BACTEREMIA, ASSOCIATED FACTORS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG FEBRILE PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE IN MWANZA, TANZANIA

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By

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A Dissertation Submitted in (Partial) Fulfillment of the Requirements for the Degree of Master of Science (Epidemiology and Laboratory Management) of the

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for the acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: **''Bacteremia, associated factors and antibiotic susceptibility pattern among febrile pediatric sickle cell disease patients in Mwanza, Tanzania",** in (partial) fulfillment of the requirements for the degree of Master of Science (Epidemiology and Laboratory Management) of Muhimbili University of Health and Allied Sciences.

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Date: _____

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I, **Sued Yassin**, 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.

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ABSTRACT

Background: More than 300,000 infants are born annually with Sickle Cell Disease (SCD) and sub-Saharan Africa accounts for 75% of the global burden. The patients with SCD are prone to bacterial infections due to compromised immunity resulting in significant morbidity and mortality. Routine use of penicillin prophylaxis for SCD patients under the age of five and routine use of conjugate vaccines against invasive *Streptococcus pneumoniae* have been widely deployed. There are however reports of increasing rates of penicillin resistance globally.

Objectives: The study determined the prevalence of bacteremia, antibiotic susceptibility patterns (AST) and associated factors among febrile SCD patients in Mwanza, Tanzania.

Methodology: This was a hospital-based cross-sectional study conducted among febrile pediatric patients with SCD between January and June 2021. Three health facilities in Mwanza, Nyamagana District Hospital (NDH), Sekou-Toure Regional Referral Hospital (SRRH) and Bugando Medical Centre (BMC) were conveniently selected. Blood culture samples from assented children and/or consented parents/guardians of the febrile SCD patients were collected aseptically by a trained research assistant. Independently associated factors for bacteremia among febrile SCD patients were determined by univariate and multivariate logistic regression analysis using odds ratios, 95% confidence intervals and p-value cut-off of less than 0.05.

Results: A total of 321 febrile pediatric SCD patients were included in the study. The median age (IQR) of the study participants was 5 (0.7-17) years. Females accounted for more than half 175/321 (54.5%) of the study participants. A total of 32/321 (10.0%) bacteria species strains were isolated from the blood culture specimens. The gram-positive micro-organisms constituted 22/32 (68.8%) with a predominance of *S. aureus* (81.8%). The gram-negative bacteria were predominated by *K. pneumoniae* (50%). The *S. aureus* strains isolated from the blood culture specimens were more resistant to erythromycin, penicillin and co-trimoxazole

with resistance rates ranging from 55.6 -77.8% whereas gram-negative bacteria showed high resistance rate (80 to 100%) to ceftriaxone, amoxicillin-clavulanic and cefepime.

Conclusion: Our findings have shown that a large proportion of children with SCD still acquire bacterial infection with *S. aureus* constituting to the majority of the bacteremic episodes. High rate of resistance has been observed towards penicillin (a prophylactic antibiotic) along with other commonly used antibiotics. However, ciprofloxacin, gentamicin and clindamycin are still sensitive and can therefore be used as alternative antibiotics. Nevertheless, the high resistance rates towards the commonly used antibiotic calls for need to introduce routine culture and AST at health facilities that lack the services.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
API 20E	Analytical Profile Index 20E
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
BMC	Bugando Medical Centre
EDTA	Ethylene Diamine Tetra-acetic Acid
ESBL	Extended Spectrum Beta-Lactamases
FELTP	Field Epidemiology and Laboratory Training Program
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
KIA	Kliger's Iron Agar
MDR	Multidrug Resistance
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MUHAS	Muhimbili University of Health and Allied Sciences
NDH	Nyamagana District Hospital
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SIM	Sulphur Indole Motility
SRRH	Sekou-Toure Regional Referral Hospital
UK	United Kingdom
USA	United States of America

DEFINITION OF TERMS

Antimicrobial resistance (AMR) – The ability of microbe (germ) to resist the effects of antimicrobials that were previously capable to treat it.

Bacteremia- Is the presence of viable bacteria circulating in the blood as evidenced by a positive blood culture.

Febrile illness – The onset of fever and symptoms such as headache, chills or muscle and joint pains.

Fever- Refers to a rectal temperature higher than 38°C (100.4°F) or armpit (axillary) temperature higher than 37.5°C (99.5°F).

Multi-drug resistant – Bacterial resistance to at least one antimicrobial in three or more classes of antibiotics.

Sickle cell disease (SCD) - An autosomal recessive genetic disease that is caused by the substitution of valine to glutamic acid in the sixth codon of the β-globin subunit.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Sickle cell disease (SCD) is an autosomal recessive genetic disease that is caused by the substitution of valine to glutamic acid in the sixth codon of the β-globin subunit(1). It is estimated that 300,000 infants are born annually with SCD with sub-Saharan Africa accounting for 75% of the global burden (2,3). With an annual incidence of 11,000, Tanzania ranks fourth in the world with the highest estimated number of newborns with SCD a year behind Nigeria (85,000), the Democratic Republic of Congo (42,000) and India (38,000), respectively (3). In sub-Saharan Africa which carries the largest portion of the global burden, 90% of children with SCD die before the diagnosis can be made (4).

As a result of compromised immune function, SCD patients are prone to several health problems including attacks of pain, anaemia, swelling in the hands and feet, stroke and bacterial infections such as *Streptococcus pneumoniae, Haemophilus influenzae* and *Salmonella spp* causing significant morbidity and mortality (5,6). Bacteremia remains the leading cause of morbidity and mortality in SCD patients with studies reporting the proportion of deaths from infections as high as 38% and 29% in the United States and Jamaica, respectively; interventions with penicillin and vaccination have however reduced mortality in these settings (7,8).

In efforts to reduce morbidity and mortality due to bacterial infections in patients with SCD, measuring the magnitude is very critical (9). Several studies in Africa show that gram-negative bacteria account for >50 % of the bacteremic episodes in children with SCD (2). However, because of delays in establishing the diagnosis in most of the African countries, studies confined to known cases will consequently under-report events in young children (4).

There is also a tremendous variation in epidemiology at instances where bacteremic episodes occur that may be greatly attributed to the higher carriage of other microorganisms, liberal use of antibiotics before hospital admission that influences results and difficulty in establishing the cause of infections by routine culture in resource-limited settings (2). Several interventions have been widely deployed over the past three decades including screening of newborns for SCD, routine use of penicillin prophylaxis for sickle cell disease patients under the age of five and routine use of conjugate pneumococcal against invasive *Streptococcus pneumoniae* and *Hemophilus influenzae* type b vaccines that have resulted in a drastic decline in mortality due to sepsis (10).

Although SCD patients routinely receive penicillin prophylaxis there are increasing rates of penicillin resistance all over the world (11). Studies have also found ampicillin, tetracycline and nalidixic acid to be resistant (12). Infections remain the major cause of morbidity and mortality in both low- and middle-income countries due to increased co-morbidities such as malnutrition, lower levels of vaccination and reduced access to care thus; strong emphasis on measures likely to prevent infection including better hygiene with hand-washing, avoidance of food contamination and nutritional supplementation should be highly warranted (2,13).

A systematic review conducted in 2003 found that washing hands with soap can reduce the risk of acquiring diarrheal diseases by 42–47% and henceforth, measures to promote this intervention could save million lives (14). Similarly, another study conducted in 2008 found that a higher volume of alcohol-based hand-rub use was significantly associated with a lower incidence of extended-spectrum beta-lactamases (ESBL) producing strains (15). The burden of ESBL colonization among the pediatric population are conflicting; however, studies conducted in Tanzania and Gabon have observed a prevalence of 34.3 and 45%, respectively (16,17).

1.2 Problem Statement

Bacteremia remains the leading cause of morbidity and mortality in SCD patients in Africa (4,9). Children with SCD are at increased risk of invasive bacterial infections, particularly from encapsulated organisms like *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (18). Studies done in Tanzania and Nigeria have reported bacteremia prevalence of 4.8% and 13.8%, respectively among SCD patients (6,9).

In efforts to reduce morbidity and mortality, SCD patients usually receive prophylactic oral penicillin and pneumococcal conjugate vaccines. However, increasing penicillin resistance along with other antibiotics have been reported elsewhere (11). A study by Dayie et al in Ghana in 2018 found a prevalence of penicillin resistance of 37.4% among pneumococcal isolates and resistance to other antibiotics including cotrimoxazole (85%), levofloxacin (2.5%) and multi-drug resistance (MDR) of 34.3% (19). The ESBL producing bacteria has also been reported among the SCD population (20). Nevertheless, treatment of the most common bacterial infections in SCD is based on consensus guidelines, clinical experience or adapting treatment applied on other diseases leading to wide variations in treatment among institutions (13).

In most cases, treatment of bacterial infection is usually done empirically giving more room for the emergence of antibiotic resistance. Most of the studies done in East Africa particularly on the prevalence of bacteremia among SCD patients did not look into the antimicrobial resistance (AMR) (4,9). The vast majority of SCD patients live in low-income countries that have inadequate hygiene, increased risk of food contamination, high prevalence and transmission rates of infections and are therefore prone to acquiring bacterial infections and thus periodic monitoring of antimicrobial susceptibility patterns in clinical settings is important to ascertain potency and re-establish empirical treatment (2,13).

Although the SCD prevalence of 21% among neonates born annually in Northwestern Tanzania including Mwanza is one of the highest in the world, studies regarding bacteremia and antimicrobial susceptibility patterns are however limited (9,21). This study aimed to assess the magnitude of bacteremia, the antibiotic susceptibility patterns and identify factors associated with bacteremia in febrile pediatric patients with SCD in Mwanza, Tanzania.

1.3 Conceptual Framework

The conceptual framework below summarizes various factors that are associated with bacteremia among SCD. SCD patients are at an increased risk of acquiring bacterial infections due to compromised immune function. The presence of underlying diseases and conditions such as Human Immunodeficiency Virus (HIV), malaria, ESBL colonization and malnutrition have been implicated in increasing chances of bacterial infection acquisition and consequently worsening outcomes. Other factors including the parents'/guardians' level of education, smoking or presence of a smoker in the family, number of family members in a household, sanitation and type of food taken have also been linked to the acquisition of bacterial infections (2,4,9,13).

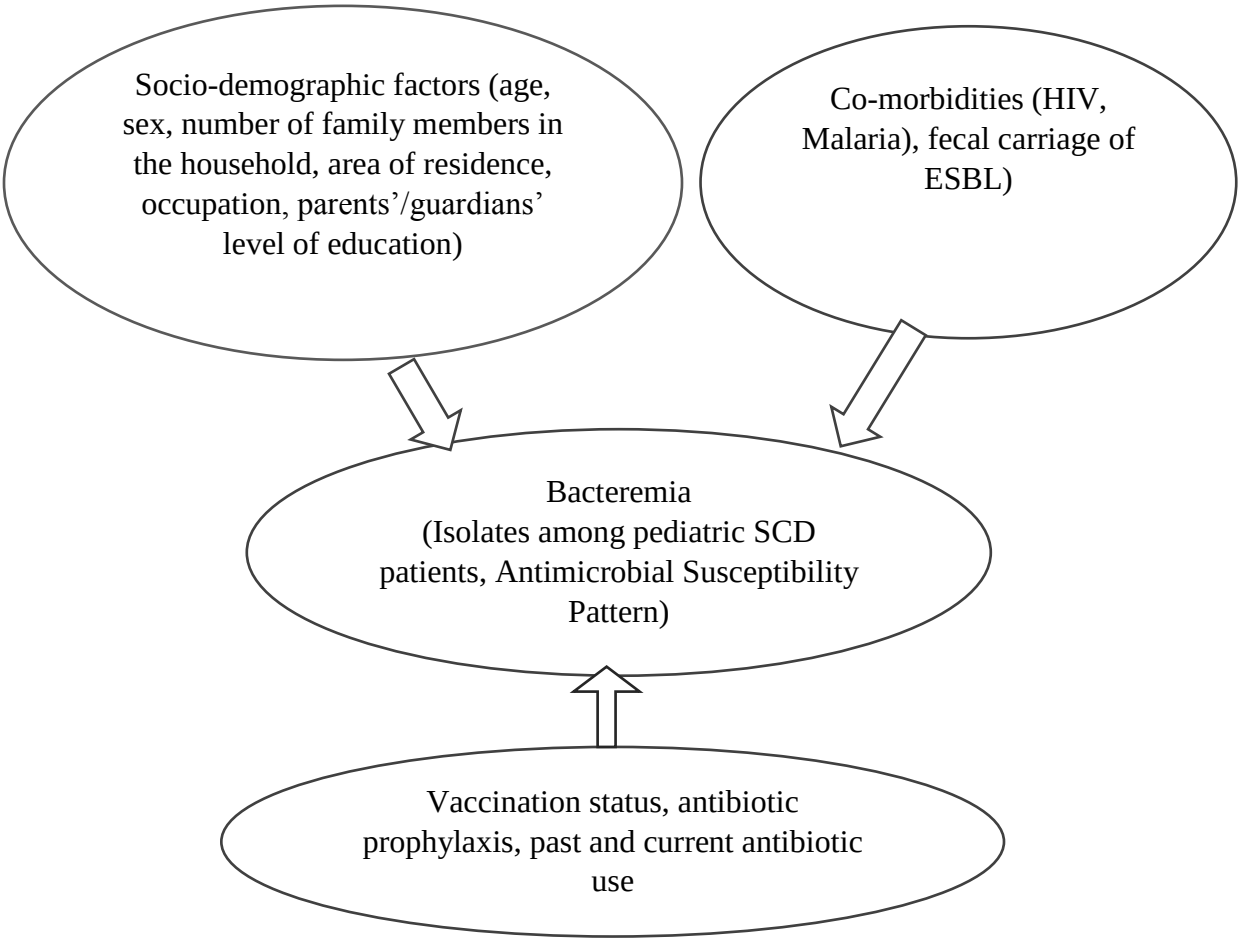


Figure 1: Conceptual frame for bacteremia among febrile SCD children

1.4 Rationale

Data from this study has helped to identify the aetiological agents and AST patterns among febrile children with SCD adding to the pre-existing knowledge in Tanzania. This information obtained will help to guide the selection of prophylactic antibiotics and to provide informed evidence for revising the guidelines for managing febrile children with SCD.

1.5 Research Questions

i. What is the magnitude of bacteremia among febrile pediatric patients with SCD?
ii. What is the antimicrobial susceptibility pattern of commonly isolated bacteria among febrile pediatric patients with SCD?
iii. What are the factors associated with bacteremia among febrile pediatric patients with SCD?
iv. Is there an association between bacteremia and ESBL colonization among patients with SCD?

1.6 Objectives

1.6.1 Broad Objective

To determine the magnitude of bacteremia, antibiotic susceptibility patterns and associated factors among febrile pediatric patients with SCD in Mwanza, Tanzania.

1.6.2 Specific Objectives

i. To determine the magnitude of bacteremia among febrile pediatric SCD patients in Mwanza, Tanzania.
ii. To determine the antibiotic susceptibility pattern of bacteria recovered from febrile pediatric patients with SCD in Mwanza, Tanzania.
iii. To determine factors associated with bacteremia among febrile pediatric SCD patients in Mwanza, Tanzania.
iv. To determine the association between ESBL colonization and bacteremia among children with SCD in Mwanza, Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bacteremia among febrile pediatric patients with SCD

Before the introduction of penicillin prophylaxis, morbidity and mortality from invasive pneumococcal disease in the sub-Saharan Africa among children with SCD was very high (18). Studies indicate that before the use of prophylactic oral penicillin, the case fatality was as high as 35% with *S. pneumoniae* infections showing a rapid progression to death in less than 24 hours from onset (22). However, the identification of children with SCD through newborn screening and prophylactic treatment with penicillin, and augmented immunization schedule, had led to a decline of rates of bacteremia to <1% in the developed nations (2). Thus children in the developed nations now have a better chance of surviving to adulthood (13). Nevertheless, the combination of suboptimal compliance and resistance to prophylactic antibiotics, non-vaccine serotypes of *S. pneumoniae* and splenism still render children with SCD at an increased risk of infections (23).

Previous studies conducted in the US indicate that febrile children with SCD have a 3% to 5% risk of becoming bacteremic due to compromised immune function although the risk may now have sharply declined due to the introduction of prophylactic antibiotics and pneumococcal vaccines (10). In sub-Saharan Africa, SCD is still an under-recognized global health problem that contributes substantially to mortality in children younger than 5 years of age (24). Bacteremia in Africa falls between 14% and 32% with a spectrum of organisms that is different from the rest of the world. Studies from the Western world have found pneumococcal infection as the significant contributor to morbidity and mortality contrary to African where gram-negative bacteria like *Klebsiella spp* and *Salmonella spp* constitute more than 60% of all isolates (13).

Even within the African continent where instances of bacterial episodes occur, the epidemiology of isolates varies greatly. A study done in Cameroon in 2017 found a bacteremia prevalence of 9.7% among SCD children with *Salmonella* (28.1%), *S. aureus* (18.8%),

Klebsiella spp (17.7%) and *E.coli* (10.4%) (25) appearing as the commonest isolates. However, the spectrums of organisms were different from a study done in Kenya in 2009 where the prevalence of bacteremia was low (6%) with *S. pneumoniae*, non-typhi salmonella and *Hemophilus influenzae* type b emerging as prevalent causative agents (4). In Tanzania, one study conducted six years ago found a bacteremia prevalence of 4.8% among sickle cell disease patients with *S. aureus* (28%), *non-typhi Salmonella* (21%), *S. pneumoniae* (7%) and *salmonella typhi* (5%) as the main contributors (9). The prevalence of neonates born annually with SCD in Northwestern zone of Tanzania and in particular Mwanza is one of the highest in the world (26), yet no recent published data on the magnitude and aetiology of the bacteremic episodes in children with SCD in the region. This highlights urgent need for additional studies to ascertain the magnitude and pattern of the causative agents for the bacteremic episodes in children with SCD.

2.2 AMR patterns of bacteria isolated among febrile pediatric SCD patients

Any fever is treated as a medical emergency in children with SCD since it can be the first sign of bacteremia and thus prompts administration of broad-spectrum parenteral antibiotic soon after collecting samples for blood culture (2,13). The initial antibiotic selected for patients presumed to be bacteremic should be broad and active against *Streptococcal spp*, *Hemophilus* and *Salmonella* as well as staphylococci and other gram-negative enteric bacteria. Further consideration should be on the local epidemiology and antibiotic resistance patterns (2). The epidemiology of antimicrobial susceptibility patterns varies with geographical location and socio-economic status (2).

A study in Nigeria in 2018 found that most of the isolates from sickle cell disease patients were resistant to a variety of antibiotics with the highest rates on cotrimoxazole (53%) and ampicillin (43%) (20). There are also increasing reports on penicillin resistance, a prophylactic antibiotic that has been widely used over the years (11). Reports of penicillin-resistant organisms are conflicting among patients receiving penicillin prophylaxis, however, reports from North America indicate that there was an increase from 5% in 1989 to more than 35% in 1997 (27).

A study conducted in Dar es Salaam, Tanzania a year ago among hospitalized children with fever found MDR resistance of 54% among the gram-negative bacteria with *K. pneumoniae, Acinetobacter* spp *and E. coli* having resistance rates of 90.3, 40 and 36%, respectively. The same study also found 51% ESBL phenotype among the gram-negative bacteria isolated (28). In another similar study conducted in Mwanza, Tanzania among the general pediatric population, MDR was found in over three quarters (78%) of the bacterial isolates with gram-negative bacteria the leading contributor. This study also found that majority of the bacteria were resistant to ampicillin and trimethoprim-sulfamexathole with rates ranging from 67-100% (29). Although there are few studies in Africa including Tanzania especially on the aetiological agents for bacteremia and AST patterns among children with SCD, increased resistance to commonly used antibiotics has been reported; ciprofloxacin and some third-generation cephalosporins are still active (13). However, the majority of children with SCD live in low-income countries that have inadequate access to healthcare, high prevalence and transmission rates of infections and therefore prone to acquiring bacterial infections that warrants periodic monitoring of AST to ascertain potency and re-establish empirical treatment (2,13).

2.3 Factors associated with bacteremia among patients with SCD

An increase in the morbidity and mortality of bacteremia in the austere environment can be linked to deficiencies in vaccination, antibiotic prophylaxis, blood supply and critical care capacity (2). Studies also show a relationship between tobacco smoke (both first and second-hand exposures) and SCD through infections as well as the influence of socio-economic status, increased poverty and deficiencies in micro-nutrients on the course of the disease (13).

Furthermore, studies indicate that not only do HIV-infected children with SCD experience more complicated hospitalizations but they also have an increased risk of developing sepsis (13,30). The scenario is worse especially in Africa where both conditions endemically co-exist and resources are scarce (31). Although studies also show that homozygous SCD confers higher resistance to malaria, their co-existence has also been associated with increased morbidity and mortality (13).

However, nutritional supplementation with zinc has shown considerable benefits in reducing the risk of infection among children with SCD (31). Studies also indicate that simple measures such as hand hygiene, proper cooking of food especially eggs and chicken, keeping items refrigerated and avoiding contamination have the potential to reduce infection among children with SCD (13,31).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

This was a hospital-based cross-sectional study.

3.2 Study Area

The study was conducted at Bugando Medical Centre (BMC), Sekou-Toure Regional Referral Hospital (SRRH) and Nyamagana district hospital (NDH) in Mwanza city. Mwanza City is found in the Northwestern zone of Tanzania and along the shores of Lake Victoria. It has eight districts (Nyamagana, Ilemela, Misungwi, Magu, Sengerema, Buchosa, Ukerewe and Kwimba). BMC is a zonal referral hospital whereas SRRH is the regional referral hospital for Mwanza city. Furthermore, Nyamagana District hospital is the district hospital for Nyamagana District council. The healthcare facility catchment population for BMC is 16,252,410 where as SRRH and NDH serves 2,772,509 and 363,452, respectively (29). There is an SCD clinic at BMC that operates on every Monday and Thursday that serves 35-40 patients per day while SRRH runs a clinic every Thursday that serves 15-20 patients a day. NDH has no specific SCD clinic but serves a large proportion of these clients on daily basis.

3.3 Study Population

Pediatric patients with SCD aged 0-17 years

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion criteria

All pediatric patients with SCD aged 0-17 years with history of fever at the time of presentation to the healthcare and whose parents/guardians voluntarily consented to participate on their behalf.

3.4.2 Exclusion criteria

All pediatric patients with SCD aged 0-17 years with a history of fever but whose parents/guardians declined to participate and those who were captured just after they were subjected on antibiotic medication.

3.5 Sample size

The sample size was estimated using the Kish Leslie formula using the prevalence of 4.8% for bacteremia among sickle cell disease patients reported in Tanzania in 2015 (9).

$$n = \frac{z^2 p(1-p)}{e^2}$$

Where; n = Sample size, z = at 95% confidence interval z value = 1.96, p = 4.8% from a study done in Tanzania (2015), e=Margin of error at 2.4% (0.024),

$$n = \frac{1.96^2 \times 0.048\ (1-0.048)}{0.024^2} = 305$$

Assuming a non-response rate of 5%, the adjusted sample size results to 321

3.6 Sampling method

A convenient sampling technique was used. All three high volume health facilities in Mwanza municipal council, Nyamagana District hospital, Sekou-Toure Regional Referral hospital and BMC were selected. The number of participants enrolled from each facility was calculated based on probability proportional to size sampling. Thus, from 321 febrile SCD patients that were enrolled into the study, BMC contributed 195 whereas SRRH and Nyamagana district contributed 84 and 42 febrile patients with SCD respectively. At the study sites, upon obtaining assent from the children or consent from the parent/guardians, all febrile children with SCD were recruited.

3.7 Study Variables

3.7.1 Dependent/Outcome variables

Bacteremia among pediatric patients with SCD

3.7.2 Independent variables

Past and current antibiotic use, other co-morbidities (malaria, HIV) ESBL colonization etc), age, sex, socio-economic status, housing and occupation, vaccination status, penicillin prophylaxis.

3.8 Study duration

The study was conducted for 6 months between January and June 2021.

3.9 Data collection

Data regarding socio-demographic, clinical, vaccination status and past or current antibiotic use was collected using a structured questionnaire. Data on vaccination history was collected from the study participants' Reproductive and Child Health card number one (RCH 1). Body temperature was measured and any patient with readings above 37.5^{o}C was selected for blood sample collection. Stool samples from febrile SCD patients were also collected to look for ESBL colonization rate. Samples were then collected from the patients and taken to BMC laboratory for processing and analysis.

3.10 Samples collection and Laboratory procedure

3.10.1 Blood collection and Bacterial isolation.

Blood samples for culture were collected aseptically by trained laboratory personnel using sterile syringes before antibiotics were given. About 5 ml of venous blood was collected from all pediatric SCD patients with febrile conditions. About 2-3 ml was placed in blood culture broth and sent to the laboratory for processing. The remaining 2 ml of blood in the drawing syringe was dispensed into an Ethylene Diamine Tetraacetic Acid (EDTA) tube for malaria testing. The HIV testing was performed by SD Bioline HIV 1/2 test (Standard Diagnostics Inc., California, USA) and Unigold HIV test (Trinity Biotech, Bray, Ireland) using the approved National HIV testing algorithm (32) and SD Bioline malaria Rapid Diagnostic Test (mRDT) (Standard Diagnostics Inc., California, USA) for malaria as per the standard operating procedures (33). The HIV and mRDT testing targeted study participants with

unknown status but those with known status results were retrieved from the patient files. The HIV rapid tests were performed by nurse counsellors after pre-counselling sessions were done and completed whereas mRDT was performed by trained laboratory personnel at the participating health facilities. Blood culture and stool/rectal swab specimens were processed at BMC laboratory. Blood culture was performed using the BACTEC system (Becton Dickinson BACTEC FX Blood Culture System, USA) according to the manufacturer's instructions. Aerobic blood culture bottles were incubated at 37°C with agitation overnight in a BACTEC. Upon alerts of a positive sample by the BACTEC within five (5) days, the samples were picked and sub-cultured on blood and MacConkey agar (Oxoid, Hampshire, United Kingdom) plates. Inoculated media was incubated under aerobic and 5% CO2 conditions at 37°C for 24 hours.

3.10.2 Stool samples collection and processing

3.10.2.1 Stool sample collection

Appropriate instructions on stool sample or rectal swab collection were given to the parents /guardians. Parents or caregivers were then given a clean, dry plastic container and asked to collect the stool sample. In case stool sample wasn't available then rectal swabs were collected and placed into a transport medium. All samples were then transported to the laboratory for processing and analysis. All the stool samples/ rectal swabs were processed at BMC laboratory.

3.10.2.2 ESBL screening from stool/rectal swab specimens

Stool samples or rectal swabs were inoculated onto MacConkey agar (MCA) (Oxoid, Hampshire, United Kingdom) without antibiotic and one supplemented with ceftazidime 2µg/ml and plates were then incubated aerobically at 37°C for 24 hours. The presence of ceftazidime allowed the growth of ESBL strains and inhibited the growth of non-ESBL.

3.10.3 Bacteria identification

Isolated bacteria were identified based on colonial appearance, Gram stain reaction, biochemical tests and serological methods. A catalase test was performed on the Gram-

positive cocci to differentiate between *Staphylococcus* species and *Streptococcus* species. Further identification of the catalase-positive gram-positive cocci was done using a Staphylase kit ProlexTM Latex Agglutination System (Pro-Lab Diagnostics, Canada) to differentiate between *Staphylococcus aureus* and other *Staphylococcus* species. Gram-negative bacilli were identified by performing Gram's staining, motility and standard biochemical tests (oxidase, Kliger's Iron Agar (KIA), citrate utilization, urease and Sulphur Indole Motility (SIM)). For identification of members of *Enterobacteriaceae* which could not be identified by biochemical tests, API 20 E system (Bio-Merieux, France) was used according to the manufacturer's instructions.

3.10.4 Antimicrobial Susceptibility Testing (AST)

The AST was done by the conventional Kirby–Bauer disk diffusion method on Muller Hinton Agar (Oxoid, Hampshire, United Kingdom) according to the Clinical and Laboratory Standards Institute guidelines, 2020 (34). The following antibiotics were included ciprofloxacin (30 μg), gentamicin (10μg), ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), ceftriaxone (30 μg), meropenem (10 μg), cefotaxime (30 μg), Cefepime (30 μg) and ceftazidime (30 μg) (Bioanalyse, Turkey) for gram-negative organisms and erythromycin (15 μg), penicillin G (10 μg), ciprofloxacin (30 μg), clindamycin (2 μg), gentamicin (10μg) and trimethoprim-sulfamethoxazole (1.25/23.75 μg) (Bioanalyse, Turkey) for gram-positive organisms.

3.10.4.1 Screening of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

MRSA was screened by the use of cefoxitin disc (30 μg) and strains showing a zone of inhibition of ≤ 21 mm were labelled as MRSA (35).

3.10.4.2 Screening of ESBL-E from blood culture specimens

The phenotypic screening of ESBL-E was done in Muller Hinton agar (Oxoid, Hampshire, United Kingdom) along with other discs using a cut-off zone inhibition of ≤ 25 mm for ceftriaxone (30 μg) and ≤ 22 mm for ceftazidime (30 μg) (35).

3.10.4.3 Phenotypic Confirmation of ESBL-E

Confirmation of ESBL-E production among isolates was done in Muller Hinton agar (Oxoid, Hampshire, United Kingdom) by the double-disc synergy method (36). Isolates presumed to be ESBL-E producers based on screening test results were picked up and emulsified in saline to a 0.5 McFarland turbidity standard. By the use of a cotton swab, the broth was evenly spread on test sensitivity agar. Discs of ceftazidime (30 μg), cefotaxime (30 μg) and amoxicillin -clavulanic acid (20/10 μg) were placed 20mm apart on the plate in a straight line, with amoxicillin-clavulanic acid (20/10 μg) in the middle. Any increase in the inhibition zone towards the disk of amoxicillin–clavulanic acid was considered a positive result for ESBL enzyme production.

3.10.5 Quality Control

E. coli ATCC 25922 and *S. aureus* ATCC 25923 were used as reference strains for gram-negative and gram-positive bacteria respectively in quality control of culture media, biochemical identification tests and AST. For ESBL bacteria, ESBL producing *Klebsiella pneumonia* ATCC 700603 was used. Two separate readers were involved both during plate reading and identification to minimize bias. Results were then verified by a senior laboratory scientist and the principal investigator before release.

3.11 Data analysis

Data was cleaned and evaluated for completeness and consistency before analysis. Data analysis was done by using STATA version 15.0 software (College Station, Texas, USA). Proportions of children with culture-confirmed bacteremia, bacterial species, and resistance to various antimicrobial agents were determined. Univariate logistic regression analysis was done on all variables but only those with a p-value cut-off of ≤ 0.2 were subjected to multivariate logistic regression analysis. Independently associated factors for bacteremia among febrile children with sickle cell disease were determined by univariate and multivariate logistic regression analysis using odds ratios, 95% confidence intervals and p-value cut-off of less than 0.05.

3.12 Ethical considerations

Ethical clearance was obtained from the Muhimbili University of Health and Allied Sciences (MUHAS) Senate, Research and Publications Committee and authorization to conduct the study was requested and granted from the hospital management. Parents and/or guardians on the other hand were given the consent forms to read and consent on behalf of the child before samples were taken. For those whose children were found with bacteremia and/or co-infection (malaria and HIV), results were communicated to both the guardian and/or parent and the attending clinician for patient management. Furthermore, HIV test results were communicated to the parent/guardian by a nurse counsellor only after counselling was done. All information and issues relating to patients in the study were treated confidentially, no unauthorized persons had access to personally identifiable information. Furthermore, to protect the confidentiality, study numbers were used instead of patient names.

3.13 Plan for dissemination of results

Data from the study will be disseminated through presentations in symposia, workshops and conferences. Additionally, the dissertation will be kept at the MUHAS repository and findings will be shared with the participating health facilities and published in peer-reviewed journals and thus contributing information to the wider scientific community.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic characteristics of the febrile pediatric patients with SCD

A total of 321 febrile pediatric SCD patients were included in the study. Females accounted for more than half 175/321 (54.5%) of the study participants. The median age of the study participants was 5 years ranging from 8 months to 17 years. The majority of the study participants lived with their parents 285 (88.8%) in a household that had a mean of 5.4 (95% CI: 5.2-5.7) dwellers. The majority of the parents/guardians 196/321 (61.1%) had attained a primary level education. The socio-demographic information is summarized in table 1.

Table 1: Socio-demographic characteristics of the febrile pediatric patients with SCD

Characteristic	**Description**	**Number (%)**
Sex	Males	146(45.5)
	Females	175(54.5)
Age group **(years)**	≤ 5	187(58.3)
	> 5	134(41.7)
Parents/guardian level of education	Informal	20(6.2)
	Primary	196(61.1)
	Secondary	86(26.8)
	University/college	19(5.9)
Who the patient lives with	Parent(s)	285(88.8)
	Guardian	36(11.2)

4.2 Clinical characteristics of the febrile pediatric SCD patients

The majority of the children 241/321 (75.1%) had evidence of pneumococcal conjugate vaccination (PCV) when verified from the Reproductive and Child Health card number one (RCH 1). Of the 321 febrile SCD patients, 33/321 (10.3%) tested positive for malaria while HIV infection was observed in 8 (2.5%) of the children. A history of prior use of prophylactic antibiotics was observed in 66/196 (33.7%) and all reported to have used penicillin V as a prophylactic antibiotic. The previous history of antibiotic use in the past three weeks before or at the time of presentation to a healthcare facility was reported in 80/321 (24.9%) of the study children of which 33/80 (41.3%) used penicillin V. The information is summarized in table 2.

Table 2: Clinical characteristics of the febrile pediatric SCD patients

Characteristic	Description	Number (%)
PCV vaccination status	Yes	241 (75.1)
	No	80 (24.9)
Prophylactic antibiotic use	Yes	66 (33.7)
	No	130 (66.3)
Antibiotic use in the past three weeks	Yes	80 (24.9)
	No	241 (75.1)
Co-infection	Malaria	33 (10.3)
	HIV	8 (2.5)

PCV-Pneumococcal Conjugate Vaccine.

The antibiotics used in the past three weeks: amoxicillin, ampiclox, penicillin V and Ceftriaxone and cotrimoxazole.

4.3 Hygienic practices of the febrile pediatric patients with SCD

Of the 321 study participants, 286/321 (89.1%) acknowledged not using soap and water after visiting the latrines while all the study participants 321/321 (100%) acknowledged washing hands before eating. On the other hand, 288/321 (89.7%) reported having been sharing toilet(s) with other members of the same household. The hygienic practices are summarized in table 3.

Table 3: Hygienic practices of the febrile pediatric patients with SCD

Characteristic	Description	Number (%)
Handwashing after latrine use	Yes	35 (10.9)
	No	286 (89.1)
Handwashing before eating	Yes	321 (100%)
	No	0 (0.0%)
Sharing of toilets with other members of the same household	Yes	288 (89.7%)
	No	33 (10.3%)

4.4 Prevalence of bacteremia among the febrile pediatric patients with SCD

A total of 32/321 (10.0%) bacteria strains were isolated from the blood culture specimens of febrile children with SCD. The gram-positive bacteria constituted 22/32 (68.8%). Of the gram-positive bacteria strains recovered, 18 (81.8%) were *S. aureus*. The MRSA strains were detected in 4/18 (22.2%) of the *S. aureus* isolated. Bacteremia was higher in males 21/32 (65.6%). Furthermore, the gram-negative bacteria strains constituted 10/32 (31.3%) of the isolates recovered with a predominance of *K. pneumoniae* 5/10 (50%). The age-specific bacteremia prevalence for children below and above five years were 26/196 (13.3%) and 6/125 (4.8%), respectively. (Figure 2)

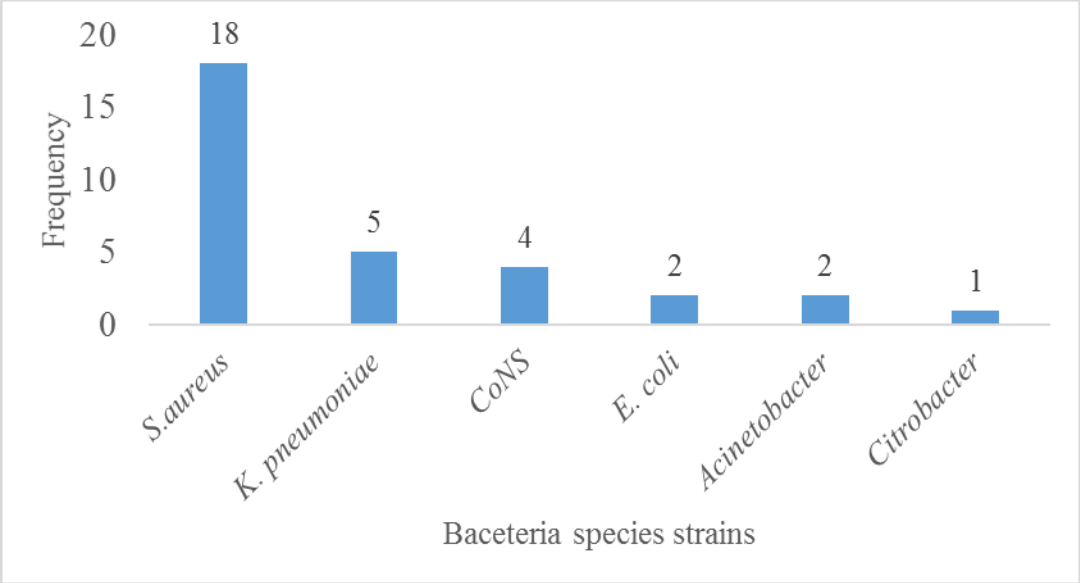


Figure 2: Frequency of Bacteria species isolated from febrile patients with SCD

4.5 AMR patterns of bacteria causing bacteremia among the febrile pediatric patients with SCD

The *S. aureus* strains isolated from the blood culture specimens were more resistant to erythromycin, penicillin and co-trimoxazole with resistance rates ranging from 55.6 to 77.8% whereas gram-negative bacteria were more resistant to ceftriaxone, amoxicillin-clavulanic and cefepime with resistance rates ranging from 80 to 100%. All the gram-negative bacteria showed no resistance to meropenem. The third-generation cephalosporin resistance was noted in *K. pneumoniae* (100%) and *E. coli* (100%). The *Citrobacter spp* isolated showed no resistance to third-generation cephalosporin. The proportion of MRSA among *S. aureus* was 4/18 (22.2%). The antimicrobial resistance pattern is summarized in table 4.

Table 4: AMR patterns of bacteria causing bacteremia among the febrile pediatric patients with SCD

Bacteria (n)	Antimicrobial resistance (n%)												
	FOX	P	SXT	GN	DA	E	AMC	CIP	AMP	CRO	CAZ	CPM	MEM
S. aureus (18)	4(22.2)	14(77.8)	14(77.8)	6(33.3)	4(22.2)	10(55.6)	NA	4(22.2)	NA	NA	NA	NA	NA
CoNS (4)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	NA	NA	NA	NA	NA
K. pneumoniae (5)	NA	NA	2(40.0)	1(20.0)	NA	NA	2(40)	3(60.0)	5(100)	4(80.0)	4(80.0)	4(80.0)	0(0)
E. coli (2)	NA	NA	2(100)	2(100)	NA	NA	2(100)	2(100)	NA	2(100)	2(100)	2(100)	0(0)
Acinetobacter (2)	NA	NA	2(100)	2(100)	NA	NA	2(100)	2(100)	NA	2(100)	2(100)	2(100)	0(0)
Citrobacter (1)	NA	NA	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	NA	0(0)	NA

GN Gentamycin, CIP Ciprofloxacin, AMC Amoxycillin-clavulanate, E, Erythromycin, CAZ Ceftazidime, CRO Ceftriaxone, SXT Trimethoprim-sulfamethoxazole, MEM Meropenem, P Penicillin, AMP Ampicillin, CPM Cefepime, DA Clindamycin, FOX Cefoxitin.

4.6 MDR bacterial strains causing bacteremia among febrile pediatric patients with SCD

MDR was observed in 15/32 (46.9%) of the specimens analysed with a predominance of gram-negative bacteria 8/15(53.3%). The ESBL producing bacteria was observed in 3/10 (30%) of all the gram-negative bacteria isolated from the blood culture specimens. Also, 4/18 (22.2%) of the *S. aureus* isolated were MRSA (Table 5).

Table 5: MDR, ESBL and MRSA isolates recovered from the blood culture specimens of the febrile pediatric patients with SCD

Bacteria (n)	ESBL	MRSA	MDR
K. pneumoniae (5)	2	NA	3
E. coli (2)	1	NA	2
Acinetobacter (2)	0	NA	2
Citrobacter (1)	0	NA	1
S. aureus (18)	NA	4	7
CoNS (4)	NA	NA	0

4.7 ESBL colonization among the febrile pediatric patients with SCD

4.7.1 Characteristics of the febrile pediatric patients with SCD colonized with ESBL

The screening testing revealed that 113/321(35.2%) of the children were colonized with ESBL. Additionally, all the positive samples on the screening test were confirmed to be ESBL positive on the confirmatory test 113/113 (100%). This study also found that, more than half 59/113 (52.2%) of the female participants were colonized with ESBL. Also, the children aged five years and below 70/113 (61.9%) were more colonized with ESBL. Furthermore, the colonization with ESBL did not confer an increased likelihood of acquiring bacteremia (P=0.096). Characteristics for the children colonized with ESBL are summarized in table 6.

Table 6: Characteristics of the febrile pediatric patients with SCD colonized with ESBL

Variable	Description (n)	ESBL (%)	**cOR (95% CI)**	p-value
Age category	≤ 5 years (196)	70 (35.7)	1.1 (0.7-1.7)	0.810
	> 5 years (125)	43 (34.4)	Ref	
Sex	Females (175)	59 (33.7)	0.9 (0.5-1.4)	0.541
	Males (146)	54 (37.0)	Ref	
Prophylactic antibiotic use	Yes (66)	18(27.3)	0.6 (0.3-1.2)	0.177
	No (130)	48(36.9)	Ref	
Washing hands after latrine use	Yes (35)	11 (31.4)	0.8 (0.4-1.7)	0.620
	No (286)	102 (35.7)	Ref	

4.7.2 ESBL strains isolated from the stool/rectal swab specimens the febrile pediatric patients with SCD

The majority of the ESBL strains isolated among children with SCD were *E. coli* 73/113 (64.6 %). Results are summarized in Table 7.

Table 7: ESBL strains isolated from stool/rectal swab specimens of the febrile pediatric patients with SCD

Bacteria species strains	ESBL (n)	Percentage (%)
E. coli	73	64.6
K. pneumoniae	36	31.9
Acinetobacter spp	4	3.5

4.8 Associated factors for bacteremia among febrile pediatric patients with SCD

Univariate and multivariate logistic regression analysis was done to ascertain how these associated factors predict the likelihood of acquiring bacteremia. The study **revealed** that, even after adjusting age by sex, febrile children with SCD aged 0-5 years had twice the odds of acquiring bacteremia (AOR=2.1, 95% CI=1.2-6.9) compared to those aged above five years, p =0.04. Furthermore, the odds of acquiring bacteremia among male participants were 1.5 times (AOR=1.5, 95% CI=1.1-3.9) compared to females, p =0.03. Associated factors for bacteremia are summarized in Table 8.

Table 8: Associated factors for bacteremia among SCD patients

Variable	Description	BSIs (n, %)	Univariate OR (95%CI)	p-value	Multivariate OR (95%CI)	p-value
Sex	Males (146)	21(14.4)	2.5 (1.2-5.3)	0.015	1.5(1.1-3.9)	0.03
	Females (175)	11(6.3)	Ref			
Age	≤ 5 years (196)	26(13.3)	3.0 (1.1-7.6)	0.013	2.1(1.2-6.9)	0.04
	> 5 years (125)	6(11.2)	Ref			
Education level of caretaker	Informal (20)	3 (15.0)	0.4 (0.5-3.2)	0.404		
	Primary (196)	22 (11.2)	0.1 (0.0-3.8)	0.462		
	Secondary (86)	6 (7.0)	0.9 (0.6-16.3)	0.97		
	University/College (19)	1 (5.3)	Ref			
Occupation of the caretaker	Formal employment (25)	2(8.0)	2.3 (0.8-6.8)	0.139		
	Self-employed (191)	15(7.9)	2.1 (0.4-13.6)	0.324		
	Informal employment (76)	10(13.2)	1.4 (0.4-4.4)	0.594		
	Not employed (29)	5(17.2)	Ref			
PCV vaccination history	Yes (241)	22 (9.1)	0.9(0.4-2.0)	0.742		
	No (80)	10(12.5)	Ref			
Prophylactic antibiotic use	Yes (66)	5(7.6)	0.8(0.3-2.1)	0.688		
	No (121)	27(22.3)	Ref			
Antibiotic use within three weeks	Yes (80)	7(8.8)	1.0(0.4-2.2)	0.924		
	No (241)	25(10.4)	Ref			
Co-infection with HIV	Yes (8)	1(12.5))	1.2(0.1-10.1)	0.808		
	No (313)	31(9.9)	Ref			
Co-infection with malaria	Yes (33)	1(3.0)	0.3(0.0-1.9)	0.160		
	No (288)	31(10.8)	Ref			
ESBL Colonization	Yes (113)	7(6.2)	1.9 (0.4-4.4)	0.301		
	No (208)	25(12.0)	Ref			

CHAPTER FIVE

5.0 DISCUSSION

5.1 The magnitude of bacteremia among febrile pediatric patients with SCD

The findings from our study suggest that large proportion of children with SCD still acquire bacterial infections with *S. aureus* the leading aetiological agent. Consequently, there are high levels of penicillin (a prophylactic antibiotic) resistance along with other antibiotics. Furthermore, our findings have shown that, female children with SCD and those aged five years or below are more prone to bacterial infections.

This study has found that, the overall magnitude of bacteremia among paediatric patients with SCD was 10.0% which is higher compared to three previous studies done in Kenya in 2009, Tanzania in 2015 and Cameroon in 2017 which found a bacteremia prevalence of 6%, 4.8% and 7.0%, respectively (4,9,25) but slightly lower than a study in Nigeria in 2017 (13.8%) (6). Moreover, the magnitude of bacteremia obtained this study is lower than obtained from a systematic review conducted in 2020 which found that bacteremia in the African region ranges from 14-32% (2). The slightly lower prevalence in our settings may be accounted for by the consumption of antibiotics before attending a healthcare facility. At least a quarter (24.9%) of SCD patients had used antibiotics in the past three weeks before attending a healthcare facility. More than a quarter of those SCD patients had used penicillin and amoxicillin (41.3%, 37.5%). These antibiotics might have inhibited the isolation of some bacteria including *S. pneumoniae* and consequently lowering the magnitude. This study found that gram-positive bacteria were predominant (68.8%) contrary to a study conducted in Nigeria three years ago which found that the majority of the bacteremic episodes (73.5%) in children with SCD were due to gram-negative bacteria (20). A similar study conducted in Dar es Salaam, Tanzania in 2015 reported that *S. aureus* was the leading cause of bacteremia among children with SCD (9). These findings are in congruence with this study which also found *S. aureus* (56.3%) the main aetiological agent but different from a study in Kenya which found *S. pneumoniae* the leading cause (4). The *K. pneumoniae* strains were more prevalent among the gram-negative organisms obtained in this study. The spectrum of bacteria strains obtained in this study is

almost the same as that observed in studies conducted in Nigeria and Uganda which found *K. pneumoniae, S. aureus* and *Salmonella* spp the most prevalent strains (37) but different from another similar study conducted in Kenya which reported *S. pneumoniae*, *non-typhi salmonella* and *H. influenza type b* the leading causes of bacteremia (4). The prevalence of *Coagulase-negative staphylococci* was lower (18.2%) than that obtained from a study conducted among adults with SCD (34.9%) more than a decade ago (38). The lower isolation rate of *Coagulase-negative staphylococci* in this study may be accounted for by a lower contamination rate due to compliance to aseptic procedures during blood collection. There were no *S. pneumoniae or H. influenzae* strains isolated from the blood culture specimens in this study contrary to other similar studies in Kenya where *S. pneumoniae* was isolated in 23% and 41% of the bacteremic episodes (4,39). A similar study that researched bacteremia among children below five years in the same region two years ago did not obtain *S. pneumoniae* or *H. influenzae* strains as well (29). Three possible reasons may be attributed to; first, this study focused on children with an existing diagnosis of SCD only. Studies suggest that *S. pneumoniae* and *H. influenzae* predominantly occur in children below five years who often die before the diagnosis of SCD is made (4). The other reasons could be the result of PCV vaccination and fastidious properties of *S. pneumoniae* and *H. influenzae*. A study conducted in Kenya found that PCV vaccination was beneficial in preventing the acquisition of invasive bacteria including pneumococcal strains in children with SCD (4). Although the PCV coverage obtained in this study was slightly lower (75.1%) than that obtained from a study in Moshi, Tanzania (78.5%) two years ago (40) it is tempting to assume that not being able to isolate any *S. pneumoniae* could be linked to exposure to PCV vaccination.

5.2 Antibiotic resistance patterns of bacteria pathogen causing bacteremia among febrile pediatric patients with SCD

This study found a proportion of MDR strains of (46.9%) with a predominance of gram-negative bacteria (53.3%). The majority of the isolates were resistant to penicillin, co-trimoxazole, ceftriaxone, amoxicillin-clavulanic and cefepime with resistance rates ranging from 80 to 100%. All the gram-negative organisms were sensitive to meropenem. These

findings are in agreement with results obtained from a Nigerian study in 2018 which found resistance to ceftriaxone, co-trimoxazole and ampicillin ranging from 56-78% (20). Also, this study found that majority of the gram-positive isolates were sensitive to gentamicin, clindamycin and ciprofloxacin with patterns ranging from 67-78%. These findings are in congruence with other similar studies which found that treatment with ciprofloxacin was still promising (13,20). These antibiotics (gentamicin, clindamycin and ciprofloxacin) can therefore be used for antimicrobial therapeutic or prophylaxis purposes. Furthermore, the widely recommended penicillin prophylactic antibiotic has shown a high resistance rate (78%) contrary to a study conducted more than a decade ago which found a resistance rate of 33% and an intermediate resistance of 64% (41). This highlights the need for selecting and administering a prophylactic antibiotic based on culture results. Furthermore, additional studies comprising of a larger population of children with SCD should be conducted to ascertain the efficacy of this prophylactic antibiotic and whether it should still be used for prophylaxis purposes. The current study also found that only 61.2% of the children with SCD on penicillin prophylactic antibiotics had optimal compliance. It is thus tempting to assume that the varying resistance pattern that was observed may be accounted for by the sub-optimal compliance. Other studies that probed into the parents' own reported compliance on penicillin prophylaxis found compliance of 67.5% and 43.1% when measured objectively (31). Furthermore, this study found that the majority of the gram-negative isolates were more resistant to gentamycin with resistance rates reaching up to 100% than it was with gram-positive isolates (33.3%). A similar study conducted in Nigeria found resistance to gentamycin of 76.3% among the gram-negative isolates recovered (20). The difference in the resistance rates may be accounted for by a slightly higher proportion of gram-negative MDR isolates (53.3%) among bacteria strains isolated in this study. The proportion of ESBL recovered from the blood culture specimens of children with SCD in this study was seemingly lower (30%) compared to findings (79%) from a study conducted on children below five years in the same region two years ago (29). The proportion of MRSA was also lower (22.2%) compared to a study done in the same region which found a higher (34.7%) proportion among children with bacteremia (29). The slightly lower prevalence obtained in this study may probably be

accounted for by the improvement in the infection and prevention control (IPC) practices which may have reduced the chances of acquiring nosocomial infection and PCV vaccination that may have reduced the frequency of hospital attendances.

5.3 Associated factors for bacteremia among febrile pediatric patients with SCD

This study assessed the association between various clinical and socio-demographic characteristics of the children with SCD and bacteremia but only age and sex was directly associated with the acquisition of bacteremia. This study found that even after adjusting age by sex, children with SCD five years and below had twice the odds of acquiring bacteremia than it was in children above five years of age. These findings are in agreement with a study conducted in Nigeria three years ago which also found a higher bacteremia prevalence in children with SCD aged 1-2 years (20). The higher chances for recovering a bacterial isolate in children with SCD below five years than with the subsequent group may be accounted for by the development of immunity as the child grows. It was also observed that males had more odds (1.5 times) of acquiring bacteremia than it was with females. Studies suggest that females mount stronger humoral and cellular responses than their male counterparts which could be beneficial in the protection and clearance of a proportion of pathogens (42). Furthermore, this study found that not all children with SCD who had fever were due to bacteremia. For instance, it was noted that 2.5% and 10.3% of the children with febrile episodes had HIV infection and malaria, respectively but when these co-infections were assessed they did not significantly increase the likelihood of acquiring bacteremia. Various studies pinpoint that, little information is available regarding the impact of co-existent HIV infection and SCD (13,31). Despite high ESBL colonization among the children with SCD, this study found no association between ESBL colonization and bacteremia. Therefore, the ESBL strains recovered from the blood culture specimens of the children may be accounted for by the irrational use of antibiotics that may have led to high selection pressure of resistant bacteria. Additionally, only two ESBL strains were isolated from both blood and stool samples collected from the same patients. However, this study did not characterize the two ESBL strains to ascertain for concurrence in both samples analyzed.

5.4 Study Limitations

Recall bias was encountered during data collection for some factors associated with bacteremia including prior consumption of antibiotics in the past three weeks before hospital attendance. To minimize recall bias, where possible details of the antibiotics used were retrieved directly from the patients' files. Furthermore, second blood culture to ascertain the possibility of contamination for CoNS was not done due to difficulties in tracing the patients upon discharge for in-patients and on completion of treatment for the out-patients. Lastly, this study did not involve the isolation of anaerobic bacteria due to financial constraints and might have been missed consequently lowering the magnitude.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

Our findings have shown that a large proportion of children with SCD still acquire bacterial infection with *S. aureus* constituting to the majority of the bacteremic episodes. Additionally, a high rate of resistance has been observed towards penicillin (a prophylactic antibiotic) along with other commonly used antibiotics. However, ciprofloxacin, gentamicin and clindamycin are still sensitive and can therefore be used as alternative antibiotics. Nevertheless, the high resistance rates towards the commonly used antibiotic calls for need to introduce routine culture and AST at health facilities that lack the services.

REFERENCES

1. Fitzsimmons R, Amin N, Uversky VN. Understanding the roles of intrinsic disorder in subunits of hemoglobin and the disease process of sickle cell anemia. Intrinsically Disord Proteins. 2016;4(1):e1248273.

2. Ochocinski D, Dalal M, Black LV, Carr S, Lew J, Sullivan K, et al. Life-Threatening Infectious Complications in Sickle Cell Disease: A Concise Narrative Review. Front Pediatr. 2020;8.

3. Makani J, Tluway F, Makubi A, Soka D, Nkya S, Sangeda R, et al. A ten year review of the sickle cell program in Muhimbili National Hospital, Tanzania. BMC Hematol. 2018;18(1):1–13.

4. Williams TN, Uyoga S, Macharia A, Ndila C, McAuley CF, Opi DH, et al. Bacteraemia in Kenyan children with sickle-cell anaemia: a retrospective cohort and case-control study. Lancet. 2009;374(9698):1364–70.

5. What are the signs and symptoms of sickle cell disease (SCD)?.. Available from: https://www.medscape.com/answers/205926-15252/what-are-the-signs-and-symptoms-of-sickle-cell-disease-scd

6. Brown B, Dada-Adegbola H, Trippe C, Olopade O. Prevalence and etiology of bacteremia in febrile children with sickle cell disease at a Nigeria tertiary hospital. Mediterr J Hematol Infect Dis. 2017;9(1):3–10.

7. Leikin SL, Gallagher D, Kinney TR, Sloane D, Klug P, Rida W. Mortality in children and adolescents with sickle cell disease. Pediatrics. 1989;84(3):500–8.

8. Lee A, Thomas P, Cupidore L, Serjeant B, Serjeant G. Improved survival in homozygous sickle cell disease: lessons from a cohort study. BMJ. 1995 Dec 16;311(7020):1600-2.

9. Makani J, Mgaya J, Balandya E, Msami K, Soka D, Cox SE, et al. Bacteraemia in sickle cell anaemia is associated with low haemoglobin: A report of 890 admissions to a tertiary hospital in Tanzania. Br J Haematol. 2015;171(2):273–6.

10. Baskin MN, Goh XL, Heeney MM, Harper MB. Bacteremia risk and outpatient management of febrile patients with sickle cell disease. Pediatrics. 2013;131(6):1035–41.

11. Steele RW, Warrier R, Unkel PJ, Foch BJ, Howes RF, Shah S, et al. Colonization with antibiotic-resistant Streptococcus pneumoniae in children with sickle cell disease. J Pediatr. 1996;128(4):531–5.

12. Duncan Adjato SF, Oduro Amoako E, Abaka-Yawson A, Agbodzakey H, Apraku Tawiah P. Bacterial Profile and Antimicrobial Sensitivity Patterns in Asymptomatic Bacteriuria: A Cross-sectional Study of Sickle Cell Disease Patients in the Ho Municipality, Ghana. Asian J Med Heal. 2019;15(2):1–7.

13. Cannas G, Merazga S, Virot E. Sickle cell disease and infections in high- And low-income countries. Mediterr J Hematol Infect Dis. 2019;11(1):1–9.

14. Curtis V, Cairncross S. Effect of washing hands with soap on diarrhoea risk in the community: a systematic review. Lancet Infect Dis. 2003 May 1;3(5):275–81.

15. Kaier K, Frank U, Hagist C, Conrad A, Meyer E. The impact of antimicrobial drug consumption and alcohol-based hand rub use on the emergence and spread of extended-spectrum β-lactamase-producing strains: A time-series analysis. J Antimicrob Chemother. 2009;63(3):609–14.

16. Tellevik MG, Blomberg B, Kommedal Ø, Maselle SY, Langeland N, Moyo SJ. High prevalence of faecal carriage of esbl-producing enterobacteriaceae among children in Dar es Salaam, Tanzania. Vol. 11, PLoS ONE. 2016.

17. Schaumburg F, Alabi A, Kokou C, Grobusch MP, Köck R, Kaba H, et al. High burden of extended-spectrum β-lactamase-producing enterobacteriaceae in Gabon. J Antimicrob Chemother. 2013;68(9):2140–3.

18. Mcgann PT, Hernandez AG, Ware RE. Sickle cell anemia in sub-Saharan Africa. Am Soc Hematol. 2018;129(2):155–62.

19. Dayie NTKD, Tetteh-Ocloo G, Labi AK, Olayemi E, Slotved HC, Lartey M, et al. Pneumococcal carriage among sickle cell disease patients in Accra, Ghana: Risk factors, serotypes and antibiotic resistance. PLoS One. 2018;13(11):1–14.

20. Bello N, Kudu ATD, Adetokun AB, Taura DW, Jobbi YD asabe, Umar M, et al. Characterization and antimicrobial susceptibility profile of bacteraemia causing pathogens isolated from febrile children with and without sickle cell disease in Kano, Nigeria. Mediterr J Hematol Infect Dis. 2018;10(1):1–9.

21. Ambrose EE, Makani J, Chami N, Masoza T, Kabyemera R, Peck RN, et al. High birth prevalence of sickle cell disease in Northwestern Tanzania. Pediatr Blood Cancer . 2018 Jan 1;65(1).

22. Cober MP, Phelps SJ. Brief Review Article Penicillin Prophylaxis in Children with Sickle Cell Disease. J Pediatr Pharmacol Ther. 2010;15(3):152–9.

23. Chakravorty S, Williams TN. Sickle cell disease: A neglected chronic disease of increasing global health importance. Arch Dis Child. 2015;100(1):48–53.

24. McGann PT. Sickle cell anemia: An underappreciated and unaddressed contributor to global childhood mortality. J Pediatr. 2014;165(1):18–22.

25. Alima Yanda AN, Nansseu JRN, Mbassi Awa HD, Tatah SA, Seungue J, Eposse C, et al. Burden and spectrum of bacterial infections among sickle cell disease children living in Cameroon. BMC Infect Dis. 2017;17(1):1–7.

26. Ambrose EE, Smart LR, Hokororo A, Charles M, Beyanga M, Hernandez AG, et al. Prevalence and mapping of sickle cell disease in northwestern Tanzania. Blood Adv. 2017;1:26–8.

27. Pai VB, Nahata MC. Duration of penicillin prophylaxis in sickle cell anemia: Issues and controversies. Vol. 20, Pharmacotherapy. Pharmacotherapy Publications Inc.; 2000. p. 110–7.

28. 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).

29. Seni J, Mwakyoma A, 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 Jan 26;19.

30. Sobota A, Sabharwal V, Fonebi G, Steinberg M. How we prevent and manage infection in sickle cell disease. Br J Haematol. 2015;170(6):757–67.

31. Booth C, Inusa B, Obaro SK. Infection in sickle cell disease: A review. Int J Infect Dis. 2010;14(1):2–12.

32. NACP. National Comprehesive Guidelines on HIV testing services. Guideline. 2019;53(9):1689–99.

33. Private U, Rdt S, Of O, Sops THE, Rdts FOR, Required M, et al. STANDARD OPERATING PROCEDURES (SOPs) FOR RDTs. 2014;

34. Limbago B. M100-S11, Performance standards for antimicrobial susceptibility testing. Clin Microbiol Newsl. 2001;23(6):49.

35. Dolinsky AL. M100 Performance Standards for Antimicrobial Susceptibility Testing. Vol. 8, Journal of Services Marketing. 2017. 27–39 p.

36. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: Review and bench guide. Clin Microbiol Infect. 2008;14(SUPPL. 1):90–103.

37. Serjeant GR, Ndugwa CM. Sickle cell disease in Uganda: A time for action. East Afr Med J. 2003;80(7):384–7.

38. Chulamokha L, Scholand SJ, Riggio JM, Ballas SK, Horn D, DeSimone JA. Bloodstream infections in hospitalized adults with sickle cell disease: A retrospective analysis. Am J Hematol. 2006;81(10):723–8.

39. Ellison AM, Ota K V., McGowan KL, Smith-Whitley K. Epidemiology of bloodstream infections in children with sickle cell disease. Pediatr Infect Dis J. 2013;32(5):560–3.

40. Emgård M, Msuya SE, Nyombi BM, Mosha D, Gonzales-Siles L, Nordén R, et al. Carriage of penicillin-non-susceptible pneumococci among children in northern Tanzania in the 13-valent pneumococcal vaccine era. Int J Infect Dis. 2019;81:156–66.

41. Di Nuzzo DVP, Fonseca SF. Sickle cell disease and infection. J Pediatr (Rio J). 2004;80(5):347–54.

42. Muenchhoff M, Goulder PJR. Sex differences in pediatric infectious diseases. J Infect Dis. 2014;209(SUPPL. 3).

APPENDICES

Appendix I: Informed consent/Assent form (English version)

Title: Bacteremia, associated factors and antibiotic susceptibility pattern among febrile pediatric patients with sickle cell disease in Mwanza, Tanzania

Aim of the study

This study aims to determine bacteremia, associated factors and antibiotic susceptibility patterns among febrile pediatric patients.

Procedure

This is consent for a research study. Your child is being invited to participate in this study because your child has sickle cell disease. Your child has fever as well; fever is a medical emergency in children with sickle cell disease because it is the first sign that the child may have acquired a bloodstream infection. This form is intended to give you and your child information to help you decide if you and your child wish to participate in this study. You should read this form and ask any questions you or your child may have before agreeing to be in this study.

Children with sickle cell disease are born with a defect in haemoglobin and they are at an increased risk of acquiring infections including bacterial infections due to compromised immune function. The bacterial aetiological agents causing infections can be treated with commonly available antibiotics. The aetiological agents are however becoming more resistant to these commonly used antibiotics and consequently hampering optimal treatment outcomes. This study will help to identify the aetiological agents causing bloodstream infections and associated factors and determine the antibiotic susceptibility pattern that will help guide the selection of empiric treatment among febrile patients with SCD.

Confidentiality

All information and issues relating to your participation in the study will be treated confidentially, no unauthorized persons will have access to your personally identifiable information. As soon as this process is complete, the information provided will be treated as confidential. Furthermore, to protect the confidentiality, your name will not appear in the written copy of the discussion.

Right to refuse or withdraw

Your participation in this study is voluntary, you have the right to withdraw at any time you feel you no longer wish to participate. However, your participation will help us obtain results that will enable us to identify the aetiological agents and their antibiotic susceptibility pattern that can be adapted for selecting empiric treatment in the management of sickle cell disease patients at our locality.

Benefits

There is no direct material benefit as such for individuals who volunteer to participate in this study. However, it is hoped that any subsequent information obtained as a result of this study will eventually contribute to fostering optimal treatment outcomes among sickle cell disease patients.

Risks

There will be no risk because your information is confidential and there is no way of linking you to your Questionnaire.

Contact details

In case of any questions about this study, please contact the principal investigator Sued Yassin Zuberi or you may also reach the chairperson of the Senate of Research and Publications Committee, PO BOX 65001, Dar es Salaam. Tel: +255 22 215 2489

Principal Investigators Mobile: 0753 15 82 48 Email: suedmtula@gmail.com

Appendix II: Informed consent form (Kiswahili version)

(Fomu ya Kukubali Kushiriki kwa Hiari)

Kichwa cha habari cha utafiti: "BACTEREMIA, ASSOCIATED FACTORS AND ANTIBIOTIC SUSCEPTIBILITY PATTERN AMONG FEBRILE PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE IN MWANZA, TANZANIA."

Madhumuni ya utafiti huu

Utafiti huu unalenga kubaini vijidudu (bakteria) vinavyosababisha maambukizi katika mzunguko wa damu kwa watoto wenye selimundu, mazingira yanayoweza kuletelea tatizo hilo na kubaini dawa inayoweza kutibu maambukizi hayo.

Kuhusu utafiti huu

Hii ni fomu ya ridhaa kwa ajili ya utafiti. Mtoto wako amechaguliwa kushiriki katika utafiti huu kwa sababu ana selimundu. Lakini pia mtoto wako ana homa; homa kwa mtoto mwenye selimundu ni kiashiria cha hatari kwa kuwa ni kiashiria cha kwanza kuwa huenda mtoto amepata maambukizi ya bakteria kwenye mzunguko wake wa damu. Fomu hii ina dhamira ya kukupa wewe na mwanao taarifa inayoweza kusaidia muweze kuona kama mngependa kushiriki au la. Tafadhali soma fomu hii kwa makini na kama wewe au mwanao mtakuwa na maswali msisite kuuliza kabla ya kukubali kushiriki katika utafiti huu.

Watoto wenye selimundu huzaliwa na tatizo kwenye chembechembe za damu na hivyo wapo katika hatari kubwa ya kuambukizwa magonjwa mbalimbali ikiwemo ya bakteria kwa kuwa kinga yao ya mwili haiko imara. Habari njema ni kwamba bakteria hao huweza kutibika kwa dawa za kawaida kabisa. Habari mbaya ni kwamba bakteria hao wameanza kujenga usugu kwa dawa hizo ni hivyo kupunguza ufanisi wa dawa hizo muhimu.

Kupitia utafiti huu tutaweza kutambua ni aina gani ya wadudu wanaosababisha maambukizi katika mzunguko wa damu kwa watoto wetu wenye selimundu na kusaidia kuchagua aina ya dawa yenye ufanisi mzuri inayoweza kutumika wakati wa dharura pindi mtoto anapokuja na dalili za hatari ikiwemo homa

Usiri

Taarifa zote zihusuzo ushiriki wako katika utafiti huu zitahifadhiwa katika hali ya usiri, hakuna mtu au watu wasioruhusiwa wataweza kuona taarifa zako ziwezazo kukutambua binafsi. Zaidi, ili kuimarisha usiri, jina lako halitaoneshwa kwenye majadiliano/chapisho lolote la utafiti huu.

Haki ya kutotaka kushiriki ama kujitoa

Ushiriki wako katika utafiti huu ni wa hiari. Unaruhusiwa kujitoa wakati wowote utakapojisikia kufanya hivyo. Unaombwa kushiriki katika utafiti huu kwani matokeo yatakayopatikana yatawezesha kutambua bakteria wanaosababisha magonjwa na kujua ni aina gani ya dawa inaweza kuwa na ufanisi mzuri pindi mtoto mwenye selimundu anapokuja kwenye kituo cha huduma wakati tukisubiri matokeo ya vipimo vingine. Hii pia itatusaidia kutambua aina ya dawa inayoweza kutumika kwa dharura katika ukanda wetu indi mtoto anakuja na dalili za hatari.

Faida

Utafiti huu hauna faida ya moja kwa moja ya mali au fedha kwa wanaojitolea kushiriki. Hata hivyo, ni matumaini yangu kuwa matokeo yatakayopatikana katika utafiti huu yatasaidia kuboresha huduma hususani watoto wenye selimundu.

Madhara

Hakuna madhara yoyote ya kushiriki kwani taarifa zako za kibinasfi hazitadhihirishwa kwenye dodoso la utafiti huu.

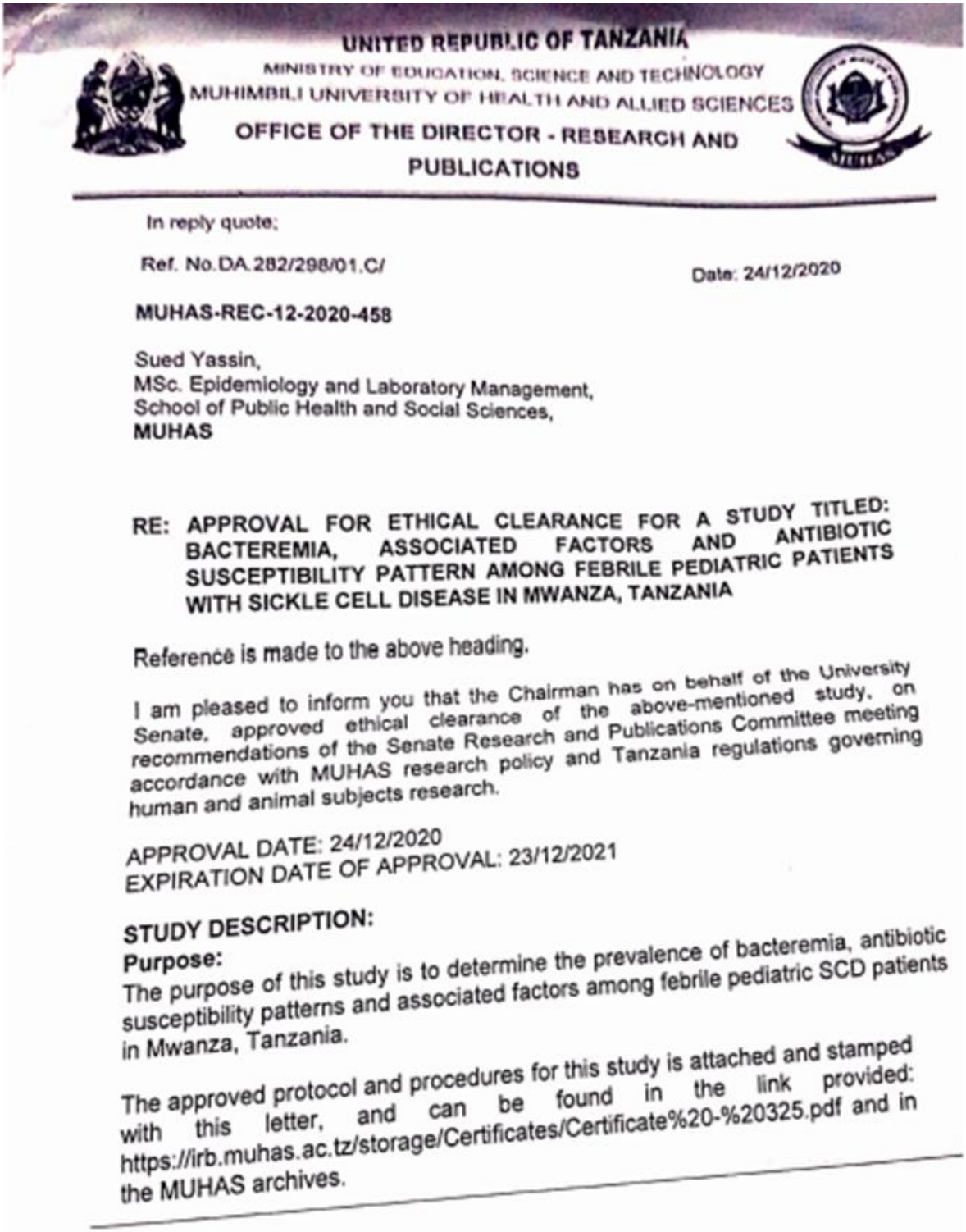
Appendix III: Questionnaire

TITLE OF THE STUDY: BACTEREMIA, ASSOCIATED FACTORS AND ANTIBIOTIC SUSCEPTIBILITY PATTERN AMONG FEBRILE PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE PATIENTS IN MWANZA, TANZANIA.

No	QUESTIONS	**RESPONSES**
A. SOCIO-DEMOGRAPHIC FACTORS		
1	Interviewer's name	
2	Date of interview	
3	Date of admission	
4	Healthcare facility name	
5	Patient registration number	
6	Ward/department	In-patient/out-patient
7	Contact phone number	
8	Date of birth	
9	Sex	Male/female
10	How many family members are in a household?	
11	Parent, guardian and/or caretaker's highest level of education completed	1. Informal 2. Primary school 3. Secondary school 4. University/college
12	Who does the patient live with (Relationship not name)	
13	Occupation of the parent, guardian and/or caretaker	1. Formal employment(Specify) 2. Self-employed(Specify)

		3. Student 4. Others(Specify)
14	Commonly eaten food (How is it processed?)	
B.	**CLINICAL CHARACTERITICS**	
15	Vaccination status	
16	Prophylactic antibiotics on use	
17	Patient's history of antibiotic use in the past three weeks	
18	Details of antibiotics used in the patient	
19	Patient's current body temperature	
20	Underlying diseases (HIV, Malaria)- Status	
C. LABORATORY TESTS (SAMPLE COLLECTION, ANALYSIS AND RESULTS)		
21	Sample type	1. Blood 2. Stool
22	Date of sample collection	
23	Bacterial culture	Positive/Negative
24	Bacterial species isolated	
25	Phenotypic AST	1. Sensitive…… 2. Intermediate…… 3. Resistant ……
26	ESBL isolated	Yes/No

Appendix IV: Letter for ethical clearance



UNITED REPUBLIC OF TANZANIA
MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR - RESEARCH AND PUBLICATIONS

In reply quote;

Ref. No.DA.282/298/01.C/ Date: 24/12/2020

MUHAS-REC-12-2020-458

Sued Yassin,
MSc. Epidemiology and Laboratory Management,
School of Public Health and Social Sciences,
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: BACTEREMIA, ASSOCIATED FACTORS AND ANTIBIOTIC SUSCEPTIBILITY PATTERN AMONG FEBRILE PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE IN MWANZA, TANZANIA

Reference is made to the above heading.

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 24/12/2020
EXPIRATION DATE OF APPROVAL: 23/12/2021

STUDY DESCRIPTION:

Purpose:
The purpose of this study is to determine the prevalence of bacteremia, antibiotic susceptibility patterns and associated factors among febrile pediatric SCD patients in Mwanza, Tanzania.

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided: https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%20325.pdf and in the MUHAS archives.

The PI is required to:

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented.
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain data transfer agreement (DTA) from NIMR if data will be transferred to a foreign country.
7. Apply for and obtain data transfer agreement (DTA) from NIMR if data will be transferred to a foreign country.
8. Apply for and obtain material transfer agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country.
9. Any researcher, who contravenes or fail to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III section 10 (2)
10. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
11. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://drp.muhas.ac.tz/.

Dr. Emmanuel Balandya
Ag. Chairman, MUHAS Research and Ethics Committee

Cc: Director of Postgraduate Studies, **MUHAS**

9 United Nations Road; Upanga West; P.O. Box 65001, Dar Es Salaam: Tel. G/Line: +255-22-2150302/6; Ext. 1038; Direct Line:+255-22-2152489;Telefax:+255-22-2152489;E-mail:drp@muhas.ac.tz;Web:https://www.muhas.ac.tz