BACTERIAL AETIOLOGY AND FACTORS ASSOCIATED WITH POOR TREATMENT OUTCOME AMONG CHILDREN WITH BLOODSTREAM INFECTION AT MUHIMBILI NATIONAL HOSPITAL

Paschal Stanley, BSc-BLS

MSc. (Microbiology and Immunology) Dissertation Report at

The Muhimbili University of Health and Allied Sciences

October 2021.

Muhimbili University of Health and Allied Sciences

Department of Microbiology and Immunology

TITLE: BACTERIAL AETIOLOGY AND FACTORS ASSOCIATED WITH POOR TREATMENT OUTCOME AMONG CHILDREN WITH BLOODSTREAM INFECTION AT MUHIMBILI NATIONAL HOSPITAL

By

Paschal Stanley

A Dissertation submitted in (Partial) Fulfillment of the Requirements for the Degree of Master of Science (Microbiology and Immunology) of

Muhimbili University of Health and Allied Sciences

October, 2021

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled: "bacterial aetiology, and factors associated with poor treatment outcomes among children with bloodstream infection at Muhimbili National Hospital", in (partial) fulfilment of the requirements for the degree of Master of Science (Microbiology and Immunology) of Muhimbili University of Health and Allied Sciences.

…….

Dr Joel Manyahi

(Supervisor)

……..

Ms Upendo Kibwana

(Co-supervisor)

…….

Date

DECLARATION

I, **Paschal Stanley,** hereby declare that this dissertation is my 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.

Signature…… Date…….

ACKNOWLEDGEMENTS

Primarily, I express my genuine thankfulness to Almighty God for his grace throughout this study. Secondly, I appreciate my supervisor Dr Joel Manyahi and my co-supervisor Madame Upendo Kibwana for their assistance and support in every step throughout this study. Their guidance helped was important all the time of research and writing this dissertation. Thanks to all academic members and supportive staff of the Department of Microbiology and Immunology mentioned Prof. (E. Lyamuya, M. Matee, and S. Aboud), Dr. (A. Joachim, M. Majigo, F. Msafiri, and D. Kamori) and Ms L. Nkinda for their encouragement and support during my residency time.

Mine acknowledge and appreciation is to staff members of the Muhimbili National Hospital specifically of the pediatric unit, Neonatal unit, Microbiology laboratory department (Central Pathology Laboratory), and of the Library at Muhimbili National Hospital (MNH) for their support for data collection and sample processing and the results from the documentation.

DEDICATION

This dissertation is dedicated to my parents Mr and Mrs Stanley P. Salala and the children of Mr and Mrs Paschal Stanley

ABSTRACT

Background

Bloodstream infections (BSI) are a significant cause of morbidity and mortality of children, especially in resource-limited settings. The objective of this study was to **assess** proportion of BSI, bacterial aetiology, antimicrobial susceptibility patterns, and factors associated with poor treatment outcomes in children with BSI at Muhimbili National Hospital.

Materials and methods: This was a cross-sectional study design conducted at the Muhimbili National Hospital between June and October 2020. A total of 650 Blood culture **samples** from children of age 0–15 years were studied. Microbiological data of bacterial, social-demographics and clinical data were captured summarized in descriptive tables and analyzed by using Stata software. A statistical tool STATA version 12 was used during analysis and a *p*-value of <= 0.05, at 95% CI was considered significant.

Results: Out of 650 blood culture samples, 289 (44.5 %) were culture positive. The proportion of bacteria pathogens was 21.5% (140/650) of the total blood culture samples. The proportion of BSI based on age (years) group was <1, 1–24, 25-48, 49-72, 73-96, 97-120 and >120 were 33.2%, 15.6%, 14.5%, 21%, 10.5%, 13.6% and 11.8%, respectively. Gram-negative bacteria (GNB) were mostly isolated (102/140, 72.9%), and the common isolated bacteria was *Klebsiella spp.* (35.0%). Other isolated bacteria were *Staphylococcus aureus* (22.1%), *Escherichia coli* (15.7%), *Acinetobacter baumannii* (8.6%), *Pseudomonas aeruginosa* (7.1%), *Streptococcus spp* (5.0%) and other Gram-negative bacteria 9 (5.9%). The overall resistance of bacteria to ampicillin and Gentamycin were 72.1% and 56.4% respectively. Gram-negative bacteria were resistant to ampicillin (79.4%), Gentamycin (60.8%), Ceftriaxone (73.5%), ciprofloxacin (55.9%), ceftazidime (80.2%) and was less when tested to amoxicillin-clavulanic acid (33.3%) and Meropenem (19.6%). The analysis shows that, in-hospital mortality rate following BSI was 10.3%, and was associated with Gram-negative bacteria infection. The length of stay in the hospital was associated with MDR, admission in the oncology ward, initially prescription of antibiotics.

Conclusions: The proportion of BSI from blood culture samples obtained from hospitalized children was 21.5%. Majority of isolated bacteria showed high resistance to the commonly use

antimicrobial agents in children (especially in neonates and under five children) for first-line and second-line. Therefore, more studies are required to be done so as to extract more information and hence to come out with a valuable decision on treatment and management of children with BSI.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

AMR	Antimicrobial Resistance
API 20E	Analytical Profile Index 20E
HAI	Hospital-Acquired Infection
CAI	Community-Acquired Infection
BSI	Bloodstream Infection
CLSI	Clinical Laboratory Standard Institute
CPL	Central Pathology Laboratory
BA	Blood Agar
DDST	Double Disc Synergy Test
CA	Chocolate Agar
AIDS	Acquired Immunodeficiency Syndrome
ESBL	Extended Spectrum Beta-Lactamase
ESBL-E	Extended Spectrum Beta-Lactamase producing *Enterobacteriaceae*
HIV	Human Immunodeficiency Virus
GPB	Gram-Positive Bacteria
GNB	Gram-Negative Bacteria
HAI	Hospital-Acquired Infection
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit

KIA	Kligler Iron Agar
MCA	MacConkey Agar
MDR	Multidrug Resistance
MHA	Mueller Hinton agar
MNH	Muhimbili National Hospital
MRSA	Methicillin-resistant Staphylococcus aureus
MUHAS	The Muhimbili University of Health and Allied Sciences

DEFINITION OF TERMS

According to this stud, BSI is going to be defined as the presence of bacteria microorganisms in the bloodstream.

Blood culture: Collection and inoculation of blood into the culture medium to grow pathogenic bacteria or fungi for diagnostic purposes.

Bacteremia: The presence of viable bacteria in the bloodstream which may be transient (e.g. following dental procedures), intermittent (e.g. undrained abscesses), or continuous (e.g. endovascular infection).

Contaminations of blood cultures refer to blood cultures positive for growth due to organisms that were not present in the bloodstream

Fever is a human's body temperature above the normal range of 36–37° Centigrade (98–100° Fahrenheit).

A contaminant is a non-pathogenic bacterium (mostly from the skin) such as coagulase-negative *Staphylococcus* species, *Corynebacterium* species, alpha- or gamma-hemolytic *Streptococci*, *Micrococcus* species, *Bacillus* species, and *Propionibacterium* species found in the blood.

Hospital-Acquired bloodstream infection that is first identified (culture has drawn) ≥ 48 h after hospital admission and within 48 h following hospital discharge

Community-Acquired Infection- Are those that occur in outpatients or are first identified ≤ 48 h after admission to the hospital

Sepsis is defined as "life-threatening organ dysfunction caused by a deregulated host response to infection.

Inappropriate antibiotic-initially prescribed antimicrobial agent which was not able to provide the intended purposes as observed during in vitro antimicrobial susceptibility test.

Poor treatment outcome: is when death or increased length of hospital stay by a patient when the initially prescribed with antimicrobial agent fail to provide the intended purpose

CHAPTER ONE

1.0 Introduction

1.1 Background:

Bacteria bloodstream infections (BSI) are a significant cause of morbidity and mortality in children (1). It can be community-acquired bloodstream infections (CA-BSI), or healthcare-associated bloodstream infections (HA- BSI) depending on the time of onset of the sign and symptom and bacteria isolation. HA- BSI is associated with morbidity, prolonged length of hospitalization, and mortality among children (2). In countries with limited resources, empiric management is applied for managing children with clinical signs and symptoms of BSI using broad-spectrum antimicrobial agents(3). However, this may lead to inappropriate management due to infection with multidrug-resistant bacteria and may cause the development of antimicrobial resistance of bacteria (4,5).

It should be noted that the aetiology of BSI is not static/same, but it changes with time, region, country, level of development, age, weight, nutrition status, immunologic maturity, co-morbidity, the physiologic state of the children, and the location of the child (home, hospital ward, intensive care, community) (6–8). Children of a young age are prone to infection since their immune system is immature and has less protection against infection (9). Neonates and children with co-morbidities have challenged immunity remaining susceptible to infectious agents (10). Recently, reported profiles of bacteria that cause BSI among under-five children in our settings are not limited to *Klebsiella pneumonia*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Salmonella spp. Enterococcus faecium*, *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* (CoNS), and *Enterobacter spp*.) (1,11–13). Children of higher age get an infection due to pathogenic bacteria, however, the information is still limited.

In Tanzania, Gram-negative bacteria such as *K. pneumonia*, *E. coli*, and *Salmonella spp* are the most common cause of BSI among children of different ages (6–10). Among neonates, the isolation rate of *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitides* in Tanzania have decreased due to the introduction of prenatal

screening and immunization (14). Furthermore, the emerging of multidrug resistance bacteria and Extended-spectrum better lactamase *Enterobacteriaceae* (ESBL) among pathogenic bacteria that cause BSI in children of different even to WHO recommended antimicrobial agents for first-line and second-line treatment options of children (3).

Therefore, this study aimed to demonstrate the current proportional, aetiology, the antimicrobial susceptibility pattern and to determine the factors associated with poor treatment outcomes of BSI in children at MNH.

1.2 problem Statement

Bloodstream infection is a major public health problem associated with high morbidity and mortality among children worldwide (15). Previous studies are reporting on the burden, aetiology, and antimicrobial susceptibility pattern of bacteria that cause BSI among children. (7–10). *E. coli, S. aureus* and *Klebsiella* spp., *Streptococcus pneumoniae (S. pneumoniae)*, *Klebsiella* spp. and non-typhoidal *Salmonella* (15) are among of the reported bacteria in neonates. In older children, *Salmonella typhi*, *E. coli*, *Staphylococcus aureus and Klebsiella spp.* are Some of the bacterial pathogens that can be isolated (16–18). However, there is a shortage of data on the current situation concerning BSI in children at Muhimbili national hospital (MNH). A periodic clinical surveillance is required to update the data and the information that would help to establish the best antibiotic management options for children with BSI at (MNH). In 2007, Blomberg and others reported on the burden and aetiology of BSI among under five children (1).

The aetiology that cause BSI in children changes from time, region, location and health condition of a child and with age groups. Furthermore, there is an increased emerging of multidrug-resistant (MDR) bacteria and ESBL-E that cause BSI in children and imposes a great challenges on treatment and management of BSI among children at MNH.

Therefore, this study was aimed at demonstrating out the current magnitude, the aetiology and their resistance pattern. Also, the factors associated with poor treatment outcomes among children with bloodstream infection at MMNH.

1.3 Conceptual framework

BSI among children can be associated with a health status of a child, age, sex, prematurity, location birth weight, nutrition status, aetiology agent, HIV status, hospitalization, and other co-morbidities. It also shows the outcome after treatment including death, prolonged time of stay in the hospital (1,2,11).

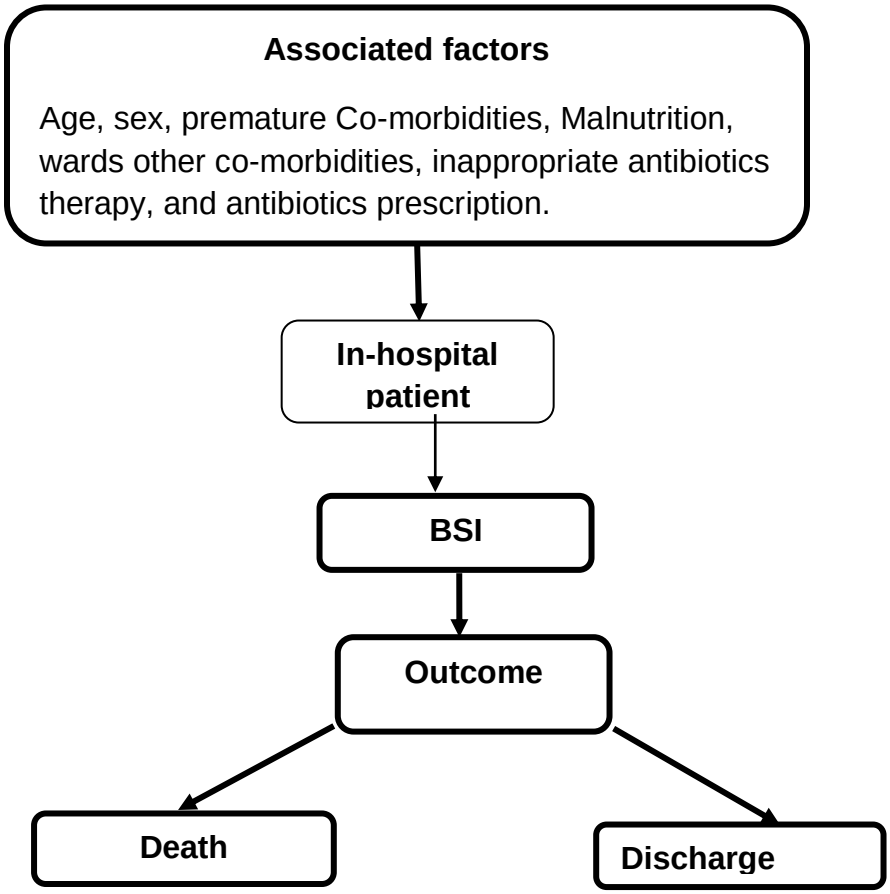


Figure 1: Conceptual framework

1.4 Rationale

The results of this study revealed the current magnitude of BSI, a profile of bacterial aetiology and their antimicrobial susceptibility pattern among isolated bacteria from positive blood culture samples collected from children at MNH. The study also revealed the factors associated with BSI and the factors associated with poor treatment outcomes among children with BSI at MNH.

Furthermore, the findings of this study also provide the opportunity to update the information on the aetiology of BSI in older children. The information on antimicrobial susceptibility patterns in this study can also be used to review and appraise the current practice of antimicrobial agent prescriptions for children with BSI during empiric management and the establishment of antimicrobial stewardship at the study settings MNH.

1.5 Research questions

1. What is the current magnitude of bloodstream infection in children admitted at MNH?
2. What is the current aetiology of BSI in children admitted at MNH?
3. What is the antimicrobial susceptibility pattern of isolated bacteria from children with BSI at MNH?
4. Which factors contribute to poor treatment outcomes in children with BSI admitted at MNH?

1.6.0 Objectives

1.6.1 Main objective

To determine the proportion of positive blood cultures, bacterial aetiology, antimicrobial susceptibility pattern, and factors associated with poor treatment outcomes in children with BSI, at Muhimbili National Hospital in Tanzania.

1.6.2 Specific objectives

1. To determine the proportion of positive cultures among the samples processed at CPL.

2. Determine bacterial aetiology of BSI among the samples processed at CPL.
3. To determine antimicrobial susceptibility pattern of bacteria isolated from blood culture samples of children admitted at MNH.
4. To determine factors associated with poor treatment outcomes in children with BSI admitted at MNH.

1.7.0 Literature review

1.7.1 Overview

Bacteria bloodstream infections (BSI) are a significant cause of morbidity and mortality among children (1). Samples for blood culture from children of age 0 to 15 years old suspected to have BSI were studied. BSI can either be community-acquired bloodstream infections (CA-BSI), or healthcare-associated bloodstream infections (HA-BSI) depending on the time of symptom and bacteria isolation. HA-BSI is associated with in-hospital children morbidity, prolonged length of hospitalization, and mortality among children (2). In 2012 about 6.9 million (uncertainty range 5.5 – 8.3 million) of death among neonates was due to bacterial infections in South Asia, sub-Saharan Africa, and Latin America (20). Children's BSI can be caused by either Gram-negative bacteria or Gram-positive bacteria or both of the two at the same time (21). Treatments of BSI requires a proper diagnosis which involves blood culture techniques for isolation identification of pathogenic bacteria and sensitivity to direct the correct patient treatment options (22–24). However, in low and middle-income countries, this is still a challenge and Mostly, treatments of BSI is based on empirical management using board spectrum antimicrobial agents using clinical diagnosis (3, 22). Fever and tympanic or auxiliary temperatures of ≥38.0 or ≤36°C are the most common clinical sign which is used for a suggestion of BSI in children (26,27).

1.7.2 Aetiology of bloodstream infection in children globally.

The spectrum of bacteria that cause BSI in children is widely changing by age, presenting symptoms, the resistance of the pathogen to antibiotics, and immune status (21). The introduction of vaccines for bacteria such as *Haemophilus influenzae*, *Neisseria meningitides* and *Streptococcus pneumoniae* has shifted the aetiology of BSI aetiology in

children especially among neonates and infants (8). The commonest bacteria that causes BSI in neonatal and infants are *E. coli, S. aureus* and *Klebsiella* spp., *Streptococcus pneumoniae (S. pneumoniae), Klebsiella* spp. and non-typhoidal *Salmonella* (15). In older children, *Salmonella typhi, E. coli, Staphylococcus aureus and Klebsiella spp.* are Some of the bacterial pathogens that can be isolated (16–18).

Among the study conducted in the United State including children of ≤19 years old, reported the Gram-positive bacteria to be the leading cause of bloodstream infection in children and CoNS was reported to be the common bacteria (28). Furthermore, a study from England and Wales reported on CoNS as the most common cause of BSI in children. Other bacteria reported in the study were *Neisseria meningitides, Escherichia coli* and *Staphylococcus aureus* (8), and there was a higher proportion of cases in children aged 1–11 months due to E. coli compared to children aged 1-4 years and 5-15 years (8). Furthermore, *Staphylococcus aureus* was reported as the common bacterial pathogen among bacteria isolates in South Africa followed by *Escherichia coli* among young children included in the study (29).

1.7.3 Aetiology of bloodstream infection among children in Tanzania.

In Tanzania, ***Klebsiella pneumoniae***, s*almonella spp, E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococci spp.* and CoNS have been reported to be the most common bacteria isolated from children especially in under five years (1,11,12,30,31). Some of these bacteria are reported to be MDR, ESBL producing pathogen, MRSA, and CRE (12,32,33). Studies have reported that Gram-negative bacteria from children with BSI are mostly isolated than Gram-positive bacteria and predominated by *K. pneumoniae* (1,14, 20). This study was aimed at determining the current magnitude, aetiology of BSI, antimicrobial pattern and the factors associated with poor outcomes among children of 0 to 15 years old at MNH.

1.7.4 Burden of BSI in children

The magnitude of BSI differs with time, place (geographical locations), age, comorbidities, development, and availability of health care facilities for providing health services and management to patients (35,36). In developed countries, children morbidity and mortality associated with BSI is low compared to that in low and middle-income countries like those

in Sub-Saharan **Africa where morbidity** and mortality are significantly higher (39,38). By improving the health care services and health facilities will reduce the rate of morbidity and mortality among children (37). Many studies in African countries have reported a higher prevalence of BSI, for example, Guinea-Bissau, reported a prevalence of 12% in 2014, Uganda 19.1% in 2015 and Ethiopia 20.6% in 2011 (39–41). In Tanzania, the burden of BSI among children is still higher ranging from 13.9% to 19.2%, and differs with study **area** and study population (1,11,12,14).

1.7. 5 Antimicrobial susceptibility pattern

The susceptibility pattern among bacteria that cause BSI is reported to change with increased resistance rate toward antimicrobial agents including the commonly used broad-spectrum antimicrobial agents as recommended by WHO for first-line and second-line options for children treatments and management (1,3,14). Many studies have reported on the changing of antimicrobial patterns among bacteria that cause BSI in children (12,42–44). The reported emergence of multidrug resistance bacteria including MRS and ESBL-E bacteria has changed the susceptibility pattern and favour resistance profile (1,14,45).

1.7. 6 Risk factors associated with BSI in children.

BSI can be influenced by many factors including age, immune status, nutrition status (malnutrition), premature birth, hospitalization time, birth weight and co-morbidities (1,2). Neonates and infants have immature immune systems to resist infection (1). Malnutrition suppresses different components of the immune system resulting in increased vulnerability and severity to infections in children of all ages (46). BSI caused by drug resistance, including MDR and ESBL bacteria has complications in management and treatments (7). Cancer patients and HIV-infected children have a higher chance of contracting BSI since their immune system is challenged. Furthermore, HIV-infected children are at higher risks of bacterial infection compared to HIV-uninfected children, including those with HIV-infected children on antiretroviral therapy (ART) (47).

1.7.7 Poor treatment outcome among children with BSI

It has been reported that death, increased length of hospital stay and health care costs, are among **the** poor outcome following children treatment and management (7,47). BSI is

among the cause of poor treatment outcomes and has been reported to cause death, increased hospital time and affects the social and income state of the family (49). Studies have reported a high rate of morbidity and mortality in neonates, infants, young children, and older children (31). Prolonged hospital stay is also attributed to factors such as co-morbidity, malnutrition, inappropriate antibiotic therapy (1,5).

The situation becomes complicated if BSI **is** caused by drug resistance bacteria, including MDR, CRE, and ESBL as they increase the morbidity of children. Co-morbidity is also reported to accelerate the poor outcome in children with positive culture blood (8). The situation becomes more worth in Low and Middle-Income countries where there are limited resources and health care facilities for providing good health care to patients. Incorrect antibiotic therapy may lead to drug resistance, prolonged hospital stay, higher health care costs and death (5).

CHAPTER TWO

2.0 Study Material and Methods:

2.1 Study design

A designed cross-sectional study was carried out on 650 blood culture samples collected from children of age 0 to 15 years old from May to October 2020 at MNH.

2.2 Study site

This study was conducted at Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania. MNH is the largest National tertiary health care facility in Tanzania and is a University teaching hospital with 1,500-bed capacities where 295 pediatric beds and approximately 1800 children get health care at MNH per month. It has 2700 employees where about 300 are doctors and specialists, 900 registered and enrolled nurses and the rest are supporting operations employees.

Geographically, MNH is located in Dar es Salaam at Upanga West administrative ward in Ilala district of Dar es Salaam region. It is located at 6°48' South, 39°17' East (−6.8000, 39.2833), on a natural harbour of the eastern coast of East Africa. The hospital saves referred patients from the regional, referral and zonal hospital of Tanzania and those within the Dar es Salaam region.

2. 3 Study population

The study involved blood culture specimen**s** collected from children aged 0-15 who were admitted at MNH with clinical signs and symptoms of BSI as indicated by medical doctors. Some of these clinical signs and symptoms are temperature (of > 38 °C or < 36 °C), age-specific tachycardia, age-specific tachypnea, convulsions, altered state of consciousness and abnormal feeding (24).

2.4.0 Selection criteria

2.4.1 Inclusion criteria:

1. All blood culture samples were collected from children of age 0-15 yrs.
2. All blood culture samples were obtained from children received at Central Pathology Laboratory (CPL) during the period study.

2.4.2 Exclusion criteria

1. All blood culture samples were collected from children hospitalized apart from Muhimbili National Hospital.
2. All repeated blood culture samples.

2.5 Study duration

This study was conducted for five months after the ethical clearance was given by the Muhimbili University of Health and Allied Sciences.

2.6 Sample size

The required sample size was estimated by the Kish Leslie formula.

- $n = \frac{Z^2 p(1-p)}{\mathcal{E}^2}$

Where,

n = sample size, p = (20.6%) prevalence at Jimma University Hospital, Ethiopia (40), Z = Standard normal deviation 95% ($\alpha = 1.96$) and $\mathcal{E}$ = Margin of error 0.03.

$$n = \frac{1.96^2 * 0.206\ (1 - 0.206)^2}{0{,}03^2}$$

n = 554

Adjusted sample size 1/0.85*554

≈ 652 blood culture samples.

2.8 Sampling technique`

The Conservative sampling technique was used to obtain blood culture samples, whereby every blood culture collected and meets inclusion criteria was enrolled in the study until the sample size was achieved.

2.9.0 Variables of the study

2.9.1 Independent variables

Sex, age, clinical conditions (malignancy, congenital malnutrition), prematurity, isolated organisms (MDR, Gram reaction), history of antibiotics use. All of this information ware obtained and collected from patient's files and databases at MNH.

2.9.2 Dependent variables

Bloodstream infection and outcomes (death or recovered) and duration of hospitalization

2.10.0 Data collection

2.10.1 Information collection

Social demographical and clinical information about patients were collected using a structured form from the hospital database and patient's files.

2.10.2 Specimen collection

Non-repetitive routine blood culture samples, collected by medical doctor paediatrician/nurses/medical person from children in hospital wards ware received at CPL, then identified to meet selection criteria before enrolled in the study for laboratory procedure.

2.11.0 Laboratory procedures

BD BACTEC machine was used to incubate and detect positive blood culture vial within 5 (five) days. Gram staining technique was performed (using standard procedure) on positive blood cultures samples to detect bacteria gram reaction before it was sub-cultured on media. The CLSI 2019 will be used as a standard guideline during isolation, identification and antimicrobial susceptibility testing of bacteria (50).

2.11.1 Culture technique and procedure

Positive blood culture **vials** with Gram-positive bacteria were sub-cultured on solid agar plates; blood agar media (BA) and chocolate agar media (CA). Optochin disk was included on BA plate and the plate was inoculated at 37^{o}C up to 48 hours in a 5-10% CO_2 incubator. On the other hand, positive blood-cultures vials with Gram-negative bacteria were sub-cultured on solid agar plates; MacConkey agar media (MCA) and the plate were incubated aerobically at 37 ℃ up to 24 hours. Blood agar media (BA) and chocolate agar (CA) were inoculated and incubated in a 5-10% CO2 incubator at 37℃ up to 48 hours to recover fastidious bacteria.

2.11.2 Identification

Preliminary identification based on colonial morphology and other features on the culture plate after the growth of the bacteria was performed. Hemolytic effect and lactose fermentation were amongst the observable effects on the plate. A purity plate was prepared from a mixed growth plate to obtain a pure colony before the further **test** was performed. Gram stain was performed and examined microscopically at 100x objective lens.

2.11.3 Biochemical test

Conventional biochemical methods were used during the identification of microorganisms after they have been recovered from culture plates. Oxidase on Gram-Negative bacteria isolates followed by SIM (Hydrogen Sulphide, Indole and Motility production test), Urease, citrate test, and (KIA) Kligler Iron Agar were carried out. The standard control strain for the oxidase and Indole test was *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, respectively. Gram-positive bacteria were tested for Catalase production using catalase reagent. A catalase-positive bacterium was then tested for both Slide and tube Coagulase test to differentiate Coagulase-positive *Staphylococcus spp.* A standard bacteria control strain of *Staphylococcus aureus* ATCC 25923 was used (45).

2.11.4 Antimicrobial Susceptibility Test (AST)

Identified bacteria were tested with antimicrobial agents to find their antimicrobial susceptibility pattern **for** different antimicrobial agents. Kirby-Bauer's Disk diffusion method using Muller Hinton Aga**r** media (MHA) was performed. AST for *Streptococcus spp* was performed by following the Clinical & Laboratory Standards Institute's Guidelines recommendations (50). Briefly, a colony of identified bacteria from a pure culture was transferred into a test tube with approximately 5ml of normal saline to make a turbid suspension. The turbid suspension was then compared to 0.5 McFarland standards before it was inoculated and distributed evenly on MHA using sterile swabs.

Sterile forceps were used to place the antibiotic disks at a distance not closer than 24 mm (centre to centre) on the MHA plate. The plate was then incubated aerobically for up to 24hrs at 37◦C. Zone of inhibition around each disk was measured using a ruler to obtain the diameter distance in millimetres (mm) and was recorded. Depending on the size of the

diameter of a zone of inhibition, isolated bacteria were classified as either susceptible, intermediate, or resistant to the tested antimicrobial agent as directed and described by the CLSI guidelines (50).

2.11.5 Gram-positive organism (Staphylococcus aureus and Streptococcus spp.)

Disks tested were; penicillin (10units), ampicillin (10µg), gentamicin (10µg), erythromycin (15µg), tetracycline (30µg), ciprofloxacin (5µg), clindamycin, (2µg), Vancomycin (2µg), trimethoprim/ sulphamethaxazole (1.25/23.75µg) and chloramphenicol (30µg).

2.11.6 Gram-negative organism (Klebsiella spp. Escherichia coli, Acinetobacter baumannii and etc.)

Disks tested were; Amoxicillin-clavulanic acid (20/10µg), Ceftriaxone (30µg), Ceftazidime (30µg), Cefotaxime (30µg) gentamicin (10µg), Piperacillin/Tazobactam, Aztreonam, ciprofloxacin (5µg), chloramphenicol (30µg), Meropenem (10µg).

2.11.7 P. aeruginosa:

Disks tested were; Ceftazidime (30µg), gentamicin (10µg), Amoxicillin/clavulanate (20/10µg), and ciprofloxacin (5µg, Piperacillin (100µg), Aztreonam (30µg) and meropenem (10µg).

2.11.8 Acinetobacter spp:

For Acinetobacter spp discs tested were; Ceftazidime (30µg), gentamicin (10µg), ciprofloxacin (5µg), tetracycline (30µg), Amoxicillin/clavulanate (20/10µg), Piperacillin (100µg), Aztreonam (30µg) and meropenem (10µg).

2.11.9 Streptococcus spp:

The following agents were tested; Penicillin, Vancomycin, Clindamycin, Ampicillin, Oxacillin, and Erythromycin.

Plates were incubated aerobically at 35◦C for 24hour in an enriched CO_2 incubator and zone oh inhibition was measured. The interpretation was sensitive, intermediate, or resistant as explained in the CLSI of 2019.

2.7 Data Management and Analysis

Social demographics, clinical information, and Laboratory results concerning samples of children were crosschecked, coded, and entered into excel computer software before analysis was performed by a Statistical tool STATA version 12. Frequency distribution and two-way tables were used to summarize the data. Descriptive analyses such as frequencies and mean were included. The fisher's exact test was employed to determine the association between BSI and children's poor outcomes. Univariate and multivariate Logistic regression was performed on associated factors. A *p*-value of < 0.05 was considered statistically significant.

2.8 Ethical Considerations

Ethical approval was obtained from the Muhimbili University of Health and Allied Sciences (MUHAS) senate Research and Publications Committee. Permission was requested from the MNH administration to carry out the Hospital. There was no consent form since there was no researcher-patient interaction in this study as collected information was obtained from the patient's database and files. The patient's registration number was used instead of names to maintain the confidentiality of the collected information.

2.9 Plan for the dissemination of results

Results obtained from this study were disseminated to MNH health care providers and the MUHAS community. **These** findings will be published in international peer-reviewed journals, contributing information to the wider scientific and health provider in the community.

CHAPTER THREE

3.0 RESULTS

3.1 Demographic and clinical information

Of 650 blood culture samples collected from children of 0-15y, 21.5% (140/650) were positive blood cultures. The majority of these samples were collected from children of less than one month (36.7%; 238/650), and many samples were of male children (58.3%, 379/650) (Table 1). The median length of stay (LOS) in the hospital among children was 17 days (IQR: 8.00-33.00) and the majority of children were prescribed one or more antimicrobial agents before blood culture collection (87.5%; 569/650). The frequently prescribed antimicrobial agent during empiric management was Ceftriaxone (61.0%; 347/650), Ampicillin (29.0%; 165/650), and Gentamycin (33.2%; 189/650) (Table 1). The mortality rate among children prescribed with an antimicrobial agent and resistant after in-vitro AST was 49.5% (48/97).

.

Table 1: Socio-demographic and clinical characteristics among children

Variable	Frequency (%)
The median length of hospital stay = 17 days [IQR:8.00 33.00]	
Age (month)	
<1	238(36.7)
1–24	201(30.6)
25-48	69(10.6)
49-72	33(5.1)
73-96	38(5.9)
97-120	21(3.2)
>120	51(7.9)
Sex	
Female	271(41.7)
Male	379(58.3)
Wards	
Neonatal ward	245(37.7)
General ward	227(34.9)
PICU	59(9.1)
Oncology	91(14.0)
Burn Unit	28(4.3)
antibiotics prescription before blood culture	
No	81(12.5)
Yes	569(87.5)
Co-morbidities	
Sickle cell	46(7.1)
Malignancy	106(16.5)
Malnutrition	46(7.1)
Congenital	81(12.5)
Others co-morbidities	115(10.6)
Infection	348(53.5)
Premature babies	134(20.6)

3.2: The proportion of culture-positive blood samples.

The proportion of BSI among the collected sample for blood culture was 21.5% (140/650). With age-specific, the proportion of BSI for age group of <1, 1–24, 25-48, 49-72, 73-96, 97-120 and >120 were 33.2%, 15.6%, 14.5%, 21%, 10.5%, 13.6% and 11.8%, respectively. Also, the proportion of BSI among wards Neonatal ward, General ward, PICU, Oncology and Burn Unit were 33.1%, 11.5%, 18.6%, 9.9% and 21.4%. Furthermore, the reported proportion of BSI in children who were prescribed antibiotics drugs before sample for blood culture collection was 23.3% while the proportion of 8.8% was observed from children who did not receive any antibiotics before sample for blood culture was collected.

.

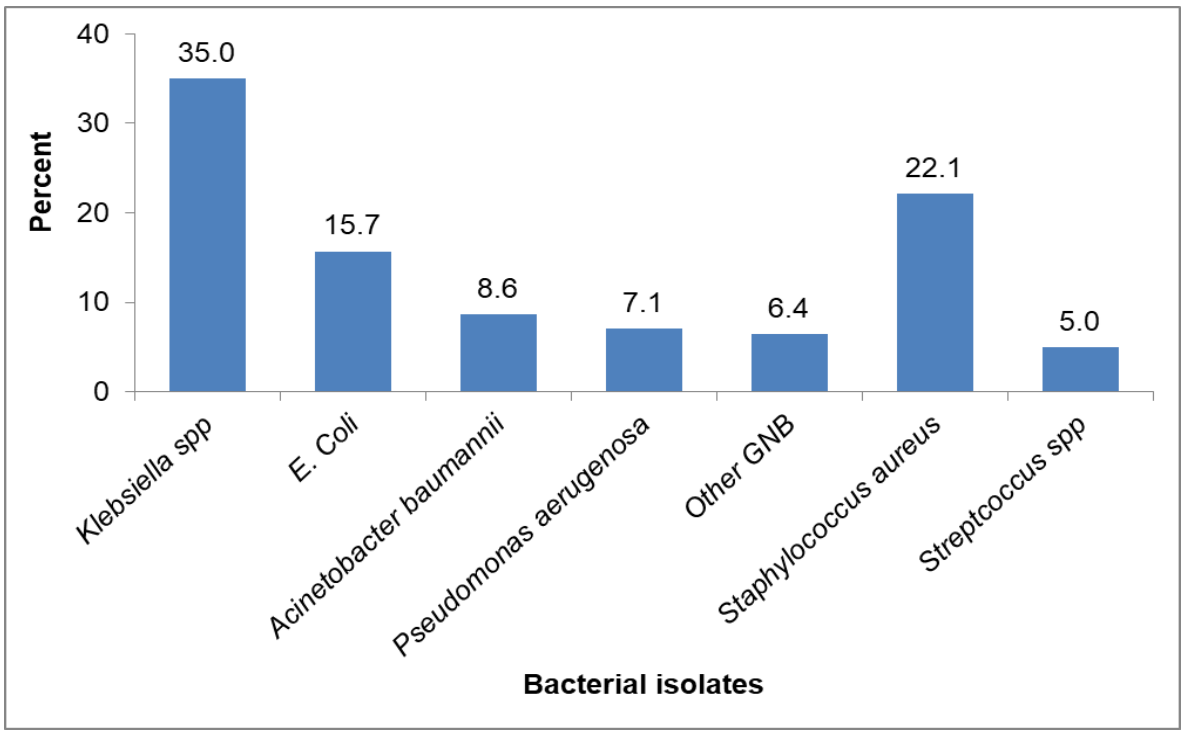
Table 2: Proportion of confirmed bloodstream among children

Variable	Frequency (%)	Proportional
Confirmed bloodstream infection		
Yes	140	**21.5**
Age (month)		
<1	79(56.4)	33.2
1–24	31(22.1)	15.6
25-48	10(7.1)	14.5
49-72	7(5.0)	21.2
73-96	4(2.3)	10.5
97-120	3(2.1)	13.6
>120	6(4.3)	11.8
Sex		
Female	59(42.1)	20.7
Male	81(57.9)	20.3
Length of hospital stay (days)		
<7	22(15.7)	16.9
7-24	27(19.3)	16.8
15-21	23(16.4)	24.0
22-28	25(17.9)	32.9
>29	43(30.7)	23.0
Wards		
Neonatal ward	83(59.3)	33.1
General ward	26(18.6)	11.5
PICU	13(9.3)	18.6
Oncology	12(8.6)	9.9
Burn Unit	6(4.3)	21.4
Antibiotics prescription before blood culture		
No	7(5.0)	8.8
Yes	133(95)	23.3
Outcome		
Alive	73(52.1)	15.0
Death	67(47.9)	41.9

3.3: Bacteria aetiology among children

The current study reported a total of 140 (21.5%) bacteria pathogens from the sample of blood collected for blood culture at MNH during the study period. The majority of bacterial pathogens isolated were Gram-negative bacteria (GNB), 102/140 (72.9%). This includes *Klebsiella spp.* (35.0%), *Escherichia coli* (15.7%), *Acinetobacter baumannii* (8.6%), *and Pseudomonas aeruginosa* (7.1%). Other Gram-negative bacteria (GNB) isolates accounted for 6.4% (9/140) of which *Serratia marcescens* = 3, *Haemophilus influenzae* = 2, *Protease spp.* = 1, *Salmonella Typhi* = 1, *Citrobacter spp* = 1 and *Aeromonas hydrophila* = 1 (Figure 1). Gram-positive bacteria accounted for 27.1% (38/140). The common organism among Gram-positive bacteria was *Staphylococcus aureus* (22.1%) and the rest (5.0%) was *Streptococcus spp.* (Figure 1).

Figure 2: Bacteria species from children with blood stream infections.

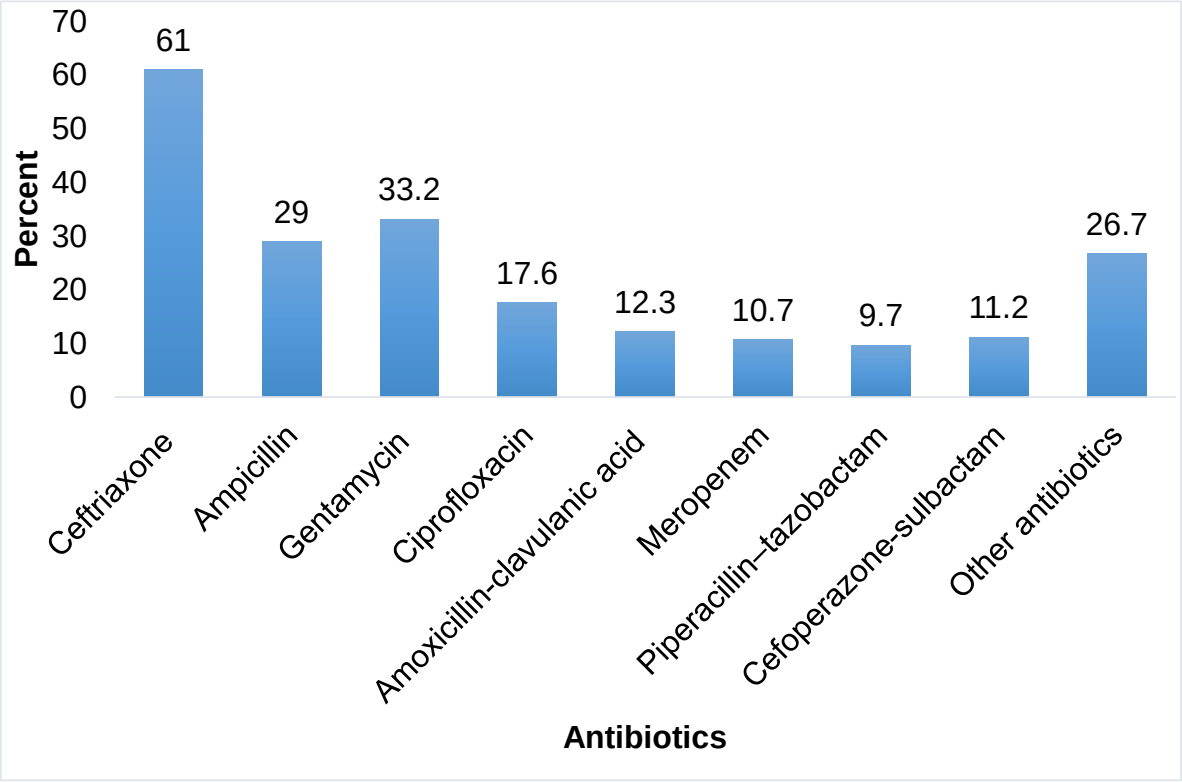


Other Gram-negative bacteria (GNB) includes; *Serratia marcescens* = 3, *Haemophilus influenzae* = 2, *Protease spp.* = 1, *Salmonella Typhi* = 1, *Citrobacter spp* = 1 and *Aeromonas hydrophila* = 1

3.4 Antimicrobial drug prescription among children

Among the 650 children, **569 (87.5%)** were prescribed antimicrobial drugs before blood culture collection. The median (IQR) age of children prescribed antimicrobial drugs was 10 ± (0.2-48) months. The frequently prescribed antimicrobial agent was Ceftriaxone 347(61.0), Ampicillin 165(29.0), Gentamycin 189(33.2), Ciprofloxacin 100(17.6), Amoxicillin-clavulanic acid 70(12.3), Meropenem 61(10.7), Piperacillin–tazobactam 55(9.7), Cefoperazone-sulbactam 64(11.2) and other antibiotics 33(26.7) **Table 1**. The resistance rate among prescribed antibiotics (inappropriate antimicrobial drug prescription) was 72.9%.

Figure 3 Antimicrobial agent prescribed to children suspected to have BSI



Other Antimicrobial agents includes; pen V, Metronidazole, Carbapenem, Vancomycin, Erythromycin, Chloramphenicol and Amikacin

3.5 Antimicrobial resistance Pattern

Table 3: represents the resistance pattern of bacteria isolates. There was a greater resistance of bacteria isolates even to mostly prescribe (WHO recommended) first-line antimicrobial agents, including ampicillin (72.1% 101/140) and Gentamycin (56.4%; 79/140). The majority of Gram-negative bacteria were resistant to 3rd Gen. Cephalosporin antimicrobial agents by 73.5% and 80.4% (Ceftriaxone and Ceftazidime respectively), **Table 3**. The moderate to high-level resistance was reported to Meropenem (19.9%) chloramphenicol (18.6%), Piperacillin-tazobactam 38.2%), amoxicillin-clavulanic acid (33.3%) and Amikacin (36.3%). The study also reported a small resistance rate of Gram-positive bacteria to Clindamycin and Vancomycin antimicrobial agents (2.6% and 10.5% respectively). Furthermore, the isolated bacteria were resistant by 55.9% to ciprofloxacin.

Table 3: Resistant patterns of bacteria isolates and their percentage

Antibiotics tested	Gram-positive (% of resistance)			Gram-negative (% of resistance)					
	S. aureus **N=31)**	***Streptococcus*** **spp. N=7**	**Total N=38**	***A. baumannii*** **N=12**	***E. coli*** **N=22**	***Klebsiella spp*** **N=49**	***P. aeruginosa*** **N=10**	**Other GNB N=9**	**Total =102**
Cefoxitin	18(58.1)	7(100)	25(65.8)	NT	NT	NT	NT	NT	
Erythromycin	16(51.6)	3(42.9)	19(50.0)	NT	NT	NT	NT	NT	
Clindamycin	1(3,2)	0(0)	1(2.6)	NT	NT	NT	NT	NT	
Doxycycline	6(19.4)	1(14.3)	7(18.4)	NT	NT	NT	NT	NT	
Vancomycin	2(6.5)	2(28.6)	4(10.5)	NT	NT	NT	NT	NT	
Ampicillin	16(51.6)	4(57.1)	20(52.6)	11(91.7)	19(86.4)	39(79.6)	5(50.0)	7(77.8)	81(79.4)
Gentamycin	13(41.9)	4(57.1)	17(44.7)	9(75.0)	13(59.1)	31(63.3)	4(40.0)	5(55.6)	62(60.8)
Ciprofloxacin	12(38.7)	4(57.1)	16(42.1)	10 (83.3)	10(45.5)	30(61.2)	3(30.0)	4(44.4)	57(55.9)
Meropenem	9(29.0)	0(0)	9(23.7)	8(66.7)	3(13.6)	4(8.2)	3(30.0)	2(22.2)	20(19.6)
Chloramphenicol	2(6.5)	0(0)	2(5.3)	2 (16.7)	4(18.2)	6(12.2)	5(50.0)	2(22.2)	19(18.6)
Amikacin	10(32.3)	1(14.3)	11(29.0)	4(33.3)	9(40.9)	16(32.7)	3(30.0)	5(55.6)	37(36.3)
Ceftriaxone	NT	NT		11 (91.7)	18(81.8)	39(79.6)	3(30.0)	4(44.4)	75(73.5)
AMC	NT	NT		9(75.0)	10(45.5)	11(22.5)	2(20.0)	2(22.2)	34(33.3)
Ceftazidime	NT	NT		11 (91.7)	18(81.8)	42(85.7)	5(50.0)	6(66.7)	82(80.4)
Cefepime	NT	NT		6(50.0)	12(54.6)	22(44.9)	2(20.0)	4(44.4)	46(45.1)
Pip-Tazobactam	NT	NT		6(50.0)	6(27.3)	20(40.8)	2(20.0)	5(55.6)	39(38.2)
Aztreonam	NT	NT		7 (58.3)	15(68.2)	27(55.1)	4(40.0)	3(33.3)	56(54.9)

Key: AMC = Amoxicillin clavulanic acid, NT = note tested

3.6. Bivariate and Multivariate logistic regression analysis on factors associated with BSI at MNH

Table 4 presents a summary of bivariate and multivariate logistic regression model analysis. Factors with a p-value of <0.2 were included in the multivariate analysis to determine the independencies. **A large per cent of bacteria isolates were found from children who were premature children** [OR (95%CI): 3.13(1.67-5.87), p = 0.040. In addition to that, age of 1–24, 25-48, 73-96, >120, sickle cell, malignancy, general ward and oncology ward were associated with bloodstream in the univariate logistic regression analysis.

Table 4: Bivariate and Multivariate logistic regression analysis on factors associated with BSI among children

Variable	Frequency (n=650)	BSI (%) (N = 140)	Bivariate analysis		Multivariate analysis	
			cOR(95%CI	p-value	aOR(95%CI	p-value
Age						
<1month	238	79(33.2)	1		1	
1–24	199	31(15.6)	0.37(0.23-0.59)	0.000	0.72(0.39-1.32)	0.289
25-48	69	10(14.5)	0.34(0.23-0.59)	0.004	0.79(0.33-1.86)	0.59
49-72	33	7(21.2)	0.54(0.23-1.30)	0.174	1.37(0.50-3.79)	0.544
73-96	38	4(10.5)	0.24(0.08-0.69)	0.008	0.55(0.17-1.81)	0.324
97-120	22	3(13.6)	0.32(0.08-0.69)	0.072	0.79(0.20-3.08)	0.738
>120	51	6(11.8)	0.27(.09-1.106)	0.004	0.55(0.20-1.51)	0.246
Sex						
Female	271	59(21.8)	1		1	
Male	379	81(21.4)	0.98(0.94-0.67)	0.936	1.08(0.71-1.61)	0.721
Co-morbidities						
Sickle cell	46	5(10.9)	0.30(0.11-0.79)	0.014	0.70(0.24-1.10)	0.503
Malignancy	106	14(13.2)	0.38(0.20-0.70)	0.002	0.79(0.37-1.66)	0.526
Malnutrition	46	8(17.4)	0.52(0.23-1.16)	0.11	0.97(0.40-4.2.35)	0.947
Congenital	81	16(19.8)	0.61(0.33-1.11)	0.105	1.01(0.51-2.02)	0.969
Others	69	10(14.5)	0.41(0.20-0.86)	0.017	0.48(0.23-1.01)	0.054
Infections	302	87(28.8)	1		1	
Prematurity						
No	516	81(15.7)	1		1	
Yes	134	59(44.5))	4.22(2.79-6.40)	0.000	3.13(1.67-5.87)	0.000

Key: cOR = crude odds ratio, aOR = adjustable odd ration, 95%CI = 95% Confidence interval, p = 0.2 for inclusion of factors on multivariate logistic regression analysis.

3.7. Bivariate and Multivariate logistic regression analysis on factors associated with overall mortality among children at MNH

The analysis of the factors associated with mortality among children in this study was done using bivariate and multivariate logistic regression model analysis**,** Table 5. A p-value of <0.2 was used to include a factor in the multivariate analysis to determine the independencies of the factors. Confirmed BSI, Antibiotic status, age of 1–24 months and age of 49-72 months, were factors found to be associated with mortality among children hospitalized at MNH [OR (95%CI): 3.49(2.30-5.28), p = 0.000, 2.88(1.326.27), p = 0.008, 0.30(0.12-0.73), p = 0.008, and 0.10(0.01-0.88), p = 0.038, respectively].

Table 5: Bivariate and Multivariate logistic regression analysis on factors associated with overall mortality

Variable	Frequency (N)	Death n (%)	Bivariate analysis		Multivariate analysis	
			cOR(95%CI	p-value	cOR(95%CI	p-value
Age						
<1month	238	88(36.6)		1		1
1–24	199	35(17.6)	0.36(0.23-0.57)	**0.000**	**0.30(0.12-0.73)**	**0.008**
25-48	69	11(15.9)	0.32(0,16-0.65)	**0.001**	0.27(0.10-1.57)	0.185
49-72	33	5(15.2)	0.30(0.11-0.82)	**0.018**	**0.10(0.01-0.88)**	**0.038**
73-96	38	6(15.8)	0.32(0.13-0.79)	**0.014**	0.56(0.07-4.40)	0.579
97-120	22	3(13.6)	0.27(0.08-0.94)	**0.039**	0.29(0.02-3.37)	0.322
>120	51	14(27.5)	0.64(0.33-1.26	0.199		
Sex						
Female	272	70(43.2)		1		
Male	378	92(56.8)	0.92(0.65-1.33)	0.685		
Confirmed BSI						
No	510	95(18.6)		1		1
Yes	140	67(47.9)	4.01(2.69-5.98)	**0.000**	**3.49(2.30-5.28)**	**0.000**
MDR						
No	32	11(16.4)		1		
Yes	108	56(83.6)	1.67(0.90-4.67)	0.085		
Antibiotic status						
Appropriate	43	19(19.4)		1		**1**
Inappropriate	97	48(80.6)	2.89(1.34-6.22)	**0.006**	**2.88(1.326.27)**	**0.008**

Key: MDR = multidrug resistance, GNB = Gram-negative bacteria, GPB = Gram positive bacteria, p = 0.2 for inclusion of factors on multivariate logistic regression analysis.

3.8. Bivariate and Multivariate logistic regression analysis on factors associated with poor treatment outcome BSI.

In the table below is a summary of the bivariate and multivariate logistic regression analysis on associated factors with poor treatment outcomes following BSI. The mortality rate among children with BSI and initially prescribed with resistance antimicrobial agents was 49.5% (48/97), (Table 1). The infection with Gram-negative was found to associate with poor treatment outcome among [OR (95%CI): 4.55(1.26-16.57), p = 0.002]. The crude old ratios multidrug-resistant bacteria (MDR), hospital-acquired infection (HAI) and prematurity in the Univariate logistic regression model analyses appear to associate with poor treatment outcomes among children but not independently (Table 6).

Table 6: Bivariate and Multivariate logistic regression analysis on factors associated poor treatment outcome among children following BSI

Variable	Frequency (n)	Death (%)	Bivariate analysis		Multivariate analysis	
			cOR(95%CI	p-value	aOR(95%CI	p-value
Age						
<1month	79	36(45.6)	1		1	
1–24	31	6(19.4)	0.29(0.11-0.78)	0.014	0.27(0.03-2.86)	0.278
25-48	10	3(30.0)	0.51(0.12-2.12)	0.356	0.27(0.01-7.32)	0.434
49-72	7	1(14.3)	0.20(0.2-1.73)	0.144	0.46(0.01-22.71)	0.695
73-96	4	1(25.0)	0.40(0.04-4.00))	0.434	0.60(0.01-45.33)	0.816
97-120	3	1(33.3)	0.60(0.05-6.86)	0.679	3.01(0.04-223.48)	0.616
>120	6					
Sex						
Female	59	20(33.9)	1		1	
Males	81	28(34.6)	1.03(0.51-2.09)	0.934	1.09(0.452.65)	0.849
Prematurity						
No	81	10(23.5)	1		1	
Yes	59	29(49.2)	3.15(1.53-6.51)	0.002	1.32(0.34-5.14)	0.681
Organism type						
GPB	38	4(10.5)	1		1	
GNB	102	44(43.1)	6.45(2.13-19.52)	0.001	4.55(1.26-16.57)	0.021
MDR						
NO	32	6(18.8)	1		1	
Yes	108	42(38.3)	2.76(1.05-7.26)	0.040	1.02(0.28-3.75)	0.974

Key: MDR = multidrug resistance, GNB = Gram-negative bacteria, GPB = Gram positive bacteria, p = 0.2 for inclusion of factors on multivariate logistic regression analysis.

3.9. The association between factors and length of stay in the hospital among children.

Table 7. Summarizes the length of hospital stay among children with BSI and their associated factors. This study observed that children prescribed antibiotics before blood culture collection stayed in the hospital for a long time than those without prescription of antibiotics before collection of blood sample [median ± (IQR): 18.00± (8.00-33.00), p = 0.003. In addition, children whose blood culture samples were positive were observed to stay in the hospital compared to those children whose blood culture samples were negative after laboratory tests [median ± (IQR): 18.00 ± (9.00-33.00), p = 0.049, (Table 7). On top of that children with confirmed BSI spent more time in the hospital than children whose samples were not laboratory-confirmed to have bacterial infection 19.5 ± (9.25-34.00), 0.043]. However, children with confirmed to have an infection due to multidrug-resistant bacteria (MDR) has a short median length of stay in the hospital [median ± (IQR):19.00 ± (9.00 - 28.75)], p = 0.043. Furthermore, the median time of hospital stay among children with malignancy was longer than those children with other co-morbidities. [Median ± (IQR): 31.50 ± (12.00-55.25)], p = 0.001. And there was no statistically significant difference in the length of stay in the hospital for females children and males children [Median ± (IQR): 18.00 ± (8.00-34.00], p = 0.508, (Table 7).

Table 7: Factors association with length of stay in the hospital among children

Table 6

Variable	Category	Median (IQR)	p-value	Test
Age				
	<1month	18.00±(10.00-32.25)		
	1–24	15.00±(6.00-28.00)	0.048	Kruskal-Wallis
	25-48	19.00±(6.50-53.00)		test
	49-72	18.00±(7.00-41.50)		
	73-96	12.00(5.00-19.50)		
	97-120	19.50±(8.75-42.00)		
	>120	20.00±(8.00-38.00)		
Sex				
	Female	18.00±(8.00-34.00)	0.508	Mann-Whitney
	Male	16.00±(8.00-32.00)		test
Antibiotics prescription				
	No	10.00±(5.00-27.75)	0.003	Mann-Whitney
	Yes	18.00±(8.00-33.00)		test
Co-morbidities				
	Sickle cell	8.00±(5.00-18.25)	0.001	Kruskal-Wallis
	Malignancy	31.50±(12.00-55.25)		test
	Malnutrition	17.50±(8.00-27.25)		
	Congenital	16.00±(9.00-29.50)		
	Other co-morbidities	14.00±(5.00-24.00)		
	No co-morbidity	18.00±(8.00-.00)		
Blood culture				
	Negative	15.00±(7.00-32.00)	0.049	Mann-Whitney
	Positive	18.00±(9.00-33.00)		test
Confirmed BSI				
	No	16.00±(8.00-32.00)	0.043	Mann-Whitney
	Yes	19.5±(9.25-34.00)		test
MDR				
	No	31.00±(15.00-53.25)	0.043	Mann-Whitney
	Yes	19.00±(9.00-28.75)		test

MDR (Multidrug resistance), BSI (Bloodstream infection)

CHAPTER FOUR

4.0 DISCUSSION.

Bloodstream infection is a major public health problem associated with high morbidity and mortality among children worldwide. Both Gram-negative and Gram-positive bacteria cause BSI in children of all ages (8). However, in resource-limited countries like Tanzania, treatments are mostly done empirically using broad-spectrum antimicrobial agents (27). The current proportion of BSI, the aetiology, the antimicrobial susceptibility pattern and the factors associated with poor treatment outcome of children with BSI at MNH was reported in this study.

The majority of blood samples were collected after antibiotic prescription. However, 21.5% had bacterial pathogenic bacteria that cause BSI. This proportion was greater than 13.9% reported in the previous study at the same setting in 2007 by Blomberg et al (1). It was also higher than the prevalence of BSI reported by Moyo SJ et al in 2020 in the region of Dar es salaam (12). In addition, this proportion was significant dissimilar to the overall prevalence of 14.2% which was reported in a study conducted in North-Western Tanzania by Seni et al in 2019 (11) but was comparable to the specific prevalence at Bugando medical centre in the same study. Furthermore, the current proportion of BSI in children at MNH was comparable to 20.6%, a prevalence of BSI among malnourished children at Jimma hospital in Ethiopia, but was less than 29.1% prevalence of BSI in reported from India (40,51).

The findings of this study reported that *Klebsiella species, E. coli* and *Staphylococcus aureus* were the commonest Gram-negative and Gram-positive bacteria pathogens. The aetiology of BSI in children at MNH remains almost the same and comparable to that reported in previous studies in the same setting (1,14). The results, however, differed from those reported in other previous studies which report that Coagulase-negative staphylococci species and *S. aureus* were the common bacteria (8,29,40). Gram-negative bacteria pathogen dominated among the isolates. They are associated with morbidity and mortality among children of different ages (33). A larger per cent of the aetiology was resistant to commonly used antibiotics for the first line and second-line treatment options during empiric managements at the settings.

The findings of this study present the overall pattern of resistance to the tested isolates. It was reported that the resistance to ampicillin and Gentamycin was 72.1% and 56.4% respectively.

The majority of Gram-negative bacteria were resistant to 3rd Gen. Cephalosporin antimicrobial agents by 73.5% and 80.4% (Ceftriaxone and Ceftazidime respectively. The resistance pattern of bacteria to the mostly used antibiotic agent is increasing as reported by other previous studies in the same setting (1,14,33).

Furthermore, this study reported that, Gram-negative bacteria were associated with in-hospital stay and higher mortality rate among hospitalized children. Treatment and management of children with BSI require a proper diagnosis to reveal the right causative agent (21,27). BSI due to Gram-negative and Multidrug Resistance (MDR) bacteria have complications on treatments due to poor response to multiple antimicrobial agents used (52). The resistance pattern of Gram-negative bacteria is widening as reported in this study and other previous studies from different parts (1,12,14,31,32). In addition, a previous study on Gram-negative bacteria reported that Gram-negative bacteria was associated with mortality among children with BSI at MNH (33).

When the length of hospital stay (LOS) was studied, we found that children with malignancy and BSI in the oncology ward were likely to stay in the hospital for a long time than in other wards. Children with malignancy have challenged immune systems and are prone to bacterial infection (35). In the case of antibiotic prescription before blood sample for blood, children with prescription were found to stay for a long time than those with no prescription. It should be noted that resistant bacteria may lead to inappropriate antibiotic use. (4,5,42). Children with positive blood cultures positive and confirmed to have BSI were found to stay for a long time in the hospital since infection is known to complicate the treatment and management process (4).

Limitations: It was difficult to deal with anaerobic bacteria for isolation and identification due to a lack of material and equipment. The insufficient fund was a big treble and had made me fail to perform further identifications of the isolated bacteria.

Conclusions: The proportion of BSI among the collected sample from children of age 0 to 15 years, was 21.5% which was higher to be reported at the setting. The most isolated bacteria pathogen were *Klebsiella species, E. coli* and *Staphylococcus aureus* of which, large per cent of bacteria pathogens were resistant to recommended antimicrobial agents for first-line and second-line treatment options on children with BSI. In addition, this study found Gram-

negative bacteria to be associated with poor treatment outcomes in children with bloodstream infections at MNH. Therefore, more studies are required to be done so as to extract more information and hence to come out with a valuable decision on treatment and management of children with BSI.

Recommendation: Due to the observations of this study, further studies on BSI among children are recommended to be done at MNH to gain more information which will help to review the treatment guideline for the management of children suspected to be with BSI and establishment of antimicrobial stewardship at the Muhimbili national hospital.

REFERENCES

1. Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DSM, Jureen R, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: A prospective cohort study. BMC Infect Dis. 2007;7:1–14.
2. Aiken AM, Mturi N, Njuguna P, Mohammed S, Berkley JA, Mwangi I, et al. Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: A prospective cohort study. Lancet [Internet]. 2011;378(9808):2021–7. Available from: http://dx.doi.org/10.1016/S0140-6736(11)61622-X
3. Nadjm B, Amos B, Mtove G, Ostermann J, Chonya S, Wangai H, et al. WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense Plasmodium falciparum transmission: Prospective study. BMJ. 2010;340(7751):848.
4. Ceyhan M, Yildirim I, Ecevit C, Aydogan A, Ornek A, Salman N, et al. Inappropriate antimicrobial use in Turkish pediatric hospitals: A multicenter point prevalence survey. Int J Infect Dis. 2010;14(1).
5. Shorr AF, Micek ST, Welch EC, Doherty JA, Reichley RM, Kollef MH. Inappropriate antibiotic therapy in Gram-negative sepsis increases hospital length of stay. Crit Care Med. 2011;39(1):46–51.
6. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. Lancet [Internet]. 2012;379(9832):2151–61. Available from: http://dx.doi.org/10.1016/S0140-6736(12)60560-1
7. Mathers C, Stevens GA, Retno Mahanani W, Ma Fat D, Hogan D, Gretchen Stevens MA, et al. Global Health Estimates 2015: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2015. 2000;(March):90.
8. Henderson KL, Johnson AP, Muller-Pebody B, Charlett A, Gilbert R, Sharland M. The changing aetiology of paediatric bacteraemia in England and Wales, 1998-2007. J Med Microbiol. 2010;59(2):213–9.
9. Strunk T, Richmond P, Simmer K, Currie A, Levy O, Burgner D. Neonatal immune responses to coagulase-negative staphylococci. Curr Opin Infect Dis. 2007;20(4):370–

5.

10. Lauffer RB, Brock JH. Iron and the Immune System. Iron Hum Dis. 2018;(May):161–78.
11. Seni J, Mwakyoma AA, Mashuda F, Marando R, Ahmed M, Devinney R, et al. Deciphering risk factors for blood stream infections, bacteria species and antimicrobial resistance profiles among children under five years of age in North-Western Tanzania: A multicentre study in a cascade of referral health care system. BMC Pediatr. 2019;19(1):1–11.
12. Moyo SJ, Manyahi J, Blomberg B, Tellevik MG, Masoud NS, Aboud S, et al. Bacteraemia, Malaria, and Case Fatality Among Children Hospitalized With Fever in Dar es Salaam, Tanzania. Front Microbiol. 2020;11(September).
13. Christopher A, Mshana SE, Kidenya BR, Hokororo A, Morona D. Bacteremia and resistant gram-negative pathogens among under-fives in Tanzania. Ital J Pediatr. 2013;39(1):1–8.
14. Mkony MF, Mizinduko MM, Massawe A, Matee M. Management of neonatal sepsis at muhimbili national hospital in dar es salaam: Diagnostic accuracy of c - reactive protein and newborn scale of sepsis and antimicrobial resistance pattern of etiological bacteria. BMC Pediatr. 2014;14(1):1–7.
15. Downie L, Armiento R, Subhi R, Kelly J, Clifford V, Duke T. Community-acquired neonatal and infant sepsis in developing countries : ef fi cacy of WHO ' s currently recommended antibiotics — systematic review and meta-analysis. 2013;146–54.
16. Ali SA. Frequency and Etiology of Community-Acquired Bloodstream Infection in Hospitalized Febrile. 2016;5(January).
17. Nimri LF, Rawashdeh M, Meqdam MM. Bacteremia in Children : Etiologic Agents , Focal Sites , and Risk Factors. 2001;47(December).
18. Hosp JR. J. A. Stockman III, MD. 2002;245–7.
19. Viscoli C. Bloodstream Infections: The peak of the iceberg. Virulence. 2016;7(3):248–51.
20. Seale AC, Blencowe H, Manu AA, Nair H, Bahl R, Qazi SA, et al. Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and

Latin America for 2012: A systematic review and meta-analysis. Lancet Infect Dis [Internet]. 2014;14(8):731–41. Available from: http://dx.doi.org/10.1016/S1473-3099(14)70804-7

21. Bard JD, TeKippe EME. Diagnosis of bloodstream infections in children. J Clin Microbiol. 2016;54(6):1418–24.
22. Kibuuka A, Byakika-Kibwika P, Achan J, Yeka A, Nalyazi JN, Mpimbaza A, et al. among febrile ugandan children treated with antimalarials despite a negativBacteremiae malaria test. Am J Trop Med Hyg. 2015;93(2):276–80.
23. Khurram MSA, Reddy UN, Khan KA. A study of predictors of occult bacteremia in febrile children 3 months to 36 months of age groups. Int J Contemp Pediatr. 2018;5(5):1892.
24. Ntusi N, Aubin L, Oliver S, Whitelaw A, Mendelson M. Guideline for the optimal use of blood cultures. South African Med J. 2010;100(12):839–43.
25. Nichols C, Cruz Espinoza LM, Von Kalckreuth V, Aaby P, Ahmed El Tayeb M, Ali M, et al. Bloodstream infections and frequency of pretreatment associated with age and hospitalization status in Sub-Saharan Africa. Clin Infect Dis. 2015;61(Suppl 4):S372–9.
26. Riordan A. duration of treatment for bacteraemia or an example of ‘ antimicrobial bingo ’ ? :117–8.
27. Fuchs A, Bielicki J, Mathur S, Sharland M, Anker JN Van Den. Antibiotic Use for Sepsis in Neonates and Children : 2016 Evidence Update. 2016;
28. Larru B, Gong W, Vendetti N, Sullivan K V., Localio R, Zaoutis TE, et al. Bloodstream Infections in Hospitalized Children: Epidemiology and Antimicrobial Susceptibilities. Pediatr Infect Dis J. 2016;35(5):507–10.
29. Crichton H, O’Connell N, Rabie H, Whitelaw AC, Dramowski A. Neonatal and paediatric bloodstream infections: Pathogens, antimicrobial resistance patterns and prescribing practice at Khayelitsha District Hospital, Cape Town, South Africa. South African Med J. 2018;108(2):99–104.
30. Crump JA, Ramadhani HO, Morrissey AB, Msuya LJ, Yang LY, Chow SC, et al. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-

uninfected children and infants in northern Tanzania. Trop Med Int Heal. 2011;16(7):830–7.

31. Mhada T V., Fredrick F, Matee MI, Massawe A. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; Aetiology, antimicrobial sensitivity pattern and clinical outcome. BMC Public Health [Internet]. 2012;12(1):1. Available from: BMC Public Health
32. Marando R, Seni J, Mirambo MM, Falgenhauer L, Moremi N, Mushi MF, et al. Predictors of the extended-spectrum-beta lactamases producing Enterobacteriaceae neonatal sepsis at a tertiary hospital, Tanzania. Int J Med Microbiol [Internet]. 2018;308(7):803–11. Available from: https://doi.org/10.1016/j.ijmm.2018.06.012
33. Blomberg B, Jureen R, Manji KP, Tamim BS, Mwakagile DSM, Urassa WK, et al. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. J Clin Microbiol [Internet]. 2005;43(2):745–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15695674%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC548071
34. Kiemde F, Spijker R, Mens PF, Tinto H, Boele M, Schallig HDFH. Aetiologies of non-malaria febrile episodes in children under 5 years in sub-Saharan Africa. Trop Med Int Heal. 2016;21(8):943–55.
35. Viscoli C, Castagnola E, Giacchino M, Cesaro S, Properzi E, Tucci F, et al. Original Paper Bloodstream Infections in Children with Cancer : a Multicentre Surveillance Study of the Italian Association of Paediatric Haematology and Oncology. 1999;35(5):770–4.
36. Rodrı M, Alcala L, Mun P, Cercenado E, Vicente T, Bouza E. Bloodstream Infections. 2008;87(4):234–49.
37. Heaton P, Bendel-stenzel E. 1, 4, 5. 2018;2009–16.
38. Hamer DH, Darmstadt GL, Carlin JB, Zaidi AKM, Yeboah-Antwi K, Saha SK, et al. Etiology of bacteremia in young infants in six countries. Pediatr Infect Dis J. 2015;34(1):e1–8.

39. Isendahl J, Manjuba C, Rodrigues A, Xu W, Henriques-Normark B, Giske CG, et al. Prevalence of community-acquired bacteraemia in guinea-bissau: An observational study. BMC Infect Dis. 2014;14(1):1–9.

40. Abrha A, Abdissa A, Beyene G, Getahun G, Girma T. Bacteraemia among severely malnourished children in jimma university hospital, ethiopia. Ethiop J Health Sci [Internet]. 2011;21(3):175–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22434997%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3275866

41. Kibuuka A, Byakika-Kibwika P, Achan J, Yeka A, Nalyazi JN, Mpimbaza A, et al. Bacteremia among febrile ugandan children treated with antimalarials despite a negative malaria test. Am J Trop Med Hyg. 2015;93(2):276–80.

42. Williams PCM, Isaacs D, Berkley JA. Antimicrobial resistance among children in sub-Saharan Africa. Lancet Infect Dis [Internet]. 2018;18(2):e33–44. Available from: http://dx.doi.org/10.1016/S1473-3099(17)30467-X

43. Musicha P, Cornick JE, Bar-Zeev N, French N, Masesa C, Denis B, et al. Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study. Lancet Infect Dis [Internet]. 2017;17(10):1042–52. Available from: http://dx.doi.org/10.1016/S1473-3099(17)30394-8

44. Bielicki JA, Lundin R, Sharland M. Antibiotic resistance prevalence in routine bloodstream isolates from children's hospitals varies substantially from adult surveillance data in Europe. Pediatr Infect Dis J. 2015;34(7):734–41.

45. Mahende C, Ngasala B, Lusingu J, Butichi A, Lushino P, Lemnge M, et al. Bloodstream bacterial infection among outpatient children with acute febrile illness in north-eastern Tanzania. BMC Res Notes. 2015;8(1):1–8.

46. Trehan I, Manary MJ. Management of severe acute malnutrition in low-income and middle-income countries. Arch Dis Child. 2015;100(3):283–7.

47. Jallow S, Madhi SA. Pneumococcal conjugate vaccine in HIV-infected and HIV-exposed, uninfected children. Expert Rev Vaccines [Internet]. 2017;16(5):453–65. Available from: http://dx.doi.org/10.1080/14760584.2017.1307740

48. Pittet D, Tarara D, Wenzel RP. Nosocomial Bloodstream Infection in Critically III Patients: Excess Length of Stay, Extra Costs, and Attributable Mortality. JAMA J Am Med Assoc. 1994;271(20):1598–601.

49. Folgori L, Bielicki J, Heath PT, Sharland M. Antimicrobial-resistant Gram-negative infections in neonates: Burden of disease and challenges in treatment. Curr Opin Infect Dis. 2017;30(3):281–8.

50. Dolinsky AL. M100 Performance Standards for Antimicrobial Susceptibility Testing [Internet]. Vol. 8, Journal of Services Marketing. 2020. 27–39 p. Available from: http://www.emeraldinsight.com/doi/10.1108/08876049410065598

51. Dharmapalan D, Shet A, Yewale V, Sharland M. High Reported Rates of Antimicrobial Resistance in Indian Neonatal and Pediatric Blood Stream Infections. 2017;6.

52. Ahmed M, Mirambo MM, Mushi MF, Hokororo A, Mshana SE. Bacteremia caused by multidrug-resistant bacteria among hospitalized malnourished children in Mwanza, Tanzania: A cross sectional study. BMC Res Notes. 2017;10(1):1–5.