resistant to third line therapy (Imipenem).

Table 3.5.1: Rates of AMR of the most prevalent pathogens isolated in NICU at UTH

Antibiotic class	**Anti-Microbial**	***Klebsiella* species[a]**	***Staphylococcus aureus***	***Escherichia coli***
Penicillins	Penicillin/Ampicillin	99% (68/69)	33% (1/3)	100% (5/5)
	Amoxcillin/Clavulinic acid	93% (50/54)	100% (4/4)	50% (1/2)
	Oxacillin	NT	0% (0/3)	NT
Aminoglycoside	Gentamicin	96% (70/73)	50% (2/4)	100% (5/5)
Sulfonamide	Cotrimoxazole	100% (54/54)	67% (2/3)	100% (5/5)
Macrolide	Erythromycin	N/A	33% (1/3)	N/A
Chloramphenicol	Chloramphenicol	71% (52/73)	20% (1/5)	60% (3/5)
Quinalone	Ciprofloxacin	71% (51/72)[b]	0% (0/5)	80% (4/5)
Cephalosporins	Ceftriaxone	94% (48/51)	33% (1/3)	100% (5/5)
	Cefotaxime	96% (71/74)	0% (0/5)	100% (5/5)
	Ceftazidime	97% (64/66)	50% (1/2)	100% (5/5)
Carbapenem	Imipenem	1% (1/73)	0% (0/3)	0% (0/5)

[a] *Klebsiella pneumoniae* (76), *Klebsiella oxytoca* (1)

[b] For Ciprofloxacin testing there were 10 intermediate results

NT = not tested

N/A=Not applicable

The patterns of resistance shown in the table 3.5.2 below illustrates an array of resistance profiles from the organisms causing sepsis in neonates as isolated in this study. The majority were Enterobacteriaceae, among which *Klebsiella pneumonia* was the most predominant species isolated at 74.8%. Ampicillin susceptibility testing on *Klebsiella pneumoniae* (although intrinsically resistant to ampicillin unlike other gram negatives) was otherwise carried out for confirmatory purposes and all *K. pneumoniae* isolates were resistant as expected. Multi-resistance in *K. pneumoniae* was observed, with one isolate (1/77) showing resistance to seven (7) antimicrobials including Ampicillin. These included ampicillin, gentamicin, ciprofloxacin, cotrimoxazole, chloramphenicol, cefotaxime and imipenem. A majority of *K. pneumoniae* isolates (51/77) were resistant to six (6) antimicrobials which included all of the above without imipenem. There were two sets of *K. pneumonia* isolates

that showed resistant to five (5) antimicrobials. Whilst one set was resistant to chloramphenicol (6/77) the other was resistant to ciprofloxacin (16/77). However they both showed resistance to other four antibiotics (ampicillin, gentamicin, cotrimoxazole and cefotaxine). Only (2/77) of the *Klebsiella* isolates showed pan-susceptibility to all antimicrobials tested.

The table 3.5.2 below also shows that all five (5) isolates of *E. coli* were resistant to six (6) antimicrobials. Susceptibility was universally observed to Imipenem.

Multi AMR was also observed in *S. aureus* isolates with one (1/6) isolate showing resistance to as many as seven (7) antimicrobials that included a penicillin, oxacillin, chloramphenicol, erythromicin, cotrimoxazole, cefotaxime and imipenem. While 3/6 *S. aureus* isolates showed resistant to three antimicrobials that included a penicillin, gentamicin and erythromicin and two isolates were susceptible to all the antibiotics tested for *S. aureus*.

Table 3.5.2: Patterns of AMR of the predominant Pathogens Isolated from NICU at UTH

Isolate	**Pattern of resistance**	**Percentage**
Klebsiella Pneumoniae	AMP, GEN, CIP, CHL, COT, CTX, IMP	1/77 (1.3%)
	AMP, GEN, CIP, CHL, COT, CTX	51/77 (66.2%)
	AMP, GEN, CIP, COT, CTX	16/77 (20.7%)
	AMP, GEN, CHL, COT, CTX	6/77 (7.8%)
E. Coli	AMP, GEN, CIP, CHL, COT, CTX	5/5 (100%)
Staphylococcus aureus	PEN, OXA, CIP, ERY. COT, CTX, IMP	1/6 (16.7%)
	PEN, GEN, ERY	3/6 (50%)

Ampicillin (AMP); Penicillin (PEN); Gentamicin (GEN); Ciprofloxacin (CIP); Chloramphenicol (CHL); Erythromicin (ERY); Cotrimoxazole (COT); Cefotaxime (CTX); Imipenem (IMP); Oxacillin (OXA).

3.6 Risk Factors associated with neonatal sepsis

Increasing neonatal age (OR=1.06), p = 0.021, and irritability (OR=1.66), p = 0.038, were neonatal characteristics that were associated with an increased risk of neonatal sepsis while increased parity (OR= 1.18), p = 0.032 was a maternal characteristic that was a risk factor for neonatal sepsis. The odds of neonatal sepsis were significantly reduced in children born to HIV-infected mothers (OR 0.46), p = 0.029, independent of age, parity, hepatomegaly, irritability and poor feeding, which were flagged by univariate analysis as shown in table 8 below. Nasal flaring and pallor were independently associated with reduced odds of neonatal sepsis. Analysing HIV-exposed and HIV-unexposed children separately did not reveal any additional risk factors. The odds of neonatal sepsis did not differ significantly between neonates born at the referral centre and those born in community clinics. There was also no significant odds in sepsis dependent on birth weight, prematurity, and delivery type as shown in the table 3.6.

Table 3.6: Risk factors for neonatal sepsis in NICU at UTH

	Odds of positive blood culture			
	Univariate		**Multivariate**	
	OR (95%CI)	**P-value**	**OR (95%CI)[a]**	**P-value**
Infants				
Male sex	1.00 (0.63-1.61)	0.989	0.97 (0.59-1.59)	0.965
Infant age (days)	1.06 (1.01-1.11)	0.031	1.07 (1.01-1.13)	0.021
Birth weight	0.83 (0.63-1.10)	0.201	0.87 (0.64-1.18)	0.360
Prematurity	1.23 (0.77-1.98)	0.385	1.15 (0.69-1.91)	0.591
Born in referral centre	0.61 (0.35-1.06)	0.082	0.69 (0.38-1.24)	0.214
Delivery type[b]				
Caesarean Section	0.56 (0.29-1.10)	0.091	0.49 (0.23-1.01)	0.054
Instrument	1.89 (0.53-6.73)	0.323	2.01 (0.49-8.29)	0.332
Clinical presentation[c]				
Poor Feeding	0.60 (0.37-0.97)	0.036	0.68 (0.41-1.13)	0.132
Nasal Flaring	0.65 (0.38-1.10)	0.106	0.54 (0.31-0.96)	0.034
Pallor	0.47 (0.2-1.13)	0.090	0.36 (0.14-0.94)	0.037
Irritability	1.66 (1.03-2.67)	0.038	1.36 (0.82-2.26)	0.228
Hepatomegally	0.12 (0.02-0.91)	0.040	0.17 (0.02-1.39)	0.098
Mothers[d]				
Parity	1.18 (1.02-1.37)	0.023	1.18 (1.01-1.37)	0.032
Maternal HIV infection[e]				
HIV infected	0.49 (0.25-0.96)	0.038	0.46 (0.23-0.93)	0.029
HIV status unknown	0.60 (0.12-3.01)	0.530	0.20 (0.02-1.97)	0.158

a Multivariate analysis controlling for age, poor feeding, irritability, hepatomegally, parity and maternal HIV status indicated by univariate analysis

b compared to spontaneous vaginal delivery (SVD) as reference category

c No significant associations were observed for: auxiliary temperature, fever, hypothermia, vomiting, difficulty breathing, tachypnoea, chest recessions, jaundice, cyanosis, lethargy, distension, umbilical discharge, eye infection, bulging fontanel, convulsions and splenomegaly.

d No significant associations were observed for: age, fever, Abdominal pain, urination pain, vaginal discharge, vaginal bleeding, or pain during sex.

e compared to HIV uninfected as reference category

Chapter 4: Discussion

4.1 Discussion

The objectives of this study were to determine the prevalence, aetiology, AMR and risk factors for neonatal sepsis in NICU at UTH. The study population was fairly representative of the whole neonatal population in NICU as the minimum sample size required was exceeded. Furthermore, within the study population, sex was representative and the median age of the neonates that participated in the study was 2 days old (IQR 1-5 days). However, these neonates had a higher mean birth weight and were less likely to be premature in comparison to the entire neonatal population in NICU. This observation could be explained by the idea that mothers to premature babies were less likely to give consent to enable their new born child to take part in a study or may still be recovering and may be too weak to consent as previously reported (Korotchikova *et al*, 2010).

The overall prevalence of culture positive sepsis was 33% (103/313), a probable under-estimate as serial blood cultures were not undertaken on each neonate recruited in this study. However, this overall prevalence is 50% higher than the pooled prevalence of 22% from 14 previous studies conducted in Africa (Musoke and Revathi 2000; Mokuola *et al*, 2002; Ayoola *et al*, 2003; Simuyu 2005; Ojukwu *et al*, 2006; Shitaye *et al*, 2010; Ogunlesi *et al*, 2011; Kohli-Kochlar *et al*, 2011; Aquah *et al*, 2013; Kiwanuka *et al*, 2013; Hamer *et al*, 2014; Mkony *et al*, 2014; Morkel *et al*, 2014; Tann *et al*, 2014). *K. pneumoniae* accounted for 75% (77/103) of all positive cultures, and correlated with an increase in mortality, both within the study population and within all neonatal admissions during the study period. This suggested that similar factors impacting mortality in our study group may have been the same as in the whole neonatal population in NICU.

During December 2013 to March 2014, there was a dramatic increase in infections that correlated with mortality. The identity of the implicated pathogen (*K. pneumoniae*) from blood cultures and surface swabs on the labour ward, caesarean theatre and NICU (UTH, 2015. Unpublished data), and the median duration of admission being 4 days (IQR1-5 days) were indicative of a nosocomial outbreak of EOS. However, the incidence of pathogens other than *Klebsiella* spp. appeared not to correlate with mortality indicating that their contribution to mortalities in neonates may have been minimal, if any. Molecular typing to establish the relationship between isolates from blood cultures and the surface swabs was beyond the scope of this study and hence cannot confirm any relatedness between the organisms isolated from these sources. Persistently high mortality rates on the unit prior to and after the study period (Figure 3.2) suggest that such outbreaks may be the *status quo*, interspersed with short periods of lower prevalence, likely influenced by multiple factors such as enhanced infection control activities and staff rotations. Efforts to promote safe birthing practices and infrastructures may be inadvertently putting neonates at risk, if corresponding efforts are not also focussed on developing birthing centre capacity and ensuring rigorous infection control (Wall *et al*, 2010).

Near universal resistance to both the first and second-line therapy, which could have been driven by plasmid mediated ESBLs, was observed for *K. pneumoniae* and *E. coli* strains that were isolated. Although, this level of resistance is higher than that observed in earlier studies conducted in other parts of Africa (Zaidi *et al*, 2005; Thaver *et al*, 2009), it is consistent with with more recent studies (Mumbula *et al*, 2015; Lukac *et al*, 2015). These recent studies have also suggested that ESBLs are an 'ever-growing burden' in Zambia and other parts of Africa. Plasmid mediated resistance ESBLs may be transferred between Enterobacteriaceae in Hospitals such as was observed in *E. coli* and *K. pneumonia* in this study. Recommended

WHO first and second-line antibiotic therapy are impotent in the face of outbreaks of ESBL Enterobacteriaceae. Therefore, focus must be on improved infection control and early diagnosis of neonatal sepsis to reduce mortality. Although this study found only one *K. pneumonia* isolate to be resistant to Imipenem, there is a window of opportunity to reduce the burden of neonatal infections before levels of carbapenem resistance mirror what is being observed with ESBLs (Manenzhe *et al*, 2014). Hence, more attention should be rendered in preventing the development and spread of carbapenem resistance in *K. pnemoniae* strains as well as other Enterobacteriaceae such as *E. coli*. Although all Enterobacteriaceae isolates were resistant to third generation Cephalosporins, approximately 20% of the *Klebsiella* tested were sensitive to Chloramphenicol and 7.8% to Ciprofloxacin providing alternatives to prevent Imipenem gross overuse and prevent development of carbapenem resistance.

The finding that blood culture diagnosis had minimal impact on treatment and outcome, since only 25% of the neonates had received results for culture and drug sensitivity before discharge/death, underscores the need for simple and scalable infection control interventions as well as novel diagnostic tools. Whilst bacterial culture is a valuable research tool, it is expensive, slow, and here offered little tangible benefit for high mortality-risk neonates. A recently developed real-time PCR assay has been shown to increase the rate of detection of sepsis, leading to early treatment and reduction of mortality in neonates (Aittakorpi *et al*, 2012)..

In this study, the mortality rate amongst culture negative neonates was very high (31.7%). This was similar to mortality rates among culture positive cases in other previously documented ESBL- *Klebsiella* outbreaks (30-36%) (Velaphi *et al*, 2009; Iregbu and Heath 2013). Therefore, at this centre, whilst infection is clearly important, it is recommended that

efforts to control, diagnose and treat these infections in neonates need to be synergized with improved obstetric and nursing care of new-borns. This may contribute to the reduction of the observed high mortality rates.

There was a low burden of gram-positive infections in this study and the absence of GBS suggested that these infections are either less common or are out-competed by AMR *Klebsiella* infections. GBS are a common cause of neonatal sepsis and meningitis in high-income regions where they are an established priority for vaccine development (Le Doare and Heath 2013). The absence of GBS in this study, and low prevalence in other African studies (Mulholland *et al*, 1999; Ojukwu *et al*, 2006; Kohli-Kochlar *et al*, 2011; Mhada *et al*, 2012; Kiwanuka *et al*, 2013), raise questions over the possible impact of a GBS vaccine in the African setting (Madhi *et al*, 2013). GBS vaccine is given to mothers colonised with GBS before labour and has been shown to be effective in prevention of neonatal GBS infection. In this study, data on reception of GBS vaccine in mothers was not collected. The most important gram positive bacteria isolated in this study was *S. aureus* which showed relative susceptibility to WHO second line therapy but resistant to first line therapy. However, one isolate which showed phenotypic resistance to oxacillin indicated a possible Methicillin Resistant *S. aureus* (MRSA) infection and this warrants further molecular investigation and calls for Health practitioners at UTH to be vigilant and prevent further spread of MRSA.

There has been huge progress over the last decade in reducing MTCT of HIV, initially through single dose nevirapine, but increasingly through more extensive antiretroviral regimens (Govender and Coovadia, 2014). The dramatic reduction in the rate of MTCT has led to a growing number of infants who are HEU (Filteau, 2009). This group, despite remaining HIV-uninfected, are known to suffer from increased morbidity and mortality, and

from impaired growth and development (Makasa *et al*, 2007; Filteau 2009) and are at greater risk of sepsis and other infections (Huson *et al*, 2015). In this study, however, HIV-exposed neonates were at a decreased risk of neonatal sepsis. This sizeable effect (a halving of the odds) was counterintuitive. This finding was mirrored by a South African study that also found rates of neonatal sepsis to be marginally lower in HIV-exposed children (Cutland *et al*, 2012). The most obvious therapeutic difference between HIV-exposed and unexposed neonates is the use of Nevirapine prophylaxis in HIV-exposed neonates, and probable maternal Cotrimoxazole prophylaxis. A previous study looking at the incidence of gastrointestinal and respiratory infections over time, demonstrated reduced mortality in the Nevirapine arm, in both HIV-infected and HEU neonates (SWEN *et al*, 2008). *In vitro* testing of Nevirapine against a panel of commercial strains and clinical isolates did not identify any antimicrobial activity, although *Klebsiella* species were not included in that study (Jackson *et al*, 2009). Reverse transcriptase enzymes which may be targets for Nevirapine have been identified in *E. coli* (Lim and Maas 1989), and Azidothymidine has been shown to have antimicrobial activity to a range of gram-negative bacteria including *K. pneumoniae* (Elwell *et al*, 1987). Both these studies may partly explain why HIV exposed neonates were less likely to develop sepsis. Although, data on maternal antibiotic history was not collected, WHO and UTH local guidelines recommend Cotrimoxazole prophylaxis in all HIV-infected women throughout pregnancy and whilst breastfeeding (Church *et al*, 2015). As all but one of the bacterial pathogens tested in this study were resistant to Cotrimoxazole, it may be speculated that immunomodulatory effects of the drug might be offering some level of protection from neonatal sepsis.

Contrary to a study carried out in South Africa which found that preterm neonates were at an increased risk of EOS (Schrag *et al*, 2012), this study found that older neonates were at a

significantly greater risk of developing neonatal EOS as the odds of having a positive blood culture was 1.06, p=0.031. This difference may be because the neonatal EOS that was observed in this study, predominantly occurred after 72 hours, hence, was more likely to be nosocomial than maternally acquired. The other possible explanation may be the population bias observed in this study, where neonates who participated were less likely to be premature in comparison to the neonatal population in NICU at UTH. Although the increasing age was associated with increased odds of positive culture sepsis, HIV exposed neonates who were more likely to be older still maintained a reduced risk of development of culture positive sepsis. A similar phenomenon was observed with increasing parity having an increased risk of culture positive sepsis even though HIV infected mothers had a significantly higher parity than HIV uninfected mothers. This counterintuitive finding that HIV exposure had a negative correlation to the development of sepsis has been reported previously in a South African study (Cutland *et al*, 2012). Neonates who were also easily irritable were at an increased risk of sepsis which may have been a symptom rather than a risk factor of sepsis. Other clinical features such as poor feeding, hepatomegaly, nasal flaring, and pallor were significant negative predictors of sepsis. These could have been symptoms of other infections in the neonates and could explain the high mortality rate (31.5%) in culture negative cases.

4.2 Conclusion

4.2.1 Among the key findings from this study was a high prevalence of neonatal sepsis that was associated with an apparent nosocomial outbreak of multi-drug resistant *K. pneumoniae*. The study further showed that approximately 85% of the neonates in NICU at UTH acquired neonatal EOS which calls for immediate efforts to reduce the spread on infection. The study therefore, showed that the most predominant aetiological agents of sepsis were *K. pneumoniae, E. coli* and *S. aureus.* Among these three predominant pathogens, increasing incidence of *K. pneumoniae* correlated with a rise in neonatal mortality.

4.2.2 For Enterobacteriaceae, resistance to WHO-recommended first and second-line antibiotics was almost universal. Multi-drug resistance was suspected to be as a result of ESBL producing *K. pneumoniae* that may have also exchanged with *E. coli*, the plasmids implicated in ESBL transfer.

4.2.3 Since results of blood cultures and speciation were available prior to discharge or death in only 25% of cases, the gold standard of sepsis diagnosis had minimal, if any, impact on treatment and management of sepsis. In the multivariate analysis of potential risk factors, maternal HIV infection was associated with a 2-fold reduction in the odds of neonatal sepsis, whilst neonatal age of 2 or more days and increasing parity were the neonatal and maternal factors, respectively, associated with an increased risk of neonatal sepsis. The finding that a factor unique to HIV-exposed neonates is associated with reduced risk of neonatal sepsis requires further detailed investigation.

4.3 Limitations of the Study

This study was undertaken at a single site (NICU-UTH) with all neonatal referrals from Lusaka district. Therefore, the findings may not be representative of other neonatal units in the country or sub-Saharan Africa as a whole. Other limitations of this study included the study design, which did not allow thorough investigation of the impact of neonatal sepsis on mortality, whilst controlling for the presence of competing risk factors in cases and controls. There was also a selection bias against babies with lower birth weight, who were under-represented in the study population. Obtaining specimens for analysis and maternal consent are both more challenging for premature neonates. Gestational age was not recorded preventing more detailed analysis comparing small for gestational age with acceptable gestational age neonates. Another limitation is that mothers of the neonates recruited in this study did not undergo blood collection and vaginal swabs for culture analysis hence reducing chances of picking up more risk factors associated with sepsis and those associated with mortality due to sepsis. The lack of serial blood cultures on each patient was another major limitation of this study, hence, one spot blood culture could have underestimated the sepsis burden in this population (Pultorak *et al*, 2013).

4.4 Future Directions

4.4.1 Hospital based epidemiological studies are needed in order to identify the pathogens and associated resistance that may cause subsequent sepsis in the hospital setting. These epidemiological studies may be used to form a basis for development of novel diagnostic tools. These tools can then be used to rapidly diagnosis neonatal sepsis and may impact on therapy which may subsequently reduce neonatal mortality.

4.4.2 Antimicrobial stewardship programmes need to be implemented at the hospital in order to reduce the development of AMR strains and prevent infection. Therefore, implementing an Antimicrobial Stewardship programme to monitor the use of antibiotics at UTH and implement vigorous infection strategies and providing many points for disinfection will aid in the reduction of neonatal sepsis and development of resistance. Further research in drugs/compounds that can kill these multi-resistant strains causing sepsis is also needed.

4.4.3 Clinical intervention trials involving HIV-uninfected and –infected mothers on prophylaxis for reducing MTCT should be done carefully and completely to evaluate the negative association this study found between development of sepsis and maternal HIV status. Others may be immunological studies looking at the immune-modulatory effects of Nevirapine on the infant/maternal pair. These may partially explain the intervention that may help reduce the risk of sepsis in neonates.

References

Acquah S.E., L. Quaye, K. Sagoe, J.B. Ziem, P.I. Bromberger, and A.A. Amponsem., 2013. "Susceptibility of bacterial etiological agents to commonly-used antimicrobial agents in children with sepsis at the Tamale Teaching Hospital." BMC infectious diseases. 13: 89.

Aiken A.M., N. Mturi, P. Njuguna, S. Mohammed, J.A. Berkley, I. Mwangi, S. Mwarumba, B.S. Kitsao, B.S. Lowe, S.C. Morpeth, A.J. Hall, I. Khandawalla, and J.A. Scott; Kilifi Bacteraemia Surveillance Group., 2011. "Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: a prospective cohort study." Lancet; 378(9808): 2021-7.

Aittakorpi A., P. Kuusela, P. Koukila-Kähkölä, M. Vaara, M. Petrou, V. Gant, and M. Mäk., 2012. "Accurate and rapid identification of *Candida* spp. frequently associated with fungemia by using pcr and the microarray-based prove-it sepsis assay." Journal of Clinical Microbiology; 50(11): 3635–3640.

Akter L., R. Haque, and M.A. Salam., 2014. "Comparative evaluation of chromogenic agar medium and conventional culture system for isolation and presumptive identification of uropathogens." Pakistan Journal of Medical Science. 30(5):1033-8

Artero A., R. Zaragoza and J. M. Nogueira., 2012. “Epidemiology of Severe Sepsis and Septic Shock, Severe Sepsis and Septic Shock.” - Understanding a Serious Killer, Dr Ricardo Fernandez (Ed.), ISBN: 978-953-307-950-9, InTech, Available from: http://www.intechopen.com/books/severe-sepsis-and-septic-shock-understanding-a-serious-killer/epidemiology-of-severe-sepsis-and-septic-shock

Auriti C., A. Maccallini, G. Di Liso, V. Di Ciommo, M.P. Ronchetti, and M. Orzalesi., 2003. “Risk factors for nosocomial infections in a neonatal intensive-care unit.” Journal of Hospital Infections. 53(1): 25-30.

Ayoola O.O., A.A. Adeyemo, and K. Osinusi., 2003. “Aetiological agents, clinical features and outcome of septicaemia in infants in Ibadan.” West African journal of medicine. 22(1): 30-4.

Ballot D.E., T. Nana, C. Sriruttan, and P.A. Cooper., 2012. “Bacterial bloodstream infections in neonates in a developing country.” ISRN paediatrics; 2012.508512.

Bateman S.L. and P. C. Seed., 2010. “Procession to Pediatric Bacteremia and Sepsis: A Series of Covert Operations and Failures in Diplomacy.” Pediatrics; 126(1): 137–150.

Bates M., M. Kabwe and A. Zumla., 2014. “Neonatal sepsis and antibiotic resistance in developing countries.” Pediatric Infectious Disease Journal; 33(10)

Cailes B., S. Vergnano, C. Kortsalioudaki, P. Heath, and M. Sharland., 2015. “The current and future roles of neonatal infection surveillance programmes in combating antimicrobial resistance.” Early Human Development. Septemeber 16. Epub ahead of Print.

Calitri C., M. Denina, C. Scolfaro, S. Garazzino, F. Licciardi, E. Burdino, T. Allice, F. Carraro, G. De Intinis, V. Ghisetti, P.A. Tovo and the Regina Margherita Children's Hospital Bloodstream Infections Study Group., 2015. “Etiological diagnosis of bloodstream infections through a multiplex real-time polymerase chain reaction test in pediatric patients: a case series from a tertiary Italian hospital.” Infectious Diseases (London); 47(2):73-9

Chiabi A., V. Takou, E. Mah, S. Nguefack, H. Siyou, V. Takou, P. Tchokoteu, and E. Mbonda., 2014. “Risk Factors for Neonatal Mortality at the Yaounde Gynaeco-Obstetric and Pediatric Hospital, Cameroon.” Iran Journal of Pediatrics; 24 (No 4), Pp: 393-400

Church J.A., F. Fitzgerald, A.S. Walker, D.M. Gibb, and A.J. Prendergast., 2015. “The expanding role of co-trimoxazole in developing countries.” Lancet Infect Dis. 15(3):327-39

Couto R.C., E.A. Carvalho, T.M Pedrosa, E.R. Pedroso, M.C. Neto, and F.M. Biscione., 2007. “A 10-year prospective surveillance of nosocomial infections in neonatal intensive care units.” American Journal of Infection Control. 35(3): 183-9.

Cutland C.L., S.J. Schrag, E.R. Zell, L. Kuwanda, E. Buchmann, S.C. Velaphi, M.J. Groome, P.V. Adrian, and S.A. Madhi; PoPS trial team., 2012. “Maternal HIV infection and vertical transmission of pathogenic bacteria.” Pediatrics. 130(3): e581-90.

Dellinger RP, M.M. Levy, J.M. Carlet, J. Bion, M.M. Parker, R. Jaeschke, K. Reinhart, D.C. Angus, C. Brun-Buisson, R. Beale, T. Calandra, J.F. Dhainaut, H. Gerlach, M. Harvey, J.J. Marini, J. Marshall, M. Ranieri, G. Ramsay, J. Sevransky, B.T. Thompson, S. Townsend, J.S. Vender, J.L. Zimmerman, and J.L. Vincent., 2008. “Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock.” Intensive Care Med, 34, 17-60.

Downie L., R. Armiento, R. Subhi, J. Kelly, V. Clifford, and T. Duke., 2013. “Community-acquired neonatal and infant sepsis in developing countries: efficacy of WHO's currently recommended antibiotics--systematic review and meta-analysis.” Archives of disease in childhood. 98(2): 146-54.

Elwell L.P., R. Ferone, G. A. Freeman, J. A. Fyfe, J. A. Hill, P. H. Ray, C. A. Richards, S. C. Singer, V. B. Knick, and J. L. Rideout., 1987. “Antibacterial activity and mechanism of action of 3'-azido-3'-deoxythymidine (BW A509U).” Antimicrobial agents and chemotherapy. 31(2): 274-80.

Fernández-Cruz A., M. Marín, M. Kestler, L. Alcalá, M. Rodriguez-Créixems, and E. Bouza., 2013. “The value of combining blood culture and SeptiFast data for predicting complicated bloodstream infections caused by Gram-positive bacteria or Candida species.” Journal of Clinical Microbiology. 51(4):1130-6.

Filteau S., 2009. “The HIV-exposed, uninfected African child.” Tropical Medicine of International Health. 14(3): 276-87.

Govender T., and H. Coovadia., 2014. “Eliminating mother to child transmission of HIV-1 and keeping mothers alive: recent progress.” The Journal of infection. 68 Suppl 1: S57-62.

Grif K, I. Heller, W. M. Prodinger, K. Lechleitner, C. Lass-Flörl, and D. Orth., 2012. “Improvement of detection of bacterial pathogens in normally sterile body sites with a focus on orthopedic samples by use of a commercial 16S rRNA broad-range PCR and sequence analysis.” Journal of Clinical Microbiology; 50(7):2250-4.

Hamer D.H., G.L. Darmstadt, J.B. Carlin, A.K. Zaidi, K. Yeboah-Antwi , S.K. Saha, P. Ray, A. Narang, E. Mazzi, P. Kumar, A. Kapil, P.M. Jeena, A. Deorari, A.K. Chowdury, A. Bartos, Z.A. Bhutta, Y. Adu-Sarkodie, M. Adhikari, E. Addo-Yobo, M.W. Weber and Young Infants Clinical Signs Study Group., 2015. “Etiology of bacteremia in young infants in six countries.” Pediatric Infectious Disease Journal. 34(1):e1-8

Huson M.A., M.P.Grobusch, and T. van der Poll., 2015. “The effect of HIV infection on the host response to bacterial sepsis.” Lancet Infectious Diseases. 15(1): 95-108.

Iregbu K.C., and U. Anwaal., 2007. “Extended spectrum Beta-Lactamase-producing *Klebsiella pneumoniae* septicaemia outbreak in the Neonatal Intensive Care Unit of a tertiary hospital in Nigeria.” African journal of medicine and medical sciences. 36(3): 225-8.

Jackson J.B., J. Dick, T. Tekle, A. Simmons, and K.C. Carroll., 2009. "Lack of antimicrobial activity by the antiretroviral drug nevirapine against common bacterial pathogens." Antimicrobial agents and chemotherapy. 53(8): 3606-7.

Kiwanuka J., J. Bazira, J. Mwanga, D. Tumusiime, E. Nyesigire, N. Lwanga, B.C. Warf, V. Kapur, M. Poss, and S.J. Schiff., 2013. "The microbial spectrum of neonatal sepsis in Uganda: recovery of culturable bacteria in mother-infant pairs." PLoS One. 8(8): e72775.

Kohli-Kochhar R., G. Omuse, and G. Revathi., 2011. "A ten-year review of neonatal bloodstream infections in a tertiary private hospital in Kenya." Journal of infection in developing countries. 5(11): 799-803.

Korotchikova I., G.B. Boylan, E.M. Dempsey, and C.A. Ryan., 2010. "Presence of both parents during consent process in non-therapeutic neonatal research increases positive response." Acta Paediatrica (Oslo, Norway: 1992); 99(10):1484-8.

Kumar S., L. Wang, J. Fan, A. Kraft, M.E. Bose, S. Tiwari, M. Van Dyke, R. Haigis, T. Luo, M. Ghosh, H. Tang, M. Haghnia, E.L. Mather, W.G. Weisburg, and K.J. Henrickson., 2008. "Detection of 11 common viral and bacterial pathogens causing community-acquired pneumonia or sepsis in asymptomatic patients by using a multiplex reverse transcription-PCR assay with manual (enzyme hybridization) or automated (electronic microarray) detection." J Clin Microbiol., 46: 3063-72.

Landre-Peigne C., A.S. Ka, V. Peigne, J. Bougere, M.N. Seye, and P. Imbert., 2011. "Efficacy of an infection control programme in reducing nosocomial bloodstream infections in a Senegalese neonatal unit." The Journal of hospital infection. 79(2): 161-5.

Le Doare K., and P.T. Heath., 2013. "An overview of global GBS epidemiology." Vaccine. 31 Suppl 4: D7-12.

Liesenfeld O., L. Lehman, K.P. Hunfeld, and G. Kost., 2014. "Molecular diagnosis of sepsis: New aspects and recent developments." European Journal of Microbiology and Immunology (Bp). (1):1-25.

Lim D., and W.K. Maas., 1989. "Reverse transcriptase in bacteria." Molecular microbiology. 3(8): 1141-4.

Liu L, H.L. Johnson, S. Cousens, J. Perin, S. Scott, J.E. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, C. Mathers, and R.E. Black., 2012. "Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000." Lancet, 379, 2151-61.

Lukac P.J., R.A. Bonomo, and L.K. Logan., 2015. "Extended-Spectrum beta-Lactamase-Producing Enterobacteriaceae in Children: Old Foe, Emerging Threat." Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 60(9): 1389-97.

Madhi S.A., Z. Dangor, P.T. Heath, S. Schrag, A. Izu, A. Sobanjo-Ter Meulen, and P.M. Dull., 2013. "Considerations for a phase-III trial to evaluate a group B Streptococcus polysaccharide-protein conjugate vaccine in pregnant women for the prevention of early- and late-onset invasive disease in young-infants." Vaccine. 31 Suppl 4: D52-7.

Makasa M., L. Kasonka, M. Chisenga, M. Sinkala, C. Chintu, A. Tomkins, and S. Filteau., 2007. "Early growth of infants of HIV-infected and uninfected Zambian women." Tropical Medicine of International Health. 12(5): 594-602.

Manenzhe R.I., H.J. Zar, M.P. Nicol, and M. Kaba., 2014. "The spread of carbapenemase-producing bacteria in Africa: a systematic review." Journal of Antimicrobial Chemotherapy. 70(1):23-40

Mhada T.V., F. Fredrick, M.I. Matee, and A. Massawe., 2012 "Neonatal sepsis at Muhimbili National Hospital, Dar Es Salaam, Tanzania; aetiology, antimicrobial sensitivity pattern and clinical outcome." BMC public health. 12: 904.

Ministry of Health. 2007 "Microbiology Laboratory Standard Operating Procedure." Zambia.

Mkony M., M. Mizinduko, A. Massawe, and M. Matee., 2014"Management of neonatal sepsis at Muhimbili National Hospital in Dar Es Salaam: diagnostic accuracy of C inverted question mark reactive protein and newborn scale of sepsis and antimicrobial resistance pattern of etiological bacteria." BMC paediatrics. 14(1): 293.

Mokuolu A.O., N. Jiya, and O.O. Adesiyun., 2002. "Neonatal septicaemia in Ilorin: bacterial pathogens and antibiotic sensitivity pattern." African journal of medicine and medical sciences. 31(2): 127-30.

Morkel G., A. Bekker, B.J. Marais, G. Kirsten, J. van Wyk, and A. Dramowski., 2014. "Bloodstream infections and antimicrobial resistance patterns in a South African neonatal intensive care unit." Paediatrics and international child health. 34(2): 108-14.

Mulholland E.K., O.O. Ogunlesi, R.A. Adegbola, M. Weber, B.E. Sam, A. Palmer, M.J. Manary, O. Secka, M. Aidoo, D. Hazlett, H. Whittle, and B.M. Greenwood.,1999. "Etiology of serious infections in young Gambian infants." Pediatric Infectious Disease Journal. 18(10 Suppl): S35-41.

Mumbula E.M, G. Kwenda, T.M Samutela, A. Kalonda, J.C.L Mwansa, D. Mwenya, L. Koryolova, T. Kaile, C.C Marimo, and C.M. Lukwesa., 2015. "Extended Spectrum β-Lactamases Producing *Klebsiella pneumoniae* from the Neonatal Intensive Care Unit at the University Teaching Hospital in Lusaka, Zambia." Journal of Medical Science and Technology. , 4(2); 85-91.

Musoke R.N., and G. Revathi., 2000. "Emergence of multidrug-resistant gram-negative organisms in a neonatal unit and the therapeutic implications." Journal of tropical paediatrics. 46(2): 86-91.

Mwaniki M.K., M. Atieno, J.E. Lawn, and C.R. Newton., 2012. “Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review”. Lancet. 379(9814): 445-52.

Nagata E., A.S. Brito, and T. Matsuo. 2002. “Nosocomial infections in a neonatal intensive care unit: incidence and risk factors.” American Journal of Infection control. 30(1): 26-31.

Ogunlesi T.A., O.B. Ogunfowora , O. Osinupebi, and D.M. Olanrewaju., 2011. “Changing trends in newborn sepsis in Sagamu, Nigeria: bacterial aetiology, risk factors and antibiotic susceptibility.” Journal of paediatrics and child health. 47(1-2): 5-11.

Ojukwu J.U., L.E. Abonyi, J. Ugwu, and I.K. Orji., 2006. “Neonatal septicemia in high risk babies in South-Eastern Nigeria.” Journal of perinatal medicine. 34(2): 166-72.

Orszag P., C. Disqué, S. Keim, M.G. Lorenz, O. Wiesner, J. Hadem, M. Stiesch, A. Haverich, and C. Kühn., 2014. “Monitoring of patients supported by extracorporeal membrane oxygenation for systemic infections by broad-range rRNA gene PCR amplification and sequence analysis.” Journal of Clinical Microbiology; 52(1):307-11.

Phillips P., M. Cortina-Borja, M. Millar, and R. Gilbert. 2008. “Risk-adjusted surveillance of hospital-acquired infections in neonatal intensive care units: a systematic review.” The Journal of Hospital Infections. 70(3): 203-11.

Pultorak E.L., R.G. Maggi, P.E. Mascarelli, E.B. Breitschwerdt. 2013. “Serial testing from a 3-day collection period by use of the Bartonella Alphaproteobacteria growth medium

platform may enhance the sensitivity of Bartonella species detection in bacteremic human patients.” Journal Clinical Microbiology; 51(6):1673-7.

Quick J., A.R. Quinlan and N.J. Loman, 2014. “A reference bacterial genome dataset generated on the MinION™ portable single-molecule nanopore sequencer.” Gigascience; 3: 22.

Reddy EA, A.V. Shaw, and J.A. Crump. , 2010. “Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis.” Lancet Infectious Diseases. 10(6):417-32.

Rogina P., M. Skvarc, D. Stubljar, R. Kofol, and A. Kaasch., 2014. “Diagnostic utility of broad range bacterial 16S rRNA gene PCR with degradation of human and free bacterial DNA in bloodstream infection is more sensitive than an in-house developed PCR without degradation of human and free bacterial DNA.” Mediators of Inflammation:108592.

Schrag S.J., C. L. Cutland, E. R. Zell, L. Kuwanda, E. J. Buchmann, S. C. Velaphi, M. J. Groome, S. A. Madhi, and the PoPS Trial Team., 2012. “Risk Factors for Neonatal Sepsis and Perinatal Death Among Infants Enrolled in the Prevention of Perinatal Sepsis Trial, Soweto, South Africa.” Pediatric Infectious Disease Journal; 31: 821–826.

Seale AC, H. Blencowe, A. Zaidi, H. Ganatra, S. Syed, C. Engmann, C.R. Newton, S. Vergnano, B.J. Stoll, S.N. Cousens, and J.E. Lawn., 2013. “Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010.” Pediatric Research; 74 Suppl 1:73-85.

Shitaye D., D. Asrat, Y. Woldeamanuel, and B. Worku., 2010. “Risk factors and etiology of neonatal sepsis in Tikur Anbessa University Hospital, Ethiopia.” Ethiopian medical journal. 48(1): 11-21.

Sigaúque B., A. Roca, I. Mandomando, L. Morais, L. Quintó , J. Sacarlal, E. Macete, T. Nhamposa, S. Machevo, P. Aide, Q. Bassat, A. Bardají, D. Nhalungo, M. Soriano-Gabarró, B. Flannery, C. Menendez, M.M Levine, and P.L. Alonso. , 2009. “Community-acquired bacteremia among children admitted to a rural hospital in Mozambique.” Pediatric Infectious Disease Journal; 28: 108-13.

Simuyu D.E., 2005. “Neonatal septicaemia in low birth weight infants at Kenyatta National Hospital, Nairobi.” East African medical journal. 82(3): 148-52.

Six Week Extended-Dose Nevirapine (SWEN) Study Team, A. Bedri, B. Gudetta, A. Isehak, S. Kumbi, S. LulsegedS, Y. Mengistu, A.V. Bhore, R. Bhosale, V. Varadhrajan, N. Gupte, J. Sastry, N. Suryavanshi, S. Tripathy, F. Mmiro, M. Mubiru, C. Onyango, A. Taylor, P. Musoke, C. Nakabiito, A. Abashawl, R. Adamu, G. Antelman, R.C. Bollinger, P. Bright, M.A. Chaudhary, J. Coberly, L. Guay, M.G. Fowler, A. Gupta, E. Hassen, J.B. Jackson, L.H. Moulton, U. Nayak, S.B. Omer, L. Propper, M. Ram, V. Rexroad, A.J. Ruff, A. Shankar, and S. Zwerski.,2008. “Extended-dose nevirapine to 6 weeks of age for infants to prevent HIV transmission via breastfeeding in Ethiopia, India, and Uganda: an analysis of three randomised controlled trials.” Lancet. 372(9635): 300-13.

Skvarc M., D. Stubljar, P. Rogina, and A.J. Kaasch., 2013. "Non-culture-based methods to diagnose bloodstream infection: Does it work?" European Journal of Microbiology and Immunology (Bp). 3(2):97-104.

Schrag SJ, C.L Cutland, E.R. Zell, L. Kuwanda, E.J. Buchmann, S.C. Velaphi, M.J. Groome, and S.A. Madhi; PoPS Trial Team., 2012. "Risk factors for neonatal sepsis and perinatal death among infants enrolled in the prevention of perinatal sepsis trial, Soweto, South Africa." Pediatric Infectious Disease Journal; 31(8):821-6.

Storberg V., 2014. "ESBL-producing Enterobacteriaceae in Africa - a non-systematic literature review of research published 2008-2012." Infection ecology & epidemiology. 4.

Tann C.J., P. Nkurunziza, M. Nakakeeto, J. Oweka, J.J. Kurinczuk, J. Were, N. Nyombi, P. Hughes, B.A. Willey, A.M. Elliott, N.J. Robertson, N. Klein, and K.A. Harris., 2014. "Prevalence of bloodstream pathogens is higher in neonatal encephalopathy cases vs. controls using a novel panel of real-time PCR assays." PLoS One. 9(5): e97259.

Thaver D, S.A. Ali, and A.K. Zaidi., 2009. "Antimicrobial resistance among neonatal pathogens in developing countries." Pediatric Infectious Disease Journal, 28, S19-21.

University of Zambia-University College London medical School Research and Training Programme, 2014. "Clinical Baseline data in the Neonatal Intensive care unit (NICU) at the University Teaching Hospital (UTH) in Lusaka. Zambia." Unpublished

University Teaching Hospital, Bacteriology Laboratory., 2015. "Neonatal Intensive Care Unit (NICU) surface swabbing." Unpublished.

van der Zwet W.C., A.M. Kaiser, R.M. van Elburg, J. Berkhof,W.P. Fetter, G.A. Parlevliet, and C.M. Vandenbroucke-Grauls., 2005. "Nosocomial infections in a Dutch neonatal intensive care unit: surveillance study with definitions for infection specifically adapted for neonates." The Journal of Hospital Infection. 61(4): 300-11

Velaphi S., J. Wadula, and F. Nakwa., 2009. "Mortality rate in neonates infected with extended-spectrum beta lactamase-producing *Klebsiella* species and selective empirical use of meropenem." Annals of Tropical Paediatrics. 29(2): 101-10.

Wall S.N., A.C. Lee, W. Carlo, R. Goldenberg, S. Niermeyer, G.L. Darmstadt, W. Keenan, Z.A. Bhutta, J. Perlman, and J.E. Lawn., 2010. "Reducing intrapartum-related neonatal deaths in low- and middle-income countries-what works?" Seminars in perinatology. 34(6): 395-407.

West B.A. and O. Peterside., 2012. "Sensitivity pattern among bacterial isolates in neonatal septicaemia in port Harcourt." Annals of clinical microbiology and antimicrobials. 11: 7.

World Health Organization., 2014. "World Health Organization. Antimicrobial resistance: global report on surveillance." (In IRIS). Geneva: World Health Organization.

Wyres K.L., T. C. Conway, S. Garg, C. Queiroz, M. Reumann, K. Holt, and L.I. Rusu., 2014. "WGS Analysis and Interpretation in Clinical and Public Health Microbiology Laboratories:

What Are the Requirements and How Do Existing Tools Compare?" Pathogens (Basel, Switzerland); 3(2):437-458.

Zaidi A.K., D. Thaver, S.A. Ali, and T.A. Khan., 2009. "Pathogens associated with sepsis in newborns and young infants in developing countries." Pediatric Infectious Diseases Journal. 28(1 Suppl):S10-8.

Zaidi AK, W.C. Huskins, D. Thaver, Z.A. Bhutta, Z. Abbas, and D.A Goldmann. 2005. "Hospital-acquired neonatal infections in developing countries." Lancet, 365, 1175-88.

Appendices

Appendix A: List of Biochemical Tests.

Tripple Sugar Iron (TSI)

TSI was used for the identification of Enterobacteriaceae depending on the reaction of slant or butt to the fermentation of sugars. It was used for suspected *Salmonella* and *Shigella* isolates using the algorithm developed by the clinical laboratory Standards institute (CLSI) to identify the isolate. A suspected colony was inoculated onto the agar and production of acidic products was observed by colour change.

Lysine Iron Agar (LIA)

LIA was used to differentiate gram negative bacilli aiding in the identification of Enterobacteriaceae. This was done by inoculating a colony of bacteria on the slant and colour changes were observed when bacteria that is able to degrade lysine was present.

Coagulase Test

The coagulase test was used to identify *S. aureus* that produced the enzyme coagulase. This also helped in the identification of coagulase negative *Staphylococci*. In this study both the slide test and the tube method was used by using EDTA anticoagulated pooled plasma which was previously HIV and Hepatitis tested to ensure no cross-reactions.

Sulphur Indole Motility Test

Production of Sulphur and Indole was done using the Sulphur Indole Motility with indole test using the Kovacs reagent. Motility was also observed and blackness was used as an indicator for sulphur production of the inoculated organism.

Citrate Test

Citrate was prepared using a Simmon's citrate agar and gram negative organisms tested for their utilization of citrate. This test assisted in the identification of species belonging to Enterobacteriaceae. A change to bright blue was indicated as citrate positive and negative was recorded if there was no colour change in the Simmon's citrate agar.

Catalase test

The Catalase test was used to differentiate those bacteria such as *Staphylococci* that produce the enzyme catalase. Catalase is the catalytic enzyme that breaks down hydrogen peroxide (H_2O_2) producing water and oxygen. Bubbles of oxygen produced in the reaction indicated a Catalase producer. A colony of suspected bacteria were placed in a tube containing colourless solution of Hydrogen peroxide and production of bubbles observed.

Gram Stain

The Gram-stain was performed by adding Crystal Violet for one minute on an air dried and heat fixed smear of the positive blood culture and suspect colonies after inoculation on solid media. The slide was then washed with distilled water. Lugol's iodine was applied for 1 minute and washed off using water. Decolourisation was done by adding acetone for about 10-15 seconds and then washing with water immediately. Dilute carbol fuchsin was used to counter stain the slides for about 30 seconds and rinsed thoroughly with water. The slides were air dried and then examined using a microscope**.**

Appendix B: Informed consent form

You and your child are welcome to the SERCH study. This study is collecting information from children admitted to UTH with suspected sepsis (infection in the blood) who have had a blood culture performed. The study will identify the common pathogens (germs) that cause sepsis in children and also help identify factors associated with poor outcomes. Knowing the specific pathogens that cause sepsis and what drugs they are resistant to will help the doctors to know the correct medicine to give to children who will present with similar problems in future and therefore save their lives. A routine blood culture has already been collected and we seek your consent to use the results of the culture and for you to answer a few questions about your family, for this study. Your participation in the study will help us prevent deaths as a result of sepsis.

BENEFITS TO PARTICIPANTS:

All children with suspected sepsis at UTH are benefiting during the course of the study, which is providing blood culture bottles and other laboratory reagents which are often in short supply. The study personnel are also working closely with the laboratory staff at UTH, to ensure that test results are reported promptly. Participants will receive the same standard of care as all other patients.

BENEFITS TO THE COMMUNITY:

The study will define the common causes of sepsis locally, and will also define levels of drug resistance. The study will also seek to identify risk factors linked with poor outcomes to inform on policy. UNZA-UCLMS is a Zambian-led research group with all staff employed locally, and with several registered for post-graduate research degrees.

POSSIBLE DISADVANTAGES TO PARTICIPANTS:

The recruitment questionnaire will take roughly 10 minutes. All participants are requested to attend a follow-up visit 2 weeks after being discharged.

COMPENSATION: Participants and their guardian will be compensated with K20 to help with transport costs to attend the follow-up visit.

For further information about the study, you are free to contact the following on their mobile numbers:

Principal Investigator- Dr Matthew Bates, Study Coordinator, SERCH study Mobile: 0974044708, UNZA SOM Ethics Committee 260-1-256067

I have read, and understand the study and agree to take part.

Signature of Parent/Guardian: _____ |__||__|/|__||__|/|__||__|

Signature of Witness : _____ |__||__|/|__||__|/|__||__|

Appendix C: Study Questionnaire and Clinical examination form

	Date of Interview	|__|__|/|__|__|/|__|__|	
	Full Name of Patient		
	Study ID Number	|__|__|__|__|__|__|□□□	
	Hospital Number	|__|__|__|__|__|__|□□□	
	Study Category	**1=Paediatrics** □ **2=Obstetric, gynaecological** □ **3=Neonate**	
	Full Name of Caretaker	**Name:_____** **_**	
	Phone	**Tel:** |__|__|__|__|__|__|__|__|__|__|__|__| _|	
	Physical Address	**_____**	
	Date of Birth	|__|__|/|__|__|/|__|__|	
	Age	**Years_____ Months_____** □□	
	Sex	**M F**	

General Information

	How many people live in the same household with the patient?	**Number** **Adults =** **Children =**	
	What is the level of education of the mother	**1=Primary Education** **2=Secondary Education** **3=Tertiary** **9=None**	

	Where was the child delivered?	**1=At home □** **2= health centre □** **3=Hospital □**	
	Who assisted with the delivery?	**1=Trained midwife** **2=Nurse or other medical personnel** **3=Relative (mother, grandmother, sister) 9=Don't Know□** **5= self□** □ □	
	Was it a normal delivery or were there complications?	**1=Normal (Spontaneous vaginal delivery)□** **2=Prolonged labour** **3=Preterm labour** **4=Caesarian Section** **5=Multiple pregnancy** **6=Obstetric, gynaecological death □** **8=Others □** **Please specify _____** **9=Don't know □**	
	Any obstetric, gynaecological illness during pregnancy?	**1=No** **2=Yes**	
	If obstetric, gynaecological illness present, specify the illness	**Illness/s_____**	
	Which rank is the child?	**Number**	
	Parity	**Gravida Para**	

Physical Examination

	Weight	□□,□**kg**	
	Height	□□□,□**cm**	
	Mid upper arm circumference	□□,□**cm**	
	Temperature (ear)	□□,□**°C**	
	Respiration Rate	**_____ / min**	
	Blood pressure	|__||__||__|/ |__||__||__| mmHg	**N/A**

General Appearance

		Yes = 1 **No = 2**	
	Pallor / anaemia	**1 2**	
	Dehydration	**1 2**	
	Jaundice	**1 2**	
	Cyanosis	**1 2**	
	Clubbing	**1 2**	
	Dyspnoea	**1 2**	
	Oedema	**1 2**	
	Other	**1 2**	
		If yes, specify: _____	

Ear/Nose/Throat

		Yes = 1 **No = 2**	
	Inflamed tonsils	**1 2**	
	Nasal discharge/Epistaxis/ sinuisitis	**1 2**	
	Ear discharge	**1 2**	
	Ear ache	**1 2**	
	Other	**1 2** **If yes, specify:** _____	

Respiratory system

	Chest deformity	**1=Yes, 2=No**	
	Auscultation	**1=Normal** **3=Crepitations** **4=Wheezes** **6=Reduced air entry** **7=Other** **Please specify** _____	
	Conclusion	**1=Normal** **2=Upper respiratory tract infection** **3=Lower respiratory tract infection** **4=Others**	

		Please specify _____	

Cardiovascular System

	Pulse rate	**_____ / min**		
	JVP	**Yes = 1, No = 2**		
	Apex Beat	**1 = Normal** **2 = Displaced**		
	Heart Sounds	**1 = Normal** **2 = Abnormal** **Specify_____**		

Abdominal system

		Yes = 1, No = 2	
	Abdominal tenderness	**1 2**	
	Ascites	**1 2**	
	Palpable mass	**1 2**	
	Hepatomegalie	**1 2** **If yes, specify: _____cm below rib cage**	
	Splenomegally	**1 2** **If yes, specify :** **palpable □** **_____cm below rib cage □**	
	Other abnormalities	**1 2** **If yes, specify: _____**	

Appendix D: Letters of Support

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH
University Teaching Hospital

P/Bag Rw 1X
Lusaka - Zambia

Fax: +260 211 250305
e-mail: mduth@yahoo.com

Tel: +260 211 253947 (Switch Board)
+260 211 251451

OFFICE OF THE SENIOR MEDICAL SUPERINTENDENT

Our Ref:
Your Ref:

24/07/2012

Dr Matthew Bates
Adult Centre of Excellence Building
University Teaching Hospital
Lusaka.

Dear Dr Bates,

Thank you for the study protocols you sent and your interest to conduct the Sepsis (SERCH) study in our institution. The hospital offers full support for this study and will be available to assist in any way possible should you face any problems.

Yours Sincerely,

Dr L. Kasonka,

MD UTH

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE

Department of Paediatrics and Child Shealth

Telephone: 254965
252641 (UTH) 254824 *(Pre-Clinical) Ridgeway* Campus

P.O Box 50110
LUSAKA, Zambia

Institute of Medical Research and Training
Adult Centre of Excellence Building
University Teaching Hospital
Lusaka.

1st August 2012

Dear Dr.Bates,

Thank you for the study protocols you sent and your interest to conduct the Sepsis (SERCH) study in our department. The department is aware our two members of staff, Dr.Kapasa and Dr Chabala are co- principal investigators on the study. This letter serves to confirm the department's full support and will be available to assist in any way possible should you face any problems.

Yours sincerely,

Dr Somwe wa Somwe

Head of Department

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm
Assurance No. FWA00000338
IRB00001131 of IORG0000774

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

24th May, 2013.

Your Ref: 004-09-12

Dr. Mathew Bates
University Teaching Hospital
Adult Centre of Excellence
P/Bag RW 1X
Lusaka

Dear Dr. Bates,

RE: RE-SUBMITTED RESEARCH PROPOSAL: **"PILOT STUDY TO DEFINE THE AETIOLOGY OF OBSTETRIC, GYNAECOLOGICAL, NEONATAL AND PAEDIATRIC SEPTICAEMIA, INCLUDING DRUG RESISTANCE AND TO EVALUATE RISK FACTORS ASSOCIATED WITH MORBIDITY AND MORTALITY AT UNIVERSITY TEACHING HOSPITAL, ZAMBIA: SEARCH"**

The above mentioned research proposal was re-submitted to the Biomedical Research Ethics Committee for ethical review on 30th April, 2013. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- This waiver does not release you from the obligation of ensuring confidentiality.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours Sincerely,

Dr. J.C Munthali
CHAIRPERSON

Date of approval: 24 May, 2013 **Date of expiry**: 23 May, 2014