SURVEILLANCE OF THE COMMUNITY ACQUIRED SEPTICAEMIA AMONG CHILDREN IN MBITA SUBCOUNTY, SOUTH NYANZA, KENYA

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P150/25834/2013

A thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Science (Infectious Diseases) of Kenyatta University

APRIL 2020

DECLARATION

This thesis is my original work and has not been presented for the academic award of a degree in any other university.

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This thesis is dedicated to my wife Billa Okotu Jillo and children Habiba, Zamzam, Yassin, Hussein and Huka whose support and encouragement made me accomplish my goals. May Almighty Allah bless you all.

ACKNOWLEDGEMENT

All thanks go to the Almighty God for His gift of nature, the abundance of good health and the means through which I have been successful. Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my wife Billa Okotu Jillo and children Habiba, Zamzam, Yassin, Hussein and Huka whose love and guidance are with me in whatever I pursue.

I am grateful to all of those with whom I have had the pleasure to work during this and other related projects. I would especially like to thank Prof. Yoshio Ichinose, Prof. M. Karama and Dr. George Gachara; as my teachers and mentors, they have taught me more than I could ever give them credit for here. They have shown me, by their example, what a good scientist (and person) should be. I am especially indebted to Prof. Yoshio Ichinose, the head Nagasaki University Institute of Tropical Medicine – Kenya Research Station, who have been supportive of my career goals and who worked actively to provide me with the protected academic time to pursue those goals.

I would like to thank Dr. Martin Bundi, Dr. Musa Ngayo, Erick Odoyo, and Amina Galata among other workmates and family of NUITM-KEMRI who as good friends, were always willing to help and give their best suggestions. I have benefited greatly from their wealth of knowledge in the laboratory techniques and from their statistical analysis acumen which contributed to the success of this work. It would have been a lonely scientific pursuit without them. I am greatly indebted and grateful to all the study participants for their time and willingness.

This selfless contribution has put a mark on the community acquired septicaemia in Kenya, may God bless you all.

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ABBREVIATIONS

MDR	– Multi drug resistance
DNA	- Deoxyribonucleic Acid
DNTPs	– Dinuclear triphosphate
XLD	- xylose lactose dextrose
BA	- Blood agar.
CBA	- Chocolate Blood agar.
PCR	– Polymerase chain reactions
AST	– Antimicrobial Sensitivity Testing
AMR	–Antimicrobial Resistance
EPEC	-Entero Pathogenic *E. coli*
ETEC	– Entero Toxigenic *E.coli*
LMIC	-Low and middle income countries
HIC	–High income countries.
CMOH	- County Medical Officer

ABSTRACT

Septicaemia is a systemic disease associated with presence of pathogenic microorganisms or their toxins in the blood. Septicaemia remains the leading cause of mortality and morbidity, especially in sub-Saharan Africa and leads to complications characterized by inflammation throughout the body referred as sepsis which are caused by viral, parasitic and bacterial agents. Studies have shown that bacterial septicaemia is the most fatal and prevalent in hospitalised cases. According to the UNICEF, 76% of children under five years of age globally die due to septicaemia. In East Africa a mortality rate of 40% have been reported. In these studies, septicaemia has normally been found to be community acquired. In Kenya, South Nyanza regions have reported higher morbidity and mortality cases among children. This has been associated with higher cases of immunosuppressive diseases in the region. This hospital based descriptive cross-sectional study aimed to determine the aetiology, antibiotic susceptibility and pathogenic profile of bacteria causing septicaemia cases in Mbita district hospital South Nyanza. Blood samples were obtained from 248 children who met the recruitment criteria and their guardian consented to participate in the study. A detailed sociodemographic questionnaire was administered to the guardian to gather information relevant to this study. Bacterial isolation and characterization was done using the automated BACTEC 9240 system while the antimicrobial susceptibility testing was done using the disc diffusion technique. Molecular characterization for resistance markers was done using PCR and plasmid profiling methods. The mean age of the participants was 27.9 (SD ±20.7) months with the majority (30.6%) aged between 1 to 12 months. The majority of the participants were males (50.8%), were from Rusinga (48%) while only 8.1% were HIV seropositive. The mean body temperature of the participants was 38 (SD 0.5) ^{O}C and the majority (58.9%) had body temperatures above 37.6 ^{O}C. The mean white blood cells (WBC) of the participants were 17720.9 (SD 8929.1) cells/ml and majority, 25.4%, had WBC above the normal levels. The mean heart rate (HR) of the participants was 111.7 (SD 12.2) beat/min and the majority (34.3%) had HR between 101 to 110 beat/min. A total of 84 of the 248 (33.9%) of the children had septicaemia with the majority (28.6%) caused by *Staphylococcus epidermidis* followed by *S. aureus* and *E. coli* each at 13.1%. The majority of the Gram negative bacteria causing septicaemia in this study were resistant to penicillin (Ampicillins) at 100% followed by tetracycline at 96.1%, sulphonamides (Trimethoprim/sulfamethoxazole) at 84.6%, Aminoglycosides (Gentamicin) at 73.1% while they were least resistant to Quinolones (Ciprofloxacin) at 19.2%. For the Gram positive bacteria, majority (96.7%) were resistant to sulphonamides (Trimethoprim/sulfamethoxazole) followed by tetracycline at 76.7%, penicillin (Oxacillin) at 73.3% and least resistant to Quinolone (Ciprofloxacin) at 30%. In conclusion, this study reported significantly higher proportion of the children with septicaemia. Majority of the cases were caused by Gram positive bacteria. Compared to Gram positive, majority of Gram negative bacteria were resistant to penicillin, tetracycline, Trimethoprim/sulfamethoxazole and Gentamicin. This community could benefit from rapid testing and etiological characterisation of

children with suspected symptoms of septicaemia in order to institute appropriate treatment and management.

CHAPTER ONE

1.1 INTRODUCTION

Septicaemia remains the leading cause of morbidity and mortality among children, especially in sub-Saharan Africa (Bryce *et al.,* 2005). The World Health Organization (WHO) defines septicaemia as an acute illness characterized by a rise in body temperature. It is also known as blood poisoning. Untreated septicaemia leads to complications characterized by inflammation throughout the body. Viral, bacterial and parasitological septicaemias are the commonest causes of the febrile illness in developing countries (WHO, 2014). Feverish illness in young and adult indicates an underlying cause or infections and these conditions are of concern to parents or the carer. Similarly, septicaemia presents a diagnostic challenge both clinical and definitive, hence becoming one of the infectious diseases that remain a major cause of childhood, adult morbidity and mortality.

In East Africa, bacterial septicaemia is the most common cause of hospitalized cases. More cases have been reported in immune suppressive cases such as HIV infection than in non-immunocompromised individuals (Jacob *et al.*, 2009). A high mortality rate has been associated with bacteria as the causative agent (Brent *et al.,* 2006), therefore bacterial septicaemia is the major cause of life threatening diseases among immunosuppressed and equally otherwise. A study conducted at Muhimbili National Hospital in Tanzania reported a mortality of 40% in children patients associated with bacterial septicaemia. In a study at Kilifi district hospital in Coastal Kenya, high prevalence of community acquired septicaemia as a result of *Streptococcus pneumonia* and *Staphylococcus aureus* was detected (Berkley *et*

al., 2005). Invasive bacteria are important etiologies of septicaemia in African children and population (Ikumapayi *et al.,* 2007).Worldwide 76% (4.6 millions) of the under-fives deaths occurs due to undiagnosed invasive bacterial as an etiology (WHO lancet 2014).

Bacterial infections and malaria are co-endemic in south Nyanza regions with young children most affected (Beier *et al.,* 1994). The most common illness documented were respiratory and gastrointestinal infections (Paxton *et al.,* 1998). In malaria endemic area 11% of children admitted are found to have bacteraemia, of this about 12% dies due to misdiagnosis of the etiological agents associated with febrile illness. The misdiagnosis and mistreatment associated with malaria significantly contributes to elevated fatality rates. Bacterial culture to diagnose the infections is not routinely done in most of the primary and secondary health facilities and this leads to unguided empirical treatment with antibiotics which eventually promotes antimicrobial resistance emergence or re-emergence, an observation that informed the design of the present study. Varieties of bacteria have been associated with septicaemia in both children and adults; these are classified generally as gram negative and positive bacteria (Bronzan *et al.,* 2007).

The choice of the right therapeutic agent is extremely important for several reasons. First, infections constitute the only medical conditions that may be susceptible to true cure rather than amelioration. Second, bacteria may develop resistance to an antibiotic with a frequency roughly proportional to the degree to which the antibiotic is used. Therefore, injudicious use of broad-spectrum antibiotics may shorten the time during which they remain useful by hastening the

evolution of a new population of resistant microorganisms. Third, use of broad-spectrum antimicrobial therapy perturbs the normal flora, promoting overgrowth of opportunistic organisms such as fungi. The physician treating a patient with an infectious disease therefore has the capacity to alter the pathogenesis of diseases not only in the patient but also in the hospital population and the community at large (Bloomberg *et al.*, 2007).

The policy of the guided empirical treatment, timely and appropriate response in various health set up to assist clinician in clinical diagnosis prepared as recipe in the study findings. The finding from this study will contribute to an already existing database of the various results in different studies in bacterial cause of febrile illness. Study will also contribute to understanding the wider picture of the significant differences in etiologies as far as underlying cases, demographic and sero-variability of the study populations will be concluded.

1.2 Statement of the problem

Neonatal bacterial septicemia are among leading cause of death in developing countries including Kenya, and the risk of bacterial resistance emergence and dissemination is exacerbated by poor strains and antimicrobial diagnosis, poor antibiotic control and precarious empirical treatment. Poor living conditions in regional populations are evident and could be major contributing factors. Without data to evaluate the burden of disease in question and antibiotic resistance in this population, the public health problem will undoubtedly remain undocumented. This study focuses on different factors in children with septicaemia as study

population that will give an insight information on the understanding the circulating contaminants and the real world pathogens. The various recorded and reported hospital based diagnosis septicaemia yields more information of the morbidity, mortality with poor prognosis as well, which remains public health problems in both community and hospital set up, besides advancing sciences of prognosis, diagnosis and drugs.

1.3 Research questions

1. What are the major dominant etiologies in septicaemia cases in Mbita, South Nyanza?
2. What are the antimicrobial susceptibility patterns of isolated etiologies of septicaemia in Mbita?
3. Which antibiotic resistance conferring genes are present in the isolated etiological agents?

1.4 Objectives of the study

1.4.1 General objective

To determine the prevalence of septicaemia, characterize the etiological agents and their antimicrobial susceptibility patterns in Mbita, South Nyanza.

1.4.2 Specific objective

1. To isolate and characterize the etiological agents causing septicaemia among the study population

2. To determine the antimicrobial susceptibility pattern of commonly used drugs against isolated septicaemia etiological agents.
3. Molecular characterization of the isolated multi Drugs Resistant etiological agents.

1.5 Justification

Despite the recent global awareness of bacterial resistance issues and indications of the growing antibiotic resistance in developing Countries, epidemiological evidence remains limited and available data are not sufficient to draw a true, recent, and accurate picture of antibiotic resistance in developing Countries among neonates and particularly in the community. Antibiotic resistance is one of the most serious public health concerns worldwide and is increasing at an alarming rate, making daily treatment decisions more challenging. This study is aimed at identifying local bacterial isolates and their antimicrobial susceptibility patterns to avoid irrational antibiotic use, especially in settings where unguided management occurs and febrile illnesses are predominant. Promote advisory to county and National health policy formulations in septicaemia cases in known and opportunistic pathogens. The study will be beneficial to clinicians in rural hospitals to aid in judicious clinical diagnosis and management of febrile illness due to different aetiologies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Prevalence of septicaemia

It has been estimated that every year, tens of millions of people worldwide get infections which progress to sepsis, calling for the need to involve a variety of business sectors to ensure that the pandemic is defeated. (Czura, 2011). The term *sepsis* has been used interchangeably with septicemia, but while septicemia refers to an infection affecting the bloodstream, sepsis is when inflammation occurs due to such an infection. Septicaemia and sepsis are serious devastating bloodstream infections that are difficult to diagnose early and for which treatment options are limited hence can rapidly become life-threatening.

Several previous studies also reveal community acquired septicaemia results from respiratory, gastrointestinal tract infections and enhanced by malnutrition (Perkins *et al.,* 1997). The chances of being septicemic increase when a patient has an indwelling catheter (intravenous or otherwise), is immune-compromised (e.g. when suffering from leukaemia or HIV), is extremely old or extremely young, and is mechanically ventilated or is undergoing chemotherapy, which weakens his/her immune system (O'Connell & Cafasso, 2015).

Although everybody is at potential risk of developing sepsis from minor infections (e.g. flu, urinary tract infections, gastroenteritis, etc), sepsis is most likely to develop in people who: are very young (e.g. premature babies) or very old, have a weakened (compromised) immune system, (often because of treatments such as

chemotherapy for cancer, steroids (e.g. cortisone) for inflammatory conditions, etc), have wounds or injuries, such as those from burns, a car crash, or a bullet, have certain addictive habits, such as alcohol or drugs, Are receiving certain treatments or examinations (e.g., intravenous catheters, wound drainage, urinary catheters, are more prone to develop sepsis than others because of genetic factors.

While the sepsis incidence rate is estimated to be 150-300 cases per 100,000 inhabitants, among patients with chronic diseases, the rate increases to 700 cases per 100,000 patients. In patients with HIV infection, the rate reaches 1,000 cases per 100,000 patients. In spite of this, HIV-positive patients with severe sepsis are less often admitted compared to patients with a similar clinical condition or higher expected lethality. Clinical manifestations of sepsis in immunosuppressed patients can be minimal or non-specific, and the systemic host response to infection is expected to be blunted.

In the US alone, sepsis affects more than one million people per annum (O'Connell & Cafasso, 2015). Classified as a Category A disease (Ryan, 2016), septicemic plague is a ubiquitous disease that is found in Asia, America and Africa (Tyring, Burnett, & Mwesigye, 2017). Septicaemia cases continue to be on the increase in many countries doubling over the last 10 years. Studies show that 20-40% of sepsis is related to community acquired septicaemia, slightly above to nosocomial related infections (Sigauque, *et al.*,2009). Antiretroviral therapy has improved life expectancy and reduced morbidity and mortality of HIV-positive patients. However, the decline in AIDS-related diseases has increased the importance of illnesses not directly related to HIV infection (Silva and dos Santos,

2013). Patients with AIDS acquire more infections and more atypical infections than does the normal population (Haddy *et al.,* 2012).Among them, septicaemia has been responsible for 12-31% of HIV-positive patients' admissions and is associated with a worse prognosis (Silva and Dos Santos, 2013), They arise from various infections, including those of the skin, lungs, abdomen, and urinary tract (Hall, 2011). Several studies from sub-Saharan Africa (SSA) reveal an association between HIV infection and an increased likelihood of bacteraemia and mortality (Jacob, S.T *et al.,* 2009).

Despite the significant increases in survival and quality of life, HIV/AIDS patients have been systematically excluded from sepsis studies, limiting the understanding of the impact of sepsis in this population (Japiassú *et al.,* 2010). Sepsis in high income countries (HICs) accounts for a significant burden of disease and mortality and has been implicated as the leading cause of non-cardiac death amongst critically ill patients in these settings. Similar epidemiologic data on sepsis in low and middle income countries (LMICs) are unavailable In LMICs, data regarding the management and outcomes of sepsis syndromes are limited (Becker JU *et al.,* 2009). The scarcity of data on this topic in developing countries necessitates further studies to evaluate cost-effective approaches to sepsis in these settings (Cheng *et al.*, 2008).

2.2 Etiology of septicaemia

Bacterial septicemia is a major cause of morbidity and mortality in children in Africa. Due to undeveloped health systems .the epidemiology of community acquired septicemia is not well represented in Africa.

Whereas there are many types of organisms that might cause septicemia, the agents known to cause urogenital, pulmonary, and renal infections have been found to be culpable in as far as septicemia is concerned (O'Connell & Cafasso, 2015)

Fleas infected with *Yersinia pestis* have been known to be the conduits of septicemic plague. In children, the septicemic plague might present as an atypical pneumonia (Wilmott, et al., 2012)Therefore, it's one of the infectious diseases that require health researchers' intervention to give outfits data to promote the mitigation measures.

In developed countries, septicemia are commonly due to *Escherichia coli*, other *Enterobacteriaceae, Listeria monocytogenes*, coagulase negative *Staphylococci* and group B *Streptococcus*. Studies have been conducted in developing countries to establish the etiology of septicaemia. A review of culture-positive cases in India, Africa, the Middle East and West Indies revealed that *Klebsiella spp*. was the most frequently isolated pathogen. *Staphylococcus aureus, Escherichia coli and Pseudomonas spp*. were also commonly isolated. Among children below the age of five, septicemia is most often caused by *Staphylococcus aureus, Neisseria meningitidis,* and *Streptococcus pneumoniae.* Septicemia may occur as a result of

other infections such as otitis media, bacterial rhinosinusitis, skin infections and meningitis, the latter of which is most commonly caused by *Streptococcus pneumoniae* and *Neisseria meningitidis* (Chamberlain, 2017). Skin infections are most commonly caused by *Staphylococcus aureus* while otitis media and bacterial rhinosinusitis are caused by *Streptococcus pneumoniae*. *Escherichia coli, Klebsiella species, Enterobacter* species as well as *Streptococcus agalactiae* are also known to be etiological agents of septicemia among children below the age of five (Chamberlain, 2017).

Information available in Kenya is principally from studies conducted at public hospitals, where the cause is largely attributed to community acquired bacteraemia, because a significant number of neonates are born outside the hospital setting (Berkley, *et al.,* 2005). Several work documented the predominant cause of community acquired septicaemia as *Staphylococcus aureus* (Tornheim *et al.,* 2007). The Coagulase negative *Staphylococcus spp* (coNs) were found to be leading causative agents of community acquired septicemia facts which has been proved and documented by other work done elsewhere (R.p Wenzel et al 2006). Therefore, it is necessary to understand more fully the particulars of sepsis in the community population via medical research to increase the survivors from this manageable infectious disease.

2.3 Antimicrobial resistance

Despite advances in understanding the biological processes involved, there is still no effective treatment beyond supportive therapy. Expert management of sepsis in patients is needed to predict and establish the correct diagnosis and to choose

appropriate empiric and specific antimicrobial agents and improve prognosis (Silva and Dos Santos 2013). Correct and timely identification of infectious agents of septicaemia as well as their antibiotic sensitivity patterns are essential as they guide both empiric and definitive treatments (ASM 10th edition, 2011).

Research evidences demonstrate both human and veterinary usage of antimicrobial compounds without any public health guidelines has majorly contributed to the emergence and dissemination of antimicrobial resistance in both pathogenic and commensals bacteria (Houser B.A et al 2009). Literatures describing drug resistance involves mainly phenotypic characteristic with genotypic resistance characterisation are limited (Hoyle D.V et al 2005). More research advances have reported that main vehicles for drug resistance transmission includes plasmids, transposon, intergrons and bacteriophages (Caratolli A. et al 2009). It's therefore prudent for resistance investigation of both pathogenic and commensals bacteria causing human infectious diseases with concerns septicemia. However, little evidence exists to guide physicians in selecting antibiotics for initial empiric therapy for suspected septicaemia (Haddy *et al.,* 2012). In addition, the wider spectrum of infectious agents could require a broader spectrum of antimicrobial regimen.

Escherichia coli has been documented as a pathogen commonly isolated in early onset infection especially among preterm babies (Stoll, et al., 2011). In a recent study, 102 isolates were recovered out of which 78% were resistant to ampicillin while resistance to third generation cephalosporins was recorded for 3% of 94

isolates (Stoll, et al., 2011). Some isolates of *Escherichia coli* are also known to be resistant to gentamicin (Stoll, et al., 2011).

In a more recent study linking *Escherichia coli* to childhood bacteremia, it has been reported that sequence type 131 is highly virulent worldwide as it is also multi drug resistant (Park, et al., 2018). From the study, it is now evident that sequence type 131 *Escherichia coli* can bear both the $bla_{\text{CTX-M-15}}$ and the $bla_{\text{CTX-M-14}}$ genes, which might explain why these isolates have a higher resistance to gentamicin, amikacin, cefotaxime and piperacillin/tazobactam (Park, et al., 2018).

The early identification of sepsis and implementation of early evidence-based therapies have been documented to improve outcomes and decrease septicaemia-related mortality (Dellinger *et al.*, , 2010). Identifying the source of the infection helps to determine what antibiotic therapy should be used and may reveal an infected site that can be drained. This process requires: Careful clinical examination, Procedures such as chest X-rays, CT scans, urine analysis etc and Collection of biological specimens (e.g., wound swabs, sputum samples, urine specimens, blood samples, etc) for bacteriological/lab laboratory analysis or testing to identify the type of microorganism causing the infection. The global health forums are at the forefront to ensure programs that increase, the probability of control, prevention, cure and eventually eradication of the sepsis predicament.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study site

This study was conducted in Mbita district hospital. Mbita District Hospital is a Ministry of Health district hospital located in Mbita Township Sub location, Gembe West Location in Mbita Constituency, Homa Bay County (Figure 3.1). Mbita district was partitioned from Suba district in 2011. The total area of both districts combined is 1055Km^{2}of this 212.6km^2 is calibrated to mark total area of Mbita district. The total population of the Mbita district is 62974. Male to female ratio is 4:5 (National Census 2009). The study area is mostly deforested and vegetation cover is uniform. The rainfall pattern is bimodal with a long rainy season occurring from March to May and short rain in November. Most inhabitants belong to the Luo and Suba ethnic groups. The main socioeconomic activities are traditional small fishing and farming.

Mbita district hospital is the main health referral facility in the district which serves the following dispensaries and health enters; Kitare, Ogongo, Usao, Tom Mboya, Wawere, Sere, Remba, Obalwanda, Soklo, Lambwe, Wakula, Takawiri, Angiu and Dhuri (Figure 3.1).The hospital has a bed capacity of 90 units. On average 120 patients are seen in the outpatient per day. It is managed by the District Medical Officer (D.M.O.H).

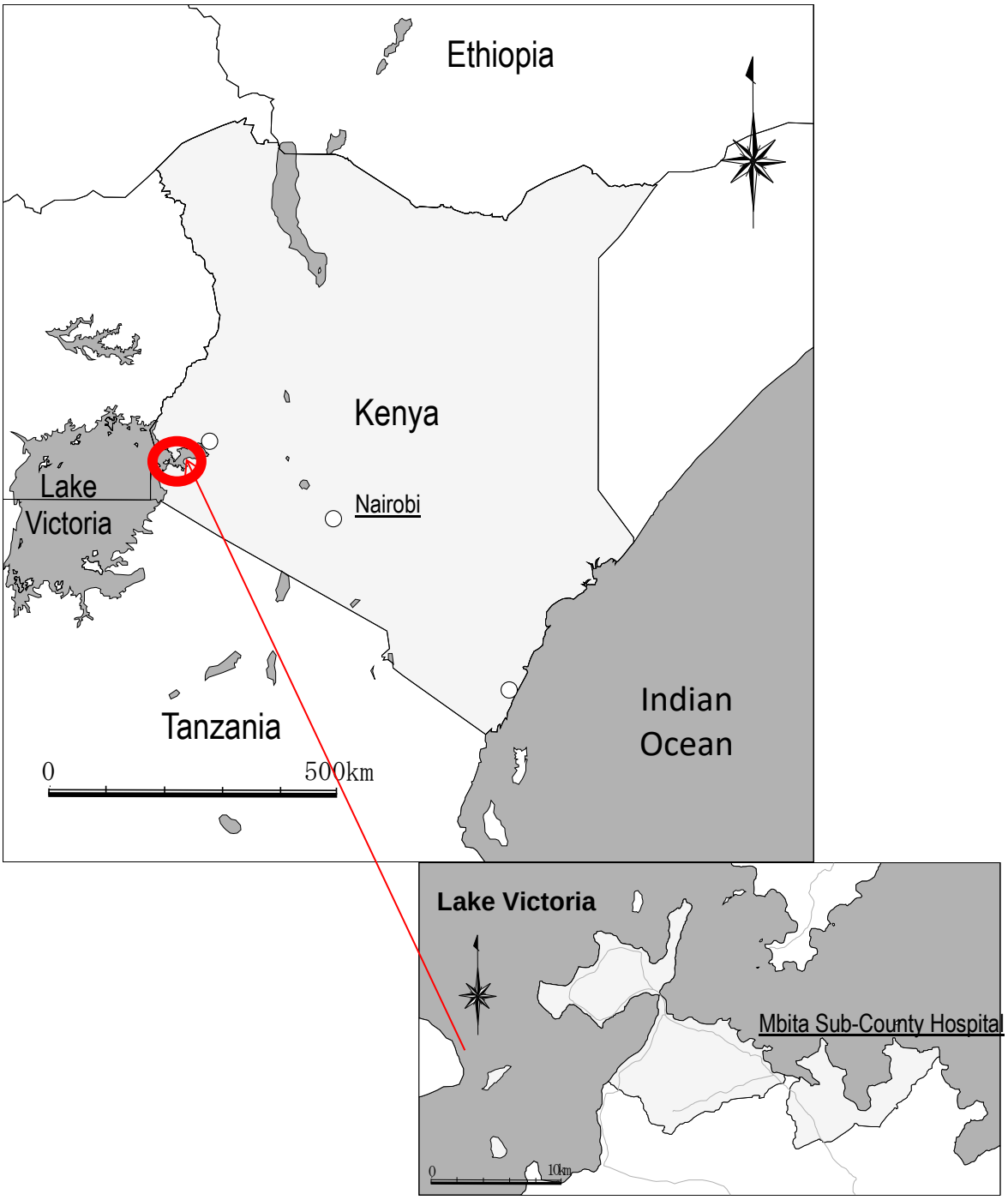

Figure 3. 1 The map showing the location of study hospital

3.2 Study design

This was a hospital based, cross-sectional study. This design was suitable in describing the prevalence of etiological agents causing septicaemia in Mbita District Hospital

3.3 Study population

The study targeted patients presenting with symptoms suggestive of septicaemia as described by the WHO (Bataar *et al*., 2010). These include: Body temp <36˚C >38˚C; Respiratory rate > 20/min; Heart Rate > 100/min; and WBC count < 4000 > 12000/mm^3.

3.4 Recruitment criteria

3.4.1 Inclusion criteria

1. Any patient with clinical symptoms suggestive of septicaemia as defined by the WHO.
2. Attending/admitted at Mbita District Hospital
3. Willing to give informed consent
4. Age from 0 months to 120 months.

3.4.2 Exclusion criteria

Patients were excluded if,

1. Had other symptoms other than those of septicaemia
2. Attending/admitted in other hospitals other than Mbita District Hospital

3. Unwilling to give informed consent.

3.5 sampling

3.5.1 Sample size determination

The Fisher *et al.,* (1998) formula was used to determine the minimum sample size required for this study

$$n = \left(\frac{z}{m}\right)^2 p(1-p)$$

Where,

- ***z*** is the critical value based on the desired confidence level (e.g., z = 1.96 for 95% confidence level);
- ***m*** is the margin of error or precision of the estimate in this case m=0.05.
- ***p*** is the estimated value of the proportion of patients with septicaemia (p = 0.76 referring to a prevalence of 76%) (WHO, 2014).

Substituting

n = 1.96 X 1.96 X 0.76X0.24/(0.05 X 0.05)

n = 281

Therefore, a minimum of 281 blood sample were required for the study to make logical conclusions of the findings.

3.6 Sampling method and recruitment

Purposive sampling method was used to recruit the desired number of patients meeting the inclusion criteria attending the Mbita District Hospital. The attending physicians/clinicians informed the patients about the study, those who consented were then enrolled. The recruited subjects (patients) were then referred to the hospital laboratory for blood collection which was done by a qualified medical lab technologist. Procedurally, the attending physician/clinician then determined the case management of the patients.

3.7 Sample collection

Blood sample*:* From each of the participants enrolled, about 2-5ml (children) blood samples were collected aseptically in aerobic and anaerobic blood culture bottles. The sampling bottles were appropriately labelled in line with pathological/request forms details i.e. name, sample code, date, time and location of the hospital and patients.

3.7.1 Sample transportation

The blood samples collected aseptically into appropriate blood culture tubes were packed in primary cases and secondary cases according to the WHO accord of transporting infectious materials. The samples were maintained in upright position in a cooler box and transported to NUITM-KEMRI Biosafety level 2 laboratory at Mbita for processing.

3.7.2 Sample reception

Samples were received in the biosafety level 2 laboratory by qualified personnel in a biosafety cabinet class II where the cooler box was opened after thorough sterilization using 70% ethanol. This was done to check for any incorrect labelling and leakage after which the samples were recorded in laboratory book capturing all details.

3.7.3 Sample processing

3.7.3.1 Microbiological analysis

Samples were incubated at 37^0C for at least 3 weeks in the BACTEC 9270 automated machine. Generally, the BACTEC indicates signals for any positive culture wells. All positive culture bottle was taken to the Biosafety level III laboratory where they were sub cultured using sterile and disposable loops on basic, differential, selective media (Oxoid type) and other appropriate media e.g. blood agar Chocolate blood agar (CBA), DHL, Salmonella– shigella agar (SS), Xylose lysine deoxycholate (XLD), and Bromol thymol blue (BTB) for other etiological agents. All plates were incubated at 37°C for 18-24hrs. Blood agar, Brucella agar and CBA were incubated in the presence of 5-10% CO2. The suspected colonies were examined and characterized by their morphology, Gram stain, biochemical identification (Sahin, *et al.*, 2008).

3.7.3.2 Antibiotic susceptibility testing

Isolates were tested for their antimicrobial susceptibility by the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute

(CLSI) guidelines (CLSI, 2016). The drugs tested included; cefoxitin (CTX), streptomycin (STR), tetracycline (TET), Ampicillin (AMP), chloramphenicol (CHLO), ciprofloxacin (CIP), Nalidixic acid (NA), gentamicin (GEN), trimethoprim-sulfamethoxazole (SXT). The antimicrobial susceptibility was classified using the CLSI guidelines, as susceptible, intermediate, or resistant to each antibiotic. In addition, isolates were also classified as either non-susceptible (including both intermediate and resistant isolates) or susceptible. Isolates found to be non-susceptible or resistant to three or more antimicrobial categories were classified as multidrug resistant (MDR).

3.7.3.3Molecular analysis PCR technique

Profiling for R-plasmids and screening for the presence of previously reported resistance genes were done using specific primers. These include among others the *TetA gene, StrA gene Str B gene, Sul2gene, Amp C gene, Gyr A gene, CatA1 gene* (Table 3.1). To accomplish this, the representative isolates belonging to various antibiograms were selected after which pure isolates were plated on Mueller-Hinton agar media overnight at 37°C. This was followed by extraction of plasmid DNA using the alkaline lysis method (Sambrook, *et al.,* 2001). Plasmids were extracted from 2-ml overnight cultures grown in Luria-Bertani (LB) broth (Difco Laboratories, Becton Dickinson, Sparks, MD). Plasmid sizes and numbers were determined using electrophoresis by comparison with previously characterized plasmids molecular markers/ladders.

Polymerase Chain Reaction (PCR) is a method for the in vitro amplification of a specific sequence of DNA. A typical PCR involves template DNA containing the target sequence to be amplified, two primers that are complementary to the target DNA sequence, nucleotides, and a thermal-stable DNA polymerase. The reaction mixture is repeatedly cycled through alternating periods of thermal denaturation, annealing, and extension, resulting in exponential amplification of the target DNA sequence (Caratolli A.et al 2009). For amplification of antibiotic resistance genes: 200µl tubes containing PuReTaq ready-to-go PCR beads (GE Healthcare UK limited, UK) were used in this study. Each reaction contained 2.5 units of PuReTaq DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 200 µM dATP, dCTP, dGTP and dTTP, stabilizers, BSA, 1µl each of forward and reverse primers (6 pmol/µl), 2µl of template DNA, and Milli-Q water to a final volume of 25µl.

The PCR was performed using icycler (Bio-rad) using the following conditions: denaturation at 94^0C, annealing at 54^0C, Extension at 72^0C going for 25-30 cycles. The PCR product was electrophoresed in 2% agarose gel. Briefly 2gm of (seaKem GTG Agarose) was dissolved in 100ml of TBE buffer, heated to dissolve, cooled in water bath at 50^0C for 15min then dispensed on gel casting tray for 15-20min to solidify. Well solidified gel was immersed in electrophoresis tank containing TBE as the running buffer, 8µl of PCR product mixed with 2µl of loading buffer and loaded in each well. Molecular marker was used for amplicon size estimation to identify plasmids and antimicrobial resistance conferring genes.A constant current of 50-100V was used for an appropriate time. Upon completion the gel

was stained with ethidium bromide (0.5µg/100ml) for 10-15min, image taken by trans illuminator for observation of positive band.

Table 3.1: gene identities, primer sequences, molecular sizes and the source: StrA - Streptomycin resistance; Sul2 - sulfamethoxazole resistance; TetA - tetracycline resistance; CTX-M –Ceftriaxone; CAtA1 gene – Chloramphenicol; AmpC- Augumentin; Gyr A – C

Target	**Forward**	**Reverse**	**Size-*bp***	**Reference**
StrA	GCTGGATAGGTTAAGGGCGG	CTCTATGGGCACTGTCCACATTG	383	Hoch hut *et al.*, (2001)
Sul2	AGGGGGCAGATGTGATCGAC	TGTGCGGATGAAGTCAGCTCC	625	Hoch hut *et al.*, (2001)
TetA	ATAAAATTCTTGAAGACGAAA	GACAGTTACCAATGCTTAATC	1080	Weill *et al.,* (2004)
CTX-M	AAA AAT GAT TGA AAG GTG GT	CAG CGC TTT TGC CGT CTA AG		Rina.k*et al.,* (2012)
CAtA1	AAGCGAACGA	GGAAGTAAAA		Hoch hut *et al.*, (2001)
AmpC	AACACACTGATTGCGTCTGAC	CTGGGCCTCATCGTCAGTTA	1870	peres-peres *et al.*,
Gyr A	TTAATGATTGCCGCCGTCGG	TACACCGGTCAACATTGAGG	648	Olga V *et al.,* (2008)
mecA	GTA GAA ATG ACT GAA CGT CCGATAA	CCA ATT CCA CAT TGT TTC GGTCTA A	533	Roche diagnostic co.

All the identified and clinical significant isolates were purified, accurately labelled and stored in 15-40% glycerol, at -80^{0}c for any future work or references. All samples were assigned a subject identification number (SID). All data entered into the study databases were de-identified and only associated with a SID in password protected files. A double entry system for the data was maintained. All paper research records were kept in a password protected, locked filing cabinet located in a restricted-access room at the research station. Data entry, cleaning and validation were performed in order to achieve a clean data.

Frequency (%), mean, standard deviation, and medium (interquartile ranges at 25% and 27%) were used to describe the qualitative and laboratory parameters. Chi-square or Fisher's exact test were used to test for significance where applicable at the significance level of $P \leq 0.05$. All statistical analyses were performed using STATA v 13 (StataCorp LP, College Station, TX, USA).

3.8 Ethical considerations

The research protocol was presented for scientific and ethical approvals by the Scientific Steering Committee and the Ethical Review Committee of Kenyatta University prior to commencement of field activities. Written informed consent was obtained from each participant (Appendix I and II).

3.9 Expected application of results

The expectation of the study was to demonstrate within an acceptable range the correlation between clinical diagnosis, laboratory diagnosis and diseases

treatment. Study expected to improve the understanding of disease pattern and etiological agents based on sex and ages, on immune status, insight significant differences in antibiotic susceptibility and resistance and circulating resistance markers.

CHAPTER FOUR

RESULTS

4.1 Baseline characteristics of the study participants

A total of 280 children met the recruitment criteria, but due to some study limitations factors only 248 were recruited into this cross sectional study. Table 4.1 shows the baseline demographic characteristics of the study participants.

TABLE 4.1: BASELINE DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

Socio-Demographic Characteristic	Sample size		$\chi 2$	df	P
	No	%			
Locality					
Gembe	107	43.1			
Lambwe	4	1.6	170.548	3	0.001
Mfangano	18	7.3			
Rusinga	119	48.0			
Gender					
Female	122	49.2	0.065	1	0.799
Male	126	50.8			
HIV status					
Positive	20	8.1	174.452	1	0.001
Negative	228	91.9			
Age (Months)					
Mean (± SD)	27.9	(± 20.7)			
Median (IQR)	24	(12-42)			
Range	118	(2-120)			
1-12	76	30.6			
13-24	74	29.8			
25-36	27	10.9	97.823	5	0.001
37-48	43	17.3			
49-60	20	8.1			
>61	8	3.2			

No - Number; % - Percentage; $\chi 2$ - Chi square; df - Degree of freedom; P - Level of significance; $P \leq 0.05$ indicates the relationship is significant

4.2 Locality of origin

Participants in this study were drawn from four different locations. There were two peaks in the distribution of participants 48% from Rusinga and 43.1% from Gembe. Other regions included 7.3% from Mfangano and 1.6% from Lambwe (Figure 4.1). There was a significant difference in the frequency of study participants based on location of origin ($\chi 2 = 170.548$; df = 3; P = 0.001)

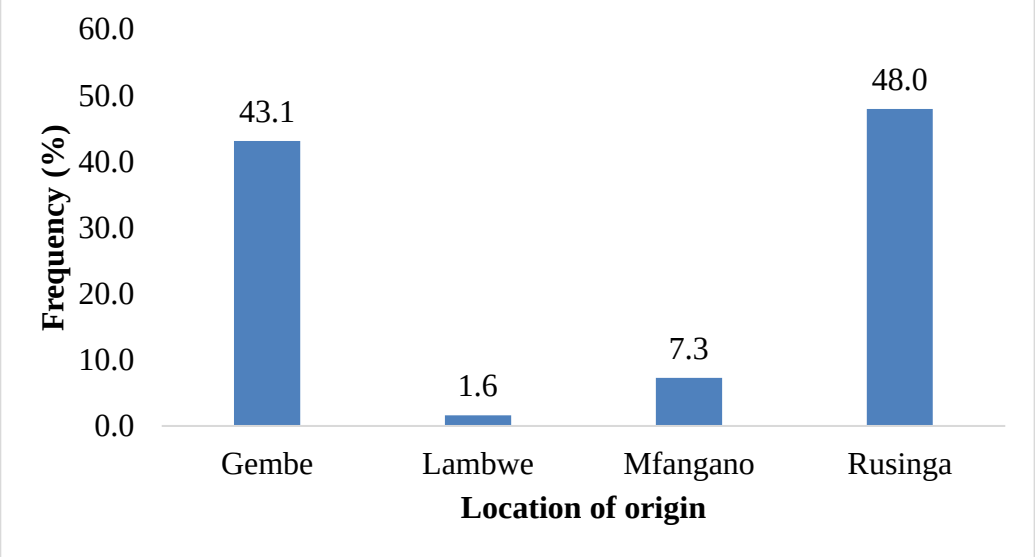

Figure 4. 1 Distribution of study participants by location of origin

4.3 Gender

There were near equal distribution between male and females; 50.8% and 49.2% respectively There was no significant difference in the frequency of study participants based gender ($\chi 2 = 0.065$; df = 1; P = 0.799) (Table 4.1).

4.4 HIV status

The majority (91.9%) of the participants were HIV seronegative while 8.1% were HIV positive. There was a significant difference in the frequency of study participants based on HIV status ($\chi 2 = 174.452$; df = 1; P = 0.001) (Table 4.1).

4.5 Age groups

The mean age of the participants was 27.93 (SD 20.6) months with median of 24 (range 2 to 120 months). The majority 30.6% of the participants were aged between 1 to 12 months followed by 29.8% aged 13 to 24 months while those aged more than 61months 3.2% were the least (Figure 4.2). There was a significant difference in the frequency of study participants based on age group ($\chi 2 = 97.823$; df = 5; P = 0.001) (Figure 4.2).

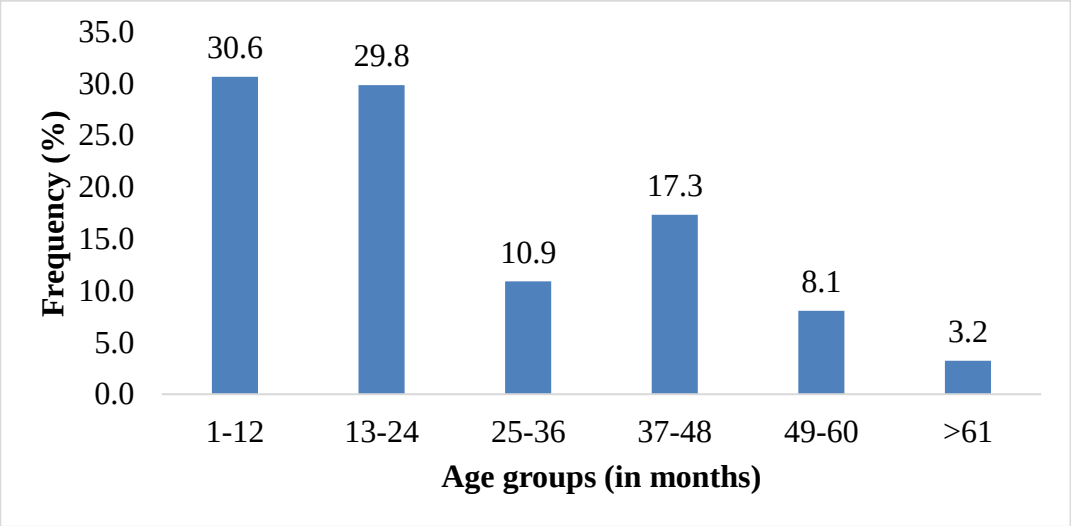

Figure 4. 2: Distribution of study participants by age group in Months

4.6 Clinical presentations

Study used four different WHO approved clinical parameters/ criteria standard to identify the septicaemia meet patients. A total of 280 children met the recruitment criteria and were recruited into this cross sectional study but only 248 samples were analysed this explains some parts of study limitations. Table 4.2 shows the summary of clinical presentations of the study participants.

TABLE 4.2: CLINICAL PRESENTATION OF THE STUDY PARTICIPANTS

Clinical outcomes	Sample size		χ2	df	P
	No	%			
Temperature (C^O)					
Mean (± SD)	38.1	(± 0.5)			
Median (IQR)	38	(37.8-38.6)			
Range	3	(37-40)			
36.5-37.5	11	4.4	111.492	2	0.001
>37.6	146	58.9			
ND	91	36.7			
WBC (Cells/ml)					
Mean (± SD)	17720.9	(± 8929.1)			
Median (IQR)	15600	(12075-22450)			
Range	45000	(-120)			
<3500	2	.8			
3500-10500	11	4.4	295.194	3	0.001
10501	63	25.4			
Not done	172	69.4			
Respiratory rate (Breaths/min)					
Mean (± SD)	17720.9	(± 8929.1)			
Median (IQR)	15600	(12075-22450)			
Range	45000	(1-45000)			
20-30	177	71.4			
31-40	37	14.9	419.258	4	0.001
41-50	11	4.4			
>51	14	5.6			
Not done	9	3.6			
Heart rate (Beat/min)					
Mean (± SD)	111.7	(± 12.2)			
Median (IQR)	110	(105.7-120)			
Range	109	(29-138)			
80-90	5	2.0			
91-100	18	7.3			
101-110	85	34.3	185.725	6	0.001
111-120	43	17.3			
121-130	19	7.7			
131-141	3	1.2			
Not done	74	29.8			

No - Number; % - Percentage; χ2 - Chi square; df - Degree of freedom; P - Level of significance; $P \leq 0.05$ indicates the relationship is significant

4.6.1 Body temperature

The mean body temperature of the participants was 38 ^{O}C (SD 0.5) with median of 38 (range 37 to 40^{O}C). The majority (58.9%) of the participants had body temperatures above 37.6 ^{O}C with only 44 % having temperatures within the normal ranges of 36.5 to 37.5 ^{O}C. There was a significant difference in the

frequency of study participants based on body temperature ($\chi 2 = 11.492$; df = 2; P = 0.001) (Table 4.2).

4.6.2 White blood cells

The mean WBC of the participants was 17720.9 Cells/ml (SD 8929.1) Cells/ml with median of 15600 (range 12075 to 22450 Cells/ml). The majority 25.4% of the participants had WBC above the normal levels of 10501 cells/ml and only 4.4% had WBC in the normal ranges of 3500 to 10500 cells/ml. There was a significant difference in the frequency of study participants based on WBC ($\chi 2 = 295$; df = 3; P = 0.001) (Table 4.2).

4.6.3 Respiratory rate

The mean respiratory rate (RR) of the participants was 30.6 (SD 10.6) breaths/min, with a median of 28 (range 18 to 96 breaths/min). The majority (71.4%) of the participants had RR between 20 and 30 breaths/min followed by 14.9% with between 31 to 40 breaths/min while 3.6% had RR of >51 breaths/min. There was a significant difference in the frequency of study participants based on RR ($\chi 2 = 419.258$; df = 4; P = 0.001) (Table 4.2).

4.6.4 Heart rate

The mean heart rates (HR) of the participants was 111.7 (SD 12.2) beats/min with a median of 110 (range 29 - 138 beats/min). The majority (34.3%) of the participants had a heart rate between 101 and 110 beat/min followed by 17.3% between 111 to 120 beats/min, while 1.2% had HR of 131 and 141 beat/min. There

was a significant difference in the frequency of study participants based on HR ($\chi 2 = 185.725$; df = 6; P = 0.001) (Table 4.2).

4.7 Prevalence of septicaemia among children from Mbita, South Nyanza

A total of 84 of the 248 (33.9%) of the children had septicaemia versus 164(66.1%) who were negative (Figure 0.1). There was a significant difference in the frequency of study participants based on the prevalence of septicaemia ($\chi 2 = 25.806$; df = 1; P = 0.001) (Figure 4.3).

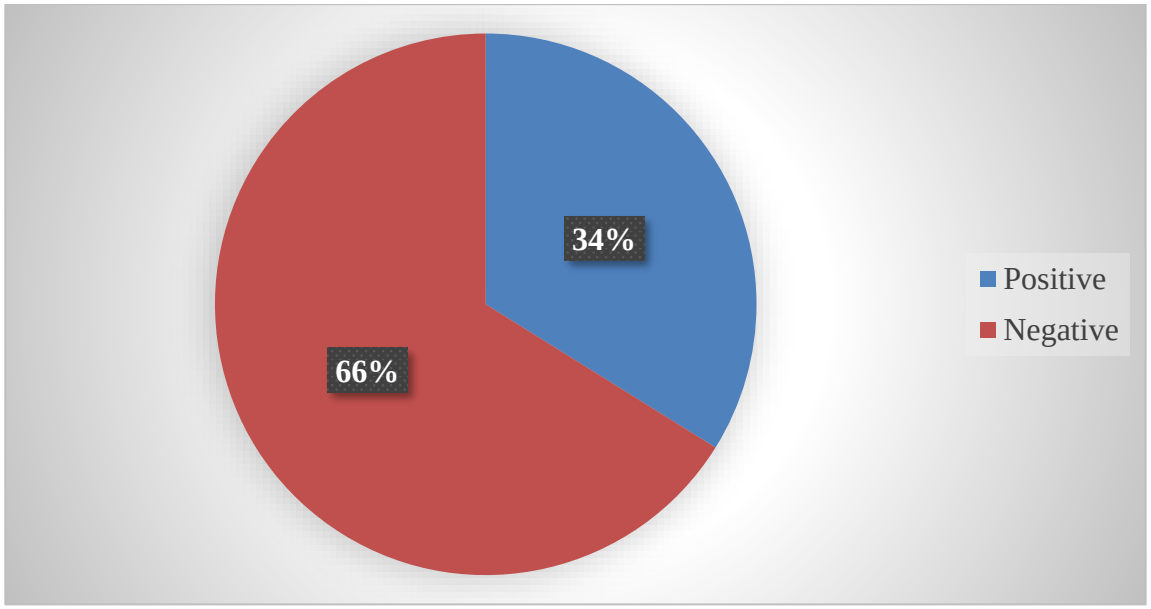

Figure 4. 3: Prevalence of septicemia in the study participants

4.8 The aetiologic agents causing septicaemia among study population

There were a total of 18 different etiological agents identified in this study. The most common causative agent of septicaemia was *Staphylococcus epidermidis* (28.6%) followed by *S. aureus* and *E. coli* each at 13.1%.

Others included *P. aeroginosa (*10.7%), *S. typhimurium (*8.3%), *S. hemolyticus* (4.8%) among others (Figure 4.4). There was a significant difference in the frequency of the septicaemia based on the etiological agent among the participants ($\chi 2 = 188.9$; df = 17; P = 0.001).

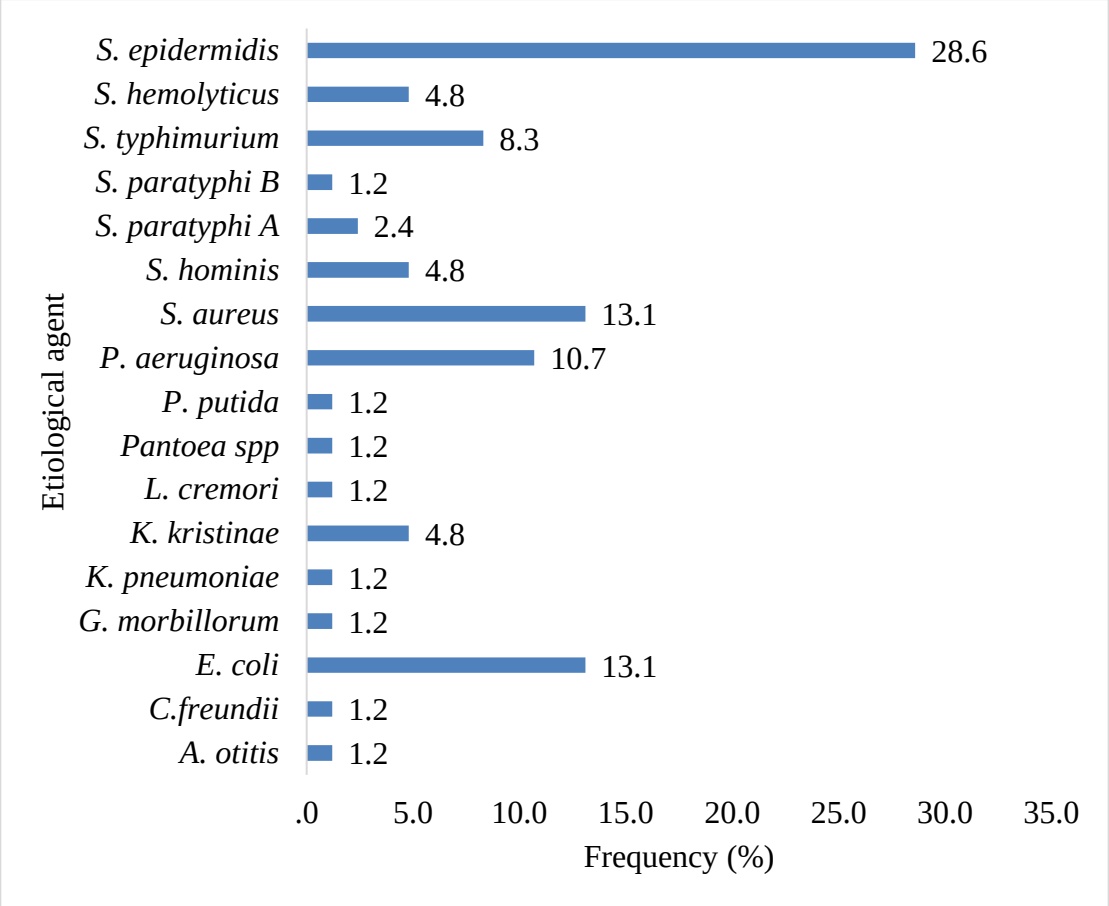

Figure 4. 4: Distribution of the etiological agents of septicemia

4.8.1 Antimicrobial susceptibility patterns of septicaemia etiological agents

Various isolates both in gram positive and negative were subjected to antibiotic susceptibility test.

4.8.1.1 Gram negative bacteria

Susceptibility testing showed that majority of gram negative isolates were resistant to penicillin(Ampicillins) 100%; followed by tetracycline 96.1%; sulphonamides (Trimethoprim/sulfamethoxazole) 84.6%; Aminoglycosides (Gentamicin) 73.1%; while they were least resistant to Quinolones (Ciprofloxacin) 19.2% (Table 4.3).

4.8.1.2 Gram positive bacteria

For gram positive bacteria majority (96.7%) were resistant to sulphonamides (Trimethoprim/sulfamethoxazole) followed by tetracycline 76.7%; penicillin (Oxacillin) 73.3% and least resistant to Quinolones (Ciprofloxacin) 30% (Table 4.3).

Table 4.3: Distribution of drug susceptibility pattern of bacteria causing septicaemia

Antibiotic class	Antibiotic tested	Susceptibility pattern					
		Sensitive		Intermediate		Resistant	
		N	%	N	%	N	%
Drug susceptibility patterns for gram negative isolates							
Penicillins	Ampicillin	0	0	0	0	26	100
	Amoxicillin/Clavulanate	16	61.5	1	3.9	9	34.6
Cephalosporin	Cefuroxime	7	26.9	1	3.9	18	69.2
	Ceftriaxone	10	38.5	2	7.5	14	53.9
Tetracyclines	Tetracycline	0	0	1	3.9	25	96.1
Quinolone	Ciprofloxacin	20	76.9	1	3.9	5	19.2
Aminoglycosides	Gentamicin	6	23.1	1	3.9	19	73.1
Sulfonamides	Trimethoprim/sulfamethoxazole	3	11.5	1	3.9	22	84.6
Drug susceptibility patterns for gram positive isolates							
Penicillins	Oxacillin	8	26.7	0	0	22	73.3
	Amoxicillin/Clavulanate	14	46.7	0	0	16	53.3
Macrolides	Erythromycin	16	53.3	0	0	14	46.7
Tetracyclines	Tetracycline	6	20	1	3.3	23	76.7
Quinolone	Ciprofloxacin	20	66.7	1	3.3	9	30
Aminoglycosides	Gentamicin	14	46.7	4	13.3	12	40
Sulfonamides	Trimethoprim/sulfamethoxazole	0	0	1	3.3	29	96.7

N - Frequency; % - Percentage

4.8.1.3 Drug resistant genes among the multi drug resistant isolates

The number of multi-drug resistant bacterial isolates included 4 *S. epidermidis,* 4 *S. aureus,* 7 *E. coli* (4 *Enterotoxigenic* and 3 *Enteropathogenic E. coli*), 4 *S. paratyphi* (3 *S. paratyphi* A and 1 *S. paratyphi* B), 3 *S. typhimurium* and 3 *P. aeruginosa* as summarized in (Table 4.4).These were subjected to genetic characterization to determine plasmid profiles and antibiotic resistance conferring genes.

TABLE 4.4: DISTRIBUTION OF RESISTANT GENES AMONG MDR *S. EPIDERMIDIS, S. AUREUS .AND E.COLI SPP.*

Sample	Isolate	No. of plasmids	Approximate sizes of the plasmids (Kb)				Pattern of drug resistance	Antimicrobial genes detected
			1500	3000	4000	>10000		
MDH/BLD/54	*S. aureus*	2	0	0	0	2	OX, Amoclav, Sxt, Tet, Ery	*mecA*
MDH/BLD/55	*S. aureus*	1	0	0	0	1	OX, Gen, Tet, Ery	*mecA*
MDH/BLD/15	*S. aureus*	1	0	0	0	1	Cip, Tet, Sxt, Ery	*SulII*
MDH/BLD/135	*S. aureus*	1	0	0	0	1	OX, Sxt	*mecA*
MDH/BLD/157	*S. epidermidis*	0	0	0	0	0	OX, Amoclav, Sxt, Tet	*none*
MDH/BLD/62	*S. epidermidis*	2	0	0	0	2	Amoclav, Sxt, Ery	*mecA*
MDH/BLD/216	*S. epidermidis*	2	0	0	0	2	OX, Cip, Gen, Tet, Sxt	*mecA*
MDH/BLD/104	*S. epidermidis*	2	0	0	0	2	OX, Amoclav, Cip, Sxt, Tet, Ery	*mecA*
MDH/BLD/183	*E. coli* (EPEC)	3	1	1	0	1	Amp, Gen, Sxt, Tet	*blaTEM, SulII*
MDH/BLD/222	*E. coli* (EPEC)	5	1	1	0	3	Amp, Amoclav, Gen, Sxt, Tet	*SulII, TetA*
MDH/BLD/229	*E. coli* (EPEC)	5	1	1	0	3	Amp, Gen, Sxt, Tet	*blaTEM, SulII, TetA*
MDH/BLD/142	*E. coli* (ETEC)	4	1	1	0	2	Amp, Amoclav, Sxt, Tet	*blaTEM, SulII, TetA*
MDH/BLD/163	*E. coli* (ETEC)	3	1	1	0	1	Amp, Gen, Sxt, Tet	*blaTEM, SulII, TetA*
MDH/BLD/215	*E. coli* (ETEC)	3	1	1	0	1	Amp, Gen, Sxt, Tet	*blaTEM, SulII*
MDH/BLD/156	*E. coli* (ETEC)	4	1	1	0	2	Amp, Amoclav, Cfm, Cfx, Gen, Cip, Sxt, Tet	*blaTEM, SulII, TetA*

Where Ox- Oxacillin; Amoclav-Amoxicillin/Clavulanate; Sxt- Trimethoprim/sulfamethoxazole; Tet- Tetracycline; Ery-Erythromycin; Gen-Gentamicin; Cip- Ciprofloxacin; mec A-Methicillin-resistant Staphylococcus aureus; blaTEM - Nonspecific TEM β-Lactamase; TetA-Tetracycline Resistant gene A; SulII - Sulfonamide resistance gene

4.8.1.3.1 Plasmid profiles and drug resistance phenotypes of *S. aureus and S. epidermidis*

At least one plasmid each larger than 10 kb in the four *S. aureus* isolates studied was detected using PCR. Three out of 4 of the *S. aureus* harboured one plasmid which was larger than 10kb in size while the remaining 1/4 *S. aureus* harboured 2 plasmids which were both larger than 10 kb in size. The antimicrobial gene, *mecA,* was detected in three of the plasmids while one of the plasmids carried *SulII* antimicrobial gene (Table 4.4).

Two plasmids each larger than 10 kb was detected in 3/4 *S. epidermidis* studied. Antimicrobial gene, *mecA,* was detected in the plasmids carried by the *S. epidermidis* isolates (table 4.4). One of the *S. epidermidis* did not carry any plasmid but showed phenotypic resistance to Oxacilin, Amoxyclavulanic acid, Sulfamethoxazole-Trimethoprim and Tetracycline. Plasmids isolated from both *S. epidermidis* and *S. aureus* species harboured resistance genes, *mecA* and *SulII,* highlighting their role in dissemination of antibiotic resistance.

High frequency of resistance was observed for Sulfamethoxazole-Trimethoprim (87.5%), Oxacilin (75.0%), Erythromycin (62.5%) and Amoxyclavulanic acid (50.0%). All the isolates were Multi resistant to between two to six antibiotics. However, there was no apparent relationship between carriage of plasmids and antimicrobial resistance in both *S. epidermidis* and *S. aureus* species (Table 4.4).

4.8.1.3.2 Plasmid profiles and drug resistance phenotypes for *E. coli*

Plasmids of sizes varying from approximately 1.5 kb to >10kb were isolated in the pathogenic *E. coli* species. Three isolates of ETEC and four isolates of EPEC pathotype of pathogenic *E. coli* species were detected (Figure 4.8 and Figure 4.9). Each *E. coli* species harboured at least three plasmids of varying sizes.(Table 4.4).

Four of the *E. coli* species harboured at least two plasmids which were larger than 10 kb in size while each of the other 3 *E. coli* species harboured one plasmid larger than 10kb (table 4.4). Antimicrobial gene, *Sul II,* was detected in all the *E. coli* isolates while *blaTEM* gene was detected in six of the seven isolates. Antimicrobial gene, *TetA,* was detected in 5 of the *E. coli* isolates (Table 4.4).

High frequency of resistance was observed for Sulfamethoxazole-Trimethoprim (100%), Ampicillin (100%), Tetracycline (100%) and Gentamycin (85.7%). All the isolates were multi resistant to between four and eight antibiotics. However, there was no apparent relationship between carriage of plasmids and antimicrobial resistance. Plasmids isolated from the *E. coli* isolates studied harboured resistance genes, *blaTEM, SullII,* and *TetA*

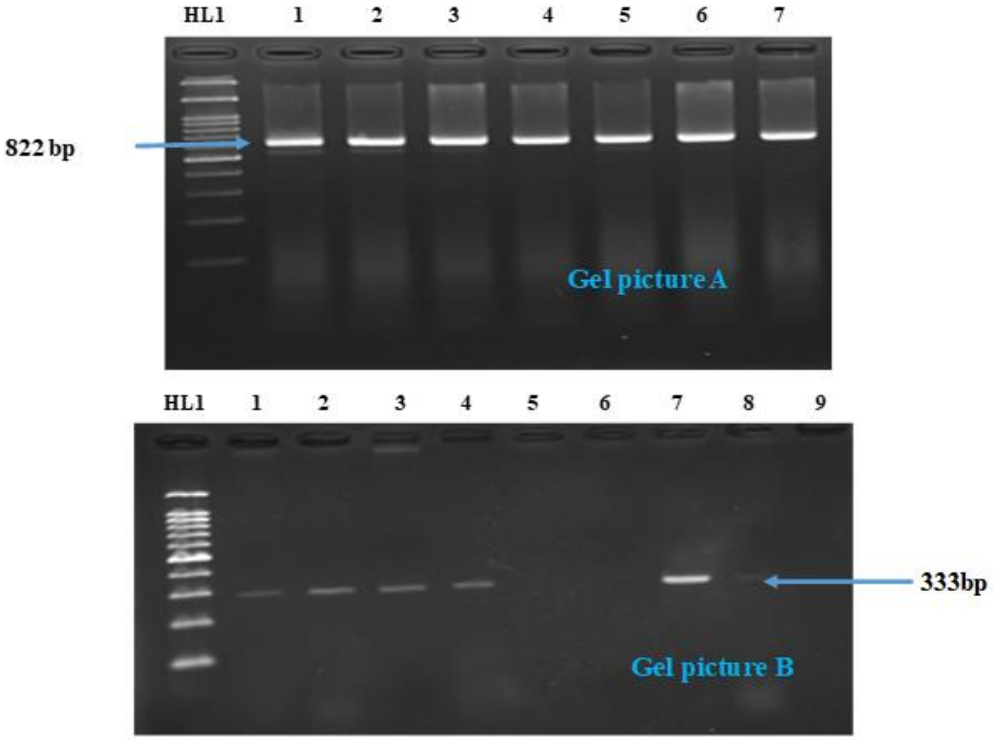

Figure 4. 5: A 2% Agarose gel (A, B) showing the amplifications of ***Sul II*** (Gel A) and ***mecA*** (Gel B) resistance genes in *S. aureus* MDR isolates.

4.8.1.4 Plasmid banding patterns demonstrated in bacterial isolates.

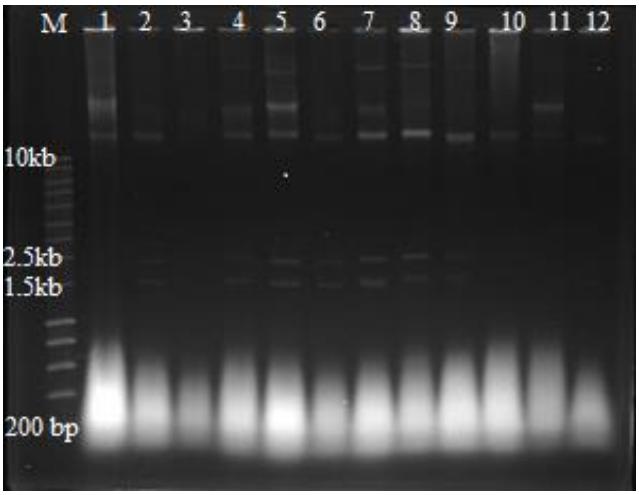

Figure 4. 6: Plasmids bands in Gram negative isolates.

Multiple plasmids of varying sizes detected among the multi-drug resistant in gram negative bacterial isolates

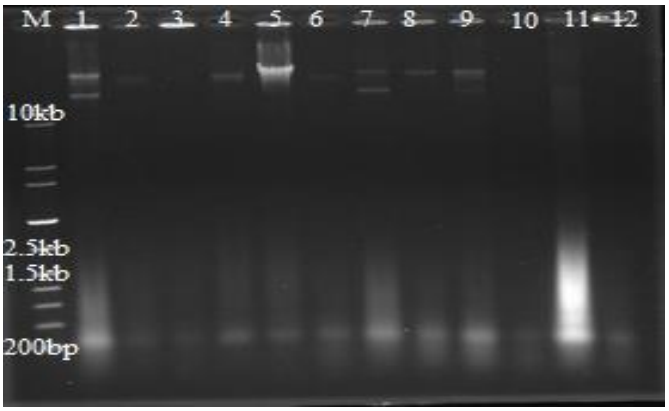

Figure 4. 7: Plasmids bands in gram positive isolates.

Multiple plasmids of varying sizes detected among the multi-drug resistant gram positive bacterial isolates

Pathotypes of the identified *E. Coli isolates*

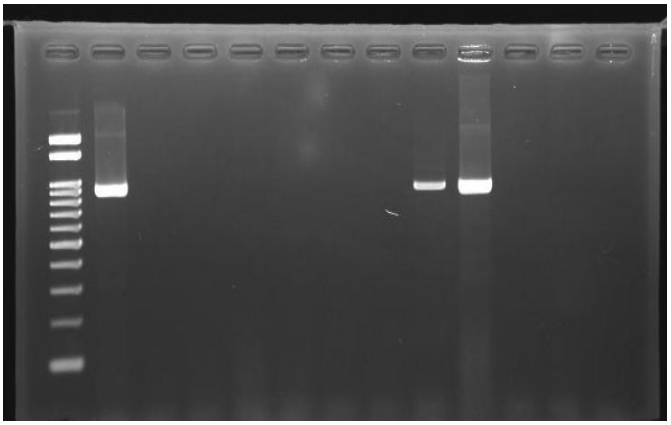

Figure 4. 8: EPEC Pathotype of *E. coli.*

Eae gene of EPEC (881 bp)

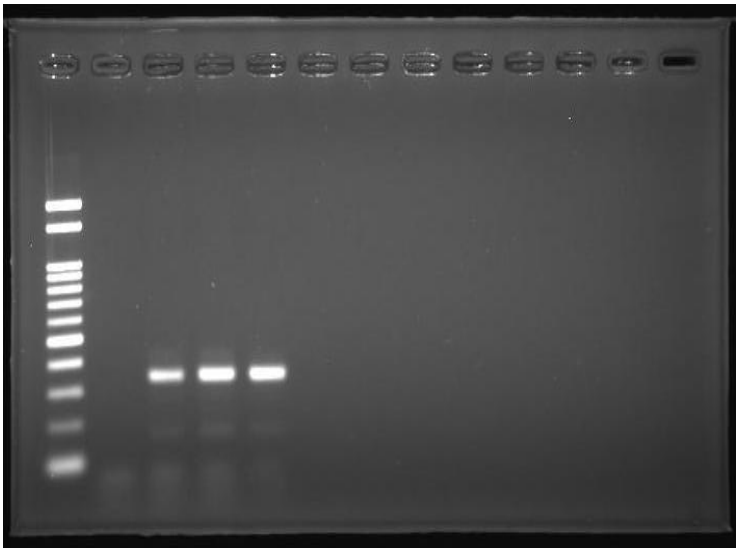

Figure 4. 9: ETEC pathotype of *E. coli.*

Elt gene of ETEC (322 bp)

Resistance genes detection

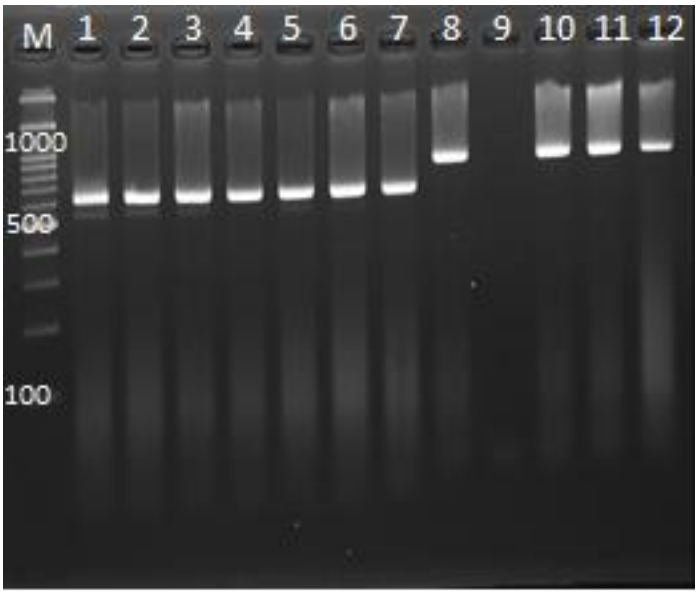

Figure 4. 10: *Sull* II **(**wells 1-7) and *blaTEM* (wells 8-12) resistance genes detection in gram negative ***(****salmonella spp)*MDR isolates.

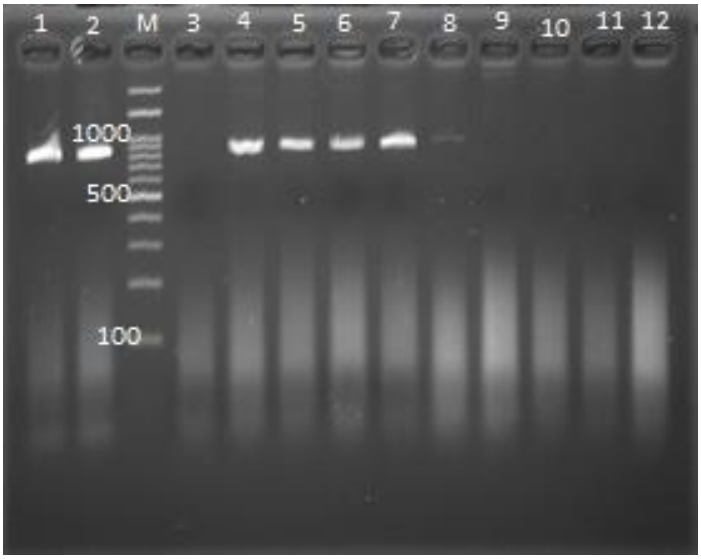

Figure 4. 11: *blaTEM* (wells 1-2) and *tetA* (wells 4-12) resistance gene detection in gram negative(*salmonella spp*) MDR isolates.

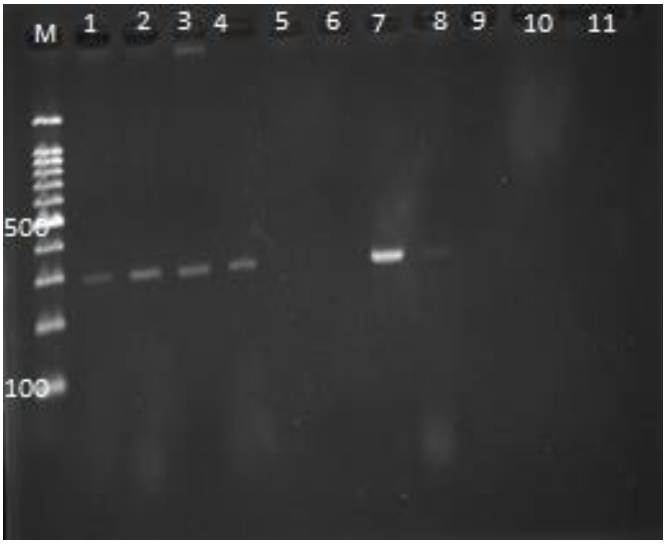

Figure 4. 12: *mecA* genes detection in gram positive (*S. aureus* and *S. epidermidis*) MDR isolates

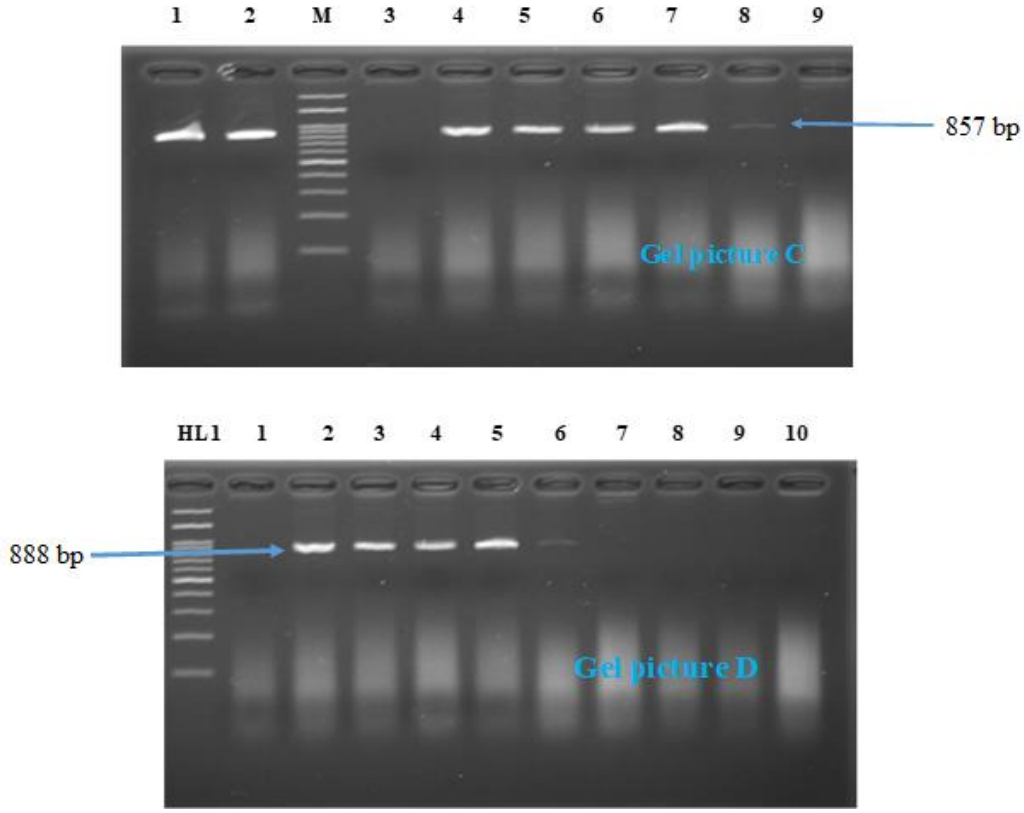

Figure 4. 13: Gel electrophoresis image (C &D) showing amplifications of *blaTEM* and *TetA* Resistance genes in gram negatives *E.coli*, Multi Drugs Resistance isolates.

Table 4.5 Distribution of resistant genes among MDR Salmonella spp and Pseudomonas spp. Isolates.

Sample	Isolate	No. of plasmids	Approximate sizes of the plasmids				Pattern of drug resistance	Antimicrobial genes detected
			1500	3000	4000	>10000		
MDH/BLD/093	*S. paratyphi* B	4	0	1	1	2	Amp, Cfm, Gen, Sxt, Tet	blaTEM, SulII, TetA, aac(3)
MDH/BLD/219	*S. paratyphi* A	3	0	1	1	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, SulII, TetA, aac(3)
MDH/BLD/238	*S. paratyphi* A	5	0	1	1	3	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, SulII, TetA, aac(3)
MDH/BLD/203	*S. paratyphi* A	3	0	1	1	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, SulII, TetA, aac(3)
MDH/BLD/133	*S. Typhimurium*	0	0	0	0	0	Amp, Cfm, Gen, Sxt, Tet	-
MDH/BLD/134	*S. Typhimurium*	5	0	1	1	3	Amp, Cfm, Gen, Sxt, Tet	blaTEM, SulII, TetA, aac(3)
MDH/BLD/189	*S. Typhimurium*	3	0	1	1	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	SulII, TetA, aac(3)
MDH/BLD/11	*P. aeruginosa*	1	0	0	0	1	Amp, Amoclav, Cfm, Cfx, Cip, Sxt, Tet	blaTEM, SulII, TetA, aac(3)
MDH/BLD/4	*P. aeruginosa*	2	0	0	0	2	Amp, Amoclav, Cfx, Gen, Sxt, Tet	blaTEM, SulII, TetA, aac(3)
MDH/BLD/8	*P. aeruginosa*	2	0	0	0	2	Amp, Amoclav, Cfm, Cfx, Cip, Tet	blaTEM, SulII, TetA
MDH/BLD/35	*P. aeruginosa*	1	0	0	0	1	Amp, Cfm, Cfx, Gen, Sxt, Tet	blaTEM, aac(3)
MDH/BLD/175	*P. aeruginosa*	1	0	0	0	1	Amp, Amoclav, Cfm, Gen, Cip, Tet	blaTEM, SulII, TetA, aac(3)

Where: Amp – Ampicillin; Cfm – Cefuroxime; Cfx – Ceftriaxone; Amoclav-Amoxicillin/Clavulanate; Sxt- Trimethoprim/sulfamethoxazole; Tet- Tetracycline; Gen- Gentamicin; Cip- Ciprofloxacin;blaTEM - Nonspecific TEM β-Lactamase; TetA- Tetracycline Resistant gene A; SulII - Sulfonamide resistance gene; aac(3) - Aminoglycoside acetyltransferase gene

4.8.1.5 Plasmid profiles and drug resistance phenotypes for Pseudomonas spp

At least one plasmid larger than 10 kb was detected in the five *Pseudomonas* isolates (Table 4.5). Three of five of the *Pseudomonas* isolates carried one plasmid which was larger than 10kb in size. Two of the *Pseudomonas* isolates harboured 2 plasmids which were both larger than 10 kb in size. The antimicrobial gene, *blaTEM,* was detected in five of the plasmids while four of the plasmids carried *SulII, TetA* and *aac(3)*antimicrobial resistance genes. High frequency of phenotypic resistance was observed for Tetracycline (100%), Ampicillin (100%), Cefixime (80%), Cetriaxone (80%), Amoxyclavulanic acid (80.0%), Gentamicin (60.0%) and Sulfamethoxazole-Trimethoprim (60%). All the isolates were multi resistant to between two to six antibiotics. (Table 4.5).

4.8.1.6 Plasmid profiles and drug resistance phenotypes for *Salmonella* spp

Plasmids of sizes varying from approximately 3.0 kb to >10kb were isolated in the seven *Salmonella* species that were studied (table 4.5). Six out of seven *Salmonella* species harboured at least three plasmids of varying sizes. Six of the *Salmonella* species harboured at least one plasmid which was larger than 10 kb in size. One of the *Salmonella* did not carry any plasmid but showed phenotypic resistance to Cefixime, Ampicillin, Sulfamethoxazole-Trimethoprim and Tetracycline. Antimicrobial genes *SulII,* was detected in the six *Salmonella* isolates that carried plasmids while *aac(3),TetA* and *blaTEM* genes were detected in five *Salmonella* isolates. High frequency of phenotypic resistance was observed for Sulfamethoxazole-Trimethoprim (100%), Ampicillin (100%), Tetracycline

(100%), Cefixime (100%), Gentamycin (100%) and Ceftriaxone (57.1%). All the isolates were multi resistant to between four to seven antibiotics. However, there was no apparent relationship between carriage of plasmids and antimicrobial resistance. Plasmids isolated from the *Salmonella* isolates studied harboured resistance genes, *blaTEM, SulII,aac(3)* and *TetA* highlighting their role in transmission of resistance (Table 4.5)

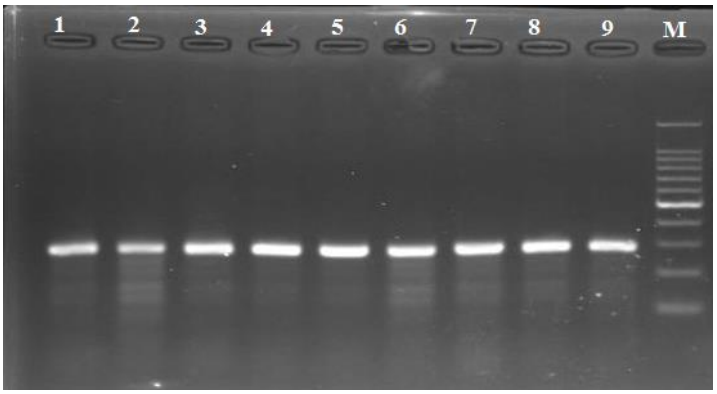

Figure 4. 14: Gel electrophoresis image demonstrating amplified *INVA* genes (284 bp) identifying *Salmonella* species.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMEDATION

5.1 Discussion

Sepsis is one of the greatest world health problems with the prevalence and death rates every year despite the gains and/or achievements in definitive diagnosis and the improved antibacterial therapy guidelines (Randolph and McCulloh, 2014). Being the most vulnerable part of the population, paediatric patients require special attention as sepsis is one of the most common causes of children's death due to infection (Kissoon *et al*., 2011). Worldwide, escalation of the awareness of sepsis is the key point to early detection and timely antibacterial treatment, which leads to better survival with both good early and late-term outcomes (Shankar-Hari*et al*., 2015).

Sepsis survival depends on timely, appropriate, and optimal antibacterial treatment. In the intensive care unit, the term "the golden hour" is applied as the treatment of critically ill children with sepsis and septic shock and is based upon early recognition and early administration of appropriate antibiotics (Khilnani *et al*., 2008). It is known that a delay in the first antibiotic administration is associated with increased morbidity and mortality (Ferrer *et al*., 2014). It's on this basis that we sought to investigate the prevalence of septicaemia, characterise the bacterial aetiological causes and to evaluate the drug resistance profiles of these agents among children visiting Mbita District Hospital, one of the region marked with a high prevalence of HIV and AIDS in Kenya.

5.1.1 Prevalence of septicaemia

The prevalence of community acquired septicaemia among the study patients was 33.9%. This prevalence was lower than that reported in Norway by Mehl *et al*., (2017) who reported a prevalence of 39.4% of community acquired septicaemia. The current prevalence was however higher than reports of other settings: In Paraguay, Guillén *et al*., (2016) reported a prevalence of 20% of community acquired septicaemia among children. In a prospective, multi-centre, hospital-based study in Italy Azzari *et al*., (2015) reported a diagnosis of sepsis in 5.3% of the children aged less than five years with fever. In Vietnam in a multi-centre point prevalence survey, Le *et al*., (2016) reported a septicemia in 26.4% of paediatric admitted at the Vietnamese pediatric ICUs. In Zanzibar, pathogenic bacteria were recovered from the blood of 14% of the patients (Onken *et al*., 2015). In Lithuania, septicaemia was diagnosed in 4% of all the patients in the paediatric ICU and was responsible for 32% of deaths (Bobelytė *et al*., 2017). In Western Cape region of South Africa, Buys *et al*., 2016 reported a prevalence of 5% of community-acquired septicaemia. Further, in Tygerberg Children's Hospital in Cape Town, South Africa, Dramowski *et al*., (2015) reported a 6.6% prevalence of septicaemia. Such variations could be attributed to heterogeneity of community set up in different regions.

Despite the difference in the prevalence, our study provided the much needed information on the burden of bacteremia among children less than five years of age with fever ≥39 °C seeking hospital care in Mbita – Suba County, a county

currently faced with one of the highest prevalence of HIV in Kenya. Thirty-nine percent cases of septicaemia among children with high fever were detected by cultural method is important for bacteremia diagnosis and should be implemented in children represented by our study population. The proportion of children with septicaemia in this region is likely an underestimation of what is expected in the entire population, since not all patients present at the hospital, and not all presenting patients were enrolled in the study.

5.1.2 Etiological agents causing septicaemia

The current study showed that *Staphylococcus epidermidis, S. aureus* and *E. coli* were the major aetiology of septicaemia among paediatrics in Mbita county hospital. Others included *P. aeruginosa, S. typhimurium, S. hemolyticus, K. kristinae, S. paratyphi A, S. paratyphi B, A. otitis, C. freundii, G. morbillorum, K. pneumonia, L. cremori, Pantoea spp, and P. putida.* This was different from those reported in other studies: Evaluating the burden of bacterial resistance among neonatal infections in low income countries, Huynh *et al.,* (2015) reported three most common bacterial isolates in neonatal sepsis were *S. aureus, E. coli,* and *Klebsiella*. In Kilifi District Hospital on the Kenyan coast Talbert *et al*., (2010) reported Klebsiella spp., Staphylococcus aureus, Streptococcus pneumoniae, Group B Streptococcus, Acinetobacter spp., Escherichia coli and Group A Streptococcus as the commonest organisms causing sepsis. In South Africa Dramowski *et al.*, (2015) reported *K. pneumonia, S. aureus* and *E. coli* as the most prevalent aetiology of septicemia. Evaluating bacteremia and invasive diseases in children aged less than five years with fever in Italy, Azzari *et al*., (2015) reported

S. pneumoniae and *H. influenzae* as the most frequently detected bacteria causing septicaemia. At the paediatric intensive care unit of the Children's Hospital, an affiliate of Vilnius University Hospital Santariškių klinikos in Lithuania, Bobelytė, (2017) reported ***N. meningitidis*** and *Staphylococcus* spp causing sepsis. In Vietnam, a study evaluating the causative agent of septicaemia showed *K. pneumoniae, P. aeruginosa, Acinetobacter baumannii,* and S. *aureus* were the major causes (Le *et al*., 2016). In Turkey Teke *et al*., (2017) reported *Pseudomonas* spp as the major causative agent of septicaemia in a tertiary paediatric hospital. In Pakistan, Mir *et al*., (2011) reported *S. aureus* as the most common pathogens causing sepsis others pathogens included *Streptococcus pyogenes* (18%); Group B *beta-hemolytic streptococci* (10 %); *Pseudomonas* spp., (8.9 %); *Aeromonas* spp. (3.2%); and *Klebsiella* spp. (2%) in that order. The results of our study together with these others show that the epidemiology of microbial pathogens causing septicaemia is divergent (changing over the years), however, majority are marked with an increase in the incidence of Gram-negative organisms (Mu˜noz *et al*., 2008; Luzzaro *et al*., 2011). This realization calls for prompt and appropriate identification in order that appropriate management is instituted so as to eliminate mortality and morbidity.

Although not the focus of the current study, existing reports shows that the annual age and gender adjusted incidence of sepsis in the USA was 0.56/1000 in all paediatric cases, and the highest age-adjusted incidence was in infants (5.16/1000), which decreased to 0.20/100 in patients aged 10—14 years (Kaplan *et al.,* 2014). In a population-based study, 56 children were identified with gram

negative bacteria caused sepsis, and the annual gender-adjusted incidence rate of gram negative bacteria caused sepsis per 100,000 persons was 129.7 in infants; this rate significantly decreased to 14.6 and 7.6in children aged 1-4 and 5-18 years, respectively (Al-Hasan *et al*., 2011). Paediatric studies on community- and hospital-acquired sepsis or bloodstream infection shows that the prevalence and aetiology depends on the various rates of underlying diseases, which vary according to the population of the health-care facility. Common underlying diseases include preterm delivery, malnutrition, hydrocephaly, meningomyelocele, convulsive diseases, neutropenia, organ transplantation, use of steroids or other immunosuppressive drugs, cancer, liver failure, congenital heart disease, neurogenic bladder, chronic kidney disease, cystic fibrosis, and malaria (Montravers*et al*., 2009; Stoesser *et al*., 2011; Marra *et al*., 2011).

5.1.3 Antimicrobial susceptibility pattern

Early diagnosis and early appropriate treatment is crucial in cases of bacterial blood infection. In severe sepsis, the case fatality increases for each hour the antibiotic treatment is delayed (Ferrer *et al*., 2014). Therefore, empirical antibiotic treatment has to be initiated before the results of blood cultures are available. However, as infections with resistant microbes is an escalating problem worldwide, it is increasingly challenging to maintain appropriate antibiotic regimens for initial empiric therapy (Nathan and Cars, 2014; WHO, 2014). Resistant pathogenic bacteria are found frequently worldwide (WHO, 2014). Studies have shown that most developing countries are home to a number of risk factors for the emergence and spread of antibiotic resistance. Misuse of antibiotics,

over-the-counter and parallel market access, and counterfeit or poor quality drugs, combined with substandard hygiene and living conditions, are the driving forces behind the emergence and spread of antibiotic resistance (Kelesidis et al., 2007). The potential for the development and rapid spread of new forms of resistance is highlighted by the recent worldwide proliferation of NDM-1-producing *Enterobacteriaceae*. The gene, which confers resistance to carbapenems, originated in India in 2009, and since 2010, NDM-1-producing *Enterobacteriaceae* have been reported in North America, Europe, and Asia (Kumarasamy et al., 2010). The World Health Organization (WHO) has recently heightened awareness of this pressing issue with calls for action to contain antibiotic resistance on a global scale (WHO. 2014). In Kenya, a regimen containing penicillin and gentamicin, plus metronidazole if an anaerobic infection is suspected has been recommended for more than thirty years in sepsis with unknown focus and etiology (Lee *et al*., 2014). In recent years, however, increasing numbers of infections with methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta-lactamase producing *Enterobacteriaceae*, and vancomycin resistant *enterococci* have been detected (Lee *et al*., 2014). Selection of inherently resistant microbes due to antibiotic use is also a challenge. Updated knowledge about the distribution of microbes in serious infection and their resistance against antimicrobial agents is needed to ensure appropriate empiric antimicrobial treatment regimens. It is also important to identify subgroups in which tailored regimens are required. This was another important aspect of this study

5.1.4 Antimicrobial susceptibility pattern of *S. aureus*

The three out of four *S. aureus* isolates causing septicaemia had the *mecA* antimicrobial gene while one carried the *SulII* antimicrobial genes demonstrating its medical significance in phenotypic characteristic resistance to Penicillins and sulfamethoxazole respectively, commonly used and available medicines in the study area. One of the four isolates was multi-drug resistant to five different drugs (Oxacillin, Amoxicillin/Clavulanate, Trimethoprim/sulfamethoxazole, Tetracycline and Erythromycin). Two isolates were resistant to four different drugs and one isolate to two different drugs. Our study further shows that 75% of the *S. aureus* were Methicillin resistant. The *S. aureus* with varying antibiotic profiles have been associated with sepsis in other settings. In Kilifi District Hospital on the Kenyan coast Talbert *et al*., (2010) reported that all the Staphylococcus aureus blood culture isolates were susceptible to methicillin. The *S. aureus* causing neonatal sepsis in Tikur Anbessa University Hospital, Ethiopia showed high-level resistance to ampicillin, cefiriaxone, cephalothin, chloramphenicol, and gentamicin (Shitaye *et al*., 2010). In Pakistan, Mir *et al*., (2011) found that all the 4.2% reported *S. aureus* were methicillin-resistant *S. aureus* (MRSA). Dramowski *et al.*, (2015), in South Africa, reported that of the *S. aureus* aetiology of septicaemia 65% of them were Methicillin-resistant. About 40% of all lethal cases of sepsis caused by *Staphylococcus spp* among children admitted at the Hospital, affiliate of Vilnius University Hospital in Lithuania were resistant to Methicillin (Bobelytė, 2017). Our study and others showing significantly high prevalence of multidrug resistant *S. aureus* is worrying given

bacteraemia due *S. aureus* are difficult to treat and are associated with 29–63% mortality (Kaasch *et al*., 2014; Fortuin-de Smidt *et al*., 2015). The emergence of new MRSA strains in the community has huge implications on patient treatment (David *et al*., 2010).

5.1.5 Antimicrobial susceptibility pattern of *S. epidermidis*

There were 3 out of 4 (75%) *S. epidermidis* that had antimicrobial gene, *mecA* and only one isolate did not carry any plasmid. All four *S. epidermidis* were resistant to between 3 and 6 different antibiotics. The strain that had no plasmid containing antimicrobial gene, *mecA* showed phenotypic resistance to Oxacilin, Amoxyclavulanic acid, Sulfamethoxazole-Trimethoprim and Tetracycline. Studies continue to report the importance of Coagulase-negative staphylococci as among the leading cause of nosocomial sepsis, especially in neonates (Marchant *et al*., 2013; Becker*et al*., 2014). Coagulase-negative staphylococci sepsis most often originates from the infection of indwelling medical devices, such as in catheter-related bloodstream infections or central line-associated blood stream infections (Vassallo *et al*., 2015). Most prominent among Coagulase-negative staphylococci infections are those due to the skin commensal *S. epidermidis* (Vassallo *et al*., 2015). However, the bacterial factors contributing to the development of sepsis, in particular in CNS, are poorly understood. Most *S. epidermidis* blood infections are caused by methicillin-resistant strains, with methicillin resistance rates even exceeding those found among *S. aureus* (Qin *et al*., 2017).

Methicillin-resistant *S. epidermidis* isolates from patients are cross-resistant to all B-lactam antibiotics, even though some might be susceptible to certain B-lactam agents by in vitro testing (Raad *et al*., 1998). Therefore, this pattern of resistance is similar to what has already been established with methicillin-resistant *S. aureus.* The prevalence of resistance has increased rapidly over the last three decades and has been attributed to the selection effect of the increasing use of b-lactam antibiotics. Studies have demonstrated an increase in the prevalence of resistant *S. epidermidis* in hospitals when isolates from 1964 were compared with isolates from 1986 or resistance and plasmid profiles (Raad *et al*., 1998). This resistance was plasmid-mediated. Some investigators attributed resistance in *S. epidermidis* to the action of the mecA gene (Raad *et al*., 1998). However, Mempel *et al*., (1994) demonstrated that *S. epidermidis* isolates could be methicillin resistant and lack mecA transcription (Fukuchi, 1994; Mempel *et al*., 1994)

5.1.6 Antimicrobial susceptibility pattern of *E. coli*

The seven pathogenic *E. coli* species harboured three types of antimicrobial gene, *SulII, blaTEM* and *TetA*. One pathogenic ETEC strain was resistant to 8 different antibiotics, 3 ETEC strains were multi-drug resistant to 4 different antibiotics. One EPEC strain was resistant to 5 different antibiotics while the remaining 2 EPEC strains were resistant to four different antibiotics. Similar studies exist showing high level antibiotic resistance to *E. coli* species isolated from neonatal septicaemia cases. Studies have showed resistance to penicillin/ampicillin ranging from 55% among *E. coli* isolates in Georgia (Macharashvili et al., 2009) to 100% among *E. coli* isolates in Uganda (Mugalu *et al.,* 2006). Resistance to gentamicin

among *E. coli* ranged from 0% in Pakistan (Mir et al., 2011), 21.7% in South Africa (Dramowski et al., 2015) and 67% in India (Jyothi et al., 2013). In Lithuania, sepsis-causing *E. coli* pathogens were characterized by the development of increasing antibiotic resistance for which initial empirical antibiotic therapy might fail (Bobelytė *et al.*, 2017).

Resistance to third generation cephalosporins ranged from 6% for *E. coli* isolates in Uganda (Mugalu et al., 2006) to 48% in India (Jyothi et al., 2013). Concerning the extended spectrum beta-lactamase production in *Enterobacteriaceae;* one reported extended spectrum beta-lactamase production in 65% of *E. coli* isolates (Jain et al., 2006). In India a study among paediatric sepsis patients attending Majeedia Hospital of Hamdard University in New Delhi showed various resistance profile of E. coli including: ampicillin (92%), amoxicillin (90.9%), amoxiclav (68.4%), cefuroxime (54.5%), cefaclor (84.2%), cefotaxime (52.9%), cefoperazone (36.8%), gentamicin (19%), amikacin (12%), netilmicin (13%), ciprofloxacin (26.1%), chloramphenicol (16.7%) and tetracycline (44.4%) (Alam et al., 2011). In Pakistan Ullah et al., (2016) reported higher level of antibiotic resistance among *E. coli* isolates including third line antibiotics; Amikacin 58%, Ciproflaxacin 67.3%, Enoxacin 83.3%, Imipenem 94.7% and Ofloxacin 77.7%. In Ghana, Obeng-Nkrumah et al. (2016) reported various antibiotic resistance among *E. coli* isolates including ampicillin (97.7%), amoxicillin clavulanic acid (53.5%), gentamicin (53.5%) and ciprofloxacin (62.1%).

5.1.7 Antimicrobial susceptibility pattern of *Salmonella* spp.

Six out of seven *Salmonella* species studied were found carrying at least three plasmids of varying sizes. Antimicrobial gene, *SulII,* was detected in the six *Salmonella* isolates while *aac(3),TetA* and *blaTEM* genes were detected in five *Salmonella* isolates. One of the *Salmonella* did not carry any plasmid but showed phenotypic resistance to Cefixime, Ampicillin, Sulfamethoxazole-Trimethoprim and Tetracycline. There was one *Salmonella paratyphi* B that was multi-resistant to five different antibiotics, three *Salmonella paratyphi* A were multi-resistant to six different antibiotics. Further, there were two and one *Salmonella typhimurium* that were multi-resistant to five and six different antibiotics respectively. Generally high frequency of resistance was observed for Sulfamethoxazole-Trimethoprim (100%), Ampicillin (100%), Tetracycline (100%), Cefixime (100%), Gentamycin (100%) and Ceftriaxone (57.1%). Previous studies confirms that the invasive forms of *Salmonella* disease include enteric fevers (typhoid and paratyphoid fevers) and non typhoidal salmonella bacteraemia and are important causes of morbidity and mortality in Asia and Africa (Brent et al., 2006; Smith et al., 2014).

In India a study among paediatrics sepsis patients attending Majeedia Hospital of Hamdard University in New Delhi showed various resistance profile in Salmonella typhi to various antimicrobials as follows ampicillin (46.4%), amoxicillin (27.3%), amoxiclav (15.4%), cefuroxime (10%), cefotaxime (25%), cefoperazone (10.5%), netilmicin (10.5%), ciprofloxacin (6.3%), chloramphenicol (9.1%) and tetracycline (37%). Salmonella typhi did not show

resistance to gentamicin and amikacin (Alam et al., 2011). On the other hand, in the same study Salmonella paratyphi A did not show resistance to antimicrobials tested (Alam et al., 2011). In Pakistan Ullah et al., (2016) reported higher levels of antibiotic resistance among *Salmonella* spp including third line antibiotics Amikacin 100%, Ciproflaxacin 66.7%, Enoxacin 66.7%, Imipenem 66.7% and Ofloxacin 83.3%. In Ghana, Obeng-Nkrumah et al. (2016) reported various levels of antibiotic resistance among *Salmonella* spp including ampicillin (63.9%) and amoxicillin clavulanic acid (23.9%). In Zanzibar, the majority of the *S. typhi* isolates (86%) were multidrug-resistant (i.e. resistant to ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol), but susceptible to cefotaxime (Onken et al., 2015).

5.1.8 Antimicrobial susceptibility pattern of *Pseudomonas* spp.

The antimicrobial gene, *blaTEM,* was detected in five of the *Pseudomonas* plasmids while four of the plasmids carried *SulII, TetA*and *aac(3)* antimicrobial resistance genes. High frequency of resistance was observed for Tetracycline (100%), Ampicillin (100%), Cefixime (80%), Cetriaxone (80%), Amoxyclavulanic acid (80.0%), Gentamicin (60.0%) and Sulfamethoxazole-Trimethoprim (60%). All the isolates were multi resistant to between two to six antibiotics. These results were similar to those in other studies. In India Alam et al., (2011) among paediatrics sepsis patients attending Majeedia Hospital of Hamdard University in New Delhi showed various resistance profile in Pseudomonas spp including: ampicillin (93.8%), amoxicillin (93.3%), amoxiclav (90.9%), cefuroxime (75%), cefotaxime (46.2%), cefazidime (38.5%),

cefoperazone (42.9%), gentamicin (33.3%), amikacin (11.8%), netilmicin (23.1%), ciprofloxacin (20%), ofloxacin (20%), chloramphenicol (40%) and tetracycline (36.4 %). In Ghana, Obeng-Nkrumah et al. (2016) reported various antibiotic resistances among Pseudomonas spp gentamicin (32.8%), ampicillin (13.1%), and ciprofloxacin (19.6%). In Pakistan Mir et al., (2011) reported no resistance to antibiotics including gentamicin among Gram-negative bacteria including *Pseudomonas* and *E. coli.* In Vietnam 56% of the sepsis causing *Pseudomonas* isolates were resistant to carbapenems (Le et al., 2016). In Turkey, 40.5% of *Pseudomonas* spp which was the major causative pathogens of sepsis in children was resistant to imipenem (Teke et al., 2016). Evaluation of the trends in antimicrobial resistance of bloodstream infections at a general hospital in Norway, showed, *Pseudomonas spp* were 100% resistant to cefotaxim and 6.9% to Imipenem (Mehl et al., 2017). This study did not evaluate these classes of antibiotics as they are unavailable or uncommon in the study setup.

5.2: CONCLUSION

1. This study identified *Staphylococcus epidermidis, S. aureus* and *E. coli* as the major aetiological agents of septicaemia among paediatrics in Mbita county hospital. Others included *P. aeruginosa, S. typhimurium, S. hemolyticus, K. kristinae, S. paratyphi A, S. paratyphi B, A. otitis, C. freundii, G. morbillorum, K. pneumonia, L. cremori, Pantoea spp, P. putida.*

2. The majority of the bacterial aetiology of septicaemia were resistant to most of the commonly used antibiotics. The majority of Gram-negative bacteria were resistant to penicillin (Ampicillins) 100% tetracycline 96.1%, sulphonamides (Trimethoprim/sulfamethoxazole) 84.6%, Aminoglycosides (Gentamicin) 73.1% while they were least resistant to Quinolone (Ciprofloxacin) 19.2%. The Gram-positive bacteria were resistant to sulphonamides (Trimethoprim/sulfamethoxazole) 96.7%, tetracycline 76.7%, penicillin (Oxacillin) 73.3% and Quinolone (Ciprofloxacin) 30%.

3. The Methicillin-resistant *Staphylococcus aureus* (mec A), Nonspecific TEM β-Lactamase (blaTEM), Tetracycline Resistant gene A (TetA), Sulfonamide resistance (SulII) genes were responsible for the development of Oxacillin, Amoxicillin/Clavulanate, Trimethoprim/sulfamethoxazole, Tetracycline, Erythromycin, Gentamicin and Ciprofloxacin among *S. aureus, S. epidermidis,* EPEC and ETEC. Further, blaTEM, TetA; SulII, and Aminoglycoside acetyltransferase gene

(aac(3) were responsible for resistance to Ampicillin, Cefuroxime, Ceftriaxone; Amoclav-Amoxicillin/Clavulanate, Trimethoprim/sulfamethoxazole, Tetracyclin, Gentamicin and Ciprofloxacin in *S. paratyphi*, *S. typhimurium* and *P. Aeruginosa*

4. The study reveals the correlation between identified mobile genetic material carrying the resistance factors and phenotypic characteristics indicating the possible spread of resistance genes among pathogenic and commensals strains.

5.3 Recommendation

1. For accurate management of Septicaemia specific aetiological agent identification is imperative given that diverse aetiological agents are implicated
2. Antibiotic resistant testing for all septicaemia agents is imperative; this will make the management of cases much easier and more accurate. It was evident that many empirical antibiotics routinely used for treatment are now facing resistance from most of the etiological agents of septicaemia in the study area.
3. Molecular markers / Genes for resistance gives an accurate picture on the possible occurrence of phenotypic antibiotic resistance. Evident enough, shown in plasmid analysis in relation to antimicrobial resistance in the

study. Further studies should consider using molecular typing of resistant genes as surrogate markers to enhance curative and diagnostic concerns.

Therefore, surveillance of prevalence, etiologies and Drug resistance is not only essential, But critical to insight of the infection and resistances plight, thus will lead to strengthen the public health as far septicemia is concerned. Future research should be able to collect quality, standardized epidemiological data along with a reliable bacteriological diagnosis and curative at the community level in order to allow for adapted public health measures necessary to combat antibiotic resistance.

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APPENDICES

APPENDIX 1: CONSENT SEEKING AND INFORMATION FORM FOR STUDY PARTICIPANTS (ENGLISH)

TITLE OF STUDY: SURVEILLANCE OF THE COMMUNITY ACQUIRED SEPTICAEMIA AMONG CHILDREN IN MBITA, SOUTH NYANZA, KENYA

Principal Investigator: GUYO H. SORA

I am a postgraduate student(Master's Degree in Infectious Diseases) at the Kenyatta University I'm carrying student research with the Mbita District Hospital and Centre for Microbiology Research, Kenya Medical Research Institute (CMR, KEMRI), which has been given the permission to study health issues affecting Kenyans with the aim of improving their health standards and welfare of Kenyans. I offer to carry out research on SEPTICAEMIA disease affecting Kenyan populations. The information from this study will be distributed to many stakeholders, Ministry of Health both county and National, KEMRI and collaborating institutions, and will also be useful to the health authorities who may form health programs in the community. This study has been approved by KENYATTA UNIVERSITY Ethical Review Committee.

RESEARCH PROCEDURES: studying the cause of septicaemia disease to know the present status of the disease between targeted groups. About 300 samples will be used to compare and analyze the clinical cause and severity of the diseases. If you agree to participate in this study, you will be asked to provide a data on yourself and consent to sample collection for study.

RISK/BENEFITS: The participants are involved in the study at their own free will and can also provide us information on their HIV status at their own free will. The information from this study is not only important for the treatment of the diseases but also will benefit your community because it will enable the different stakeholders involved in the study to recommend and design appropriate interventions to minimize the impact of the disease.

REFUSAL TO PARTICIPATE / WITHDRAWAL

It is not a must to participate in the study, your right to refuse the study or withdrawal during the study progress will be upheld. This will not interfere with the participants to request the clinician for information or treatment.

CONFIDENTIALITY: We will make every effort to protect your identity in any reports or publications of this study. Information on the participants HIV status will be held highly confidential. In order to ensure complete confidentiality of the test results, no name appear to the blood samples containers, but an ID number assigned to the participant will be used to label the sample container.

CONTACT INFORMATION: If you may have questions now or in the future regarding this study, you may ask any of the field officers involved in this study or contact above named (Principal Investigator) of NUITM-KEMRI Project at +254-20-272-0794. 0725531346.

STATEMENT: I have understood the content of this consent form, the detail of the study and the basis of the participation. I also understand that I am free to

choose to be part of the study and I can quit participating at any time if I don't want to join the study.

I have agreed to participate in this study without any force whatsoever.

Name of the participant…… Signature…….. Date……..

Name of the clinician…….. Signature…… Date……..

APPENDIX 1I: CONSENT SEEKING AND INFORMATION FORM FOR STUDY PARTICIPANTS (DHOLUO)

TITLE OF STUDY: SURVEILLANCE OF THE COMMUNITY ACQUIRED SEPTICAEMIA AMONG CHILDREN AND ADULTS IN MBITA, SOUTH NYANZA, KENYA

Thuon wach mar nonroni

En manyo yore e okang` mamalo kute makelo tuo ma ndira uradi e Kenya maimbo.

Jatelo ma tayo nonroni: Guyo H sora

Jogo makonye tayo nonroni: Wan mbalariany ma Nagasaki koa Japan kod dala maduong matimo nonro kuom weche magtouché e Kenya (**KEMRI**) Mosemi thuolo mondo otim nonro e gik machando ngima jokenya.Wayie mar timo nonro e kute makelo ndira kod touché mayudore e remo machando nyithindo mamiyo nyithindo mangeny tho. Weche mawayudo kuom nonroni wabomiyo jogo makonyo Kenya, migawo maochung ne ngima, kod jogo mawatimogo nonroni.

Chenro mar nonroni: Watimo nonro kuom kute makelo ndira,kendomondowapimchalmargi e kind jogo man kodkute mag ayakigijogomaongekute mag ayaki. Ka iyie konyowa e nonroni, wakwayi mondo ikonywa kod chalmari kuom kute mag ayaki.

Ber kata rach madibedie: Ngatnomoyiekonyowa e nonroninyalonyisowachalnekuomkute mag ayaki mana koyie ok chune.

Duokomawayudokuomnonroni ok bokonyowakuomthiethkende, kata bende onyalo konyowa kuon demawadaki enikech jogo mobirokonyo jokenya mondogilos yore maggeng`okutegimondogidogchien.Wabirotimogimoraamoramondokikngeyie andikemoroamoramawagolokuomnonroni.Duokomawayudoe chalnikuomkutemagnayakiibirokaneyomakendemaongengatoangatamanyalonge yo kata yudo.

Ornwaobok: Kainkodpenjo mora amorasani kata bange , inyalopenjongamatimononronikanyo kata inyalowuoyogi Prof. Yoshio Ichinose mar mbalariany ma Nagasaki e namba +25420272074.

Asesomoandikagiduto kata osesomna .aseyudothuolomarpenjopenjkuomnonroni kendo penjodutomasepenjoaseyudoduokomakare.Ayiemaokochunaniabirokawothuolo maramondo abed e achielkoduenonronisamoraamora ma daher.

Jachiwre:_____Seyi_____Tarik_____

Janeno_____Seyi_____Tarik_____

3.7

APPENDIX I1I: PATHOLOGICAL FORM FOR CLINICAL CRITERIA

NUITM-KEMRI Project, Nairobi Station
NAGASAKI UNIVERSITY INSTITUTE OF TROPICAL MEDICINE
Centre for Microbiology Research, KEMRI

P.O. Box 19464-00202, Next to KNH Post Office, Nairobi, Kenya TEL 020-272-5120 FAX 020-272-5144

PATHOLOGICAL INVESTIGATION (Mbita District Hospital /BLOOD SAMPLE)

Patient's Ref. No.: _____ Date of collection _____Time _____

Name of Patient/Father _____/ _____

Site of Puncture_____ Sex _____ Age _____ (year, month) Weight_____N/S _____ A (+/-) ___HDSS HOUSE ID_____

Location_____ Sub-location_____ Clan _____

Landmark (e.g. nearby school, church e.t.c) postal address_____

_____

_____Referred from_____

Clinical notes
Date of onset: _____
Symptoms: _____
Medical history: _____
Prognosis: _____

Body temp	Respiratory rate	Heart Rate	WBC count
<36˚c >38˚c	> 20/min	> 100/min	< 4000 > 12000/mm^3

NB// Two or more of the above qualities should be present or any supporting lab results or Doctors recommendation for septicaemia note.

Clinical Diagnosis _____

Specimen/Coding number _____

Dr. Name: _____ Signature _____ Date _____

APPENDIX 1V: PATHOLOGICAL RESULT FORM

REPORT OF INVESTIGATION FOR BLOOD SAMPLE

(CONFIDENTIAL) FOR MDH

DATE……

Name of patient……..Age…… (MM/YY).Sex… (M/F)……...

Collection Date……Code Number……

Blood Culture results…… (+ve)……. (-ve)……Positivity Date…….

Grams Stain Result……..

Isolates and AST conclusions……

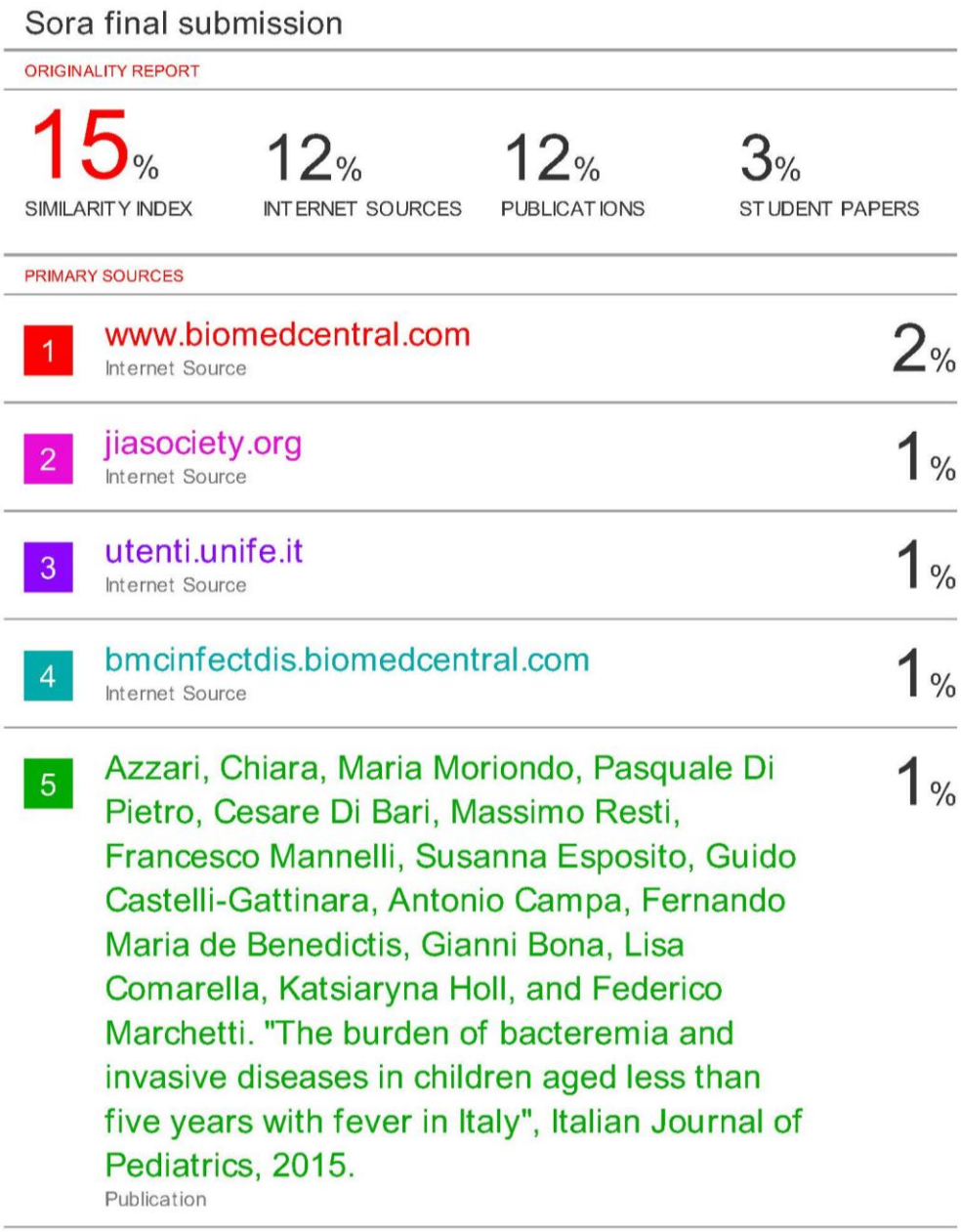
…….....

.

TECHNOLOGIST NAME……

SIGNATURE……..…… **DATE**……..……

APPENDIX V: PLAGIARISM CHECK RESULTS



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