AN EPIDERMIOLOGICAL STUDY ON NEONATAL SEPTICEMIA AT MOROGORO REGIONAL REFERRAL HOSPITAL, TANZANIA

NURU BEDA NDAWEKA

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2014

ABSTRACT

Neonatal septicaemia remains a major cause of morbidity and mortality in neonates worldwide and majority of the cases are in developing countries. This cross sectional study was conducted between December 2013 and March 2014 with the aim of determining the epidemiology of neonatal septicaemia among the neonates admitted at Morogoro Regional Referral Hospital. The study was subdivided into two sub sections which are retrospective and prospective. In retrospective section records of neonates hospitalized between month 2011 and month 2012 were used. In prospective study a questionnaire was administered to 303 mothers/caregivers on risk factors for neonatal septicaemia. Subsequently, blood samples from 303 neonates clinically suspected to have septicemia and pus swabs from 17 neonates with pus discharging umbilicus were collected for bacterial culture and sensitivity testing to commonly used antibiotics using standard methods. Retrospective results showed that the prevalence of neonatal septicaemia is 53.7% while prospective study showed a prevalence of 13.5%. The significant risk factors for neonatal septicaemia were age, weight, sex, umbilical cord discharge and poorly cared cord. Whereas, maternal factors associated with neonatal septicaemia were fever, excessive vaginal examination and caesarean section. Mortality rate due neonatal septicaemia was significantly high (12.7% retrospective and 7.9% prospective). Commonly isolated bacteria were *Staphylococcus aureus, Escherichia coli, Klebsiella* spp. and *Pseudomonas auriginosa* which showed multi-drug resistance. This study shows that the magnitude of neonatal septicaemia is high and therefore deliberate measures aimed at minimizing the problem need to be taken.

DECLARATION

I, NURU BEDA NDAWEKA, do hereby declare to the senate of Sokoine University of Agriculture that, this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Beda 12/11/2014

NURU BEDA NDAWEKA Date

(MSc. Candidate)

12/11/2014

Dr. Hezron E. Nonga Date

(Supervisor)

12/11/2014

Dr. Lucas E. Matemba Date

(Supervisor)

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ACKNOWLEDGEMENT

This dissertation is the end of my journey in obtaining my MSc in Public Health and Food Safety and I have not travelled in a vacuum. This dissertation has been kept on track and been seen through to completion with the support and constant encouragement of numerous people including my well-wishers, my friends, colleagues and various institutions of Sokoine University of Agriculture. Thanks all members of the Department of Veterinary Medicine and Public Health. I also thank the National Institute for Medical Research (NIMR) for granting ethics clearance to conduct this research in humans at Morogoro Regional Referral hospital.

I would like to thank my supervisors Dr. Hezron E. Nonga and Dr. Lucas E. Matemba for their invaluable guidance and support throughout my research, this work would not have been possible without your guidance, support and encouragement. Your unflinching courage and conviction will always inspire me, and I hope to continue to work with your noble thoughts.

Special thanks to Morogoro regional referral hospital administration for allowing me to conduct my research work in the hospital. I would like to thank Morogoro hospital, pediatrician Dr. H. Msaky, for allowing me to work in the wards, her clinical expertise and advices made this work possible. Thank you to hospital laboratory manager and technicians for technical support. Special thanks go to authorities of NIMR- MOMS laboratory for allowing me access to their laboratory

facility. Special thanks also go to all study participants involved in the study for agreeing to participate and allowing samples to be collected from their children.

My special acknowledgements go to my family; my parents deserve special mention for their inseparable support and prayers. My father, Beda Ndaweka, in the first place is the person who put the fundament to my learning character, showing me the joy of intellectual pursuit ever since I was a child. My late mother, Asha Omary Msombelli and my guardian mother Devota Mkenda are the ones who sincerely raised me with their caring and gentle love.

Finally, I would like to thank everybody who was important to the success of this dissertation, as well as expressing my apology that I could not mention personally one by one.

DEDICATION

This work is dedicated to all stakeholders of this study, special appreciation goes to Prof. B.G. Kessy, Prof. D.G. Mpanduji and Mr. L. Lugembe my friends Amina Ahmed, Makbel and David Augustus, my young brothers and sisters Maua, Goodluck senior, Josephine, Margaret, Neema, Jackline, Ikunda, Jackson, Junior, Robert, Gilbert, Victor, Gabriel and Pascal, thanks for being supportive and caring siblings.

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LIST OF ABBREVIATIONS AND SYMBOLS

AIDS	Acquired Immunodeficiency syndrome
BHI	Brain Heart Infusion
CBC	Complete blood cell count
CMV	Cytomegalovirus
CRP	C-Reactive Proteins
g	grams
HIV	Human Immunodeficiency virus
MH	Muller Hinton
Mls	Millilitres
MRCC	National Research Coordinating Committee
MRRH	Morogoro Regional Referral Hospital
n-IMCI	Integrated Management of Childhood Illnesses
NIMR	National Institute for Medical Research
OR	Odds ratio
PHCT	Primary Health Care in Team
PMTCT	Prevention of Mother to Child Transmission
PROM	Premature Rupture of Membranes
UTI	Urine Tract Infection
VDRL	Venereal Diseases Research Laboratory
SUA	Sokoine University of Agriculture
TSI	Triple Sugar Iron
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Neonatal septicaemia also known as "sepsis neonatorum" is a blood infection that occurs to infant younger than 90 days old (Stoll *et al.*, 2010). Early-onset sepsis is seen in the first week of life. Late-onset sepsis occurs between days 8 and 89 (Verani *et al.*, 2010). Also neonatal septicaemia can be any infection involving an infant during the first 28 days of life. Differentiation between early and late onset of neonatal sepsis is important in prevention and treatment because of aetiological differences (Verani *et al.*, 2010). The infection may be systemically infective to the infants or can be specific or limited to a single organ (Stoll *et al.*, 2010). This infection can be transmitted or be acquired prior to birth or while the foetus is in the uterus (intrauterine septicaemia) or after birth (extrauterine septicaemia) (Stoll *et al.*, 2011). It has been established globally that mortality of neonates account for more than one-third of deaths of children aged less than five years (Rajaratnam *et al.*, 2010). Moreover, available studies suggest that about 99% of the neonatal deaths occur in low and middle-income countries (Lawn *et al.*, 2005). Septicemia is a common cause of infants' morbidity and mortality in developing countries. Of all the deaths that occur in developing countries; half of them are reported from African regions alone (WHO, 1995).

Neonatal septicaemia infection can be caused by different organisms in different forms such as viruses like herpes and rubella virus and, rarely occasion can be caused

by fungus such as *Candida* spp. However, bacteria such as group B *Streptococcus* and *Staphylococcus aureus* are known to be among the most common cause of infection that significantly causes neonatal and early childhood admissions to hospitals. This also increases the disease morbidity in the community (Stoll *et al.*, 2010). It is estimated that about 5 million neonatal deaths worldwide occur in each year (WHO 1995; Rahman *et al.*, 2002).

Clinical presentations of neonatal septicemia vary and in most studies symptoms include fever, difficulty in breathing, tachycardia, malaise and lethargy (WHO, 1995). Other signs include inability to breast feed, convulsion, chest wall in-drawing, jaundice and umbilical redness which are strongly associated with neonatal septicemia. A study done in Kenya highlighted some of the commonest clinical signs to include difficult in feeding, unexplained pallor, cyanosis and unconsciousness (English *et al.*, 2004). Other signs associated with neonatal septicemia were fast breathing, nasal flaring, grunting and lethargy. However, there are no pathognmonic features for neonatal septicaemia which is coupled with limited availability of diagnostic facilities and medical services especially in most of the developing countries.

According to findings of a study conducted at Muhimbilli National Hospital Tanzania; the prevalence of neonatal septicemia ranged from 15.9 to 22.4% (Bloomberg *et al.* 2007; Mhada *et al.*, 2012). Another study conducted at Bugando Consultant Hospital showed that there is also high mortality rate due to neonatal septicemia summing to 19% similar to other studies in East Africa region (Kayange

et al., 2010). Kilimanjaro Medical Centre as one of Tanzanian referral hospital has been reported to have prevalence 6.5% (Klingenberg *et al.*, 2003).

World Health Organization (WHO) has made criteria for initial diagnosis of neonatal sepsis, but the sensitivity and specificity of clinical diagnosis can vary considerably. Such clinical features like inability to breast feed, lethargy, convulsion, chest wall in-drawing, respiratory rate > 60/minute, grunting, temperature >38°C, jaundice and umbilical redness strongly suggest classical presentation of neonatal septicemia (Shalini *et al.*, 2010). The same kinds of clinical signs are used by WHO (2003) as criteria for the diagnosis of neonatal septicemia. However, in most hospitals and health facilities in Tanzania including Morogoro regional referral hospital (MRRH), diagnosis of neonatal septicemia is in most cases done through clinical signs and symptoms. Treatment is always done symptomatically and its preciseness and reliability may be questionable (Mhada *et al.*, 2012). This consequently, may lead to significant increase in neonate mortalities due to septicemia and many other causes definitive diagnosis could not be established (Rajaratnam *et al.*, 2010).

In Tanzania the epidemiology of neonatal sepsis has not been extensively studied and the treatment protocols are not well established. Timely diagnosis and effective treatment is urgently needed so as to prevent further deaths and complications resulting from septicemia. Physical signs and symptoms are useful in identifying neonates and children with septicemia. These clinical characteristics can be good indictors for positive blood culture but they have limited specificity and sensitivity (WHO, 1995). Rapid immunological techniques like C-Reactive Proteins (CRP)

assays may help in the diagnosis of septicemia; though they lack the capacity to detect specific pathogens and are not available in many centres in developing countries (Mugalu *et al.*, 2006). Blood culture remains the gold standard for definitive diagnosis of septicemia (WHO, 1995) but the results of blood culture take hours to days, thus necessitating initial empirical treatment of suspected cases. Knowledge of predictors of positive blood culture and antimicrobial susceptibility pattern of common pathogens in a given area are essential in guiding local empirical choice of antibiotics (Mugalu *et al.*, 2006).

To date most of empirical treatments in developing countries have been formulated using data from developed countries (English *et al.*, 2004). In the management of neonatal sepsis, clinicians in many resource limited settings make tentative diagnosis and empirical treatment based on the new neonatal WHO Integrated Management of Childhood Illnesses (n-IMCI) guidelines (Klingenberg *et al.*, 2003). However aetiology of neonatal sepsis as well as response to antimicrobial agents may vary significantly from time to time and geographically which may affect the success of empirical management (Klingenberg *et al.*, 2003). Currently in Tanzania and especially at MRRH ampicillin, cloxacillin and gentamicin are used as the first line empirical treatment of neonatal septicemia. The decision on this regime was based on research findings from different institutions in developing countries (Signh *et al.*, 2003). Thus the current study was carried out to determine the possible causes of neonatal septicemia, characterize the common bacteria involved and determine their sensitivity profile to commonly used antimicrobial drugs. The data generated will serve as predictors of positive blood culture and are therefore useful to guide

clinicians on wise decisions on better and appropriate management of neonatal septicaemia cases at MRRH and probably in many other hospitals in Tanzania. They will also be useful in policy formulation aimed at development of best guidelines for prevention and management of neonatal sepsis not only at MRRH but many other hospitals in Tanzania.

1.2 Problem statement and study justification

1.2.1 Problem statement

The problem of neonatal septicemia is increasingly becoming a threat to survival of neonates and has been causing increased hospitalization of neonates in many hospitals in Tanzania. This necessitates an empirical approach to combat the problem so as to reduce a number of neonates who suffer from septicemia in Tanzania and specifically at MRRH. This can be only possible if the risk factors for infection are established, causative bacteria and their sensitivity to commonly used antibiotics are known and a better way to abate the problem is established. It is anticipated that the results of this study including the treatment protocol will help to reduce disease burden in neonates, reduce drug resistance to commonly used antibiotics and possibly minimize neonatal death and congestions in pediatric wards.

1.2.2 Justification of the study

Septicemia is among the common cause of neonatal death especially in developing countries. At the moment MRRH appears to have many cases whose causes have not been established. Culture and sensitivity test to the organism causing septicemia is also not routinely carried out in many of hospitals in Tanzania. Therefore, understanding the cause of neonatal septicemia would significantly help the medical practitioners to come out with clear treatment protocol and possibly identify the risk factors which will be the basis for designing of preventive measures. This will reduce the incidences of neonatal infections and subsequent development of septicemia.

1.3 Objectives of the study

1.3.1 Main objective

To determine the epidemiology of septicaemia among neonates hospitalized at Morogoro Regional Referral hospital

1.3.2 Specific objectives

(i) To estimate the prevalence of neonatal septicaemia at Morogoro regional referral hospital

(ii) To determine the risk factors for neonatal septicaemia at Morogoro regional referral hospital

(iii) To establish the common bacteria causing septicaemia in neonates

(iv) To establish antimicrobial sensitivity profiles of commonly isolated bacteria.

1.4 Research questions

(i) What is the magnitude of neonatal septicaemia at Morogoro regional referral hospital

(ii) What are the risk factors leading to neonatal septicemia in Morogoro?

(iii) What are the common microorganisms causing neonatal septicemia at Morogoro Regional Referral hospital?

(iv) Which treatment protocol can help to ameliorate this problem?

(v) Which preventive measures can be adopted to minimize occurrences of neonatal septicaemia at Morogoro regional referral hospital?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition

Neonatal sepsis is a blood infection that occurs in an infant younger than 90 days old. Early-onset sepsis is seen in the first week of life while late-onset sepsis occurs between days 8 and 89 (Stoll *et al.*, 2011). Neonatal sepsis can be explained as an infection to an infant during the first 28 days of life (Mhada *et al.*, 2012). The infection may be systemically infective to the infants or may be specific or limited to one organ like lungs. The additional features of pneumonia or meningitis may be present depending upon the localization of infection in different systems and organs of the body (French *et al.*, 2005).

2.2 Causes of the neonatal infection

Neonatal septicaemia can be caused by different bacterial species including *Escherichia coli*, *Listeria monocytogens*, *Pseudomonous, aeruginosa Staphylococcus aureus, Klebsiella* spp. and certain strains of streptococcus (Kayange *et al.*, 2010). *Escherichia coli* are Gram negative, non-spore forming rod and may be motile. The organism is a facultative anaerobe and ferments sugars to form lactic, acetic, and formic acids. The optimal growth temperature is 37°C (Doyle and Schoeni, 1984). *Listeria monocytogens* is a Gram positive anaerobic bacterium, motile, grows at 30°C and below. However *Streptococcus* spp. are Gram positive cocci bacteria that appear in chain or pairs fastidious and require an infusion medium or an enriched medium such as blood agar. *Pseudomonas aeruginosa* is a Gram negative rod, aerobe bacterium and is citrate, catalase and oxidase positive (WHO, 1995).

2.3 Risk factors for neonatal infection

Generally there several risk factors for neonatal septicaemia. However Utomo *et al.* (2010) listed a number of factors that can lead to development of neonatal septicemia which include:

(i) Maternal factors like: premature rupture of membranes (PROM) especially more than 18 hours, infection and fever of the mother during labour, foul smelling of amniotic fluid, turbidity and meconeal stained amniotic fluid, and multiple gestations (Schuchat *et al.*, 2004).

(ii) Neonatal factors like prematurity, low birth weight, asphyxia, resuscitation during delivery, invasive procedure, congenital anomaly, parenteral nutritional, long hospital stay in neonatal intensive care unit (Verani *et al.*, 2006).

2.3.1 Antenatal care and diseases screening

Woman healthy during pregnancy is important and the obstetrician has to continuously monitor the health of both the women and her foetus for any signs or symptoms that might indicate sepsis during the course of the whole pregnancy to the delivering of the foetus (Verani *et al.*, 2010). Prior to delivery, there may be many indicators or signs which can give signal of the potential infection development to pregnant women and the foetus. All women are supposed to be screened or tested for infectious diseases at their first clinic visit (antenatal care clinic) before being sent to the obstetrician or healthcare provider in the hospital or health centres. Some of the infections to be screened include HIV/AIDS, gonorrhoea, syphilis, herpes simplex, *Chlamydia*, and hepatitis B, as well as immunity to rubella (Mersch and Shiel, 2009).

Some symptoms and signs of the infection sometimes takes longer time to manifest compared to growth of the foetus such as slower than anticipated foetal growth, can be subtle indications of threatened foetal well-being (Verani *et al.*, 2010).

Measurement of uterine size by using hands through palpation (leopard manuever), the traditional tape measure or obstetric ultrasound examination of the uterus, placenta, and foetus will both provide critical information of wellbeing of mother and the foetus (WHO 1995). Throughout the pregnancy, regular hospital or clinic visits will provide the opportunity to monitor foetal heart rate. The obstetrician commonly evaluates both the actual heart rate at rest as well as the infant's cardiac response to a mild stress (for example, uterine contraction). If concerns develop, specialized evaluations can be performed ("biophysical profile") during which foetal heart rate, foetal movement and foetal tone and liquor are monitored and any objective risk assessment may be made and managed accordingly (Isaac, 2006). Maternal fever during her pregnancy warrants a timely and thorough evaluation; if the infection is not well treated equally may lead to the onset of premature labour or premature rupture of the amniotic sac (termed "premature rupture of membranes") (Verani *et al.*, 2010).

During labour, several indicators may raise concern regarding the possibility of neonatal sepsis. Abnormalities of foetal heart rate, maternal fever, premature separation of the placenta from the uterine wall (abraptio placenta), or foul smelling/cloudy amniotic fluid (chorioamnitis) all indicate the possibility of high-risk labour and delivery. These situations will commonly prompt consultation with the

paediatrician or neonatologist regarding the potential for delivery and or management of postpartum complications (Mersch and Shiel, 2009). Infants may present with neonatal sepsis by subtle signs such as poor feeding, jaundice, unusual rashes, or more obvious indicators such as seizures, projectile vomiting, or abdominal distension (Mersch and Shiel, 2009).

2.3.2 Intrauterine factors

Intrauterine or factors that increase the risk before birth include the following: poor prenatal care, poor nutrition, recurrent abortions, preterm or still birth, and substance abuse. Intrauterine infections occur when pathogenic organisms cross the placenta into the foetal circulatory system. The organisms, such as cytomegalovirus (CMV), can reside in the amniotic fluid (Schuchat *et al.*, 2004). Other organisms ascend from the vaginal track, infecting the membranes and causing them to rupture. This rupture of membranes can lead to infections of the respiratory and gastrointestinal tract of a newborn. Early onset of neonatal sepsis is most commonly caused by Group B streptococcus infection during pregnancy (Mhada *et al.*, 2012).

2.3.3 Intrapartum factors

Intrapartum factors that increase the infants chance of becoming infected during the delivery process include: prolonged rupture of membranes (>12 to 18 hours), infection of the placental tissues and amniotic fluid (chorioamnionitis), urinary tract infections, preterm birth, prolonged or difficult labour, maternal fever, colonization with Group B streptococcus (GBS), and maternal infections (Schuchat *et al.*, 2004). Most infections during the delivery process are related to the infant coming into

unavoidable contact with an infected birth canal. The birth canal can host bacteria that an infant's immune system cannot defend against.

2.3.4 Postnatal factors

Postnatal infections may be contracted after delivery, as in the case with infections contracted during resuscitation, or as a result of a nosocomial infection due to improper hand washing of the caregivers and health care providers (Mugalu *et al.*, 2006). Infections in the postnatal period are more common in those infants who require foreign objects to be introduced into their systems. Most invariably attributed to contaminated intravenous cannula retained in a blood vessel for long time, nasogastric tubes, endotracheal tubes or indwelling catheters increase the risk of an infant becoming septic (Schuchat *et al.*, 2004). Nosocomial infection is among the causes of increased infant's risk to septicemia after delivery and long-time or prolonged hospital stay for an extended period of time (Stoll *et al.*, 2011).

2.4 Clinical signs of neonatal infection

Clinical presentation of neonatal sepsis varies significantly between areas and depends on the social economic factors and there are no pathognmonic features (Seale *et al.*, 2009). However in one study, Kayange *et al.* (2010) gave an indication of features which can predict septicemia. Such clinical features like inability to breast feed, lethargy, convulsion, chest wall in-drawing, respiratory rate > 60/minute, grunting, high temperature >38°C, jaundice and umbilical redness strongly suggest of neonatal septicemia. The same kinds of clinical signs have been used by WHO (2003) as criteria for diagnosis of neonatal septicemia. Nonspecific clinical signs for

the presentation of neonatal sepsis and poor or delayed laboratory services have resulted in the provision of empirical treatments for sepsis in many developing countries (Mhada *et al.*, 2012).

2.5 Diagnosis of neonatal septicaemia

To increase the infant's chance of survival, early recognition of signs and symptoms of sepsis is imperative. Often the diagnosis of sepsis is based upon suspicion of the presenting clinical signs and symptoms. Rapid immunological techniques like C-Reactive Proteins (CRP) assays may help in the diagnosis of septicemia; however they lack the capacity to detect specific pathogens which are not available in many centres in developing countries (Mugalu *et al.*, 2010). Blood culture to isolate the offending pathogen remains the gold standard for definitive diagnosis of septicemia (WHO, 1993). Other methods are complete blood cell count (CBC) with differential, chest x-ray, urine culture, and lumbar puncture with blood being the principle fluid assessed for suspected sepsis. Ideally, all cultures should be obtained before antibiotics are started (WHO, 1993). Molecular methods like diagnostic polymerase chain reaction (PCR) and serological test like ELISA may be used in diagnosis of neonatal septicaemia but are not feasible under routine practices in hospital laboratories.

2.6 Treatment of neonatal septicemia

Antibiotic therapy is usually started before the laboratory results confirm and identify the pathogen causing the infection (Bellig, 2004). In addition to antibiotic treatment, supportive therapy may be provided that consists of circulatory, respiratory,

nutritional, and developmental support. The treatment begins with careful monitoring of the infant's vital signs and regulation of the thermal environment (Isaac *et al.*, 2010). Supportive therapy for a septic infant starts with the administration of oxygen when respiratory distress or hypoxia is observed.

In the treatment of neonatal sepsis, many clinicians in many resource limited settings do make provisional diagnosis and empirical treatment of neonatal sepsis based on the new neonatal WHO Integrated Management of Childhood Illnesses (n-IMCI) guidelines (Klingenberg *et al.*, 2003). In a study conducted in Kilimanjaro, Tanzania more than two thirds of neonates admitted to a special care baby unit received antibiotics prior to investigations and response to treatment was high (Kayange *et al.*, 2010). This empirical treatment can vary significantly from time to time. The same applies to MRRH where clinicians also use the WHO guidelines in the treatment of the neonatal septicaemia.

2.7 Prevention of neonatal sepsis

Prophylactic antibiotics therapy may be given to pregnant women who have chorioamnionitis, Group B streptococcus infection, or who have previously given birth to an infant with septicemia (Mayor-Lynn *et al.*, 2005). Preventing and treating infections in pregnant mothers, providing a clean birth environment and hygienic handling of newly born neonates, and delivering the baby within 24 hours of rupture of membranes, where possible, can all help lower the chances of neonatal sepsis (Schuchat *et al.*, 2000). Better management of umbilical cord including discouraging parents/guardians from traditional norms and taboos that insult the cord are among

the methods of prevention of neonatal septicemia. Immediate feeding of the neonates after delivery and proper counselling of inexperienced mothers about the benefits of breast feeding will also reduce the number neonates who are treated as cases of neonatal septicemia instead of dehydration fever. Reducing some of the invasive procedures to the neonates such as suction will help to reduce trauma that rate of organisms crossing to blood stream (Mugalu *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted in Morogoro Municipality in Morogoro Region at MRRH (Figure 1). The Municipal is about 195 km west of Dar es Salaam city, on foot of Uluguru Mountains with total area of 260 square kilometres. The human population of Morogoro Municipal is 315,866 according to the 2012 census (PHCT, 2012). MRRH on average attends between 360 and 400 patients in a day with the admission rate ranging between 65 and 70 patients. The hospital admits between 25 and 35 pregnant women per day with the average range of same deliveries per day. Nevertheless patients suspected of neonatal septicemia are also among the ones admitted at MRRH. A large number of such patients also come from Mafiga Health Centre in Morogoro Municipality. MRRH has a neonates ward (Prem unit) which admits between 15 and 20 neonates per day. Among them 8 to 15 neonates are admitted as cases of neonatal septicemia which ranks the second after birth asphyxia which is the major cause of admission.

3.2 Study design and setting

This was a hospital based prospective cross-sectional study conducted at Morogoro Regional Referral hospital between December, 2013 and March, 2014.

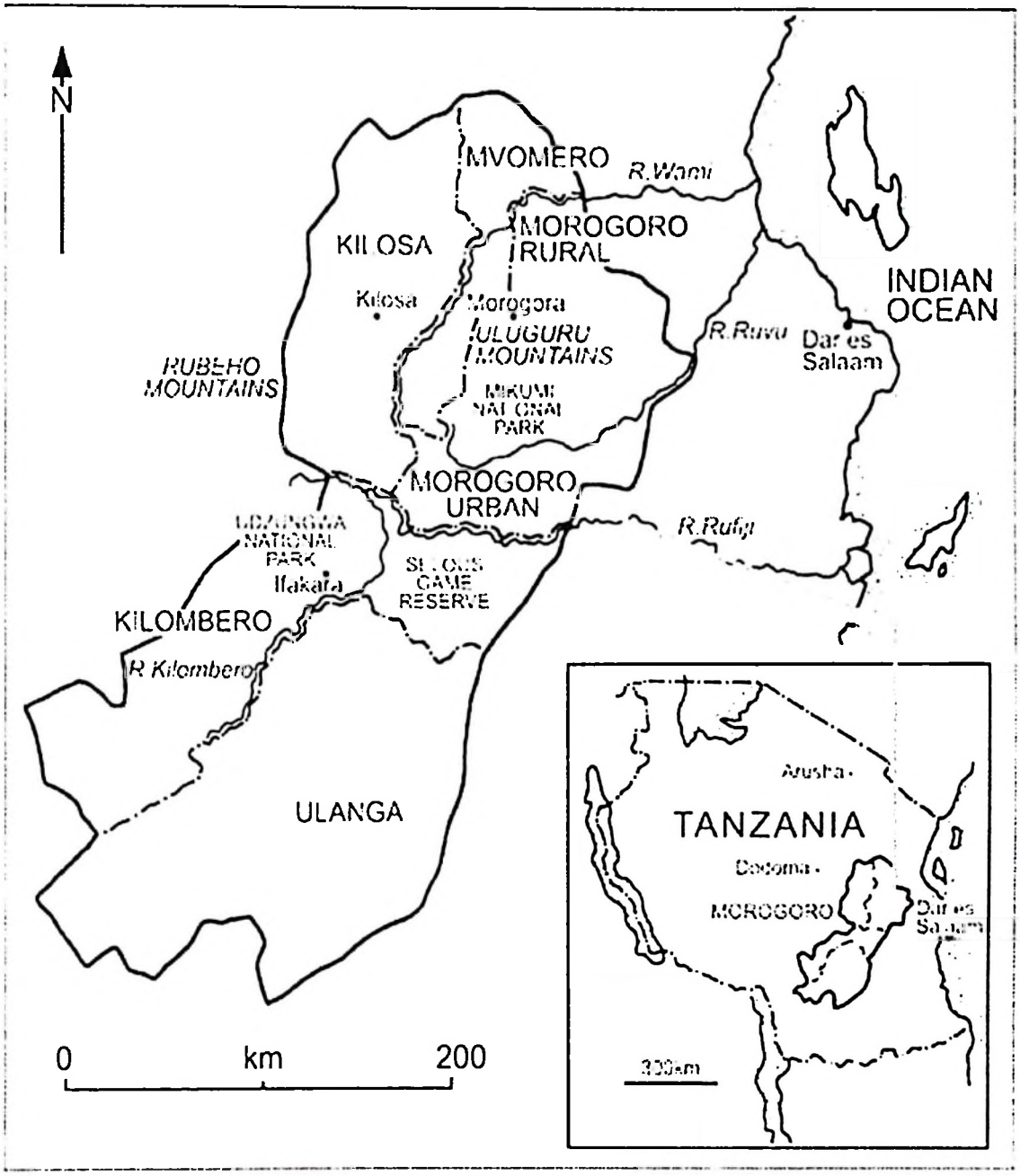

Figure 1: **A map showing location of Morogoro Municipality where the MRRH is based.**

3.3 Study population and inclusion criteria

All the neonates who were admitted at MRRH during data collection from December 2013 and March, 2014 formed a study population. The patient's inclusion criteria included patients within one month of life, patients whose mothers willingly accepted to participate in the study, patients admitted to the Morogoro regional referral hospital suspected to have neonatal septicemia at a time of study and newly admitted neonates before start of antibiotic medication. This study excluded patients above one month of age, patients whose mothers or guardians refused or were not willing to participate to the study, patients not admitted at the MRRH and patients who were already under antibiotic treatment.

3.4 Sample size calculations

The sample size was calculated using the formulae as described by Martin *et al.* (2007).

Sample size number; $n = \frac{Z^2 \times P \times 1\text{-}P}{E^2} = \frac{Z^2 \times P \times Q.}{E^2}$

Where: Z = Confidence interval 1.96, P = Prevalence proportion from the previous study was 0.224, Q – 1= Prevalence proportion from the previous study was 0.224 and E = Standard errors 5%.

Where the 95% CI = 1.96, P = Prevalence proportion = 0.24 (Mhada *et al.*, 2012), Q= 1- P = 1-0.24 and E = Standard Error = 5% = 0.05. The calculated sample size therefore was:

$$n = \frac{1.96^2 \times 0.224\ (1\text{-}0.224)}{(0.05)^2} = 271$$

Hence the minimum required sample size for this study was 271 neonates. However, to increase the precision of the study, a total of 303 neonates were involved in the study.

3.5 Ethical consideration

The permission to carry out this study at Morogoro regional referral hospital was sought from hospital authority and ethical clearance to conduct the study in Tanzania was issued by the ethics review subcommittee of the National Research Coordinating Committee (MRCC) with reference number NIMR/HQ/R.8a/Vol. IX/1626 (Appendix 1) before any of the human sampling exercise started. Participation in the study was on voluntary. Informed consent was obtained from each of the selected participating mother followed by explanation of the purpose and importance of the study prior to sample collection. Mothers who willingly agreed to participate in the study signed the consent form which abided with the rules and regulations of research in human subjects (Appendix 3). Confidentiality of the study participants was strictly observed and after processing the samples, results were given to the medical personnel in the respective paediatric ward for further management and close follow up of the neonates.

3.6 Data collection

3.6.1 Retrospective data

Patient case notes (admissions files) were retrieved from MRRH archives for the period of two years between January 2011 and December 2012. The main focus on the records was to obtain the biodata of patients (age, sex, weight, place of

residence), mode of delivery (normal spontaneous vaginal delivery or caesarean section), at term birth, premature, reasons for admission, if neonatal septicaemia; what were the clinical manifestations, was the definitive diagnosis established?, was there laboratory culture and sensitivity test?, treatment undertaken, prognosis after treatment, and any other basic information. As means of quality control of data, cases with improper recording, diagnosis and ambiguous information on dates of hospitalization were excluded.

3.6.2 Prospective data collection through questionnaires

This study targeted all neonates admitted in paediatric ward suspected to have neonatal septicaemia as per WHO (1995) guidelines. Mothers whose neonates participated in the study were randomly selected whereby, every third mother admitted to the paediatric ward (neonatal unit) were selected to participate in the study. A questionnaire containing information like biodata of patients (age, sex, weight, place of residence), biodata of mothers (age, marital status, level of education, employment, parity number, mode of delivery (normal or caesarean section, birth at term, premature), management of umbilical cord and reasons for hospitalization was administered (Appendix 2) was used for data collection. After interview with the mothers, the selected patients were carefully examined for the clinical presentation and basic health parameters. This aimed to establish any observable clinical manifestations, any observable abnormalities and the general health status of a neonate. Thereafter, the sampling of patients was done aseptically as detailed in the subsequent sections.

3.6.3 Prospective data collection for bacteriological work

3.6.3.1 Sampling and sample handling

Peripheral blood and umbilical pus swab samples were collected from neonates whose mothers or care givers willingly accepted to participate in the present study. Swab samples were collected only from neonates who had their umbilical discharging pus.

3.6.3.2 Blood samples collection

After the consent was obtained, injection site was disinfected using methylated spirit followed by povidone iodine and left to dry, venous blood sample was collected using a sterile syringe and a needle. About 2-5 mls of blood samples from the selected patients were collected aseptically and immediately inoculated into universal containers with brain heart infusion (BHI) broth in a ratio of 2 mls blood and 10 mls BHI broth and were taken to the laboratory within 30 minutes of collection for bacterial culture. The top of the container was also disinfected using methylated spirit before inoculation of the sample in BHI.

3.6.3.3 Umbilical pus swab samples

Swab samples from umbilicus stump discharging pus were aseptically collected by using a sterile swab stick. The swab sample was placed in sterile container before being taken to the laboratory within 30 minutes after collection for bacterial culture.

3.7 Laboratory procedures

3.7.1 Media preparation and storage

Before the culture was done, all the media were prepared in advance and the procedures for preparations were done according to manufacturer's instructions. After the media were prepared they were stored 8°C refrigeration until use. Different types of media were used as detailed in the subsequent sections.

3.7.1.1 Preparation of Brain Heart Infusion (BHI) broth

Brain Heart Infusion (BHI) is composed of calf brain infusion solids 12.5 g, beef heart infusion solids 5 g proteose peptone 10 g glucose 2 g sodium chloride 5 g and Di-sodium phosphates 2.5 g. The medium was prepared according to manufacturer's instructions by dissolving 37 g of (BHI) broth powder (Oxoid® Ltd., Basingstoke, Hampshire, England, UK CM0225 Lot 750364) in 1 litre of distilled water and shaken to dissolve. Then 10 ml of BHI was dispensed into sterile universal bottles and was autoclaved at 121°C for 15 minutes. The media was left to cool and stored under 8°C until use.

3.7.1.2 Preparation of MacConkey agar

MacConkey medium is composed of peptone 20 g lactose 10 g bile salt 5 g sodium chloride 5 g neutral red 0.075 g agar 12 g. The medium was prepared according to manufacturer's instructions by dissolving 52 g of MacConkey powder (Oxoid® Ltd., Basingstoke, Hampshire, England, UK CM0007 Lot 747575) into 1 litre of distilled water, followed by gentle boiling to dissolve completely. The medium was sterilized in the autoclave at 121°C for 15 minutes, cooled to around 40°C in a water bath.

50mls of defibrinated sheep blood was added mixed well gently then placed in a hot water around 80°C for 15 minutes cooled to 40°C and poured in the sterile glass petri dishes at the volume of 20 to 30 mls. The plates were left at room temperature covered for two hours for the media to solidify then incubated for 24 hours at 37°C to check for sterility. The media were stored under refrigeration temperature until use.

3.7.1.3 Preparation of blood agar

Blood agar contains 10 g of beef extract powder, 10 g tryptose, 5 g sodium chloride and 15 g agar. The medium was prepared according to manufacturer's instructions by dissolving 40 g of blood agar powder (Liofilchem® s.r.l. Bacteriology Products Italy REF 610005 lot 102612203) in 1000 ml distilled water, gentle boiled to dissolve completely and autoclaved at 121°C then cooled to 50°C. Aseptically 50 mls of defibrinated horse blood was added in the molten media, mixed thoroughly and poured in the sterile glass petri dishes at the volume of 20 to 30 mls. The plates were left at room temperature for two hours for the media to solidify then incubated for 24 hours at 37°C to check for sterility. Then the media was stored under refrigeration temperature until use.

3.7.1.4 Preparation of chocolate agar

Chocolate agar is prepared by using blood agar base which contains 10 g of beef extract powder, 10 g tryptose, 5 g sodium chloride and 15 g agar. The medium was prepared according to manufacturer's instructions by dissolving 40 g of blood agar powder (Liofilchem® s.r.l. Bacteriology Products Italy REF 610005 lot 102612203) into 1 litre of distilled water, followed by gentle boiling to dissolve completely. The

medium was sterilized in the autoclave at 121°C for 15 minutes, cooled to around 40°C and poured in the sterile glass petri dishes at the volume of 20 to 30 mls. The plates were left at room temperature for two hours for the media to solidify then incubated for 24 hours at 37°C to check for sterility. The media was stored under refrigeration temperature until use.

3.7.1.5 Triple Sugar Iron Agar (TSI)

Triple Sugar Iron Agar (OXOID® Ltd., Basingstoke, Hampshire, England, U.K.) is composed of 3 g 'Lab-Lemco' powder, 3 g Yeast extract, 20 g Peptone, 5 g Sodium chloride, 10. g Lactose, 10. g Sucrose, 1 g Glucose, 0.3 g Ferric citrate, 0.3 g Sodium thiosulphate, 0.024 g Phenol red and 12 g Agar. The medium was prepared by suspending 65 g of powdered medium in 1 litre of distilled water. It was boiled to dissolve completely, mixed well and distributed in to final petri dish. The medium was sterilised by autoclaving at 121°C for 15 minutes. After sterilization was completed, the medium was allowed to set in sloped form with a butt about 1 inch deep.

3.8 Laboratory culture of samples

3.8.1 Blood samples

Each universal bottle with blood sample in BHC was incubated under aerobic conditions at 37°C for 24 hours. Universal bottles were observed for turbidity and cloudiness. Clouded blood samples were subcultured on blood agar, chocolate agar and MacConkey agar and incubated at 37°C under aerobic conditions and assessed for bacterial growth after 24, 48, 72 and 96 hours of incubation. For isolation of

special bacteria like *Listeria monocytogens*, special microaerophilic incubation condition was used. The clouded blood samples were subcultured on blood agar and chocolate agar and after inoculation, the petri dishes were loaded in the anaerobic jars (Cold stream Engineering Ltd. 18-10, Arista Sweden) containing a lit candle and closed tightly. The jars were placed in incubators at 37°C and assessed for bacterial growth after 24, 48, 72 and 96 hours of incubation. Suspected *Listeria* spp. colonies were purified by sub culturing on blood agar base (Oxoid® Ltd., Basingstoke, U.K.) and re-incubated at the same culture environment for 24 hours before bacteria identification.

3.8.2 Swab samples

Swabs samples were first smeared on a glass slides for Gram stain identification. Gram positive bacteria appeared dark purple and Gram negative bacteria appeared pale to dark red; followed by inoculation of the sample on blood agar, chocolate agar and MacConkey agar and incubated under the same conditions as for blood samples. The culture plates were assessed for bacterial growth after 24, 48, 72 and 96 hours of incubation. Suspected bacteria colonies were purified by subculture on blood agar base and re-incubated at the same culture environment for 24 hours before bacteria identification.

3.9 Bacteria identification

3.9.1 Morphology

Assessment of bacteria colony morphology characteristics on solid agar plates was used as first stage for identification. In most cases, the common bacteria colonies

observed were medium to large with sharp borders, round and convex in shape with creamy to golden colour, and some with zones of clear beta-haemolysis. Such colonies were suspected to be of *Staphylococcus* spp. Colonies which appeared small, shiny or dry with grey-white or colourless appearance on blood agar were suspected to be of *Streptococcus* spp. Other bacteria colonies like *E. coli* colonies appeared medium to small dry flat pink reddish colonies on MacConkey plate agar. *Klebsiella* spp were large mucoid colonies with odour of fresh bread and *Pseudomonus aeruginosa* appeared small rough colonies on blood agar plate with beta hemolytic, with grape like smell.

3.9.2 Gram staining technique

The Gram stain of the bacterial colony was done on sterile glass slide as described by Hans Christian Gram in 1884. Briefly, a drop of normal saline was put on a glass slide and loop full of bacteria colony was added and made a smear which was dried in air and fixed on flame. The fixed smear was flooded with crystal violet stain for 30 – 60 seconds, washed with tap water and flooded again with Lugol's iodine for 30 – 60 seconds followed by second washing with tap water. Acetone-alcohol was used to decolorize the smear before the third washing was applied. The smear was then with counterstained with neutral red that stayed for 2 minutes then washed off with tap water. The back of the slide was wiped clean and placed on a draining rack for the smear to air dry. A drop of oil immersion was added on the smear and examined under the light microscope first at 40X objective to check the staining and the distribution of material and then at 100X objective to visualize the morphology

of the bacteria. Gram positive bacteria appeared dark purple and Gram negative bacteria appeared pale to dark red.

3.9.3 Biochemical tests

Standard biochemical tests were performed on pure colonies for identification of different bacteria isolated.

3.9.3.1 Catalase test

A thick smear of each bacteria colony was made on a sterile glass slide to which a drop of 2 – 3 ml of 3% hydrogen peroxide was added. A positive catalase reaction was based on appearance of effervescence within few seconds.

3.9.3.2 Coagulate test

This test is used to identify *S. aureus* which produces the enzyme coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. The rabbit plasma was allowed to warm to room temperature before being used. A drop of distilled water was placed on each end of a slide on two separate slides. A colony of the test organism was emulsified in each of the two drops to make two thick suspensions and a loopful of plasma to one of the suspensions was added and mixed gently. Presence of clumps of the organisms within 10 seconds means a positive for coagulase test for *S. aureus*.

3.9.3.3 Indole test

This test is very useful in testing *E. coli* through determination of the organism's ability to produce indole from deamination of tryptophan by tryptophanase. A filter paper was moistened with indole reagent, using sterile loop stick rubbed a portion of colony onto the moistened filter paper. Colour change within 2 minutes to light blue indicates the presence of indole.

3.9.3.4 Urease test

Testing for urease enzyme activity is important in differentiating enterobacterecea. Protein strains are strong urea producers. The test organism is cultured in a medium which contains urea in presence of the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea to give ammonia and carbon dioxide with the release of ammonia. The medium become alkaline by change in colour of the indicator to pink red that indicate a positive urease test.

3.9.3.5 Oxidase Test

Oxidase test was used to detect *P. aeruginosa* by its ability to produce the enzyme cytochrome oxidase. A filter paper on the petri dish was moistened with Kovac's oxidase reagent. A sterile wire loop was stickled and rubbed a portion of colony onto the moistened filter paper then colour change within 10-60 seconds confirmed a deep blue to purple colour confirming a positive reaction of *P. aeruginosa.*

3.9.3.6 Triple Sugar Iron (TSI)

Klebsiella spp. organisms were biochemically identified by using Triple Sugar Iron (TSI). A pure colon cultured was inoculated to the agar slant surface and stubbed the butt of the agar and incubated at 37°C for 24 hours. Black colour of the content in the tube meant there was production of hydrogen sulphide which confirmed presence of *Klebsiella* spp.

3.9.4 Antimicrobial sensitivity testing

Antimicrobial sensitivity testing to all bacteria isolates was performed for the treatment of neonatal sepsis in Tanzania which includes ampiclox, gentamicin, ceftriaxone, ampicillin, tetracycline, cotrimoxazole, erythromycin and ciprofloxacin. The procedure was performed on Muller Hinton (MH) Agar by agar disc diffusion method as described by Luangtongkum *et al.* (2007) with some modifications. Each of the bacteria suspensions were prepared in a sterile normal saline and the suspensions adjusted to a turbidity equivalent to a 0.5 McFarland standard. Sterile cotton-tipped swabs were used to transfer the inocula onto Mueller-Hinton plates to produce a confluent lawn of bacterial growth. After the inocula on the plates dried, the test antibiotic discs were distributed over the inoculated plates by using a sterile forceps. The plates were incubated at 37°C for 24 hours. After the incubation period, the plate cultures were examined for inhibition zones around the discs. Results were recorded as resistant or sensitive based on absence or presence of zone of inhibition respectively (Lennette, 1995) and the diameters of inhibition zones were measured with slipping callipers. For test and interpretation of the results the general guidelines of NCCLS (2002) and Gaudreau and Gibert (1997) were followed.

3.10 Data management and analysis

Collected data were verified, cleaned and followed by a double entry system and were performed by using a Microsoft excel data base. Analysis was achieved by using a logistic regression SAS interpretation of the results was done. The laboratory data was analysed by using Epi Info version 7 statistical software (Coulombier *et al.*, 2001). To compare the proportions (%) of neonatal septicaemia between categories, chi square was used at critical probability of $P<0.05$ at 95% confidence interval using Stat Calc function on Epi Info Version 7. The proportions considered in the comparisons of the neonatal septicemia included the possible risk factors like sex, age, birth weight and clinical features for neonatal septicaemia (fever, difficulty in breathing, tachycardia, malaise and lethargy). Other signs include inability to breast feed, convulsion, chest wall in-drawing, jaundice and umbilical redness which are strongly associated with neonatal sepsis were determined.

CHAPTER FOUR

4.0 RESULTS

4.1 Retrospective data

4.1.1 General results

Retrospective data were obtained from hospital records where 4051 files of neonate patients admitted between 2011 and 2012 were reviewed. It was found that 2178 (53.7%) of the admitted neonates had clinical septicaemia. A significantly higher number (P= 0.022) of clinical neonatal septicaemia cases were recorded in 2011 (55.8%, n = 1812) than in 2012 (52.1%, n = 2239).

4.1.2 Demographic and neonatal septicaemia clinical features

The demographic characteristics of the neonates with clinical septicaemia are summarized in Table 1. Age, sex and birth weight were significant factors for clinical neonatal septicaemia. Table 2 shows the clinical characteristics of the study participants admitted as septicaemia cases. The most frequently reported clinical features included fever, inability to breast feed and difficulty in breathing. These data can further be sub divided in early and late neonatal septicemia which is differentiated by the age of the neonates. Early-onset neonatal septicemia is observed during the first week of life, whereas the late-onset septicemia occurs between day 7 and 28 of life.

Table 1: Demographic characteristics of the retrospective neonatal septicaemia cases at Morogoro Regional Referral Hospital, Tanzania

Parameter	Category	In 2011 (n=1812) Number (%) affected	P value	In 2012 (n=2239) Number (%) affected	P value	Total
Age (days)	0 – 6	874 (60.9)	0.0000	1043 (58.2)	0.0000	1917 (59.4)
	7 – 28	137 (36.3)		124 (38.5)		261 (31.7)
Sex	Male	542 (56.2)	0.7702	706 (56.3)	0.0000	1248 (57.0)
	Female	469 (55.4)		461 (46.8)		930 (43.0)
Weight (g)	≤2500	178 (27.1)	0.0000	170 (27.9)	0.0000	348 (16.0)
	>2500	833 (72.2)		947 (58.1)		1780 (82.0)

Table 2: Clinical characteristics of the retrospective neonatal septicaemia cases at MRRH, Tanzania

Clinical characteristics	In 2011 (n=1011) Septicaemia number (%) with signs	In 2012 (n= 1167) Septicaemia number (%) with signs	Total (%)
Fever	876 (86.7)	988 (84.7)	1864 (85.5)
Hypothermia	135 (13.4)	51 (4.4)	186 (8.5)
Inability to feed	444 (43.9)	485 (41.6)	929 (42.6)
Difficulty in breathing	291 (28.8)	369 (31.6)	660 (30.3)
Convulsions	99 (9.8)	107 (9.2)	206 (9.5)
Umbilical pus discharge	224 (22.2)	403 (34.5)	627 (28.8)
Jaundice	10 (0.9)	29 (2.5)	39 (1.8)
Skin rashes	2 (0.2)	6 (0.5)	8 (0.4)
PMTCT - Yes	51 (2.3)	38 (1.7)	89 (4.1)
- No	949 (44.0)	1119 (51.0)	2068 (95.0)
– Unknown	11 (0.5)	10 (0.5)	21 (1.0)

PMTCT = Prevention of Mother to Child Transmission

4.1.3 Distribution of neonatal septicemia according to the outcomes of the disease (recovered or non-recovered) in 2011 and 2012 years

The distribution of cured and death cases of neonatal cases are shown in Figure 2. Analysis of the retrieved files for two years (2011 and 2012) revealed that a total of 1901 (87.3%) neonatal septicaemia cases cured while 277 (12.7%) died. In 2011, a total of 898 (88.8%) neonatal septicaemia cases cured and 113 (11.2%) died while in 2012 a total of 1003 (85.9%) cases recovered and 164 (14.1%) died. Statistically, comparison of mortality differences between the two years was not significant (P=0.052). The monthly distribution shows more neonatal septicemia cases were recorded between March and May with little increase in November and December for both years. In February of 2012 many cases were recorded compared to February of 2011.

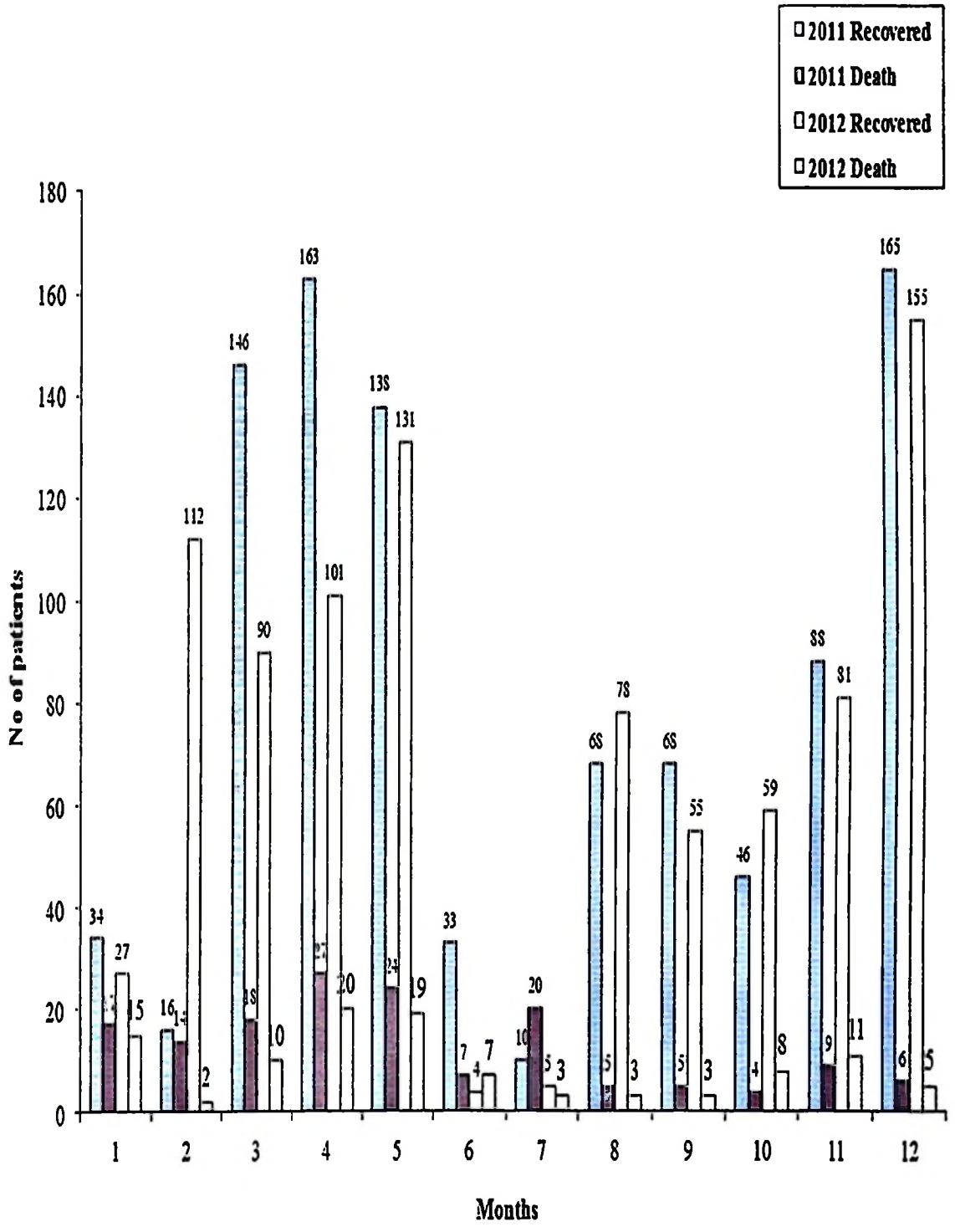

Figure 2: **Monthly distribution of neonatal septicaemia cases attended at MRRH, Tanzania for the recovered and non-recovered cases in 2011 and 2012 years**

4.1.4 Laboratory culture, drug sensitivity and treatment

During review of records in files, it was realized that all the neonatal septicaemia cases were diagnosed clinically (based on clinical signs and symptoms). No laboratory investigation was done to confirm neonatal septicaemia cases. As for the treatment, the records showed that the most commonly used antibiotic was ampiclox (ampicillin and Cloxaxcillin) (97%) and in most cases it was combined with gentamycin. About 3% of all cases were treated with ceftriaxone and 2.4% of patients received a combination of all three types of antibiotics. The records further showed that most of the neonatal septicaemia cases were clinically managed such that cases with hypoglycaemia were being managed by administration of 10% dextrose, convulsion cases were treated with antipyretic and phenobabitone and prematurity was treated with vitamin K together with a special care in incubator.

4.2.0 Prospective data

4.2.1 General results

Prospective study involved collection of data through questionnaire and sampling. A total of 303 neonates with a clinical diagnosis of septicaemia were involved in the study and their mothers/care givers were administered with questionnaires. A total of 880 neonates were excluded from the study due to the criteria stated under 3.3 above.

4.2.2 Demographic characteristics of the respondents (mothers/care givers of neonates)

The demographic data of 303 respondents involved in the study are shown in Table 3. Majority of the respondents had the age between 21 and 30 years, were married,

their ethnic group was mostly Lugulu and the level of education the majority of respondents was primary school. It was also established that many of the mothers/care givers were housewives with the monthly income below 50 000 Tanzanian shillings.

Table 3: Demographic characteristics of mothers/care givers of neonates at MRRH, Tanzania

(N=303)

Parameter	Category	Number	Percent
Age (in years)	15 – 20	74	24.4
	21 – 30	180	59.4
	31 – 40	48	15.8
	Above 40	1	0.3
Marital status	Single	83	27.4
	Married	195	64.4
	Widow/Widower	13	4.3
	Divorced	12	4.0
Level of education	No formal education	57	18.8
	Primary education	126	41.6
	Secondary education	75	24.8
	College	45	14.8
Ethnicity (tribe)	Lugulu	121	40.0
	Chagga	36	11.9
	Pogoro	16	5.3
	Ngoni	15	5.0
	Others*	115	37.8
Religion	Muslim	144	47.5
	Christian	159	52.5
Occupation	Peasants	53	17.5
	Employees	96	31.7
	Business	50	16.5
	House wife	91	30.0
	Students	10	3.3
	Pastoralists	3	1.0
Income	Below 50,000	113	37.3
	51,000 - 300,000	93	30.7
	301,000 - 500,000	60	19.8
	Above 500,000	37	12.2

Others* means tribe less than 15 were included in others category

4.2.3 Demographic and clinical characteristics of the study neonates

The demographic and clinical characteristics of neonates with septicaemia are summarized in Table 4. Majority of neonates with clinical septicaemia were in the age between zero and six days. Male neonates with clinical septicaemia constituted a larger group and most of the neonate deliveries were at term. The most frequently observed clinical features of neonates with septicaemia included fever, difficulty in breathing and inability to breast feed.

Table 4: Demographic and clinical characteristics of neonates with septicaemia at MRRH, Tanzania – prospective study

(N= 303)

Parameter	Category	Number of patients	Percentage
Age (days)	0 – 6	276	91.1
	7 – 28	27	8.9
Sex	Male	185	61.1
	Female	118	38.9
Weight (g)	≤2500	40	12.9
	> 2500	265	87.1
Maturity	Preterm	13	4.3
	Term	290	95.7
Clinical characteristics	Fever	302	99.7
	Inability to breastfeed	230	75.9
	Chest wall indrawing	33	10.9
	Convulsions	94	31.0
	Umbilical pus discharge/hyperaemia	17	5.6
	Jaundice	11	3.6
	Skin rashes with pustules	1	0.3
	Hypothermia	1	0.3
PMTCT	-No	299	98.7
	-Yes	4	1.3

PMTCT = Prevention of Mother to Child Transmission

4.2.4 Bacteria isolation in relation to age

A total of 303 blood samples were collected for culture from neonates with clinical septicaemia. In addition, 17 neonates who had their umbilical cords discharging pus were sampled and the swab samples subjected to bacterial culture. The culture results generally indicated that 41 (13.5%) neonates with clinical septicaemia whose blood and pus samples were cultured had bacterial septicaemia (Table 5). *S. aureus, E. coli* and *Klebsiella* spp. were the commonly isolated bacteria from the blood especially in cases with early-onset neonatal septicaemia while *S. aureus* and *P. aeruginosa* were commonly isolated in pus samples of early-onset neonatal septicaemia cases. Generally, the dominant bacteria causing infection in blood were *S. aureus* and *E. coli*. It was found that 8 pus swabs (47.1%) had growth of bacteria again being dominated by *S. aureus* and *P. aeruginosa*. Interestingly, all the neonates with pus swab culture positive samples had their blood also culture positive although only one neonate had the same species of bacterial infection (*S. aureus*) in pus and blood sample.

Table 5: Bacteria isolation in relation to age (days) of neonates at MRRH, Tanzania

Bacteria species	Number (%) of bacteria isolated from blood samples (n= 33)		Number (%) of bacteria isolated from Swabs samples (n=8)	
	0 – 6 days	7 – 28	0 – 6	7 – 28
Staphylococcus aureus	14 (87.5)	2 (12.5)	3 (75.0)	1(25.0)
E. coli	11 (84.6)	2 (15.3)	0 (0.0)	0 (0.0)
Klebsiella spp.	3 (75.0)	1(25.0)	1 (100.0)	0 (0.0)
Pseudomonas aeruginosa	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)

4.2.5 Factors associated with neonatal septicemia

Different factors were considered to be associated with neonatal septicaemia both on the maternal and neonatal sides (Table 6). Maternal factors like fever, per vaginal examination and history of caesarean section were found to be statistically significant factors ($P<0.05$) associated with neonatal septicaemia, confirmed through laboratory culture. Nevertheless, some of neonatal clinical characteristics like umbilical pus discharge / hyperaemia; poorly cared cord and wrapping of umbilical cord with a piece of cloth (Figure 3) were found to be statistically significant predicting factors for laboratory confirmed neonatal septicaemia. Several other maternal factors and neonatal clinical features were considered but statistically were not significant (Table 6). It was noted from the questionnaire that there was different types of umbilical cord care that were reported by mothers/care givers of the neonates namely application of methylated spirit 178 (58.7%), wrap a piece of cloth 94 (31.0%), wash with warm water 16 (5.3), apply coconut oil 13 (4.3%) and two respondents said they don't do anything.

Table 6: Maternal perinatal factors and clinical characteristics of neonates in relationship to laboratory culture results at MRRH, Tanzania

Variable	Number (%) of culture positive cases	Number (%) of culture negative cases	OR	95% CI	P value
Maternal perinatal factors					
Fever	13 (16.5)	66 (83.5)	0.439	0.199-0.967	0.0411*
PROM >=18Hrs	9 (30.0)	21 (70.0)	0.586	0.227-1.514	0.6134
Twin pregnancy	2 (40.0)	3 (60.0)	1.219	0.463-3.2121	0.4419
Primegravida	15 (13.2)	99 (86.8)	1.762	0.416-7.471	0.9918
PVE	15 (7.5)	185 (92.5)	13.9084	2.0851 - 92.7743	0.0065*
History of C/S	6 (46.2)	7 (53.9)	12.843	1.904-86.487	0.0087*
History of SVD	27 (9.3)	263 (90.7)	1.223	0.560-2.674	0.6886
Neonates clinical characteristics					
Convulsions	13 (13.8)	81 (86.2)	1.08	0.53 – 2.20	0.9730
Chest wall in drawing	4 (12.1)	29 (87.9)	0.89	0.29 – 2.70	0.9370
Umbilical pus discharge / hyperaemia	10 (58.8)	7 (41.2)	16.3	5.68 – 46.95	0.0000*
Failure to suckle	33 (14.3)	197 (85.7)	0.63	0.27 – 1.49	0.3960
Hypoglycaemia	11 (8.9)	113 (91.1)	0.50	0.24 – 1.05	0.0930
Fever	41 (13.6)	261 (86.4)	-	-	0.2760
Difficulty in breathing	4 (13.3)	26 (86.7)	1.01	0.33 – 3.07	0.7930
Jaundice	3 (27.3)	8 (72.7)	2.58	0.66 – 10.18	0.3420
Poorly cared cord	36 (28.8)	89 (71.2)	5.74	122.49 - 788.79	0.0002*
Wrap piece of cloth on umbilical cord	26 (27.7)	68 (72.3)	5.33	2.50 – 11.47	0.0000*

PROM =Premature Rupture of Membranes, PVE =Per Vaginal Examination,
C/S = Caesarean Section SVD =Spontaneous Vertex Delivery

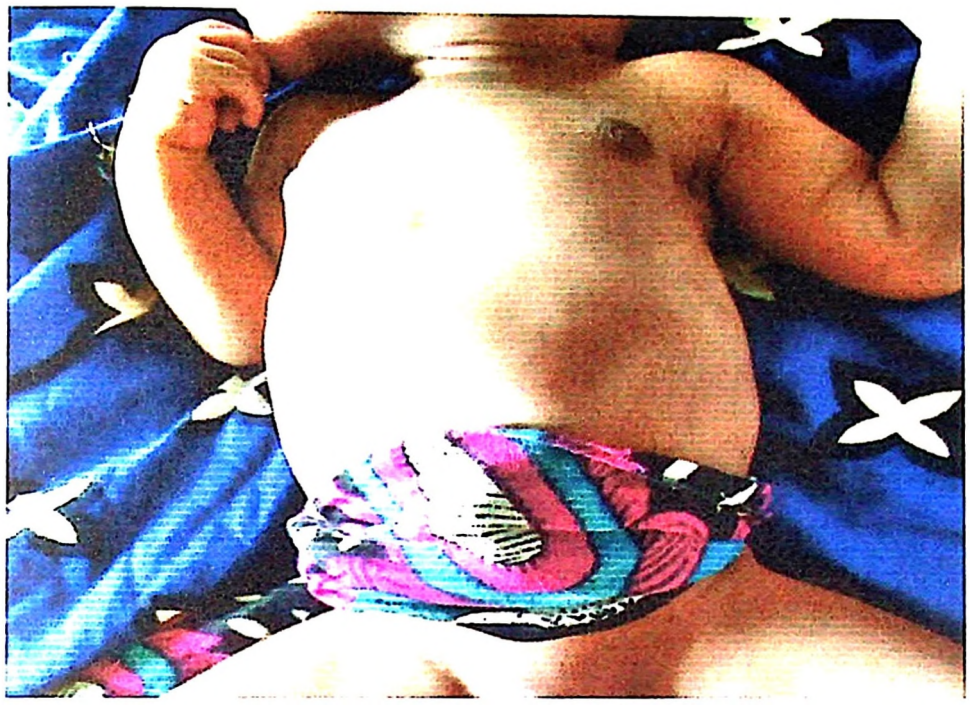

Figure 3: **A three days neonate with his umbilical cord wrapped with a piece of cloth at MRRH, Tanzania**

4.2.6 Antimicrobial sensitivity patterns of isolated bacteria from blood

Antimicrobial sensitivity test of isolated bacterial from blood and pus samples was performed against different antibiotics which are commonly used to treat neonatal septicaemia and the results are shown in Table 7. It was observed that *S. aureus* and *E. coli* were sensitive to ampicillin, cefazolin, gentamycin and ceftriaxone while they showed high rates of resistance to amikacin, cloxacillin and tetracycline. *Klebsiella* spp. and *P. auriginosa* were resistant to most of the antibiotics tested.

Table 7: Antibiotic sensitivity pattern of isolated bacteria (numbers and percentages) from neonates at MRRH, Tanzania

Bacteria	Proportion of isolates sensitive to different antimicrobial agent (%)						
	Ampicillin	Cefazolin	Gentamycin	Ceftriaxone	Amikacin	Cloxacillin	Tetracycline
Bacteria isolated from blood							
S. aureus (n=16)	12 (75.0)	14 (87.5)	13 (81.3)	16 (100.0)	12 (75.0)	10 (62.5)	8 (50.0)
E. coli (n=13)	10 (76.9)	11 (84.6)	12 (92.3%)	11 (84.6)	9 (69.2)	7 (53.9)	6 (46.2)
Klebsiella spp. (n=4)	2 (50.0)	3 (75.0)	2 (50.0)	2 (50.0)	1 (25.0)	1 (25.0)	1 (25.0)
Bacteria isolated from umbilical pus							
S. aureus (n=4)	2 (50.0)	3 (75.0)	2 (50.0)	3 (75.0)	0 (0.0)	2 (50.0)	1 (25.0)
Klebsiella spp. (n=1)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
P. auriginosa (n= 3)	0 (0.0)	1 (33.3)	2 (66.7)	1 (33.3)	3 (100.0)	0 (0.0)	0 (0.0)

4.2.7 Infection outcome of the 303 neonates of prospective study

This study involved 303 neonates who were taken samples among them 279 (92.1%) were cured from the disease and were discharged home. Unfortunately during the follow up period 24 (7.9%) died due neonatal septicaemia. Of the 24 neonates who died, 26.8% had positive blood culture compared to 5% with negative blood culture (*P = 0.001*).

CHAPTER FIVE

5.0 DISCUSSION

The purpose of this study was to determine the epidemiology of neonatal septicaemia in neonates hospitalized at Morogoro Regional Referral hospital and establish the common bacteria involved and their susceptibility profiles to antibiotics commonly used in treatment. This is in response to the fact that neonatal septicaemia is reported to be among the leading causes of neonatal morbidity and mortality in Tanzania (Kazaura *et al.*, 2003; Klingenberg *et al.*, 2003; Blomberg *et al.*, 2007; Manji, 2009; Kayange *et al.*, 2010; Mhada *et al.*, 2012) and other developing countries (Lawn *et al.*, 2005). In addition, the trend of multi-antibiotic resistance of bacteria isolates from cases of neonatal septicaemia is increasing, reduces recovery and endangers survivals of infected neonates (Blomberg *et al.*, 2007; Mshana *et al.*, 2009; Moyo *et al.*, 2010; Mhada *et al.*, 2012). Results of the current study showed that neonatal septicaemia is among the major diseases affecting neonates at Morogoro Regional Referral hospital. Retrospective data based on clinical neonatal septicaemia established a prevalence of 53.7% while prospective data based on culture positive samples established a prevalence of 13.5% with a number of bacteria showing multi-antibiotic resistance. Mortality rate due to neonatal septicaemia recorded during the retrospective study was 12.7% while during prospective study was 7.9%. This shows that neonatal septicemia is a serious problem to neonates and deliberate measures need to be taken to minimize the magnitude of the problem. The results of this study indicated a high magnitude of neonatal sepsis at MRRH, which may reflect a number of predisposing factors including the maternal factors during pregnancy and delivery

process, low quality of neonatal care especially the umbilical cord and poor hospital services.

5.1 Prevalence study

Retrospective data of the current study show that the prevalence of neonatal septicaemia was 53.7% among the admitted neonates in paediatric ward at MRRH between 2011 and 2012. This hospital records results are comparable to the previously reported study by Moyo *et al.* (2010) who established a prevalence of 48.8% at Muhimbilli National Hospital and Kayange *et al.* (2010) at Bugando Medical center who reported 38.9% cases of clinical neonatal septicemia. However, results of the prospective study of the current study showed 13.5% of neonates with clinical septicaemia were confirmed to have bacteria septicaemia. This laboratory confirmed results of neonatal septicaemia are comparable to those of Blomberg *et al.* (2007) who reported neonatal septicaemia prevalence of 13.9% at Muhimbilli National Hospital. A study by Klingeberg *et al.* (2003) at Kilimanjaro Christian Medical Center in Tanzania reported a lower infection rate (6.5%) compared to the current study. The incidence of neonatal septicemia is variable and differs from place to place, because it depends on various factors like gestational age, foetal birth weight, maternal nutrition, perinatal care and hygienic conditions, child health care facilities and many other factors. The results of the current study generally showed that the problem is big and parents have been incurring a lot of costs in terms of money and time spent when hospitalized in pediatric wards.

It was further observed that the prevalence of neonatal septicaemia recorded during retrospective study was much higher (53.7%) compared to the laboratory confirmed cases (13.5%) during prospective study. Such a big difference may be due to diagnosis of neonatal septicaemia cases based on clinical manifestations as per criteria set by WHO (WHO, 1995; English *et al.*, 2004) without laboratory confirmation. Moreover, there might be oversights of other causes of febrile illness which can easily be misdiagnosed as neonatal septicaemia. Other cause of such differences may be poor record keeping, history taking and improper final diagnosis.

Indeed, the two years records of neonatal cases visited during the current study revealed that most of the neonatal septicaemia cases were recorded between March and May, and during November and December. This is very interesting because it exactly coincided with the period of rainfall in Morogoro region. This finding was associated with possibilities for high umbilical cord infections. The current study form a basis for hypothesizing that during wet season, the wound healing rate is reduced and there is also high fly activity which may likely carry infectious bacteria to the umbilical cord and become the sources of infection. The hands of the mothers and care givers also may easily become vehicles for infectious bacteria especially during the rainy season. We propose for further studies to confirm this observation.

5.2 Clinical features

The retrospective study revealed that as septicemia cases were diagnosed based on clinical manifestations as per criteria set by WHO (WHO, 1995; English *et al.*, 2004). The most frequently reported clinical features during retrospective study

included fever, inability to breast feed, convulsions and difficulty in breathing. Similar features were also observed during prospective study. Indeed these features were used as predictors of positive blood and umbilical swab culture as also reported by Iregbu *et al.* (2006). These clinical signs and symptoms complied with those laid by WHO young infants study groups (WHO, 1999) which were recommended to be used in the areas with limited facilities to predict positive blood culture and initiation of proper empirical management.

Similar observations have been reported in several other studies of neonatal septicaemia in Tanzania and elsewhere (English *et al.*, 2004; Mugalu *et al.*, 2006; Kayange *et al.*, 2010; Mhada *et al.*, 2012; Kheir and Khair, 2014; Mustafa and Ahmed, 2014). Therefore, considering the limited availability of culture and antimicrobial sensitivity services in most of health faculties in Tanzania, the use of common clinical features for neonatal sepsis is important. In fact, even in places where the culture services are available, the clinicians are advised to start treatment with antibiotics for any of the suspected cases of neonatal septicaemia while are waiting for the laboratory results which otherwise takes not less than 24 hours.

In the present study, almost all neonates clinically diagnosed as neonatal septicaemia had fever and all confirmed culture positive cases of septicaemia had fever. This suggests that septicaemia was among the causes of fever syndrome observed. It is known that neonatal septicaemia is among the fever causing conditions which are normally misdiagnosed with other common fever causing agents and conditions to the neonates like malaria and dehydration fever. A study by Crump *et al.* (2013)

established bacteraemia to be a significant cause of fever in infants and children. Similarly a study by D'Acremont *et al.* (2014) established that 22.0% of fever cases in children were due to bacterial infections, and 10.5% had malaria. Moreover from the current study neonates who had fever observed during the current study and were not confirmed to have positive blood culture for neonatal septicaemia might have been misdiagnosed with other febrile causing syndrome like dehydration fever, malaria, non bacterial pneumonia, dehydration and other. Indeed, majority of the neonates with fever after were well fed and sometimes administered with 10% dextrose, the fever syndrome subsided. Therefore dehydration fever was likely to be the major problem affecting majority of the neonates admitted at Morogoro hospital suspected of being neonatal septicaemia and other conditions. However, the neonates with fever but culture negative might have started using antibiotics such that possibilities for isolating any bacteria became minimal.

5.3 Significant factors associated with infection in neonates

Retrospective and prospective results of this study further established that age, sex, birth weight and premature of neonates were found to be significant factors for clinical neonatal septicaemia recorded during the study. Neonates of zero to six days of age were more affected signifying that early-onset neonatal septicemia is more prevalent compared to late late-onset neonatal septicemia. The results of this work concur with the findings by Mhada *et al.*, (2012); Mustafa and Ahmed (2014) both reported significantly high prevalence of early-onset neonatal septicaemia compared to late-onset. A study conducted at Bugando hospital in Tanzania by Kayange *et al.* (2010) did not establish a significant difference on the prevalence between the early-

onset and late-onset neonatal septicemia. However a study by Mugalu *et al.* (2006) at Mulago Hospital in Uganda reported high prevalence of neonatal sepsis in neonates of more than 8 weeks of age. Generally, early onset can be due to prematurity, low birth weight and unhygienic conditions during labour. Vertical transmission of bacteria during labor or delivery may result in an invasive infection in the newborn infant during the first week of life (Mayor-Lynn *et al.*, 2005). However, the too young newly born babies (0-6 days of age) are still very tender with soft skin and prone to entry of different pathogens. In addition, the umbilical cord is still raw such that any kind of mismanagement may lead to infection.

Moreover, the current study established higher neonatal septicaemia in male neonates compared to female neonates. This is comparable to a report by Mustafa and Ahmed (2014). The reason for male preponderance is unknown, but this could be due to sex-dependent factors. The reason advanced by Khatua *et al.* (1986) is that the synthesis of gamma globulins is probably regulated by X–linked immunoregulatory genes and as males are having one X chromosome, be more prone to neonatal septicemia than females. More studies may need to be carried out because other studies did not observe any differences in neonatal sepsis between sexes (Mugalu *et al.*, 2006; Kayange *et al.*, 2010; Mhada *et al.*, 2012).

Our results further showed that the majority of neonatal septicaemia cases were those with birth weights equal or above 2500 gm which is the normal delivery weight of neonates at term. Weight less than 2500 gm which may be associated with prematurity are among the factors known to predispose a neonate to sepsis (Moro *et*

al., 1996; Utomo, 2010). It was reported that neonates with low birth weight and the premature are relatively immunodeficient, a state that predispose them to infection (Shah *et al.*, 2006; Utomo, 2010). In addition, this group of neonates normally get some invasive procedures and monitoring which may lead to nosocomial infection (Shah *et al.*, 2006; Utomo, 2010).

Maternal factors associated with confirmed neonatal septicaemia included fever, per vaginal examination and history of caesarean section. Feverish mothers in the current study had their neonates noted to have higher frequency of sepsis as compared to those with normal body temperature. Similar findings have also been reported by Schuchat *et al.* (2000). Fever in delivery mothers predisposes a neonate to septicaemia due to ascending infections. Mugalu *et al.* (2006) observed that there was no significant difference of neonatal septicaemia in neonates born from feverish and non-feverish mothers. Cimolai and Roscoe (1995) reported high possibilities for bacteremia in infants born to mothers with fever. When a mother has fever may suggest some infections which can easily be carried to a baby. In fact per vaginal examination was found to be among the possibilities that may lead to infection to mothers that could possibly be carried to neonates.

History of caesarian section to mothers was established to be among the factors for neonatal sepsis compared to the other modes of delivery which were spontaneous vertex delivery and assisted vacuum delivery. A study by Utomo (2010) also revealed that neonates born from caesarian section have a higher risk to become septic than non-caesarian section. It is believed that caesarian section may contribute

to the changes of normal flora in the infant. Normal floras in infants have a role in immunity system of infant so changes in the normal flora may lead to a risk of septicaemia (Utomo, 2010).

Per vaginal examination in pregnant or delivering mothers was also a significant factor associated with neonatal septicaemia cases. This contributes much as some of the medical personnel do vaginal examination for cervical dilation without proper hand swabbing/cleaning before examining. Sometimes the equipment used may be contaminated which ultimately may also cause infection to mothers who will vertically infect the neonates and cause early-onset neonatal septicaemia.

A history of umbilical pus discharge with hyperemia, poorly cared cord and wrapping of umbilical cord with a piece of cloth were significantly associated with neonatal septicaemia. Indeed, these neonatal clinical characteristics significantly served as predictors of laboratory confirmed neonatal septicaemia. A pus discharging umbilical cords suggests that there was infection on the umbilicus which serves as a conduit of infection to the blood. In a study by Kayange *et al.* (2010) at Bugando hospital in Tanzania and Mugalu *et al.* (2006) at Mulago hospital in Uganda established a significantly high culture positive neonatal sepsis cases from neonates with pus discharging umbilical cords. Interestingly, during the current study it was found that majority of the babies had their umbilical cords wrapped with a piece of cloth (Figure 3) in belief that they would be protected from becoming impotent once the piece of a cord touches the reproductive organs in the due process of falling off. This predisposed the umbilical cord to infection which positively contributed to the

observed prevalence of neonatal septicaemia at MRRH. Neonates whose umbilical cord were wrapped with piece of cloth on were 5.33 times ($P=0.001$) more or less likely to acquire the infection compared to the neonates who were not wrapped with the piece of cloth.

5.4 Neonatal mortality

It was further observed that during the two years of retrospective study neonatal mortality due to neonatal septicaemia was 12.7%. However, during prospective work 8.0% neonate mortality due to septicaemia was recorded of which 26.8% of these belonged to the positive blood culture neonates. These results are comparable to what was reported by Kayange *et al.* (2010) at Bugando hospital Tanzania and Mugalu *et al.* (2006) at Mulago hospital in Uganda. However, higher death rate (34.9%) due to neonatal septicaemia has been reported by Blomberg *et al.* (2007) at Muhimbilli National Hospital compared to what has been documented in the current study but within a range (25-54%) commonly reported in developing countries (Lawn *et al.*, 2005; Klingenberg *at el.*, 2003). This can be explained by relative similar management practices and similar hospital services.

It has been reported that neonatal mortality due to septicemia significantly carries much higher mortality to neonates equivalent to or more than malaria (Berkley *et al.*, 2005; Blomberg *et al.*, 2007). Indeed, as for the finding of the current study neonatal septicaemia is the second cause of death among the neonates admitted in the prem unit at MRRH after birth asphyxia. Neonates are always very delicate and their body immunity is still very weak such that blood stream infection is dangerous and may be

fatal as has been observed during this study. It is therefore recommended that whenever there is suspicion of neonatal septicemia before laboratory confirmation and antibiotic sensitivity of involved agent prompt antimicrobial treatment is imperative for the survival of patients (Kumar *et al.*, 2006). Although some of the dead cases of neonates were already under antibiotic therapy, several reasons could be considered including late commencement of treatment and problems of antibiotic resistance as was observed in the current study and elsewhere (Blomberg *et al.*, 2007; Mshana *et al.*, 2009; Kayange *et al.*, 2010).

5.6 Aetiology of neonatal septicaemia

The current study further found that *S. aureus, E. coli* and *Klebsiella* spp. were the commonly isolated bacteria from the blood especially in cases with early-onset neonatal septicaemia while *S. aureus* and *P. aeruginosa* were commonly isolated in pus samples again of early-onset neonatal septicaemia cases. This indicates that the bacteria are the common causes of neonatal septicaemia and possibly contributes to the recorded high mortality rate of neonates at MRRH. Different studies in Tanzania and other East African countries have isolated more or less similar bacteria especially *S. aureus*, *E. coli* and *Klebsiella* spp. suggesting that they are the common causes of neonatal septicaemia in African countries (Berkley *et al.*, 2005; Mugalu *et al.*, 2006; Blomberg *et al.*, 2007; Kayange *et al.*, 2010; Mhada *et al.*, 2012). Other bacteria which are also common causes of neonatal sepsis are *Enterobacter* spp., *Streptococcus* spp., *Acinetobacter* spp., *Listeria* spp., *Pseudomonas aeruginosa*, *Salmonella* spp. and *Enterococcus* spp. Infection with all these bacteria may originate from the mother during delivery or handling of the neonate. However, other

external environmental factors like other people handling the baby with contaminated hands, hospital environment and mismanagement of the umbilical cords as was observed during the current study contributes to the occurrence of the disease. *Staphylococcus aureus* was the main isolate from both blood and swabs which may be attributed to acquisition of bacteria through handling of the neonates by the mother, health care providers, family members and dirty umbilical cord.

5.7 Antibiotic resistance

Staphylococcus aureus and *E. coli* showed high rates of multi-antibiotic resistance including amikacin, cloxacillin and tetracycline. *Klebsiella* spp. and *Pseudomonus auriginosa* were also resistant to most of the antibiotics tested. Other studies in Tanzania and East Africa have also isolated bacteria which are multi-antibiotic resistant (Mugalu *et al.*, 2006; Blomberg *et al.*, 2007; Moyo *et al.*, 2010; Kayange *et al.*, 2010; Mhada *et al.*, 2012). The observed resistance may be contributed by irrational use of antibiotics. Other factors which cause development of resistance could be the easy availability and rampant use of broad-spectrum antibiotics in the presumptive treatment of infections even in health centres. Lack of enforcement of regulations on antibiotic use as a part of infection control programmes could have influenced the pattern of resistance results to a considerable degree.

Nowadays antibiotic resistance is a widespread global problem that has been causing ineffectiveness in empirical treatment against bacterial infection. As was observed in the current study, *Klebsiella* spp. and *Pseudomonus auriginosa* had high resistance to current empirical treatment protocol (ampicillin+ gentamicin). These are the first line

treatment for septicaemia according to World Health Organization (WHO) recommendation. Antibiotic resistance can cause many difficulties in the treatment of septicaemia such as increase in mortality rate, duration of hospitalization and treatment expenses. It is apparent that the current antimicrobial empirical treatment protocol of neonatal septicaemia be revisited.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the findings of this study it is concluded that:

(a) There is high prevalence of neonatal septicaemia at MRRH as depicted from retrospective data (53.7%) and laboratory confirmed cases (13.5%) during prospective study.

(b) The most frequently observed clinical features of neonatal septicaemia included fever, inability to breast feed, convulsions and difficulty in breathing which were used as predictors of positive blood and umbilical swab culture.

(c) Neonatal factors associated with neonatal septicaemia were zero to six days of age, being a male neonate and birth weight equal or above 2500 g while maternal factors included fever, per vaginal examination and history of caesarean section.

(d) Umbilical pus discharge with hyperemia, poorly cared cord and wrapping of umbilical cord with a piece of cloth were significantly associated with neonatal septicaemia.

(e) During the two years retrospective study (2011 and 2012) neonatal mortality due to neonatal septicaemia was 12.7% and during prospective work was 8% of these, 26.8% of the mortality belonged from the positive blood culture neonates.

(f) The aetiological agents of neonatal septicaemia at MRRH are *S. aureus, E. coli, Klebsiella* spp. and *P. aeruginosa.*

(g) *Staphylococcus aureus* and *E. coli* showed high rates of multi-antibiotic resistance especially to amikacin, cloxacillin and tetracycline while *Klebsiella* spp. and *P. auriginosa* were resistant to most of the antibiotics tested.

6.2 Recommendations

Based on the conclusions above, it is therefore recommended that:

(a) Physicians should keep on relying on clinical manifestation and symptoms in diagnosis of neonatal septicaemia using criteria set by WHO and laboratory blood culture and sensitivity tests should be used to confirm and reguide on the type of antibiotic to use.

(b) Since there is high prevalence of neonatal septicaemia at MRRH which was accompanied with high mortality rates, we recommend a deliberate community health interventions, engaging community members such as village health workers or community workers aimed at controlling the neonatal septicemia. This may include healthy education not only concerning with the pregnancy and safe delivery, but also social cultural norms and taboos e.g. how to handle the neonates especially cord care.

(c) Some of the neonates included in the study were from health centers being referred to MRRH while the disease was at advanced stage and have been inadequately treated resulting in mortality. Therefore, medical practitioners at lower level health facilities should be educated on WHO guidelines to diagnosis of neonatal septicaemia and the treatment approach required.

(d) *Staphylococcus aureus* and *E. coli* were the commonly isolated bacterial species in neonatal septicaemia cases and showed high rates of multi-

antibiotic resistance despite that the antibiotics are the commonly used antibiotics at the hospital. Therefore, a rational use of these antibiotics against the bacteria is important.

(e) Not every fever in neonates is neonatal septicaemia. More efforts should be done on excluding other diseases with similar presentation. Special consideration should be put on dehydration which can also cause fever in neonates rather than neonatal septicaemia. This will reduce congestions in neonatal wards also early administration of antibiotics to the neonates.

(f) It is known that there are many bacteria (*S. aureus*, *E. coli*, *Klebsiella*, *Enterobacter* spp., *Streptococcus* spp., *Acinetobacter* spp., *Listeria* spp. *Pseudomonas aeruginosa*, *Salmonella* spp. and *Enterococcus* spp., etc.) that have been reported to cause neonatal septicaemia in Tanzania, East Africa and many other developing countries. The present study isolated four types. The study recommends further studies to elucidate the involvement of other bacteria and establish their sensitivity to commonly used antibiotics, to enable development of customized treatment protocol in this region.

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Appendices 1: NIMR ethical clearance certificate

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
P.O. Box 9653
Dar es Salaam
Tel: 255 22 2121400/2900
Fax: 255 22 2121380/2121360
E-mail: headquarters@nimr.or.tz
NIMR/HQ/R 8a/Vol. IX/1629

Ministry of Health and Social Welfare
P.O. Box 9083
Dar es Salaam.
Fax: 255 22 2113849
E-mail: [illegible]

23rd September, 2013

Dr Nuru Beda Ndaweka
Sokoine University of Agriculture
Faculty of Veterinary Medicine
P O Box 302,
MOROGORO

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Epidemiology of Neonatal Septicaemia in Morogoro Regional Referral Hospital (MRRH). (Ndaweka N.B *et al*) has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Morogoro Regional Referral Hospital, Morogoro Region

Approval is for one year, 23rd September 2013 to 22nd September 2014.

Name: Dr Mweleele N Maleelo
Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Donan Mmbando
Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: RMO
DMO

Appendices 2: Questionnaire for mothers/caregivers

CONFIDENTIAL!

Date (dd/mm/yyyy): .....Respondent's ID-NO ☐☐☐☐☐

Name of interviewer:.....

Place of interview:.....

A. Respondent biodata

Address:

Hamlet:.....Village/Street:.....Ward.....

District: .....Region.....

1. Sex: (a) Male..... (b) Female.....
2. Marital status

   a) Single..... b) Married..... c) widow/widower..... d) Divorced.....
3. Age range:

   (a) 15-20..... (b) 21-30 ..... (c) 31-40 ..... (d) 41 and above .....
4. Level of education:

   a) No formal education.... b) Primary education ..... c) Secondary.....

   d) College..... e) Vocational training..... (f) Others (specify).....
5. What is your ethnicity (tribe)? .....
6. What is your religion: (a) Muslim .... (b) Christian ... c) Others (specify) ...
7. What is your main occupation?

   (a) Peasant.... (b) Employee ..... (c) Business..... (d) Housewife....

   (c) Student .... (f) Others (specify).....
8. What is your average income per month (Tshs)?

   (a) Below 50,000 ..... (b) 51,000 – 300,000 .... (c) 301,000 – 500,000....

   (d) Above 500,000....
9. What is your marital status?

   a) Married..... (b) Single..... (c) Cohabitating .....

   (d) Separated/Divorced..... (f) Widowed.....

Information of pregnancy and delivery

1. Gravid.....Parity.....Living.....

Have you ever experienced any of the following?

Stillbirth (a) Yes..... (b) No..... If Yes. how many times.....

Any abortion (a) Yes..... (b) No..... If Yes. how many times.....

What kind of management to your reproductive system after

(i) Abortion.....

(ii) Stillbirth .....

2. Did you have a normal, healthy pregnancy? (a) Yes..... (b) No.....

If answered No, what were the problems during pregnancy.....

3. Did you suffer from any diseases during pregnancy? (a) Yes..... (b) No.....

If answered yes, list down the diseases you suffered?.....

.....

4. Did you have premature rapture of membranes (PROM)? (a) Yes..... (b) No.....

(i) If yes, how long before delivery.....

(ii) Did you get any medication after (PROM) before delivery (a) Yes.....

(b) No.....

(iii) If answered Yes, what type of medication did you get after PROM.....

5. How many times were you measured for dilation of cervix.....

(a) 2-3 times (b) 4-5 times (c) above 6 times

6. What was the state/type of delivery? (a) Normal vaginal delivery .....

(b). Assisted vacuum delivery..... (c) Operation C/S..... (d) Others (please state).....

Information of the baby

1. What is: (a) age of your baby (weeks/days)..... (b) Sex.....(c) birth weight (kg).....

(d) Birth length (cm): .....

2. In which week of gestation was the baby born?.....

3. Did you have a single child (not twins)? (a) Yes..... (b) No.....

If twins are they both sick? .....

4. What is the health problem of your baby? .....

5.What are the reasons for hospitalization.....

.....

6. What signs/symptoms made you to think that your baby is sick?.....

.....

7. Has your baby ever experienced any of the following health problems ever since was born?

Symptom	Yes	No	Kind of medication/management given
Umbilical cord infection			
Fever			
Diarrhoea			
Cough or colds			
Pneumonia			
Anaemia			
Not sucking			
Convulsions (degedege)			
Others (state) (i)..... (ii).....			

8. Do you breast feed your baby? (a) YES.... (b) NO.....

(i) If answered No, why do you not breast feed the baby.....

(ii) Which other milk do you give your baby?.....

9. How did you care the umbilical cord after delivery?.....

.....

10. Did you notice any kind of umbilical cord problem/change of colour etc? (a) YES....

(b) NO.....

If answers yes, what did you do?.....

11. Did the umbilical cord problem also occur to other babies you had in previous parities? (a) YES.... (b) NO.....

12. Were there any complications during delivery? (a) YES.... (b) NO.....

If yes, did any of the following occur?

Complication	Response
Asphyxia (kuchelewa kulia)	
Resuscitation during delivery	
Invasive procedures	
Congenital anomaly	
Parenteral nutrition	
Long hospital stay	
Illness onset on day of birth	
Any change with baby movements after delivery? (a) Reduced	
(b) Normal	
(c) Increased	

Thank you for participating in the research!

Appendices 3: Kibali cha ujulisho wa kufanya mahojiano (nakala ya Kiswahili)

Namba ya mtafitiwa na mwaka ☐☐☐☐☐☐☐☐☐

Ruhusa ya kukubali kufanya mahojiano kwenye utafiti huu

Salaam! Mimi naitwa Nuru Beda Ndaweka, mwanafunzi wa kozi ya uzamili katika Chuo kikuuu cha Kilimo cha Sokoine, Morogoro katika fani ya Afya ya jamii na usalama wa chakula. Nia ya kufanya utafiti huu ni kutafuta chanzo cha 'bakteria' wasababishao maambukizi kwenye damu kwa watoto wadogo katika hospitali ya mkoa wa Morogoro nchini Tanzania. Unaombwa kushiriki katika tafiti hii kwa sababu una uelewa na ujuzi wa kutosha kuweza kukamilisha tafiti hii.

Kukubali kushiriki

Ukikubali kushiriki katika tafiti hii vitu vifuatavyo vitafanyika:

1. Utafanyiwa usaili kuhusu ujauzito wako, mtoto wako, damu kutoka kwa mtoto wako, sampuli katika pamba toka kwenye kitovu cha mtoto.
2. Zaidi ya hayo hapo juu utaulizwa kuhusu habari zihusuzo maisha yako kama umri, urefu na uzito wako, Elimu uliyofikia, kazi yako, mwenyeji wa wapi na habari za mtoto wako kama vile jinsia yake, uzito, ' wastani wa apga na urefu'

3. Jina lako halitatakiwa kuwa wazi (ni siri ya mtafiti na hospitali ya Morogoro) pamoja na mafaili yahusuyo rekodi za matibabu. mtafiti atatumia namba ambayo haitamfanya kujua jina lako.
4. Ikiwa una nia ya kufanya mahojiano katika tafiti hii au zijazo katika tafiti za namna hii tafadhali tuarifu.
5. Utafanyiwa usaili kwa muda usiozidi dakika 20 katika chumba cha faragha.

Faida

Hakutakuwa na faida ya moja kwa moja kwako; ila tafiti inajaribu kutafuta uelewa zaidi chanzo na madhara ya maambukizo ya vitovu kwa watoto wachanga katika hospitali ya mkoa Morogoro.

Usiri

Unahakikishiwa usiri mkubwa katika yote utakayoyazungumuza wakati wa mahojiano haya. Mtafiti mkuu pekee katika tafiti hii na daktari wako ndio watakuwa na nafasi ya kuona mahojiano hayo. Sehemu kubwa ya ripoti itakuwa na majibu ya maswali na vipimo vya kutoka maabara kutoka kwa watoto mbalimbali ambao watakuwa hawana majina. Hatutaweka jina lako au kitu kingine ambacho kitakutambulisha kuwa wewe ndiwe uliyetoa habari hizo.

Mambo ya hatari

Katika mahojiano haya maswali mengine yanaweza yasiwe mazuri kwa upande wako. Unaweza kutokujibu maswali hayo. Hakuna mashaka yanayohusu ukusanyaji

wa damu,ukusanyaji wa sampuli kwenye pamba kutoka katika vitovu vya watoto na pamba kidogo kutoka kwenye mikono ya watoto hao.

Ruhusa ya kutofanya mahojiano na mbadala wake

Kufanya kwako mahojiano katika utafiti huu ni wa kujitolea. Ukiamua kutofanya mahojiano katika utafiti huu wakati wowote wa mahojiano hakutakuwa na matatizo yeyote. Kutotaka kufanya mahojiano au kujitoa kwenye utafiti huu hautakufanya wewe kutopata huduma katika hospitali hii.

Endapo utaumia

Hatutarajii wakati wa mahojiano haya uumie wewe au mtoto wako.

Nani wa kuwasiliana naye

Kama utakuwa na maswali yahusuyo utafiti huu wasiliana na Msimazi wa Tafiti hii Dk. Hores Msaky, Hospitali ya Mkoa wa Morogoro, S.L.P. 110, Morogoro, Dk. H. E. Nonga na Dk. Lucas Matemba wa Chuo cha Kilimo Sokoine, S.L.P. 3000 Morogoro

Cheti cha ruhusa

Nimekaribishwa kushiriki katika tafiti hii ya "Uchunguzi wa bacteria wanaoambukiza kwenye damu ya watoto wachanga katika hospitali ya mkoa wa Morogoro, Tanzania". Nimesoma au nimesomewa na kuelewa. Nakubali kushiriki katika tafiti hii.

Sahihi

Jina la mama mshiriki (katika herufi kubwa) _____

Sahihi (au dole gumba) la mshiriki _____

Sahihi ya shahidi endapo mshiriki hawezi kusoma na kuandika _____

Sahihi ya mtafiti msaidizi _____

Tarehe ya kukubali huku _____

Je unakubali kuhusishwa na tafiti zingine zijazo?

Ndiyo ☐ Hapana ☐

Anuani: S.L.P. _____

Kitongoji_____ kijiji _____ Kata _____

Wilaya _____ Mkoa _____

Appendices 3: Informed Consent Form (ENGLISH VERSION)

Respondent's ID-NO and year ☐☐☐ ☐☐☐☐

Consent to participate in this study

Greetings! My name is Nuru Beda Ndaweka, a postgraduate student at Sokoine University of Agriculture pursuing a master of science in public health and food safety. The purpose of the research is to investigate the bacterial causes of neonatal septicemia in Morogoro regional referral hospital Tanzania. You are being invited to participate in this study because you have particular knowledge and experiences that may be important to the study.

Participation involvement

If you agree to participate in this study the following will occur:

1. You will be interviewed about your pregnancy, your baby, and a swab from the umbilical cord.
2. In addition some personal information will be gathered such as age, height and weight, level of education, occupation, ethnicity and baby information including gender, weight, apga score and length.
3. Your personal information will be stored at a confidential place in the Hospital, no names will be used, instead codes will be used and can only be recognized by the researchers.
4. In case you are willing to participate in future with further research within this or future projects, you can specify.

5. The interview will last for approximately 15- 20 minutes and will be conducted in a private place.

Benefits

There will be no direct benefit to you; however the information you provide will help to increase the understanding on the effects neonatal septicaemia in Morogoro. This will help to formulate prevention and control strategies aimed at reducing the neonatal mortality.

Confidentiality

All the information collected from you will be kept confidential. Only the main researcher in the project and the medical staff attending your child will have access to the information. The final report will contain responses and laboratory results from several neonates without any reference to individuals. We will not use your name or other identification from the records or information you provide.

Risks

You will be interviewed as described above. In case some questions make you feel uncomfortable, you are free not to respond to them. There is no risk associated with the collection of blood samples, umbilical and hand swabs from the neonates.

Rights to withdraw and alternatives

Your participation in the study is on voluntary basis. If you choose not to participate in the study or decide to stop participating in the study at any time, there will not be any problem. Refusal to participate or withdrawal from the study will not affect the quality of health service you get from the hospital.

In case of injury

We do not anticipate that any harm will occur to you or your baby as a result of participation in this study.

Who to contact

If you have questions about this study, please feel free to ask me now, alternatively you may wish to contact the Medical Officer in charge of paediatric ward Dr. Hores Msaky, Morogoro Regional Hospital, P.O. Box 110, Morogoro. Or any of my supervisors Dr. H. E. Nonga and Dr. Lucas E. Matemba, Sokoine University of Agriculture P.O. Box 3000 Morogoro.

Certification of consent

I have been invited to take part in the study titled "investigation of bacterial causes of neonatal septicemia in Morogoro regional referral hospital Tanzania". I have read the foregoing information or the information contained has been read and explained to me and I have clearly understood. I agree to participate in this study.

Signature

Name of participating mother/guardian (*in capital letters*).....

Signature (or thumbprint) of participant .....

Signature of witness (if participant cannot read) .....

Signature of research assistant .....

Date consent signed.....

Are you willing to be contacted for future research? YES ☐

NO ☐

Address: P.O. Box.....

Hamlet: .....Village: .....

Ward.....

District: .....

Region.....