BACTERIAL INFECTIONS AND ANTIMICROBIAL SUSCEPTIBILITY AND OUTCOME IN NEONATES AT MUHIMBILI NATIONAL HOSPITAL.

By

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Medicine (Paediatric and Child Health) of the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam.

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CERTIFICATION

The undersigned certify that, they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, a dissertation entitled: ***Bacterial Infections and Antimicrobial Susceptibility and Outcome in Neonates at Muhimbili National Hospital,*** in partial fulfilment of the requirements for the degree of Master of Medicine (Paediatrics and Child Health) of the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam.

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DECLARATION AND COPYRIGHT

I, **Tumaini Edward Vincent Mhada**, declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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May the Almighty God be praise for guiding me through this work and my studies at large. AMEN

DEDICATION:

I dedicate this dissertation to my beloved wife Neema and my lovely daughter Marren for their love, moral support and prayers.

To My parents for their advice and unconditional support

ABSTRACT

Background: Neonatal septicaemia remains a major cause of morbidity and mortality. More than 5 million neonatal deaths occur worldwide each year, the majority in developing countries where neonatal mortality accounts for 50–70% of the infant mortality rate.

In Muhimbili National Hospital neonatal bacterial septicaemia ranked number three among causes of neonatal mortality and morbidity in 2007/2008. The spectrum of organisms causing neonatal septicaemia changes over time, therefore it is important to know the sensitivity pattern of the commonly used antimicrobials. This information is useful in the proper management of neonatal bacteria septicaemia.

Objectives: To determine the aetiology, antimicrobial susceptibility pattern and immediate outcome of neonatal bacterial infections among neonates admitted in neonatal unit at Muhimbili National Hospital (MNH)

Methods: A hospital based cross sectional study was conducted at the neonatal ward from neonates presenting with clinical feature of infections to Muhimbili National Hospital in Dar es Salaam city from October 2009 to January 2010. A structured questionnaire was used to capture demographic information, neonates presenting of any symptoms and signs of neonatal infections were recruited consecutive daily and included into the study after obtaining a written informed consent from parents/guardians. A thorough physical examination including weight, length and occipital frontal circumference were done. Culture and sensitivity of blood, swabs, and urine were done. Pure colonies were identified based on characteristic morphology, gram stain appearance and standard commercially prepared biochemical tests, and thereafter-antibiotic sensitivities were done. Data analysis was done using *SPSS software version* 15 (Statistical Package for Social Science) for windows evaluation. A p-value of less than or equal to 0.05 was considered biostatically significant.

Results: Three hundred and thirty neonates met inclusion criteria and were recruited into the study. Out of which 170 (51.5%) were male and 160 (48.5%) were females, 253 (76.7%) were 0 to 6 days while 77 (23.3%) were 7 to 28 days old, with the mean age of 6 days. Common clinical features were umbilical pus discharge with hyperaemia (96.1%), fever (91.5%) and

inability to feed (62.4%). Skin rash with pus spots and hypothermia were found to be independent predictors of bacterial infections. The frequency of infections confirmed by cultures in blood was 74 (22.4%), umbilical swabs 285 (89.9%) and skin swabs 28 (90.3%). Overall bacterial isolated were 371 from 330 neonates, 74 (19.9%) isolates from blood and 297 (80.1%) from pus. In blood, 48.6% of bacteria were gram positive and 51.4% gram negative, of which the common aetiological agents were *Staphylococcus aureus* (36.5%), *Klebsiella spp* (29.7%) and *Escherichia coli* (18.9%). The microorganisms isolated were resistant to ampicillin (88.2%), cloxacillin (85.3%) and moderately resistant to gentamicin (58.8%). Of the 68 bacteria organisms isolated antibacterial susceptibility pattern showed 98.5% were sensitive to amikacin. Overall mortality was 13.9%, of which 14.2% was among neonates aged 0 to 6 days while 13.0% were 7 to 28 days old. In addition, participants with neonatal septicaemia have a higher proportion of mortality than those without septicaemia. (24.3% versus 10.9%, P = 0.003)

Conclusion: In this study, the common aetiological agents were *S. aureus, Klebsiella spp* and *E. coli*. In early onset septicaemia, *Klebsiella spp* were the predominant organisms, while *S. aureus* was predominant in late onset septicaemia. The microorganisms isolated were highly resistant to ampicillin and cloxacillin and moderately resistant to gentamicin (58.8%). Sensitivity to amikacin was high; the organisms were also sensitive to ceftriaxone and cefuroxime. Among the neonates with neonatal septicaemia, 11 (61.1%) died within the first 72 hours of admission

Recommendation: The use of ampicillin, cloxacillin and gentamicin as first line treatment of neonatal bacteria septicaemia in the neonatal ward of MNH needs to be re-evaluated. Amikacin should be used as an initial therapy. Antibiotic susceptibility surveillance, evaluation of local pathogens causing neonatal septicaemia and detection of any shift in their antimicrobial susceptibilities

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ABBREVIATIONS:

CLED	Cysteine Lactose-electrolyte-deficient
CMV	Cytomegalovirus
CSF	Cerebral Spinal Fluid
DIC	Disseminated intravascular coagulopathy
EDTA	Ethylene Diamine Tetra-acetic Acid
EOS	Early onset septicaemia
ESBL	Extended-spectrum beta-lactamase
GBS	Group B Streptococcus
HIV	Human Immunodeficiency virus
IL	Interleukin
LBW	Low Birth Weight
LOS	Late onset septicaemia
LPP	Lipopolysaccharide
MIC	Minimum Inhibitory Concentration
MNH	Muhimbili National Hospital
MOHSW	Ministry of Health and Social Welfare
MUHAS	Muhimbili University of Health and Allied Sciences
NCCLS	National Committee on Clinical Laboratory Standards
OFC	Occipital frontal circumference
PAF	Platelet activating factor
SPSS	Statistical Package for Social Science
TDHS	Tanzania demographic and health system survey
TNF	Tumor necrosis factor
U.T.I	Urinary tract infection
VLBW	Very Low Birth Weight
WBC	White blood cell
WHO	World Health Organization

DEFINITION OF TERMS

Neonate refers to an infant aged between 0 to 28 days of life.

Neonatal septicaemia is a clinical syndrome of bacteraemia characterized by systemic signs and symptoms of in first month of life

Neonatal Bacteraemia is defined as presence of bacterial infection confirmed by a positive blood culture in the first month of life

Early onset septicaemia (EOS) defined as disease present as a multisystem illness within 24 hours to 6 days of life

Late onset septicaemia (LOS) defined as disease most commonly recognized after the first week of life

Urinary tract infection (UTI) defined as any growth on the culture media

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background and Literature Review

Neonatal septicaemia remains a major cause of morbidity and mortality.[1] More than 5 million neonatal deaths occur worldwide each year, the majority in developing countries where neonatal mortality accounts for 50–70% of the infant mortality rate.[2] The main causes of neonatal death are prematurity birth, birth asphyxia, and infections. [3, 4]

Septicaemia in neonates refers to generalized infection documented by a positive blood culture in the first 28 days of life often associated with severe disease resulting in a systemic response leading to organ dysfunction followed by death. [5-7] In 1999, a study in Kilimanjaro, Tanzania reported of case-specific mortality of 47 neonates, of which 21.3% were due to infections. [2] Infections are frequent and important cause of morbidity and mortality in the neonatal period. [8] As many as 2% of foetus are infected in utero, and up to 10% of infants are infected in the first month of life. [3] In the study done in Kilifi Kenya in 2005, bacterial infections was found to be 12.8% in infants of which *E. coli* (14%) and GBS (11%) were the predominant organisms isolated at the age less than 60 days, whereas neonatal bacteraemia had a minimum rate of 5.46 cases per 1000 live birth. [9]

Neonatal bacterial septicaemia is caused by a variety of gram-positive as well as gram-negative bacteria. [3, 8] Bacteria commonly isolated include *Klebsiella pneumoniae*, *Group B Streptococci, Escherichia coli*, *Enterobacter* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* [3, 10-13] Commensal species found on the skin and sometimes in the intestine of the neonates may significantly cause neonatal infections. [3, 14] In 2005, J. Milledge et al reported overall isolated bacteria were 54% gram-positive and 46% gram-negative organisms. In 582 (74%) neonates with neonatal bacteraemia the commonest causes were S. aureus (15%), group B streptococcus (13%) and non-typhoidal Salmonella (13%), while other etiologies were E. coli (10%), Klebsiella spp (9%), group A streptococcus (8%) and S.

pneumonia (6%). Of which the prevalent causes of all neonatal sepsis were group B streptococcus (17%) and non-typhoidal Salmonella (14%).[15]

1.1.1 Pathogenesis

Newborn skin and mucus membrane are easily injured; in addition, their physical and chemical barriers to infection are functionally deficient. For a sick newborn, the risk of infections is much greater at an early age neonatal immunity is not fully developed neonatal PMN are less deformable therefore less able to move through the extracellular matrix of tissue to reach the site of inflammation and infection. In addition, neonates become deficiency in opsonic efficiency against GBS, *E coli*, and *S pneumoniae* following decreased levels of fibronectin. [16, 17]

Infective microorganisms are taken up by macrophages, lysed gram positive organism release peptidoglycans, while gram negative organism release endotoxins or lipopolysaccharide (LPP). LPP binds to LPP-binding protein attached to the macrophage activating it, thereby stimulates the release of arachidonic acid metabolites such as prostacyclin and thromboxanes A_2. [17] It initiates the release of proinflammatory cytokines, which in turn switch on the release of anti-inflammatory cytokines stimulated by Tumor necrosis factor (TNF), Interleukin 1 (IL 1β) and IL-6. IL-1β stimulates T helper cells to produce IL-2, which stimulates Cytotoxic T cells. [3, 16]

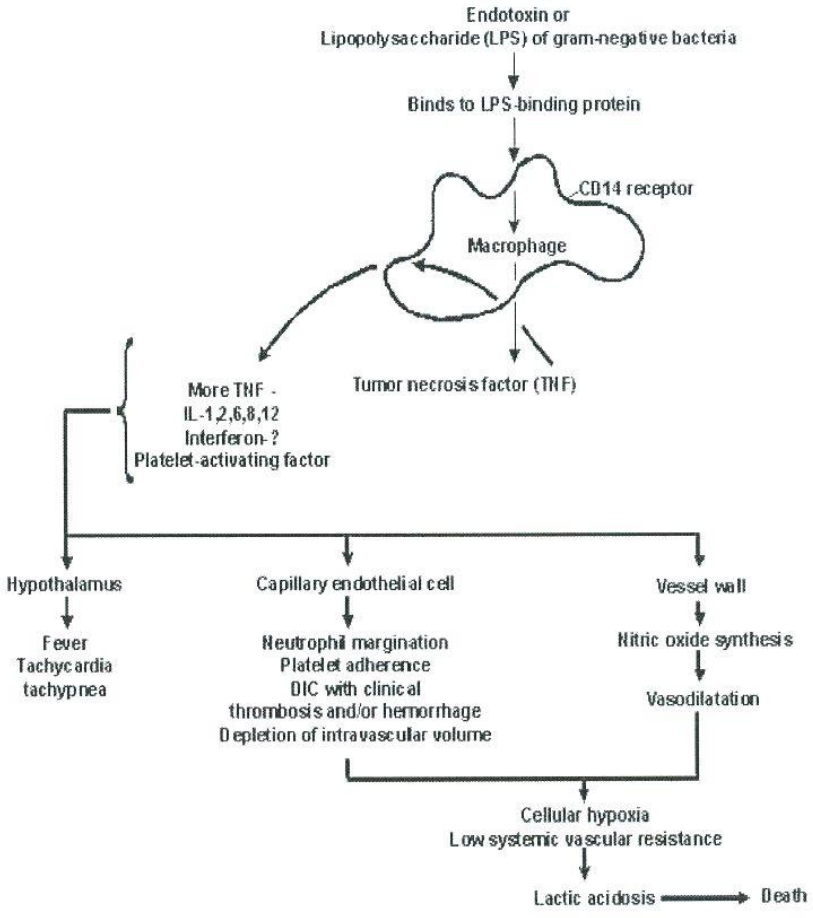
Furthermore, bacteria fragments, endo or exotoxins activates complement, coagulation and fibrinolytic cascade releasing prostaglandin E_2, nitric oxide (NO), and platelet activating factor (PAF). They also activate leucocytes releasing proteases and oxygen free radical, which causes localized tissue damage, increase capillary permeability, and hence leads to endothelial damage. [16, 17]

All the above-mentioned events working together or individually cause endothelial damage. Consequently, systemic septicaemia becomes multisystem disorder, where by the effect on the cardiovascular system leads to hypotension, in the renal system to pre-renal failure due to hypovolaemia. In the liver, cholestasis and hyperbilirubinaemia ensures following

overproduction of inflammatory cytokines and endotoxins by Kupffer cells and bacteria fragment respectively.

Infection affects the haemopoietic system causing neutropaenia, anaemia and disseminated intravascular coagulation. This results in tissue hypoxia, metabolic acidosis and acceleration of organs dysfunction proceeding to septic shock and apoptosis-death. [3, 16, 17]

Figure 1. Summary outline of gram-negative bacteria



(Source: Courtesy of Santhanam S *et al*. emedicine/infectious disease. Sepsis) [16]

1.1.2 Symptoms and signs

Neonates with septicaemia may have either non-specific signs and symptoms or focal signs of infection including temperature instability, hypotension, tachycardia or bradycardia, apnoea, respiratory distress, grunting, cyanosis, irritability, lethargy, seizures, feeding intolerance, abdominal distension, or jaundice.[3, 18] Jeena et al in Durban, South Africa reported, illnesses requiring urgent hospital management in the age group less than 7 days presented with hyperbilirubinaemia 43% and sepsis 43%, while those in the age group 7-27 days pneumonia were 26%, followed by sepsis 17% and hyperbilirubinaemia 15%. The clinical signs included not feeding well, fever more than 37.5°C and respiratory rate more or equal to sixty per minute. [19]

It is useful to classify neonatal septicaemia presentation as early onset septicaemia (EOS) and late onset septicaemia (LOS) because it helps in determining the common microbial organism, mode of transmission, presentation and treatment by age.[1, 17] Early-onset neonatal bacterial septicaemia is seen within the first week of life and has a relatively high mortality rate between 15% and 50%, while late-onset septicaemia starts after the first week of life and may result in mortality rates up to 15%. [3, 13, 20-22] Early onset septicaemia is commonly caused by microorganism acquired from the mother before and during delivery and is frequently associated with obstetric complications. [3, 17]

Bacteria responsible for late onset septicaemia include those acquired from maternal genital canal and after birth from contaminated equipments or materials and human contacts. A study done in Tanzanian children in 2002 reported gram positives organisms contributed to 32.3% of late onset septicaemia, *Staphylococcus aureus* (16.1%) were commonly isolated.[11] In another study conducted at Mulago Hospital, Kampala Uganda, gram-positive organisms were reported to be predominant in both late and early onset septicaemia, commonly isolated organism was *S. aureus* 70.9% versus 54.5%, respectively.[23] The difference in relative occurrence of microorganisms observed between these two studies could be attributed, at least

in part, to the difference specimen collection and handling methods as well as methodological and geographical differences.

1.1.3 Predisposing factors

Factors predisposing to neonatal infection include birth asphyxia, prematurity, low birth weight, underlying illness (such as galactosaemia, immune defects), premature rupture of membrane, prolonged rupture of membrane and during resuscitation at birth.[17]

In hospitalized newborns, prematurity is associated with a 3 to 10 higher incidence of infection than full term gestation responsible for significant morbidity and late mortality, explained by immaturity of immune system. [17, 21, 24] A study by C. Klingenberg et al, reported an overall mortality rate of 19%, 85% of death occurring in the first week of life, of which majority of death (66%) occurred in the first 24 hours, notably contributed by perinatal asphyxia, gestational age, low birth weight and infections. [5.] Another study in India, reported an overall fatality rate of 48.5%, factors significantly associated with death were weight, gestational age, age at onset of sepsis, and hypothermia. [25]

1.1.4 Treatment

Treatment of suspected bacterial neonatal septicaemia is determined by the antimicrobial sensitivity pattern of the aetiological agents whose composition is dependent on infant's age, and is influenced by organisms within the neonatal unit (hospital-acquired infection). The antibiotic combinations prescribed in most units are penicillin's (benzyl penicillin, ampicillin, or cloxacillin) together with an aminoglycoside, most commonly gentamicin. [3]

Most gram-negative and gram-positive organisms previously reported to be sensitive to ampicillin and cloxacillin are increasingly reported to be resistant to gentamicin. [3, 22]

The empirical use of antibiotics in neonates involves using broad-spectrum drugs to be continued for a total of 10 days, after a clinical response has occurred.[3] In a retrospective study

in India, it was reported that most of the isolates were resistant to ampicillin and amoxicillin although, most of the gram-negative organisms were sensitive to amikacin. [25]

In 2005, a study in Tanzania revealed high rates of resistance to commonly used antimicrobials. Extended-spectrum beta-lactamase, (ESBL)-producing organisms were resistant to almost all tested antimicrobials except for ciprofloxacin (contraindicated in neonates) and meropenem. [11, 26]

The emergence and spread of resistance can be due to over use or under use of broad spectrum antibiotic, over the counter sale of antibiotics, mutant forms of common bacteria and ineffective or/and poor policy in the management of neonatal septicaemia.[17] Third-generation cephalosporin's such as ceftriaxone have been valuable additions for empiric treatment of neonatal septicaemia. The reasons being i) minimal inhibitory concentrations are needed for treatment of gram-negative enteric bacilli compared with aminoglycosides and ii) Third-generation cephalosporins have excellent sensitivity against bacteria. [3, 27]

A study done in Kenya, in 2003 revealed that blood isolates were resistant to ampicillin and gentamicin but showed good *in-vitro* sensitivities to amikacin, cefuroxime and the third generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime). [28] Another study in Mulago Hospital, Kampala reported resistance of organisms to ampicillin (except *Group B Streptococcus*) and most of the organisms were sensitive to gentamicin and reported that third generation cephalosporins; ceftriaxone and ceftazidime showed high sensitivity against Gram-negative organisms like *E. coli*, *Salmonella spp.* and *Proteus spp.* These drugs however showed low sensitivity for *Group B Streptococci (GBS)* and *S. aureus.* [23.]

However, the routine use of third-generation cephalosporins for suspected sepsis in neonatal Care Units patients is inappropriate because of the potential rapid emergence of resistant organisms.[3, 27] The wide spread resistance to antibiotics calls for constant surveillance of antimicrobial susceptibility pattern of the etiological agents to reduce morbidity and mortality as well as reducing the emergence of multi-drug resistant organisms. [29]

1.1.5 Complications

In general, complications of neonatal bacterial septicaemia may result from a cascade of inflammatory processes or concomitant effects such as thrombocytopenia and disseminated intravascular coagulopathy, other complications include endocarditis, septic emboli, and abscess formation, septic joints with residual disability, osteomyelitis and death.[3, 17] A study in Asia revealed fatality rate of 33%.[30]

1.1.6 Prevention

Successful strategies to reduce rates of infection, includes clean and safe deliveries, strict postnatal cleanliness, adherence of universal precautions to all patient contact, avoiding nursery overcrowding and limiting nurse-to-patient ratios, early and exclusive breast feeding.[17, 24] Furthermore, strict compliance to hand washing, decreasing the number of vene-punctures and heel prick, and providing education to nursery personnel.[31] Hand washing before entering the neonatal unit and before each patient contact, using antimicrobial soaps or alcohol-based preparations remains the most important and effective means of reducing nosocomial infections in nurseries and prompt use of antibiotics would save newborns from septicaemia complications.[3, 12, 31]

1.2 PROBLEM STATEMENT

In Muhimbili National Hospital neonatal bacterial septicaemia ranked number three among causes of neonatal mortality and morbidity in the annual report 2007/2008.[32] Out of 7575 admissions, 1837 neonates (24.3%) died. The causes of death were prematurity (46.4%), birth asphyxia (28.3%), septicaemia (17.1%), pneumonia (4.2%), and congenital abnormalities (1.9%). Anaemia and haemolytic disease of newborn contributed to (1.0%), and others accounted for only 1.1% of all the deaths.

A study done in 1981 to determine the aetiological agents of neonatal septicaemia at MNH neonatal unit found that *S aureus*, *E.coli*, and *K. pneumoniae* were the most common.[33] While another study in 2002 in the same facility reported, *Enterobacteriaceae* (51.9%), *Klebsiella* spp (31.5%), *E.coli* (11.1%), and *Enterobacter* spp (7.4%), as being the most frequent isolates. [11, 33]

The development of resistance to all known antibiotic agents makes the selection of reliable empirical therapy difficult. In 2005 Dar es Salaam, a study reported gram-negative bacteria such as *E. coli*, *Klebsiella* spp and *Proteus* spp, showed high rates of resistance to common used antibiotics such as ampicillin (80%), tetracycline (77%) and trimethoprim-sulfamethoxazole (76%), while resistance to gentamicin was 8%. Another study in 2007 at the same institution reported *Klebsiella* spp and *S aureus* shows progressively increase in resistance to ampicillin (95%) and gentamicin (53%). [11, 26]

Hence, the knowledge about the aetiological agent in neonatal ward and their antibiotic susceptibility pattern is important.

1.3 RATIONALE OF THE STUDY

The last study to determine for aetiological agents of neonatal septicaemia and their susceptibility at MNH was conducted eight years ago.[11] It is likely that there could be changes in the relative distribution of aetiological agents with time and hence the need for continuous surveillance. WHO recommends the use of ampicillin, cloxacillin and gentamicin as first line treatment of newborn presenting with sepsis.[18] However, current studies seem to suggest increasing level of resistant to the commonly used antibiotics. [11, 34] Rational use of antibiotics is known to improve treatment outcomes and shortens the duration of hospital stay; it also reduces the cost of treatment. However, this requires continuous surveillance of antimicrobial susceptibility pattern. Generating information on the current situations of neonatal bacterial septicaemia, aetiological agents and update on their anti-microbial sensitivity pattern will contribute to improvement of management of newborns with sepsis at MNH.

1.3.1 Research question

What is the aetiological bacterial agents affecting neonates at MNH?

What is the current level of resistance of bacterial pathogens of neonatal septicaemia to the commonly used antimicrobial?

What is the immediate outcome in neonates with bacteriological confirmed neonatal septicaemia at MNH?

1.3.2 Hypothesis

Commonly used antibiotics for treating neonatal septicaemia are effective and there is no significant bacterial resistance pattern.

2.0 OBJECTIVES

2.1 Broad objective

To determine the aetiology, antimicrobial susceptibility pattern and immediate outcome of neonatal bacterial infections among neonates admitted in neonatal unit at Muhimbili National Hospital (MNH) from October 2009 to January 2010.

2.2 Specific objectives

2.2.1 To determine the aetiology agents of neonatal bacterial infections according to age and sex among neonates admitted in neonatal unit

2.2.2 To determine the antimicrobial sensitivity pattern of pathogens isolated from neonates admitted in neonatal unit at MNH

2.2.3 To determine the association between neonatal bacterial infections and immediate outcomes among neonates admitted in neonatal unit at MNH

3.0 METHODOLOGY

3.1 Study design:

This was a hospital based descriptive cross sectional study

3.2 Study area:

Neonatal ward at Muhimbili National Hospital, which receives neonates delivered at MNH labour ward and neonates, referred from other hospitals within Dar es Salaam and some from upcountry. The ward has a capacity of 70 beds, admitting approximately 30 - 40 babies per day, with a total annual admission ranging between 6000 – 8000 neonates.

3.3 Study population.

Study population were term and preterm neonates aged between 0 to 28 days who were admitted at Neonatal ward at Muhimbili National Hospital during the time of the study (October 2009 to January 2010). During the study period 1881 neonates were admitted in the ward.

3.4 Sample Size Estimation

Sample size was estimated using a software *Epi info version 6.*

Assumption as quoted from a prospective cohort study on blood stream infections at MNH by Blomberg *et al,* [11] reported confirmed blood stream infection in neonates to be 16%

Where;

p = the estimated value of laboratory confirmed blood stream infection in neonates 16%

d = estimated minimal marginal error of 4 %.

Therefore, calculated sample size **(n)** was 323; adding 10% to allow for incomplete results, thus bringing the sample size to 330.

3.5 Inclusion Criteria

The inclusion criteria are:

3.5.1 Term and preterm neonates with clinical diagnosis of neonatal septicaemia, presenting with and any one of the following; presence of fever (≥ 37.5°C) or hypothermia (≤ 36.5°C), convulsions, lethargy, inability to feed, hypoglycaemia, vomiting, bulging fontanelle, respiratory distress, jaundice and signs of infection on the skin with pus spots and umbilicus pus discharge with hyperaemia. [18, 35]

3.5.2 Neonates of parents or guardian who had granted a written consent form to participate in the study

3.6 Exclusion Criteria includes

3.6.1 Unwillingness of the parent or guardian to participate in the study

3.6.2 Very sick children in decompensate state and requiring resuscitation

3.6.3 Neonates with severe congenital malformation such as anencephaly

3.7 Training of the research team

Three research assistants (2 medical doctors and one nurse) were trained prior to the commencement of the study. The training included aim of the study, patient recruitment, filling of questionnaire, consent form administration, procedure of specimen collection, handling and delivery to the laboratory, and follow-up of recruited neonates while in the ward.

3.8 Questionnaire piloting

Three week prior to commencement of data collection a pilot was done to test the practicability of the questionnaire.

3.9 Recruitment of study participants

The caretakers of the eligible study participants were given detailed explanation of the study and were invited to participate into the study after they had given written informed consent (Refer Appendix II/III). Recruitment of participants and sample collection was consecutive done daily until a sample size of 330 neonates was reached.

The two medical doctors (research assistants) as well as the researcher were responsible to make the initial diagnosis.

3.10 Data collection

The author and research assistants assessed neonates' eligibility. Information were recorded in a structured questionnaire as indicated in Appendix I. Demographic characteristics including name, age, sex, residence, date and time of admission.

Parents/guardians were asked on the presence and onset of presenting symptoms according to WHO guidelines for the management of common illnesses. Then the investigator and research assistants (medical doctors) did a thorough clinical examination of every neonate according to the principles of paediatric assessment. This included taking weight, length, occipital frontal circumference, temperature, and respiratory rate for one minute. Gestational age assessment was done using New Ballard score. [36] (Appendix IV)

Weight

Weight was measured using SECA beam balance (CMS Weighing equipment, London, United Kingdom) to the nearest 100g, the infants were completely naked during measurement, and calibration of the scale was done before each session. For accuracy and consistent data, the scale was calibrated once weekly using a known weight of 1 kg.

Length

Parents/guardian assisted in removing neonates clothing and gently laying the infant in a supine position on the length board with their heads placed at 90° to the fixed head piece. The research assistance held the neonate's head to the headpiece while the investigator straightened the legs at the knees and ensuring that the feet were at the right angles to the sliding foot piece brought into contact to the neonate's heels. Length was recorded to the nearest 10 millimetres.

Occipital frontal circumference (OFC)

Using a non-elastic tape measure placed above the ears and midway between the eyebrows and the hairline to the occipital prominence at the back of the head recorded to the nearest 0.1 cm

Temperature

Temperature was measured by a mercury thermometer. The thermometer was shaken down to 35°C and placed in the right or left axilla. The arm on which the thermometer was placed held gently firmly for five minutes to allow the thermometer to stabilize and the temperature was recorded.

During the study period, admitted neonates presenting with clinical features that lead to presumptive diagnosis of neonatal septicaemia were fever ($\geq$ 37.5°C), hypothermia ($\leq$ 36.5°C), convulsions, lethargy, inability to feed, hypoglycaemia, vomiting, bulging fontanelle, respiratory distress, jaundice, signs of infection on the skin with pus spots and umbilicus pus discharge with hyperaemia.[18, 35]

3.11 Laboratory investigations

To determine whether or not the child had bacterial septicaemia, cultures of blood, swabs and urine were done

3.11.1 Blood Specimen Collection

Two millilitres of venous blood was drawn from anterior cubital fossa of each neonate using a sterile disposable syringe and needle. The femoral vein was used in neonates who were difficult to access the peripheral veins. The neonate's skin cleaned with 2% povidone-iodine and a 70% alcohol swab, allowed to dry before blood was drawn for a full blood picture and blood culture and sensitivity.

Two millilitres of blood were inoculated into the paediatric blood culture bottles (BacT/ALERT PF Organon-Teknika Corp., Durham, N.C.). For each neonate only one set of blood culture was drawn, the volume was marked before and after inoculation to know, the exact volume inoculated. Used needles and syringes were disposed safely into special boxes. The specimens' bottles were labelled with the name of the patient, time and date when the specimen was taken and then placed together with the completed investigation forms. The collected samples were kept in the specimen container and were taken to the laboratory for processing and testing.

3.11.2 Blood Culture

In the laboratory, each blood culture bottle was incubated at 37°C overnight. Then aliquot was taken for subculture on solid agar plates; MacConkey, blood (HIMEDIA-India) and chocolate agar for up 96 hours before being regarded as negative. Identification was based on characteristic morphology, gram stain appearance and standard commercially prepared biochemical tests; (DNase test, Novobiocin test, Streptex reagent, Indole test, Urea test, Kligler Iron agar, Citrate test).

Antimicrobial susceptibility of the bacterial pathogens isolated was determined by the Kirby-Bauer diffusion method. Antibiotics discs used were ampicillin 10µg, cloxacillin 5µg, gentamicin 10µg, amikacin 30µg, cefuroxime 30µg, and ceftriaxone 30µg. (Liofilchem Bacteriology Products - Italy)

Interpretation was done after 18 to 24 hours of incubation at 37°C on Muller-Hinton agar (HIMEDIA laboratories-India). Inhibition halos were measured comparing them with the zone diameter of the standard NCCLS inhibition halos. Sensitivity was recorded when the zone diameter (mm) was more than the standard NCCLS halos and resistant when was less for that particular antibiotic disc, a sliding callipers, held at the back of the inverted Petri plate was used to measure the diameter to the nearest whole millimetre (mm). Results were recorded as sensitive, intermediate or as resistant. In this study for convenient interpretation, intermediate results were categorized as resistant during data analysis. Also the study did not evaluate the clinical significant of coagulase negative staphylococci hence were not considered as aetiological agent in this study therefore no susceptibility test was done to organism isolated

3.11.3 Pus swab collection

Pus swab from the umbilicus cord or septic pustules was taken using a sterile swab on a stick and transported in a Stuart transport media (DELTALAB, Spain) to laboratory for processing and testing

In the laboratory, pus swab was smeared on a glass slide for gram staining identification was done. On MacConkey agar (HIMEDIA laboratories-India) inoculated pus swab by standard inoculation method then incubated at 37°C overnight. Thereafter, colonies identification and sensitivity were tested as described above.

3.11.4 Urine Collection

Urine sample collected by supra-pubic aspiration of the bladder aspirating 3 ml of urine after cleansing the skin with 2% povidone-iodine solution and a 70% alcohol swab and put into the sterile container. In an event of dry tap, the aspiration was repeated within one hour. The specimen were analysed within one hour of collection, urine not analysed in an hour of collection was kept in refrigerator at 4^0C and was analysed not later than 18 hours from collection time. Three mls of urine collected from neonates was used for urinalysis, and for culture and sensitivity. The author or his assistants assessed urine for macroscopic appearance and using Urine Reagent Strips (CYBOW ™) 1.5 mls was used for urine chemistry the remaining was taken to the laboratory.

In the laboratory, a sample of urine was inoculated using a standard wire loop was streaked on Cysteine Lactose Electrolyte Deficient culture medium (CLED; HIMEDIA-India) incubated at 37°C overnight, identification as earlier described.

Quality Control

Quality control was ensured throughout the study period, media were prepared according to the manufacturer's recommendations and after preparation plates from every plate prepared, were incubated at 37°C for 24 hours. To ensure no contamination of the media, it was checked regularly with the laboratory standard control organisms. All laboratory work was performed according to standard operational procedures in the laboratory.

Follow-up of the study participants to ascertain immediate outcome was monitored every two hours.

4.0 ETHICAL ISSUES

4.1 Ethical Clearance

Ethical clearance to conduct this study was sought and granted by the Higher Degree Research and Publication Committee of the Muhimbili University of Health and Allied Sciences, Dar es Salaam. Permission to conduct the study was granted by the Muhimbili National Hospital authorities

4.2 Ethical Consideration

Written informed consent to participate in the study was obtained from parents/guardians prior to enrolment in the study. Every neonate's parent/guardian was informed beforehand to ensure that he or she had the information needed to make an informed choice. A complete description of the aims of the study, benefits, risks, and blood collection procedures were explained to the caretakers and that data were being collected to ensure proper management in neonates with septicaemia at MNH and his or her privacy and anonymity would be respected. In this study, irrespective whether or not neonates participated, would receive appropriate management as per standard of care at MNH. Signed consent forms were obtained before data collection. Blood culture results were taken to the ward and treatment was done according to their sensitivity pattern. (Appendix IV).

5.0 DATA MANAGEMENT

Data was cleared for accuracy and then was entered into the computer and cleaned by the principal investigator. Analysis was done by Statistical Package for Social Science (SPSS) software version 15 for windows evaluation. Participants' characteristics of age and sex were summarized using frequency distribution tables. Continuous variables were summarized as means. Chi square test and Fishers exact tests were performed to examine the distribution between categorical variables. Fisher's exact test was used to compare proportions in two by two tables where expected value in a cell was less than five. p-value of less than or equal to 0.05 was considered biostatistically significant

6.0 RESULTS

During the study period, 1881 neonates were admitted to the neonatal ward. Three hundred thirty (17.5%) neonates with clinical diagnosis of neonatal septicaemia were recruited. Of these 160 (48.5%) were females, with female to male ratio of approximately 1:1. The overall mean age of the participants was 6 days. The majority of neonates were aged between 0 to 6 days (76.7%), while 77 (23.3%) aged between 7 to 28 days. Thirty three percent of neonates had low birth weight, while the remaining 67% had birth weight within the normal range. Preterm neonates accounted for 23.3% of the recruited participants.

The most frequently observed clinical features were the presence of umbilical pus discharge with hyperaemic abdominal area (96.1%) and fever (91.5%). Other clinical features reported include inability breast feed (62.4%), lower chest wall in drawing (27.0%), difficulty in breathing (22.1%), jaundice (13.3%), bulging fontanelle (10.0%), skin rashes with pus spots (9.4%), convulsion (3.0%) and hypothermia (2.4%) as shown in Table 1.

Table 1: Distribution of Demographic patient's characteristics and clin

Variables	N=330	%
Age (Days)		
0 – 6	253	76.7
7 – 28	77	23.3
Sex		
Male	170	51.5
Female	160	48.5
Weight (gm)		
≤ 2500	109	33
> 2500	221	67
Maturity		
Preterm	77	23.3
Term	253	76.7
Clinical features (signs & symptoms)		
Fever	302	91.5
Hypothermia	8	2.4
Inability to feed	206	62.4
Bulging fontanelle	33	10.0
Difficulty in breathing	73	22.1
Lower chest wall in drawing	89	27.0
Convulsions	10	3.0
Umbilical pus discharge with hyperaemia	317	96.1
Skin rash with pus spots	31	9.4
Jaundice	44	13.3

Overall bacteria isolates were 371 from 330 neonates, 74 (19.9%) of bacteria isolates were from blood and 297 (80.1%) from pus. In total, the diagnosis of septicaemia was confirmed by blood culture in 74 (22.4%) neonates, 52 neonates had early onset and 22 neonates having late onset septicaemia.

Staphylococcus aureus was the most common isolated organism from blood culture and pus swab, followed by *Klebsiella species and E. coli.* These three organisms accounted for approximately 85% of all isolates in blood culture and 94.3% of all isolates from pus swab. *Klebsiella spp (32.7%)* were predominant at the age 0 to 6 days and *S. aureus (*54.5%) were more found in neonates with 7 - 28 days of age (Table 2). *Staphylococcus aureus* (42.1%) and *Klebsiella spp* (38.9%) were the predominant isolated organisms in male and female neonates respectively. (Table 3).

Table 2: Distribution of bacteria isolates in blood according to age (N = 74)

	Age (days)		
Organism	0 – 6 n(%)	7 – 28 n(%)	Total N(%)
Staphylococcus aureus	15 (28.8)	12 (54.5)	27 (36.5)
Klebsiella species	17 (32.7)	5 (22.7)	22 (29.7)
Escherichia coli	10 (19.2)	4 (18.2)	14 (18.9)
Staphylococcus epidermidis	6 (11.5)	0 (0)	6 (8.1)
Group B Streptococci	1 (1.9)	0 (0)	1 (1.4)
Pseudomonas spp	1 (1.9)	1 (4.5)	2 (2.7)
Other *streptococcus species*	2 (3.8)	0 (0)	2 (2.7)
Total	**52 (100)**	**22 (100)**	**74 (100)**

Table 3: Distribution of bacteria isolates in blood according to sex: (N = 74)

	Sex		
Organism	Male n(%)	Female n(%)	Total N(%)
Staphylococcus aureus	16 (42.1)	11 (30.6)	27 (36.5)
Klebsiella species	8 (21.1)	14 (38.9)	22 (29.7)
Escherichia coli	9 (23.7)	5 (13.9)	14 (18.9)
Staphylococcus epidermidis	1 (2.6)	5 (13.9)	6 (8.1)
Group B Streptococci	1 (2.6)	0 (0)	1 (1.4)
Pseudomonas spp	1 (2.6)	1 (2.8)	2 (2.7)
Other *streptococcus species*	2 (5.3)	0 (0)	2 (2.7)
Total	**38 (100)**	**36 (100)**	**74 (100)**

Antimicrobial susceptibility pattern was performed for 68 bacteria and 294 bacteria isolated from blood and pus swab respectively. Highest resistance was noted against ampicillin (88.2%) and cloxacillin (85.3%) in isolated bacteria from blood culture. Of the isolated organisms *Klebsiella spp* 100% and 95.5% were resistance to ampicillin and cloxacillin respectively,while *E. coli* was 92.9% and 100% were resistant to ampicillin and cloxacillin respectively. Resistant pattern of isolated organisms were 58.8% overall against gentamicin, of the isolated bacteria resistant was 77.3% Klebsiella spp, 55.6% *S. aureus* and 42.9% *E. coli.* However, isolated bacteria were least resistant to amikacin (1.5%) and ceftriaxone (16.2%). Hence, the isolated bacteria were resistant to commonly used antibiotics, while sensitivity was noted to amikacin, ceftriaxone and cefuroxime. (Table 4)

Table 4: Antibacterial susceptibility pattern of bacteria isolated from blood (N = 68)

	Antibacterial agent					
	Amp	Clox	Gent	Amik	Cefur	Ceft
	R	R	R	R	R	R
Organism	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Staphy. Aureus	23(85.2)	22(81.5)	15(55.6)	0(0)	4(14.8)	4(14.8)
Klebsiella species	22(100)	21(95.5)	17(77.3)	1(4.5)	8(36.4)	4(18.2)
E. coli	13(92.9)	14(100)	6(42.9)	0(0)	1(7.1)	2(14.3)
Group B Streptococci	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
Pseudomonas spp	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	1(50)
Other *strept. Species*	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
Total	**60(88.2)**	**58(85.3)**	**40(58.8)**	**1(1.5)**	**14(20.6)**	**11(16.2)**

Key:

Amp: Ampicillin, Clox: Cloxacillin, Gent: Gentamycin, Amik: Amikacin, Cefur: Cefuroxime, Ceft: Ceftriaxone

R: Resistance

Among the 330 neonates, 297 (90.0%) had positive pus swab culture from both the umbilical discharge and skin pus spots. Antimicrobial sensitivity pattern was done to 294 of the different organism isolated and highest resistant was noted against ampicillin (90.8%), cloxacillin (86.0%) moderate resistance was noted against gentamicin (50.9%) to the bacteria isolated in pus swab. Of the isolated *Klebsiella spp* 97.4% and *E. coli* 88.7% and 84.5% were resistant to ampicillin and cloxacillin respectively, while resistant pattern of isolated organisms were 51.0% overall against gentamicin of the isolates 61.0% Klebsiella spp, 52.3% *S. aureus* and 39.4% *E. coli.* However, overall resistant to amikacin was 4.8% and ceftriaxone was 38.4%. (Table 5)

Table 5: Antibacterial susceptibility pattern of bacteria isolated from swabs (N = 294)

	Antibacterial agent					
	Amp	Clox	Gent	Amik	Cefur	Ceft
	R	R	R	R	R	R
Organism	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Staphy aureus	116(87.9)	106(80.3)	69(52.3)	7(5.3)	45(34.1)	55(41.7)
Kleb species	75(97.4)	75(97.4)	47(61)	2(2.6)	34(44.2)	31(40.3)
E. coli	63(88.7)	60(84.5)	28(39.4)	5(7)	24(33.8)	19(26.8)
Pseudomonas spp	7 (100)	7 (100)	4 (57.1)	0 (0)	4 (57.1)	6(85.7)
Proteus species	6 (85.7)	5 (71.4)	2 (28.6)	0 (0)	0 (0)	2 (28.6)
Total	**267(90.8)**	**253(86.1)**	**150(51.0)**	**14(4.8)**	**107(36.4)**	**113(38.4)**

Key:

Amp: Ampicillin, Clox: Cloxacillin, Gent: Gentamycin, Amik: Amikacin, Cefur: Cefuroxime, Ceft: Ceftriaxone

R: Resistance

In the current study 297 (90.0%) neonates had positive pus swabs culture result whereas 69 (23.2%) had both pus and blood culture positive result. However, neonates with both blood and pus swabs culture positive results were comparable with those blood culture positive and pus swabs culture negative (23.2% versus 17.6%, p = 0.48)

Among the neonates participated in the study, no bacteria isolates were observed from urine specimens.

In the study blood culture were collected in all 330 participants, while umbilical swabs and skin swabs were collected in 317 and 31 participants respectively. The positive rates to this sites contributes to 22.4% blood, 89.9% umbilical swabs and 90.3% skin swabs (Table 6)

Table 6: Culture isolates from different sites and their positive rates.

Culture sites	Positive rates n (%)	Total N
Blood	74 (22.4%)	330
Umbilical swabs	285 (89.9%)	317
Skin swabs	28 (90.3%)	31

Of the clinical features among the study participants; hypothermia, fever, and normal temperature was observed in 8 (2.4%), 302 (91.5%), and 20 (6.1%) neonates respectively. On the other hand, septicaemia was found in 62.2%, 21.9%, and 15% of neonates with hypothermia, fever, and normal temperature ($p = 0.02$) respectively. Sixty-eight neonates had umbilical pus discharge and hyperaemic abdominal area compared with those without umbilical pus discharge and hyperaemic abdominal area $p = 0.04$. While 16 neonates (51.6%) having skin rash with pus spots had septicaemia compared with 19.4% without skin rash with pus spots ($p < 0.001$).

In this study, the presence of septicaemia had an insignificant association with inability to feed, bulging fontanelle, convulsion, jaundice and lower chest wall in drawing. (Table 7)

Table 7: Distribution of clinical features and septicaemia **(N = 330)**

Clinical features:	Has septicaemia n (%)	No septicaemia n (%)	Total N (%)	p value
Temperature				
Hypothermia	5 (62.5)	3 (37.5)	8 (100)	0.02 ‡
Normal	3 (15.0)	17 (85.0)	20 (100)	
Fever	66 (21.9)	236 (78.1)	302 (100)	
Inability to feed				
Yes	50 (24.3)	156 (75.7)	206 (100)	0.30*
No	24 (19.4)	100 (80.6)	124 (100)	
Bulging fontanelle				
Yes	8 (24.2)	25 (75.8)	33 (100)	0.79*
No	66 (22.2)	231 (77.8)	297 (100)	
Difficult breathing				
Yes	17 (23.3)	56 (76.7)	73 (100)	0.84*
No	57 (22.2)	200 (77.8)	257 (100)	
Lower chest wall in drawing				
Yes	21 (23.6)	68 (76.4)	89 (100)	0.76*
No	53 (22.0)	188 (78.0)	241 (100)	
Convulsion				
Yes	1 (10.0)	9 (90.0)	10 (100)	0.48 ‡
No	73 (22.8)	247 (77.2)	320 (100)	
Umbilical pus discharge with hyperaemia				
Yes	68 (21.5)	249 (78.5)	317 (100)	0.04*
No	6 (46.2)	7 (53.8)	13 (100)	

Skin rash with pus spots				
Yes	16 (51.6)	15 (48.4)	31 (100)	<0.001*
No	58 (19.4)	241 (80.6)	299 (100)	
Jaundice				
Yes	12 (27.3)	32 (72.7)	44 (100)	0.41*
No	62 (21.7)	224 (78.3)	286 (100)	
Total	**74 (22.4)**	**256 (77.7)**	**330 (100)**	

*p- value for Chi square test. ‡ p-value for Fisher's exact test

Of the 330 study neonates, most of the neonates (86.1%) recovered regardless whether they had septicaemia or not. However death was more among neonates less than 7 days (14.2%) and mortality was independent of age. p = 0.78 (table 8).

Table 8: Distribution of immediate outcome by Age (N = 330)

	Outcome		
Age (days)	**Recovered n (%)**	**Died n (%)**	**P value**
0 – 6	217 (85.8)	36 (14.2)	0.78*
7 – 28	67 (87.0)	10 (13.0)	
Total	284 (86.1)	46 (13.9)	

*p- value for Chi square test

Forty-six (13.9%) neonates recruited in the study died. Among those with neonatal septicaemia 24.3% died, 11 (61.1%) of them dying within 72 hours of admission. Septicaemia was significant associated with death (p-value 0.003) as shown in table 9.

Table 9: Distribution of immediate outcome and septicaemia (N = 330)

Septicaemia	Outcome		
	Recovered n (%)	Died n (%)	P value
Has	56 (75.7)	18 (24.3)	0.003*
No	228 (89.1)	28 (10.9)	
Total	284 (86.1)	46 (13.9)	

*p- value for Chi square test

Hypothermia (OR† 0.22, p = 0.05) and skin rash with pus spots (OR† 0.26, p = 0.004) were independently significant predictors of septicaemia in multiple logistic regression analysis. (Table 10)

Table 10: Clinical feature as independent predictors of septicaemia

Variable	OR†	(95% CI)	p-value
Hypothermia	0.22	(0.05 – 1.00)	0.05
Umbilical pus discharge with hyperaemia	1.16	(0.29 – 4.54)	0.84
Skin rash with pus spots	0.26	(0.10 – 0.66)	0.004

Key: OR† = adjusted odds ratio, 95% CI = 95% Confidence interval

In this study hypothermia, skin rash with pus spots and Umbilical pus discharge with hyperaemia had no independent significant association with neonatal death (Table 11)

Table 11: Clinical feature as independent predictors of death

Variable	OR†	(95% CI)	p-value
Hypothermia	2.06	(0.38 – 11.07)	0.40
Umbilical pus discharge with hyperaemia	0.59	(0.11 – 3.01)	0.52
Skin rash with pus spots	1.19	(0.36 – 3.93)	0.77

Key: OR† = adjusted odds ratio, 95% CI = 95% Confidence interval

7.0 DISCUSSION:

The aim of this study was to determine the aetiology, anti-microbial susceptibility pattern and immediate outcome of neonatal bacterial septicaemia in neonatal unit at Muhimbili National Hospital (MNH). In addition, isolates from pus lesions were isolated for identification as well as determination of antimicrobial susceptibility pattern.

In this study, the frequency of bacterial infection in blood as revealed by culture results was 22.4%. This rate is higher compared with previous studies done at the same facility by Mchomvu JAK et al [33] and Blomberg et al.[11] In those studies, incidences of neonatal septicaemia were 8.9 per 1000 live birth and 16%, respectively. The difference in prevalence noted could be accounted for by differences in specimen collection and handling as well as study design. For example, in this study conventional agar media were used for bacterial isolation and incubation was done under facultatively anaerobic conditions. This is different from Blomberg study, in which myco/F lytic blood-culturing methods were used, which supports superior yields for the growth of bacteria, fungi and mycobacterium.

According to culture results, a number of bacteria were isolated. Among the isolates, *S. aureus* was the predominant gram-positive organisms (36.5%), while *Klebsiella species* (29.7%) and *Escherichia coli* (18.9%) were the commonly isolated gram-negative organisms. The common organism isolated in early onset septicaemia (neonates aged 0 to 6 days), was *Klebsiella species* (32.7%), followed by *S. aureus* (28.8%%) and E. coli (19.2%%). Collectively these three organisms accounted 80.7% % of all the bacterial isolates. The predominance of Klebsiella spp found in the present study is in keeping with the findings by Blomberg et al in the same facility (MNH) where *Klebsiella spp* accounted for 28.2% of the isolates. [11] Similar observations were also noted by Zaidi AK et al in Pakistan, where 25% of isolated bacteria were *klebsiella spp.*[13] Collectively these studies seem to indicate that *Klebsiella spp* is the

most frequent bacterial isolate among neonates aged 0 - 6 days at MNH, followed by *S. aureus* and *E. coli*.

For late onset septicaemia (neonates aged 7 to 28 days), *S. aureus* was the most frequent isolate (54.5%), followed by *Klebsiella spp* (22.7%), and *E. coli* (18.2%). Collectively these three organisms accounted for 94.9% of all the bacteria isolates. The predominance of *S. aureus* found in this study is in keeping with findings of another study conducted in Mulago, Uganda where *S. aureus* accounted for 70.9% of all isolates,[23] but different from the study done by Blomberg et al in MNH, in which the predominant bacteria was *Klebsiella spp* (22.6%).[11] The differences between this study and that of Bloomberg could be attributed, at least in part, to the age differences of the studied groups. In this study, children were aged up to 28 days, while in the Bloomberg study children were aged up to seven years. The difference could also be due to other factors that were not investigated e.g. HIV infection and malnutrition.[11]

Comparison of this study and that done in 2002 by Bloomberg (both conducted at MNH) seem to suggest time and age variations in the aetiology of neonatal septicaemia. These findings support the need for continuous age-specific surveillance of the aetiology of neonatal bacteria septicaemia.

In this study positive pus swab results were found in 297 (90%) of neonates. Among the isolates, *S. aureus* (44.4%) was the most frequently isolate followed by *Klebsiella species* (25.9%) and *Escherichia coli* (23.9%). Collectively these three organisms contributed to 94.3% of all bacteria isolates from pus swab.

Among the 297 neonates organisms isolated from pus swabs culture, sixty-nine cases (23.2%) had similar identity with those isolated from blood samples. *Klebsiella spp* contributed to 37.7%, followed by *S. aureus* (27.5%) and *E. coli* (20.3%). The similarity of organisms seems to suggest that bacteria found in the infected cord or septic spots were most likely the source

of infections of the blood stream. However, to confirm this DNA finger printing technique would be required to compare the isolates.

In comparing the resistant pattern of the current study with done previous by Blomberg, results shows in both studies there was higher resistance of the common isolated organism to ampicillin. Resistance pattern of *S. aureus* to cloxacillin increased considerably ranging from 81.5% (S. aureus) to 100% (E. coli) in current study compared to Blomberg study there was high-rise of resistance against S. aureus from 12% to 85%. S. aureus and Klebsiella spp had considerable increased resistant pattern to gentamicin in current study as compared to Blomberg study, while E. coli was noted in both studies no considerable change in resistant pattern.

Resistance to cefuroxime and third generation cephalosporins was much less ranging from 7.1% (E. coli) to 36.4% (Klebsiella) for cefuroxime and from 14.3% (E. coli) to 18.2% (18.2) levels that are comparable with the finding of Blomberg (12 – 21%). S. aureus and Klebsiella spp had no considerable change in resistant pattern in both studies while there was decreased resistance to E. coli in the current study as compared to Blomberg study. The resistance pattern of S. aureus, Klebsiella spp and E. coli to cefuroxime and third generation cephalosporins had no considerable change. (Table 12)

Table 12: Comparison of antibacterial susceptibility pattern in blood isolates between Blomberg study and current study

Antibacterial	Bacteria isolated	Blomberg study (%)	Current study (%)
Ampicillin	*S. aureus*	100	85.2
	Klebsiella spp	100	100
	E. coli	85	92.9
Cloxacillin	*S. aureus*	12	81.5*
	Klebsiella spp	ND	95.5
	E. coli	ND	100
Gentamicin	*S. aureus*	19	55.6*
	Klebsiella spp	47	77.3*
	E. coli	46	42.9 NCC
Cefuroxime	*S. aureus*	12	14.8 NCC
	Klebsiella spp	26	36.4
	E. coli	46	7.1**
Third generation cephalosporins (Ceftazidime for Blomberg and Ceftriaxone in current study)	*S. aureus*	20	14.8
	Klebsiella spp	21	18.2
	E. coli	12	14.3

* Increased resistance; ** Decreased resistance; ND Not tested in Blomberg study; NCC no considerable change

Resistance pattern of *S. aureus*, and *Klebsiella spp* increased appreciably to ampicillin, cloxacillin and gentamicin in the current study as compared to Blomberg study.[11] The E. coli had increased resistance to ampicillin and cloxacillin but was not resistant to gentamicin.

This represents a rapid increase in resistance to gentamicin and cloxacillin. The differences in resistance of microorganisms against tested antibiotics are most likely due to overuse, leading to evolution of resistant strains. In both studies *S. aureus*, *Klebsiella spp* and *E. coli* were noticeably sensitive to cefuroxime and third generation cephalosporin most probably due to

limited use of these antibiotics. (Table 12) Additionally the relative order of prescribed drugs in the neonatal unit decreases from ampicillin, cloxacillin, gentamicin to third generation cephalosporins.

Therefore, this has impact in the treatment of neonates which shows most of the initially used antibiotic in the treatment of neonatal septicaemia have considerable drug resistance

However, in this study the bacteria isolated from blood were most sensitive to amikacin. This sensitivity could be explained by the efficacy of the drug but also in our set up there is minimal use of drug compared to other antibacterial in the treatment of neonatal bacteria septicaemia.

In this study, no bacteria isolates were observed from urine specimens of the participants; which is consistent with findings from other studies. A retrospective study by Tamim et al. reported that no infant (n= 349) had positive urine culture at admission or during the first day of life (less than 6 days).[37] Contrary to the current study, these authors also found that approximately 25.3% of 189 infants had positive urine cultures while 18 infants (38%) with positive urine cultures had positive blood cultures. These differences could be due to study designs since the current study being prospective while Tamim et al. is retrospective study, as well as the age difference, not all isolates were from neonates.

The current study findings are also inconsistent with the results reported by Barton et al. as 51 (38%) of 135 neonates had confirmed urinary tract infection.[38] This inconsistency could be explained by the different methods of collecting urine samples such as urine bag, catheter, and suprapubic aspirate whereas the latter method was only used in this study.

Among neonates suspected with neonatal septicaemia using clinical presentation, 22.4% were confirmed to have bacterial septicaemia and no significant difference was observed among neonates with early or late onset septicaemia. ($p > 0.05$) This is comparable with results reported by Ojukwu et al in Nigeria as 23.9% of 138 neonates had confirmed neonatal septicaemia.[39] However findings were lower compared to J. Mugalu et al in Uganda in which neonatal septicaemia was 37%.[23] The difference could be explained by the vastly presentation used in current study.

Of the participants in the current study, clinical features such as hypothermia, fever, umbilical pus discharge with hyperaemia and skin rash with pus spots were found to be independently associated with septicaemia. ($p < 0.05$) These findings are supported by results of J. Mugalu et al that demonstrated the occurrence of neonatal septicaemia had significant association with history of umbilical pus discharge with reddening of the anterior abdominal wall and skin rash with pus discharge.[23] In addition, hypothermia and Skin rash with pus spots were found to be predictors of neonatal septicaemia in a multivariate logistic regression analysis. These clinical features could be used to predict neonatal septicaemia and therefore instigation of proper empirical management

In this study, the immediate outcome was measured in terms of recovery or death. Situation analysis 2009, mortality in neonates with septicaemia was 24.3%; this was lower than that reported in the TDHS report of 2004/2005 (29%).[4] However, it was higher compared to mortality noted by Blomberg et al (14.8%) [11] and by Simiyu et al (15%).[24] There was no significant difference in mortality by age group. In the current study, majority of death occurred within 72 hours of admission without significant difference in mortality by age group, and clinical features were not predictors of mortality. These findings may be in part due to severe disease at presentation, delay in seeking medical attention as well as poor supportive care. Moreover, a previous study in the same centre had shown antimicrobial resistance to be an independent predictor of mortality; this may also explain the high mortality seen in this study.[11]

7.1 STUDY LIMITATION

- In this study, isolation of anaerobic microorganism was not done due limited availability of laboratory identification tests.
- Other aetiological agents of neonatal septicaemia such as viral and fungal were not investigated and excluded due to limited resources.
- Neonates may have other co morbidities, which might have contributed to their death, were not fully investigated and followed up.
- CSF was not examined

8.0 CONCLUSIONS AND RECOMMENDATIONS

8.1 CONCLUSIONS

In this study, the common aetiological agents were *S. aureus, Klebsiella spp* and *E. coli*. In early onset septicaemia, *Klebsiella spp* and *E. coli* were the predominant organisms, while *S. aureus* was predominant in late onset septicaemia. The microorganisms isolated were highly resistant to ampicillin (88.2%) and cloxacillin (85.3%), moderate resistant to gentamicin (58.8%). The most effective antibiotic was amikacin; however, ceftriaxone as well as cefuroxime also showed to be sensitive to microorganisms isolated with a resistance of 16.2% and 20.6% respectively. Eleven neonates (61.1%) with neonatal septicaemia, died within the first 72 hours after admission

8.2 RECOMMENDATION

- The use of ampicillin, cloxacillin and gentamicin as first line treatment of neonatal bacteria septicaemia should be reviewed, due to the significant and progressive increase in drug resistance, additional studies are needed to find the depth of the problem.
- Amikacin should be used as the empirical therapy for the treatment of neonatal septicaemia and third generation antibiotic (ceftriaxone, etc) as second line.
- Neonates with clinical diagnosis of neonatal septicaemia pus swab and blood for culture and sensitivity should be done routinely for all patients with suspected septicaemia
- Antibiotic susceptibility surveillance, evaluation of local pathogens causing neonatal septicaemia and detection of any shift in their antimicrobial susceptibilities should be done at least every 5 to 10 years.

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