BLOOD CELL COUNT CHANGES AND HAEMOGLOBIN GENOTYPES IN CHILDREN WITH MALARIA AND BACTERAEMIA IN SIAYA COUNTY REFERRAL HOSPITAL, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

The study is unreservedly dedicated to my cherished wife and children, who were my source of inspiration and strength when I thought of giving up, and who continually provided me with moral, spiritual, and emotional support. To my supervisors and friends who shared their words of advice and inspiration to finish this study.

And to Almighty God, for the direction, strength, supremacy of mind, protection, and healthy life. All of these, I give to Him.

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ABBREVIATIONS AND ACRONYMS

HbAA	Normal haemoglobin
AIDS	Acquired immune deficiency syndrome
HbAS	Sickle cell trait (haemoglobin AS genotype)
DIC	Disseminated intravascular coagulation
FBC	Full blood count
Hb	Haemoglobin
Hemo-AFSC	Combination of haemoglobin A, F, S and C
HIV	Human immunodeficiency virus
NTS	Non typhoid salmonella
PCV	Packed cell volume
RBC	Red blood cell
SMA	Severe malaria anaemia
SPSS	Statistical software for social science
SCRH	Siaya County Referral Hospital
WBC	White blood cell
WHO	World health organization.

ABSTRACT

Malaria and bacteraemia co-infection in children, normally produce changes in blood cellular components. Full blood count results from children whose haemoglobin genotypes and bacteraemia is not known can greatly influence the reporting of the cellular components results from automated cell counter and this formed the aim of the on the clinical management and interpretation of the results. Nevertheless, there was missing information on the role of malaria and bacteraemia co-infection on cellular components of normal haemoglobin and sickle cell trait in children. A total number of 384 children were recruited and complete blood count test were analyzed by automated cell counter machine. Other tests done included malaria smear microscopy, blood culture by Bactec 9050 machine and haemoglobin genotype determined by Helena Haemoglobin electrophoresis method. Children were stratified into two study groups; Malaria positive and bacteraemia negative and malaria positive and bavteraemia positive. Across groups analysis against region established normal ranges, showed lymphocytopnia and thrombocytopenia. Bacteria isolated were all from children with Hb genotype AS with malaria and bacteraemia co-infection with bacteraemia prevalence of 8.1% (31,of 384). The isolated bacteria species included non-*typhi salmonella* (NTS) (32%), *Escherichia coli* (3%) *Enterobacter cloacae* (6.5%), *Staphylococcus aureus* (39), *Listeria monocytogenes* (10%), *Streptococcus pyogenes* (3%) and v*iridans streptococci* (6.5%). Obtained results indicated that *salmonella species* and *staphylococcus aureus* bacteria as the most prevalent isolates associated with bacteraemia in children with haemoglobin genotype AS with malaria infection. Haemoglobin genotypes AS children are seen to be prone to malaria and bacteraemia co-infection and lymphocytopenia and thrombocytopenia being the common cellular changes seen in full blood count results. *Staphylococcus aureus* and s*almonella spp* were the most prevalent bacterial isolates. Recommended that haemoglobin electrophoresis should be considered for all paediatric patients admitted with malaria and bacteraemia co-infection with complete blood count indicating lymphocytopenia.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

In Africa malaria and bacterial disease are the most prevalent cause of death and morbidity in children less than five years of age (Church & Maitland, 2014). In sub-Saharan Africa, Non-Typhi *Salmonella* (NTS) is among the common pathogens causing bacteraemia in adults and children. (Tack *et al.,* 2020). Children NTS bacteraemia and malaria shares in relations to regional dissemination, age sets at highest threat and occurrence of the two ailments in the tropics (Balasubramanian *et al.,* 2019). The outcome of malaria on vulnerability to NTS bacteraemia was initially noted in British Guiana in the early part of the l8th century (Sikorski, 2022).

Malaria and bacteria co-morbidity are common childhood infections among children under 3 years presenting with fever of temperature above 37°C in most hospitals in Kenya (Church & Maitland, 2014). *Streptococcus pneumonia* and *Salmonella species* have been consistently reported as causes of bacteraemia in children (Bello, 2018; Takem *et al.,* 2014) . More than half of all paediatric admissions in hospitals in Africa are due to malaria and bacteraemia (Aiken et al., 2011; Muro *et al.,* 2015). Malaria is endemic throughout most of the tropics. Most malaria cases (90%) are owed to *plasmodium falciparum* (Hassen *et al.,* 2020; McCann *et al.,* 2020). About 81% and 91% of malaria sickness and demises respectively happen in the African region, where malaria remains one of the highest causes of mortality and serious illness, particularly in youngsters and expectant women and almost 86% of malaria mortality worldwide occurs in children below 5 years of age (Mwangi *et al.,* 2014). In endemic area young children and gravid women have high chances to suffer from complicated malaria than adults and non-gravid women (Morley *et al.,* 2012; Ukaegbu, 2020;

Menendez, 2006). In these countries treatment of complicated malaria and bacteraemia in children has been shown to pose a bigger challenge to clinicians (Menendez, 2006; Crawley *et al.*, 2010). In endemic areas, clinical malaria may be asymptomatic in some children or cause severe disease which can be fatal and life-threatening (Bartoloni & Zammarch, 2012). Malaria disease is associated with destruction of the host red blood cells and eventual reduction in the haemoglobin levels that leads to anaemia (Anderson *et al.*, 2018).

Previous studies have reported invasive bacterial infections being the foremost cause of bacteraemia among children in sub-Saharan Africa (Church & Maitland, 2014; Gomez-Perez *et al.*, 2014). A Study at Kilifi County referral hospital, showed that bacterial diseases in children and infants are principal cause of hospital admittances and most of the death in these populations is linked to malaria and bacteraemia (Berkley *et al*., 2005). Clinically, children diagnosed with malaria and bacterial infection have signs of splenomegaly and have high chances of developing anaemia commonly known as severe malaria anaemia (SMA) (Gomez-Perez *et al.*, 2014; Were *et al.,* 2011). Severe malaria anaemia has been studied extensively and shown to be the foremost root cause of sickness and death in youngsters in areas where *P. falciparum* malaria is holoendemic (Patel,*et al*, 2020; Gomez-Perez *et al.*, 2014).

The high frequency of the gene for haemoglobin variants especially Hb S in areas with high malaria transmission is thought to give selective advantage due to its effects on the malaria parasites once they enter the erythrocytes (Sebastiani *et al.*, 2012; Steinberg *et al.*, 2012). Nevertheless, current qualitative and quantitative tests performed in many government hospitals in Africa does not include haemoglobin genotypes test and can only identify marked haemoglobin insufficiency.

Characterization and quantitative genotype testing in young children and in a big population has not been performed before. Malaria and bacterial co-infections are quite challenging due to the fact that both disease causes severe illness and life-threatening condition in children. Due to undeveloped immune responses, for example in young children and infants tend to react with increasing body temperatures to different disease causing agents (Dupuis & Auvin, 2015).

In most cases bacteraemia due to non-typhoid *salmonella* (NTS), usually occurs during high malaria transmission in paediatrics population (Takem *et al.,* 2014; Nielsen *et al.,* 2012; Kariuki *et al.,* 2006). There is clear similarity and correlation between bacteraemia in young children in endemic region and severe malarial anemia (SMA) (Perkins *et al.,* 2011; Bronzan *et al.,* 2007). In areas where haemoglobinopathies are very common, especially in Africa and parts of western Kenya, information on haemoglobin genotype and haematological parameter profiles of children with malaria and bacteraemia co infections remain unknown.

1.2 Problem Statement

Bacteraemia and malaria co-infection in children is normally associated with high mortality rate if not detected and treated early. Co-infection usually results in misdiagnosis, and since malaria is usually the most preferred infection when it comes to laboratory diagnosis by clinicians and therefore ignoring the element of bacteraemia in co-infection (Bassat *et al.,* 2009). This is also attributed to the fact that performance of bacteraemia diagnosis requires time in isolation of micro-organism from sample collection, culture and to identification as compared to malaria diagnosis which employs rapid diagnostic techniques, including blood smear microscopy.

Bacteraemia and malaria co-infection is normally associated with deranged blood cellular components on peripheral blood smear and complete blood count test results. Previously, it has been reported that malaria infection in children worsen symptomatically after treatment and this state has later been linked to bacteraemia co-infection (Takem *et al.*, 2014; Chen *et al.*, 2016; Bassat *et al.*, 2008). Blood cellular components are vital indicators for the detection of the infections and in monitoring disease progression and treatment. Full blood counts results from patient whose haemoglobin genotype is not known can greatly influence the reporting of the cellular count test results from automated cell counter and may significantly have a contrary effect on the clinical management and interpretation of the results, which can be confusing. Despite these, the common blood cellular components changes in bacteraemia and malaria co-morbidity in haemoglobin genotype AS and AA children in children resident in Siaya in western Kenya remains unknown. As such, the current study has evaluated common blood cellular components changes in bacteraemia and malaria co-infection in haemoglobin genotype AS and AA children in in Siaya County, Kenya.

1.3 Justification

In resource limited African health facilities, malaria and bacteraemia infections cause an enormous burden. Both diseases lead in the number of inpatients cases in hospitals and together represent majority of children deaths (Takem *et al*., 2014; Okomo et al., 2019; WHO, 2011). The two diseases are the leading cause of children illness and hospitalization, which also have been shown to cause the economic burden in sab-Saharan Africa. Many young children hospitalized with bacterial infection in malaria endemic areas have been linked to probability of testing positive with malaria

parasites during the course of antibiotic treatment (D'Acremont *et al*., 2014; Scott *et al*., 2011).

Although previous research work has shown that malaria makes children susceptible to some specific bacterial infections (Roux *et al*., 2010). Malaria and bacteraemia interactions and the seasonal distribution of pathogenic bacteria have not been clearly studied. Similarly, bacterial infection in young children has been reported to trigger a more severe disease in malaria infected children (Uche *et al*., 2017). The clinical presentation of non-Typhi *Salmonella (NTS)* bacteraemia is nonspecific and, in the absence of microbiological blood culture, has been confused with other pyretic illnesses, such as malaria. Previous studies only investigated bacteraemia in malaria, bacteraemia and haemoglobin AS, malaria and haemoglobin AS and no or limited information on cellular changes in bacteraemia and malaria co-morbidity in haemoglobin AS and AA children (Crump & Heyderman, 2015). Other studies conducted in this study areas have shown have prevalence of malaria and bactereamia in children below 36 months, with invasive nontyphoidal salmonella (NTS) being prevalent (Oneko *et al*., 2015; Were *et al*., 2011; Davenport *et al*., 2016). And in regards to the aforementioned studies, information alluded in the above publications provide adequate background information on the malaria and bactereamia co-infection burden and prevalence in children in Siaya County, and hence enabled setting up of this study to investigate the missing information regarding the role of malaria and bacteraemia co-infection on cellular components of haemoglobin genotypes AA and AS in children.

1.4 Research Questions

1. What are the cellular component changes associated with bacteraemia and malaria co-infection among children in Siaya County, Kenya?

2. What is the prevalence of haemoglobin AS and AA among children with bacteraemia and malaria co-infection in children resident in Siaya County, Kenya?

3. What are the various bacterial isolates associated with malaria co-infection in haemoglobin genotype AA and AS among children in Siaya County, Kenya?

1.5 Objectives

1.5.1 General Objective

To evaluate the blood cellular component changes in malaria and bacteraemia co-infection in haemoglobin genotype AS and AA among children in Siaya County, Kenya.

1.5.2 Specific Objectives

1. To determine blood cell count levels among children with malaria and bacteraemia co-infection in SCRH, Kenya

2. To determine the prevalence of haemoglobinopathies among children with bacteraemia and malaria co-infection in SCRH, Kenya.

3. To identify bacterial pathogens causing bacteraemia among children with malaria in SCRH, Kenya.

1.6 Significance of the Study

This study has increased and reduced knowledge gap on how bacteraemia and malaria co-morbidity alters blood cellular components in children, and also has compared cellular changes in normal haemoglobin and sickle cell traits children. The generated data has given an in-depth understanding of the role played by bacteraemia and malaria co-morbidity on blood cellular components and the information will further help in characterizing different cellular parameters that will be used in predicting the malaria and bacteraemia co-infection. Information from this study will form a basis of clinical management of the paediatric patients with haemoglobin genotype AS and AA with malaria and bacteraemia co-morbidity.

Bacteraemia and malaria co-morbidity in children causes lymphocytopenia, erythropenia and thrombocytopenia cellular changes which can be used in predicting the co-infection of bacteraemia and malaria in children with haemoglobin genotype AS.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of Malaria

Malaria in human beings is initiated by five species of protozoan parasites species of the genus *Plasmodium; P. falciparum, P. vivax, P. malariae, P. ovale and P.knowlesi (*Sato *et al.,* 2021*).* The first four species above infections are specific to human, whereas P. Knowlesi is normally maintained in macaque monkeys and causes zoonotic malaria more so in South East Asian sub continent (Sato *et al*., 2021). In Africa, *plasmodium falciparum* is wide spread, while *P.vivax*, *P.ovale*, and *P.malariae* cases are geographically restricted (Yman *et al*., 2019; Autino *et al*., 2012). The malaria parasites are spread by Anopheles mosquitoes, with An. gambiae sensu stricto, An. funestus, and An. arabiensis being the most dominant in Africa (Russell *et al*., 2011). The *Plasmodium falciparum* species is leading in Africa, and produces the most severe symptoms and is responsible for most malaria deaths (Maghendji-Nzondo *et al*., 2016).

The population mostly affected by malaria are youngsters below the age of 5 years (Mutombo *et al*, 2018). *P. falciparum* is an exclusive parasite of man spread by *anopheles* mosquitoes as the vector and causes the most severe malarial disease (Wilson *et al*., 2011; WHO, 2014; Molina-Cruz *et al*., 2016). In children, *P. falciparum* infection has varied clinical presentations characterized by different signs and symptoms. This variability is in relation to species of the infecting malaria parasite, the geographical setting, and the level of immunity an individual has acquired over time (Laishram *et al*., 2012). *P. falciparum* is conspicuously prevalent in sub-Saharan Africa where it is liable for approximately 200 million clinical cases (Shibeshi *et al*., 2020). Malaria infection in children is characterized by fever,

accompanied by headache, aches and pains completely over the body. Other severe complications that may present are generalized convulsions, anemia, kidney failure, low sugar levels, respiratory oedema, fluid cations and anions, and acid base disorder, cardiovascular collapse and shock, these features may occur discretely or in combination. The impact of the disease on an individual is assessed by noting the severity of the symptoms mentioned in the previous sentence. Over the years, provision of anti-malarial drugs, ecological sanitation, application of fly spray killers and most currently, use of mosquitoes cured bed nets have been encouraged as the best methods in the fight against malaria infection. However, global eradication programs carried out over the past several decades have been greatly hampered by the general increase of drug resistance by the parasite and insecticide fight by the host vector, the mosquito (Das *et al*., 2012).

In addition to the increasing death due to malaria infection , over 213 million death toll and malarial "attacks" cause over 2 years of ill health in African child every year (Breman *et al*., 2004). The problem is compounded further by the blow-out of drug-resistant malaria parasites. Malaria disease has caused most of the affected countries to spend large amount of money in control and prevention (Rogerson *et al*., 2020). Desperate measures have resulted to shift of attention to discovery of vaccines that will confer lasting immunity, specifically in children and non-immune individuals.

Malaria parasites infection is accompanied by moderate anaemia but it can be life threatening in high parasitaemia but the fatality of the disease may be affected by patient-specific immunity and age as well as the type of the malaria parasite (Jain *et al*., 2013). Malaria associated anaemia can cause severe sickness and death in children below 5 years of age if not well diagnosed and promptly treated. Previous studies

have shown that anaemia in Malaria is due to amplified haemolysis of infected red blood cells (Jain *et al*., 2013).

Thousands of children who suffer and die from malaria related cases each year in African countries; especially in countries where malaria is endemic have bacteria co-infection (Mutua *et al*., 2015;Takem *et al*., 2014). In malaria endemic areas adult and older children have acquired mild immunity (Barry *et al*., 2011). However, young children who have not reached the age of 5 years, malaria infection tends to be more complicated with high chances of death (Lawn *et al*., 2016). In the early child life especially the first few months after birth, children have maternal passive immunity of immunoglobin-G (IgG) protection and may have mild malaria infection manifestation and low parasitemia (Adamou *et al*., 2019). Both the innate and adaptive immunity are usually involved in malaria infection. Environmental factors may also contribute to these responses. For instance, children who live in endemic areas have some anti-malarial immunity as compared to those in malaria free areas**.** The incidence of malaria changes geologically dependent on the native malaria spread strength (Komen *et al*., 2015).

2.2 Pathophysiology of Malaria

Malarial infection commences when an individual is bitten by an infected female anopheles mosquito and sporozoites of plasmodium parasite are inoculated into the bloodstream. The sporozoites are carried via hepatic circulation to liver, and where they multiplies asexually over the next 7–10 days after transmission (Soulard *et al*., 2015). The asexual reproduction of plasmodium parasites have no symptoms. The outcomes of asexual reproduction are merozoites, which emerges from the infected hepatic cells vesicles and are then circulated through the heart to the pulmonary

system. The disintegration of infected vesicle, releases merozoites which then enters the circulatory system where they invade and multiply in red blood cells and starting erythrocytic cycle of plasmodium parasite (Laurens *et al*., 2020; Ouattara *et al*., 2015).

Red cell invasion by plasmodium parasites occurs when the merozoite freed following haemolysis of an infected red cell attaches to the surface of a naive red cell (Biryukov *et al*., 2016). Within a small period of 30 to 90 seconds the incursion process of malaria parasites is usually finished. When the infected red blood cells burst, more erythrocytes are invaded by plasmodium parasites (Yahata *et al*., 2012). In malaria clinical indicators, includes fever, which occur during the rupture of infected red blood cells and the discharge of plasmodium debris, together with malarial pigment (hemozoin) and glycophosphatidylinositol, the assumed ‘malaria toxin (Ebrahimzadeh, 2019. Common symptoms in malaria infected persons include, high temperature, inflexibilities, and chills, and most of the patients with malaria dont require hospital admission. Severe malaria advances in a minority, and more so in paediatric patients and may manifest as a fever, reduced awareness, low haemoglobin, lung distress, convulsions, and low levels of blood glucose, among other symptoms (Manning *et al*., 2011). As proven, the symptoms are related with the haemolysis of the infected erythrocytes and the discharge of assumed malaria toxins, which trigger peripheral blood mononuclear cells and stimulate the liberation of cytokines. It is understood that the balance between pro-inflammatory and anti-inflammatory cytokines, chemokines, and effector molecules defines malaria severity (Perkins *et al*., 2011).

2.3 Bacteria Infections in Children

Bacteria and other pathogens are great cause of illness in school going children, whether in endemic sub-Saharan Africa or malaria free region any infection is a great danger to human health (Park *et al*., 2016; Takem *et al*., 2014). Bacterial infection and subsequence invasion of the blood components can have fatal illness, including organ failure, complicated blood coagulation disorders, and if untreated may cause death. Blood infection in human constitutes the worst stage of disease infection and brings more complication in terms of management and treatment. Alteration in local conditions at the site of the injury allows bacteria to proliferate and seed the blood stream causing bacteraemia. Reduced killing of the bacteria cells by host phagocytes is a second possible mechanism of body defence.

Bacteraemia is connected with substantial death and sickness in children (Were *et al*., 2011). Closely earlier the institution of the pneumococcal vaccine in the United Kingdom, about 20% of infant mortality was owed to infection related, with "septicemia" the greatest commonly known cause of death (Chaturvedi *et al*., 2016). Bacteria commonly isolated from the blood cultures normally consist of; Coagulase-negative *staphylococcus* (CoNS), *Staphylococcus aureus, Viridian streptococci, Enterococcus* spp*., Beta-haemolytic streptococci, Streptococcus pneumonia, Haemophilus spp, Neisseria* spp, *Enterobacteriaceae* and anaerobic bacteria such as *Bacteroides and Clostridium* spp.

Blood is normally a sterile environment, and the detection of malaria and bacteria is always abnormal. *Salmonella* species causes vast worldwide burden of sickness and death in patients. In human, *salmonellae* infections are separated into typhoidal serotypes; *Salmonella typhi* (*Salmonella enterica* var *Typhi* and *Salmonella paratyphi*

(*Salmonella enterica* var Paratyphi A) and non-*typhi salmonella* (NTS). In developed countries, non-*typhi salmonellae* are major cause of self-limiting diarrhoeal illness in healthy individuals (Haeusler *et al*., 2013; Smith *et al*., 2016). In Africa, the serotypes of *Salmonella* that mostly cause invasive non-*typhi salmonella* infection are *S. Typhimurium* and *S. Enteritidis*, and are often connected with a wide host range and with enteric disease (Morpeth *et al*., 2009; Branchu *et al*., 2018). Non-typhi *salmonell*a (NTS) bacteraemia has been connected with other infectious diseases, especially HIV and lack of proper diet, and cases of malaria infection in young children (Kariuki *et al*., 2016; Morpeth *et al*., 2009). In several publications in Africa that preceded the human immune deficiency virus (HIV) epidemic, non-*typhi salmonella* (NTS) were considered as a predominant root cause of children bacterial infection (Ao *et al*., 2015; Mtove *et al*., 2010). And in children with malaria, invasive non-typhoidal *salmonellae* were first noted in 1987 (Feasey *et al*., 2012). Two cases of African grown-ups with AIDS and *Salmonella enterica* var *Typhimurium* (*S. Typhimurium*) bacteraemia were reported in Belgium, Just after AIDS discovery in 1983 (Feasey *et al*., 2012; Owen *et al*., 2017). An investigation done on households file cases of children with invasive non-typhoidal salmonella disease in Kenya (Kariuki *et al*., 2020), indicated that 6·9% of the study subject had non-*typhi salmonellae* in their faeces as carriers and based on molecular analysis done on the same patients, about 66% of isolates were of comparable strains (Kariuki *et al*., 2020).

Invasive salmonellosis in sub-Saharan Africa is not well understood despite much work on prevention and diagnosis. Particularly of relevance is the NTS sepsis which has not been widely studied in the past decade. This inadequacy of knowledge and capacity may be attributed lack of adequate bacteriological experts and facilities in African government hospitals. Proper and well-structured survey on invasive

Salmonellosis in developing countries is necessary to assist in diagnosis, treatment and well developed guidelines in the management of this disease (Njume *et al*., 2012). As such, the current study will give more insight and information on the common bacterial pathogens isolated from children with malaria and bacteraemia co-infection and their combined effects on blood cellular components.

2.4 Pathophysiology of Bacteraemia

Bacteraemia is well-defined as the existence of multiplying bacteria in the circulatory system and can occur in everyday undertakings like mouth washing, tooth brushing and minor medical techniques like dental work and infection (Grealy *et al*., 2011). In the first phase of bacteraemia, infection is usually a brief and biologically nonthreatening illness and the host immune mechanisms fights to eradicate the microorganism from the blood (Brady *et al*., 2020). Conversely, when the immune protection mechanisms flops or in the existence of anatomic lesions, disconcerted cardiac blood flow and foreign material, bacteraemia can result to infection and septicaemia (Christaki *et al*., 2014).

When microorganisms achieve to escape the host immune mechanisms or when the immune system flops to resist bacterial infiltration due to intrinsic or acquired immune faults that are connected with vulnerability to infection, bacteraemia may also advances to sepsis. The host genetic signature may also influence pathogenesis characteristics of bacteraemia (Van der Poll *et al*., 2009).

2.5 Cellular Components Changes Associated with Bacteraemia and Malaria Co-Morbidity

Previous finding showed that malaria predisposes infected children to invasive bacterial infections (Scott *et al*., 2011; Bassat *et al*., 2009; Church *et al*., 2014;

Lokken *et al*., 2018; Mtove *et al*., 2010) . Since malaria and bacteraemia co-infection in a host may trigger some sort of immunological reaction to exert effects on their presence in the body. Recently, infection with malaria parasites in children was linked with bacterial infection especially children living in areas where there is wide spread of malaria transmission throughout the year (Mackenzie *et al*., 2010; White, 2018). It is not well understood if there are any alteration in leucocytes and platelets morphology and counts in malaria infected children living in areas with high parasite inoculation (Schofield *et al*., 2005; {RÃ©nia *et al*., 2012).

Thrombocytopenia and anaemia with increasing parasitaemia has been noted in past studies (Kotepui *et al*., 2010; Maina *et al*., 2010). Platelets have been shown to cause formation of complexes with immune cells during malaria infection. These immune complexes proceed to bind to the vascular walls and subsequently damage the endothelial surfaces and cause vascular malfunctions. Direct interaction of malaria infected red blood cells induces platelets activation and aggregation leading to thrombocytopenia (Cox & McConkey, 2010; Guo *et al*., 2019). The lysis of these complexes may also occur and has been shown to affect normal uninfected red blood cells which may lead to anaemia (Gwamaka *et al*., 2011). These blood changes linked to malaria parasite infection have been previously studied, but other leucocytes and platelets alteration may be associated with environmental factors malaria parasite consistency in an area, red blood cells abnormalities, malnutrition and immune status (Kimbi *et al*., 2013).

Infection with malaria has displayed changes in leucocytes counts and leucogenesis especially low neutrophil count, immature and increased neutrophils counts. Malaria also affects the lymphocytes counts and morphology. Other cells which may be

affected by malaria parasite infection include monocytes and eosinophils (Kotepui *et al*., 2015). Alteration of these haematological components are part of the complex complication caused by malaria infection and has been reported to affect multiple organ functions. The most commonly affected cells include erythrocytes, white blood cells and platelets (Maina *et al*., 2010). The pathological changes associated with malaria infection in children include severe anaemia, thrombocytopenia, and leukocytosis (Akhtar *et al*., 2012; Birhanu *et al*., 2017).

Infection with malaria parasites has several key diagnostic indicators in children who have fever including low platelets count and low white blood count (Maina *et al*., 2010). The blood cellular change that occurs in malaria and bacteraemia co infection has not been fully illustrated and currently there is scanty or negligible information pointing at some common cells changes occurring in the co infections. Demonstration of cellular changes that frequently exist in children suffering from malaria and bacteraemia co-infection will be of paramount importance in aiding prompt clinical diagnosis and management of children with malaria and bacteraemia co-morbidity.

2.6 Haemoglobin Genotypes AA and AS In Malaria and Bacteraemia Co-Morbidity

Haemoglobin is a cellular protein in the red blood cells, containing 4(four) polypeptide globin chains, individually crumpled around a 'heme' molecule. The main function of haemoglobin is conveyance of oxygen from lungs to the muscle and other tissues, and carbon dioxide from tissue to the alveolar cells of the lungs. Haemoglobin genotypes normally includes normal haemoglobin HbAA and fetal haemoglobin(HbF), and other abnormal haemoglobins collectively referred as haemoglobinopathies like HbAS, HbAC, HbSS, HbAC and HbCC and SS. The study

focused on Hb AA and AS since they are the most prevalent haemoglobinopathies in the study area as illustrated by previous studies on malaria in western Kenya (Ahmed *et al*., 2020). The globin chains are determined by their respective genetic factor existing on the quantified trait locus (QTLs) on chromosome 11 and chromosome 16 and both recognized to have numerous alleles. The haemoglobinopathies which are abnormal haemoglobin genotypes arise when an affected individual inherits altered globin gene of haemoglobin S, C, D, E, from both parents.

It is estimated that the world-wide economic problem of the haemoglobinopathies on public health will surge over the coming years. Haemoglobin S in heterozygous and homozygous state, Hb AS and Hb SS respectively usually results in sickling disorders that results in sickle cell anaemia. Other haemoglobinopathies heterozygous compounds of Hb S that includes Hb SC, SD & SE also cause sickling disorders. Haemoglobin S occurs when there is substitution of valine for glutamic acid at position 6 in the β – chain of normal haemoglobin A (Kaur *et al*., 2013). Sickle cell disease (SCD) is a haemoglobinopathy initiated by autosomal recessive genetic blood disorders and deletional mutations on the haemoglobin alpha and non-alpha chains leading to Haemoglobin S (Hb S) within red blood cells. People who suffer from SCD have abnormal diminished spleen function that makes them more susceptible to infections (Vichinsky *et al*., 2016) . Haemoglobin S and other haemoglobinopathies are linked to confer some protection from malaria parasite infection or the clinical manifestation of malaria (Taylor *et al*., 2012). Haemoglobinopathy protection from malaria has been revealed in different cohorts suggesting that Haemoglobin S has a big role in the way the parasite infect and survive in the infected red blood cells and affecting the manifestation of clinical malaria (Lopera-Mesa *et al*., 2015). Currently there is shortage of data on the haemoglobin genotypes in malaria and bacteraemia

co-morbidity in Siaya county and Kenya at large, hence propelling the need for this study to bridge gap in the knowledge and also increase data relating to cellular changes occurring in malaria and bacteraemia co morbidity in children of haemoglobin AS and AA.

In some studies, it has been reported that people with HbAS are more protected from bacterial infections (Scott *et al*., 2011). Further research work done by Taylor and Parobek have presented data that Hb SS weaken the ability of *P.falciparum* to thrive in the red blood cells by reducing oxygen tension hence protective against malaria disease (Fairhurst *et al*., 2012; Taylor *et al*., 2015). The presence of sickle cell trait provides up to 60% protection against malaria in children less than 16 months of age (Gong *et al*., 2012; Uyoga *et al*., 2019). These alterations in the haemoglobin are prevalent in malaria endemic areas and are thought to be host protective mechanisms against malaria infection.

Haemoglobin AS enhances faster clearances of the infected red blood cells from circulation upon the sickling of the infected cells and their clearance from circulation and thus offering protection against malaria infection in individuals (Su *et al*., 2019). Following complications that includes chronic pains and recurrent painful incidences of musculoskeletal tissues, stroke, pulmonary hypertension and septicaemia routinely occurs in Sickle cell disease (SCD) and causes complications in several body organs. These complications often co-exist, distressing the quality of life for patients, and if untreated, they could lead to death. Musculoskeletal complications in sickle cell children is normally due to avascular necrosis, osteomyelitis, and septic inflammation of the joints (Vaishya *et al*., 2015).

2.7 Bacterial Pathogens in Malaria and Bacteraemia Co-morbidity

In earlier studies defined outcomes from their research work piloted in Nigeria and Uganda, that *S. pneumonia* bacteria was accountable for merely 10% of all bacteraemia, with *Staphylococci*, *E. coli* and *Klebsiella* accountable for the bulk of septicaemia (Bereket *et al*., 2012). An association between malaria and nontyphoidal salmonella septicaemia was first noted in British Guiana (Crump *et al*., 2015). Salmonella septicaemia was found to be more common and more severe during malaria outbreaks than other disease. In malaria parasite infections in children, the bacteria which are commonly isolated from blood included *non-typhi salmonella* (NTS) and other bacteria which have similarity in cell wall structure (Were *et al*., 2011; Crump *et al*., 2015; Mtove *et al*., 2010). The risk group that suffers from non-typhoidal *salmonella* with no associated diarrhoea, include patients who are immuno-suppressed of HIV infection, on steroid use, cancers, chronic renal or liver disease, diabetes, or Hb SS, and aged and children. The association of bacteraemia and *P. falciparum* infection in young children has not been explained in early translocation of enteric bacteria.

Low immune responses and enhanced production of red blood cells have been reported as linkage to enhanced probability to bacteraemia in *P. falciparum (CÃ©spedes et al., 2020;*,Gomez-Perez *et al*., 2014). Non-typhoid salmonellosis and *P. falciparum* infection are closely associated in terms environmental distribution and age of the infected hosts (Lokken *et al*., 2014;Crump *et al*., 2015). Identified precise risk factors to progress non typhi s*almonella(NTS)* bacteraemia include children, malaria infection, severe malarial anaemia(SMA), hepatosplenomegaly, human immunodeficiency virus(HIV) and severe malnutrition (Gilchrist *et al*., 2019). The risk of *P. falciparum* infection and susceptible infection with non-typhoid *salmonella*

bacteria was reported in parts of British Guiana (Takem *et al*., 2014). Bacteria diseases in children below 5 years is one of the common infection suspected in most public hospital, but it has been a big problem particularly when it is accompanied by bacteraemia co-morbidity. Former studies on paediatrics and bacterial infection were more closely associated with symptomatic children and were done before guidelines on screening of mothers for *group B Streptococcus* (GBS), and most of them were qualitative studies (Esposito *et al*., 2018). In susceptible population around 1.1 - 5.9 percent of febrile children with bacterial infection have been diagnosed with bacteraemia (Berkley *et al*., 2005), but the rate and co-infection with malaria parasites in this cohort remains unknown.

Children with sickle cell disease are greatly vulnerable to bacterial infections and are the major cause of children sickness, hospital admission and mortality. Understanding blood cellular changes that occur in malaria and bacteraemia co-morbidity can be a milestone in managing diseases which are responsible for most of mortality and morbidity in children below five years. The study evaluated common cellular patterns that occur in the two diseases and their eventual application in clinical diagnosis of the co-morbidity of malaria and bacteraemia. Few studies have looked at hematological changes occurring in malaria and bacteraemia in isolation and no study had so far examined blood cellular changes depicted in malaria and bacteraemia co-morbidity and this formed the basis of this study.

2.8 Laboratory Diagnosis of Malaria

Malaria effect is worldwide spread-out and comprises the extremes of the healthcare system stretching from global tourists going back to non-malaria countries with tertiary referral medical care and to citizens in hyper-endemic areas lacking or with

limited access to health care. Prompt and accurate diagnosis of malaria is required to control the escalating global impact of malaria related with ever-increasing resistance to antimalarial drugs and related death losses arising from malaria infection. Functioning treatment of malaria disease as per the WHO has concentrated on the use of insecticide dried mosquitoes nets, in-house lasting insecticide spraying, and intermittent malaria prophylaxis in pregnancy, and artemisinin-based combination therapy (ACT). In improving the treatment of patients infected with plasmodium parasites, it is important to do timely and precise testing to avert superfluous sickness and death, while circumventing unwarranted use of malaria drugs and also decreasing the emergence of malaria drug resistant parasites. Malaria diagnostic approaches need to be more effective both in resource constraints countries where malaria has a significant burden on society and also in industrialized nations where know-how in the identification of plasmodium parasites is often missing (Hofmann *et al*., 2015). Previous guidelines on malaria treatment focus on medical investigative procedures, microscopy of malaria, and empiric treatment. The exactness of medical diagnosis, which was the best commonly working system, is improper, even in countries where malaria is hyper-endemic due to coinciding clinical symptoms with other tropical diseases such as bacteraemia and the point that coinfections can occur (Harmen, 2009).

Microscopy identification of malaria remains the gold standard in ascertaining malaria parasites as it can provide evidence on both the parasite specie and parasitaemia (*Berzosa et al.*, 2018). The problems associated with malaria microscopy are expanded in non-endemic regions where use of light microscopy to detect plasmodium parasites is irregularly done, causing overlooked diagnosis, improper

identification of *Plasmodium* species, and delays in treatment (Berzosa *et al*., 2018; Mathison *et al*., 2017).

The examination of Giemsa or Field-stained thick and thin blood smears under the light microscope are some of the routinely performed procedures in the diagnosis of malaria. Finger prick blood sample is the best sample but blood gotten by venipuncture and preserved in heparin- or ethylene diamine tetra acetic acid (EDTA)-flashed coated tubes is acceptable if used shortly after blood is drawn (Mathison *et al*., 2017). Quantification and identification of malarial parasites by microscopy in most laboratories normally involves preparation of both thick and thin blood smears. In summary, blood smears is accepted as the currently blood smears are universally accepted as gold standard method, and where quantification of parasites is done by counting parasites in thick smears.

Limited clinics examining blood smears from a large number of patients suspected of having malaria are often inadequate by small numbers of trained microscopists or Medical Laboratory personnel's and required equipment, for example microscopes. Subsequently, malaria diagnosis is habitually made only on the basis of clinical symptoms whereas this is, at best, 50% accurate (WHO and CDC, 2010).

2.9 Laboratory Diagnosis of Bacteraemia

One of the most serious functions of clinical microbiology laboratories is bacteraemia diagnosis . In general, bacteraemia diagnosis can be done through application of conventional blood culture methods (manual systems such as biphasic blood culture, lysis filtration–centrifugation, manometer methods, and automatic systems, both radiometric and non-radiometric) provide results within 2 to 5 days. Incubation period of over 5 days is not usually mandatory with modern continuous automated

monitoring methods such Bac Alert or Bactec machines (Olga *et al*., 2019). The outcome of laboratory results provides a major health decision in the management of patients. Clinical chemistry and haematology sections of the laboratory provides various diagnostic tests whose results can be given out within hours of admission into the hospital (Opota *et al*., 2015). A blood culture is well-defined as growth of blood obtained from a single vein blood collection, the blood collected may be injected into one or into many bottles or tubes (Patton *et al*., 2010). When a bloodstream infection (BSI)/ or bacteraemia is suspected, microbiological culture linked analysis remains the reference standard in identifying the causal agent. When an antimicrobial treatment is commenced, blood culture is not ideal method of diagnosis, and also for diagnosis of uncultivable microorganisms. The best method to detect the implicating pathogens when a blood system infection is alleged, blood culture remains the best method of detection and also provides assurance in the choice of satisfactory antimicrobic treatment. Blood culture sensitivity and specuficity has been enhanced in the last years to reduce the turnaround time (TAT), in detection and identification of blood cultures isolates.

Bacterial infection that results in septic syndrome are associated with morbidity and mortality (Mei *et al*., 2010). Laboratory culture-based methods of diagnosis of bacteraemia remains the gold standard method, and has been used for many decades. The most significant recent advancements have occurred with antigen based assays, whereas the role of nucleic acid amplification tests has yet to be fully clarified. Routine collection of specimens for microbiological culture is carried out before the beginning of empiric antibiotic treatment to downplay fears of under treatment (Wang *et al.*, 2014; Levy *et al*., 2018).

The number of microbes presents in the circulatory system during bacteraemia ranges from one (1) to ten (10) CFU/mL [12–15] to 1×10^3 and 1×10^4 CFU/mL (Bacconi *et al*., 2014). Blood cultures, present days represent the main method in defining the aetiology of bacteraemia, because of their high sensitivity and simple to perform. The volume of the blood sample collected for culture determines the culture yield/or outcomes of isolates. For adults, one blood sample of up to 20 mL of venipuncture is used to inoculate two bottles (one aerobic bottle and one anaerobic bottle). To spot a causal agent in 80% to 96% of bacteraemia prior to antibacterial treatment, about two to four blood cultures samples are required, , i.e. blood sample volumes of between 5mL to 20 mL (Riedel *et al*., 2008). The rise in the percentage of contaminants is highly seen in small blood sample volumes collected or might be connected with difficulties in keeping germ-free conditions due to poor venous access common scenario in paediatric blood culture collection (Gonsalves *et al*., 2009). Remarkably, peripheral vein puncture, arterial access or central venous entrances are associated with different contamination rates of 36%, 10% and 7%, respectively (Gonsalves *et al*., 2009).

About one-third of growth in blood cultures due to blood culture contamination can arise when microorganisms from external environment that are not existing in the bloodstream are introduced into the culture bottle in the blood during collection process and sampling (Gonsalves *et al*., 2009). Though blood cultures are collected under aseptic conditions, contaminations may occur due to environmental organisms present on the skin as skin flora, such as coagulase-negative staphylococci (CoNS), being most prevalent microorganism identified in blood cultures in laboratory performing blood culture. Blood culture contamination is common and very expensive for the health system, and frequently complicates clinical decision in

patient treatment. To reduce the risk associated with blood culture contamination with the normal skin flora, proper skin decontamination process ought to be done thoroughly before venipuncture. Maintenance of blood culture contamination rate at less than 3% is required in routine performance of blood cultures. Blood culture contamination rate higher than 3% should be investigated with proper tool for root cause analysis and corrective action and corrected with educational efforts (Garcia *et al*., 2015). And rate of blood culture contamination can be monitored as quality indicator or improvement project as part of routine quality management system implementation in the line with ISO 15189 accreditation and certification of the Microbiology Laboratory/ or section.

Most Standard culture bottles have been designed for aerobic and anaerobic growth conditions, and contains rich culture to growth in both conditions respectively. They are intended for up to 10 mL of patient blood. However, because of the struggle of obtaining large volumes of blood, specific paediatric blood bottles have been planned for the culture of volumes <3 mL. To counterbalance antibiotics given preceding to sampling, Charcoals or resins have been introduced in specific culture bottles to counter balance antibiotics given to patients prior to blood culture collection. To promote the retrieval and growth of microorganisms that have been endocytosed by phagocytes, lysing agents are usually supplemented in some growth media. Normal culture growth time is usually 5 days, that is ample for the retrieval of the common organisms comprising the HACEK group of fastidious bacteria (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikuenella and Kingella*) bacteria and Brucella spp (Ravest *et al*., 2016).

2.10 Laboratory Diagnosis of Haemoglobin Genotypes

Haemoglobinopathies are the joint latent monogenic conditions globally, and contributes to a substantial global disease burden in the management of affected people (William *et al*., 2016). Production of the protein-globin chains of haemoglobin is directly affected bydifferent types of genes, and end in transformed synthesis of haemoglobin resulting in haemoglobinopathies that includes thalassaemia, persistent circulation of Hb F, Hb SS, haemolytic anaemia and rarely cyanosis. Most haemoglbinopathies interconnects to each other and produces a wide variety of haemoglobin abnormalities of variable degrees of severity (William *et al*., 2016). Hb S and Hb E are medically more significant than the alfa-thalassaemias are linked to beta thalassaemia and other related conditions are caused by modification of haemoglobin, and their potent forms warrants lifetime clinical treatment and management in many affected people (Traeger *et al*., 2014).

Haemoglobinopathies carrier detection and diagnosis represent valuable procedures that ascertain children with high chances of syndrome, and this offers them with choices to have healthy life. There are numerous methodologies employed to detect haemoglobinopathies. Disorders of haemoglobin gene are inimitable among all genetic diseases, in that identification of carrier traits is necessary by haematologic and clinical chemistry tests either, than with gene analysis. In most countries, including Northern and Western Europe, the conventionally non-endemic countries warrants haemoglobin traits identification and before birth finding of the haemoglobinopathies due to global migration of population. Presumptive identification of abnormal hemoglobin requires use of two methods. Haemoglobin electrophoresis at alkaline pH 8.6 using cellulose acetate films and haemoglobin

electrophoresis at acid pH 6.0 using agarose or citrate agar gel are two methods used in the identification of abnormal haemoglobin (Stephens *et al*., 2012).

Haemoglobin variants are diagnosed using DNA based testing procedures that uses polymerase chain reaction (PCR). Protein gene variations in individuals can be detected using various PCR based methods. The selection of particular PCR by a Laboratory detection of protein gene base variants is determined by selected PCR method and depends on the practical skills available and also on the form and variety of the modifications dominant in the study population (Traeger *et al*., 2014).

Detection of abnormal haemoglobin by screening methodology forms a probable base for identification of the variant haemoglobin. Phenotypic analysis does not detect some rarer condition and this has to be acknowledged during interpretation of results. Screening methods of haemoglobinopathies by haematological methods come first before genetic diagnosis (Traeger *et al*., 2014). EDTA anticoagulant is used in collection of blood samples for complete blood count, haemoglobin forms analysis and measurements (Traeger *et al*., 2015). Most clinically important Hb variants such as ; Hb S, Hb C, Hb E, Hb D-Punjab and Hb O-Arab are clinically important Hb variants which can be identified by hybridization and sequencing techniques, however Hb C which can also be detected by polymerase chain reaction(PCR) (Traeger *et al*., 2015).

2.11 Measurement of Blood Cellular Components

Electronic or automated measurement is recommended for blood cellular components, especially for red blood cell indices (Traeger *et al*., 2015). Red cell diameter width (RDW) can used to differentiate between thalassaemia carriers and iron deficiency and also between thalassaemia traits and a thalassaemia disorder or other rare causes

of microcytosis, as indicated by decreased mean cell volume (MCV) and being a quicker way of detecting anisocytosis though not comprehensive. To differentiate between iron deficiency and haemoglobin variants, red blood cells counts and morphology can also be used as differential marker. Respective laboratory should come up their own limit points for red cell indices based on the local population and sick people age group(s). Complete blood count (CBC) performed in samples more than 1 day old after blood collection ought be made with caution, since MCV changes, however no changes occurs in MCH which is stable in the blood for up to 5 days at storage temperature of 4–20 ^{0}Celsius (Buch *et al*., 2011).

2.12 Malaria in Children

Malaria is quiet a main cause of demise and severe sickness amongst children in many portions of tropical Africa, but only a small percentage of children, possibly 1–2%, who become ill with malaria develops severe disease (Carneiro *et al*., 2010). Studies based on modelling have predicted that, while the problem of malaria cases will move to children above 5 years with declines in spread, the most severe outcomes will remain to predominantly affect the under 5 years children (Carneiro *et at*., 2010). A current 25-year investigation of data in Kenya where prediction of malaria with highest positivity index was detected to rise marginally to children older than 5 years, and the median age of hospital admission doubled considerably from age of 2 to 4 years (Mogeni *et al*., 2016). Vectors of malaria are also getting more flexible in their host preference as *An. gambaie sensu stricto*, formerly thought to be highly animal preference, has been revealed to take nearly half of blood meals from other hosts including human being *(Ndenga et al.,* 2016). Malaria epidemiology varies geologically conditional on the local intensity of malaria spread or level of malaria endemicity (Getting *et al*., 2011). World health organization has estimated numbers of

deaths arising from malaria to be 395,000 in Africa in 2015, though this numbers may be uncertain due to underreporting (Idris *et al*., 2016).

Based on the overwhelming effects of malaria, the origin of the malaria parasites infecting humans has extensively been a topic of concern. Accounts of malaria-like sickness can be found in ancient texts from China, India, the Middle East, Africa and Europe, demonstrating that people have been battling *Plasmodium* infections throughout much of our recorded history (Loy *et al*., 2017). Resistance associated to *Plasmodium* infection and malaria-associated disease among human genomes, are estimated to be thousands of years old (Loy *et al*., 2017). African inhabitants have unique variant of sickle cell trait that protects against fatal *P. falciparum* malaria (Loy *et al*., 2017).

2.13 Bacteraemia in Children

Bacteraemia, pneumonia, meningitis, and septicaemia in African children are caused by invasive bacteria (Church *et al*., 2014). Bacteraemia in children below 5 years in many portions of the biosphere, including Africa is caused non-typhi *salmonella*, being the most important enteric pathogen (Morpeth *et al*., 2009; Kariuki *et al*., 2015). *Salmonella* species are a common cause extra intestinal infection resulting in severe sickness and death among young children in tropical Africa (Kirk *et al*., 2015) . Malaria and anaemia in non-typhoidal *salmonella* is often complicated in diagnosis and results in delayed clinical treatment of patients (Feasey *et al*., 2012). NTS was shown to be the most common bacteria isolate from blood culture in children who remained febrile after clearance of malarial parasitaemia in a previous study done in Central Africa Republic (Takem *et al*., 2014). Non-typhoidal *Salmonella* spp.(NTS) was the most common *Enterobacteriaceae* spp. and accounted for nearly all gram

negative bacteria isolates (86.2%; 25 of 29) in his previous study (5.7%; 29 of 506) (Were *et al*., 2011). As noted by Mulholland and Adegbola in their study in rural communities, that bacteraemia may be even more significant cause of death among children than it is in a hospital setting, as the management of bacterial illness in the community is expected to be less effective than the management of malaria (Mulholland *et al*., 2005). Risk of bacteraemia is increased among children admitted to the hospital is connected to HIV and malnutrition, however only 18 percent of children admitted with bacteraemic illness were infected with HIV, and 37 percent with malnutrition, suggesting that the latter is a more important cofactor (Berkley *et al*., 2005).

2.14 Bacteraemia and Sickle Cell Anaemia

The key cause of sickness and death in children with sickle-cell anaemia are bacterial infections. Numerous bacteria, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, and non-typhi *Salmonella* species, have been identified as important causative agents through studies undertaken in the USA (Makoka *et al*., 2012). It is also recognized that *streptococcus pneumoniae* is much higher in children with haemoglobin SS having sickle cell anaemia (Williams *et al*., 2012). The introduction of penicillin based prophylaxis and vaccination with conjugate vaccines directed against *S pneumoniae* and *H influenzae* type b have led to considerable improvements in the treatment of patients born with sickle-cell anaemia in industrialized countries and reduced mentioned bacteria prevalence (Sobota *et al*., 2015).

In Africa, about more than 80% of all children with this sickle cell disease are native (Uyoga *et al*., 2019), available data suggest that the range of organisms causing invasive bacterial disease in African children with sickle-cell anaemia might differ

from that defined in the earlier studies (Uyoga *et al*., 2019). And because of no or limited information on the bacterial pathogens in malaria and bacteraemia co-infection in children with haemoglobinopathies, calls for further studies to that will provide more information to guide policy makers in health care system.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study was done in Siaya County referral hospital in western, Kenya (Appendix 3, (Ong'echa *et al*., 2006). Siaya County experiences an equatorial climate and is located at an altitudes range between 1145m - 1420m above the sea level. Malaria is reported to be primary cause of the childhood morbidity and mortality (Ong'echa *et al*., 2006).

The mosquito vectors responsible for malaria transmission across this region are *Anopheles gambiae, Anopheles. arabiensis and Anopheles funestus (*Wamae *et al*., 2015). People living in this area receive 100–300 mosquito bites every year and are likely to be infected with malaria parasites bites (Ong'echa *et al*., 2006). In addition, recent review of hospital records in 17 communities across East Africa between 1990 to 2007 illustrated that >98% of the population residing in Siaya County experience high *P. falciparum* transmission resulting in over 40% hospital admission being malaria related sickness and mainly affecting infants and young children <5 years (Okiro *et al*., 2009). Most malaria infections and mosquito population increase in rainy seasons between months of March to late July.

3.2 Study Design

A cross-sectional study design was adopted, where children under the age of 3 years (3-36 months) and presenting with fever were recruited at Siaya County referral hospital.

3.3 Sample Size

Sample size of 384 children was determined using Naing formula (Naing *et al*., 2006)

$$n = \frac{Z^2 \times P\ (1-P)}{d^2}$$

$$n = \frac{1.96^2 \times 0.5\ (1-0.5)}{0.05^2}$$

n = size of the sample,

Z = Z statistic for a level of confidence, at 95%, standard value of 1.96

P = anticipated prevalence or proportion

(In proportion of one; if 50%, P = 0.5), and

d = precision (in proportion of one; if 5%, d = 0.05).

n=384

3.4 Sampling Procedure

Purposive (purposing) sampling method was used and children with body temperature (fever) of above 37°C were recruited into the study. Recruitment was done by a trained clinical officer in conjunction with a laboratory technician.

3.5 Inclusion Criteria

Children aged between 3- 36 months of both sexes were eligible for participation in the study, Children who had malaria parasitaemia and haemoglobin (Hb) <11.0g/dl. Presence of bacteraemia and abactereamia in children

3.6 Exclusion Criteria

Children hospitalised prior to study enrolment. Any previous history of severe malaria anaemia (SMA) prior to baseline (enrolment). Children who had history of any

acquired immunodeficiency syndrome (AIDS)-defining events and evidence of acute respiratory infections.

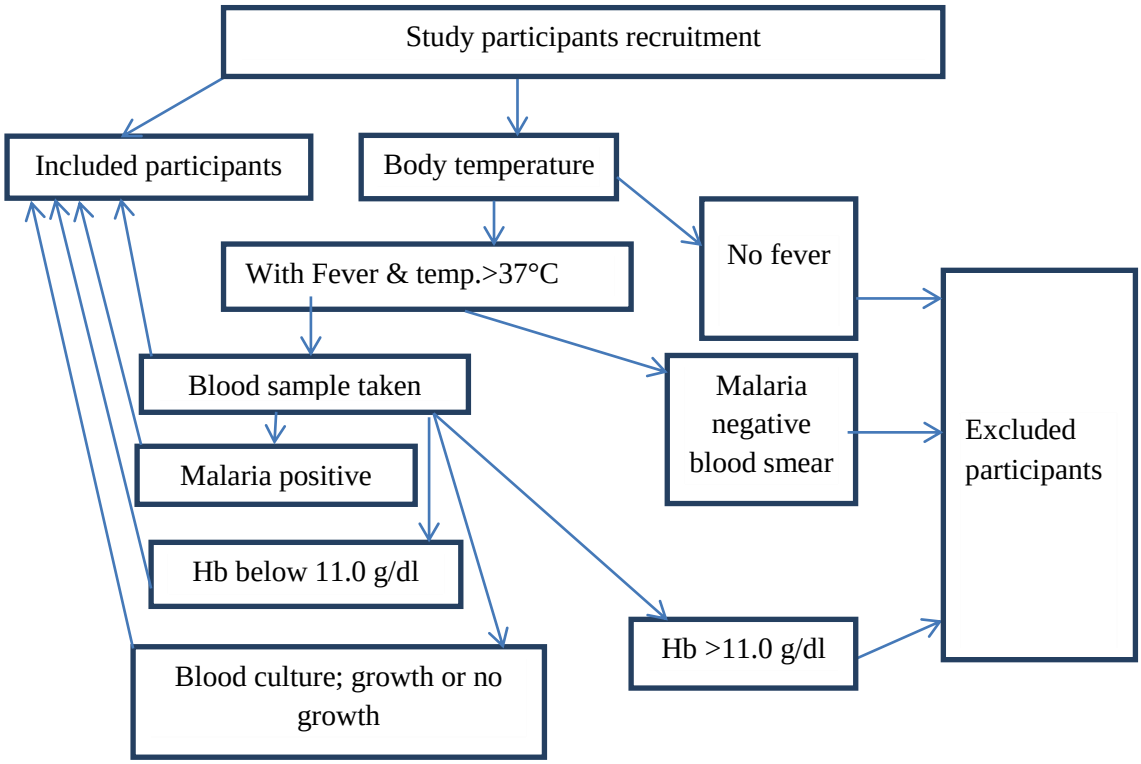

Figure 3.1: Flow Chart Showing Inclusion and Exclusion Criteria.

3.7 Laboratory Methods

3.7.1 Blood Collection

The skin around the site of venipuncture was sterilized with 70% ethanol and allowed to dry. About 5ml of vein blood was removed in syringe. After drawing blood in a syringe bactec blood culture flasks (BACTEC Peds Plus, Becton Dickinson) were first inoculated after removal of initial collection needle and new sterile needles placed to avoid contamination, without applying pressure while dispensing blood sample into bactec culture bottles, as this normally results in the haemolysis of the red blood cells and hence interfering with subsequent growth of bacteria if present. for culture. Remaining blood in the syringe after inoculation bactec bottles was placed into EDTA bottles and followed by gentle inversion to avoid formation of blood clot in the

sample. EDTA preserved blood sample were used for automated cell count estimation, blood smear preparations.

3.7.2 Hematological Measurements

Cellular blood components analysis/ haemogram/full blood count (FBC) was determined on day 0 (enrolment day) in an automated fashion with a Coulter A^{c}T diff 2 machine (Beckman Coulter® AcT diff2™, Beckman-Coulter Corporation, Miami, USA) using blood sample collected in EDTA vials. The analysis included white blood cells count, erythrocytes count, haemoglobin, packed cell volume, white blood cells differential counts, platelets count, and red blood cells indices. Peripheral blood film for each enrolled study participants was prepared for cell morphology examination.

3.7.3 Haemoglobin Phenotype Determination

Haemoglobin phenotype determination was done by electrophoresis using cellulose acetate with Titan III plates bestowing to the producer's procedures (Helena Bio-Sciences, Oxford, United Kingdom). Concisely, haemolysed sample made from blood specimen and quality control Hemo AFSC controls were allotted onto the acetate paper, and haemoglobin (Hb) variants were divided under electrophoresis process at an alkaline pH of 8.6. After timed period of electrophoresis, acetate plates with blood samples under investigation were then stained using Ponceau S stain, and haemoglobin types were scored using the Hemo AFSC control run on the same test Titan 111 plate.

3.7.4 Bacteriology Methods

About 2.5 ml of blood collected by venepuncture was inoculated into BD bacteria inoculation bottle (BD BACTEC PEDS PLUS/F) for blood culture. The inoculated bottles were incubated in a BD automated bacteria growth system (BACTEC 9050;

Becton Dickson) for a minimum of five days. The bottles were daily examined for presence of bacteria and the positive samples tested by gram staining method. The positive blood cultures samples on Bactec 9050 machine after flagging for growth were subsequently inoculated on blood agar, chocolate agar and MacConkey agar media. The chocolate agar plates were incubated in 5% carbon dioxide incubator at 37℃ for the isolation of fastidious organism likely to cause bacteraemia, whereas blood agar and MacConkey agar plates were incubated at 37℃ in aerobic incubator for 18-24 hours respectively. To distinguish and confirm suspected bacteria specie, a biochemical test kits (API, Biomerieux) was used for identification. Gram negative isolates were recognized using API® 20E for oxidase negative Enterobacteriaceae or API20NE for oxidase positive organisms (BioMerieux, Inc.), while gram positive organisms were known using various biochemical techniques (i.e., catalase of hydrogen peroxide reagent, blood agar hemolysis patterns, serologic grouping, and coagulase test). Gram positive Bacilli and Coagulase Negative Staphylococcus (CoNS) were treated as laboratory/skin contaminants and not included in analyses.

3.7.5 Malaria Diagnosis

Prepared peripheral blood films both thick and thin smears were used for malaria diagnosis. Giemsa stained blood smears (Abbas *et al*., 2018) were examined for presence or absence of malaria parasites. The prepared slides were dried, and thin blood films were fixed by dipping in methanol for around 10 seconds and stained for 20 minutes, washed in buffered water and air dried. The dried stained blood smear slides were inspected for presence of malaria parasites by a trained medical laboratory technologist. A thin blood film was examined to confirm *plasmodium* species as detected on the positive thick blood smears.

3.8 Ethical Approval

Informed consent was obtained from each parent/guardian of the child who was enrolled in this study (Appendix 1). Each parent/guardian of the child was informed about the study objectives, risks and benefits. They were given chance to decline to join the study or provide written consent to participate in the study. Ethical approval was sought from the Kenyatta University Ethical Review Committee (Appendix 4**)**.

3.9 Data Analysis

Data was checked for completeness and all queries resolved after double data entry in Microsoft excel worksheet. The data enquiry was carried out using SPSS (version 16). A cross study group differences was analysed using Kruskal-Wallis test. A pair wise comparison of haematological changes in each cellular component was calculated using Mann-Whitney U tests. Frequency procedure of SPSS was employed for the frequency distribution of bacteraemia and malaria parasitaemia in children with haemoglobin genotype AS, AA and SS. Tabulation of data was done by use of Graph Pad Prism version 6.0 (GraphPad Software, Inc) and microsoft excel Pivotal tables.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic and Clinical Characteristics of the Study Population

Study participants were stratified into two study groups; Group 1, comprised of children with malaria and bacteraemia negative, n=353, and group 2, comprised of children with malaria and bacteraemia positive, n=31, making overall study population of, n=384.

In terms of gender distribution within study sample of (n=384), Male were 53.6% (206 of 384) and Female were 46.4% (178 of 384). Contrary to previous study (Were et al., 2011), which indicated that bacteraemia had a higher proportion in female, this study shown higher proportion bacteraemia in males than female (58.1 versus 41.9) respectively. Children of the age bracket of between (8-12 months &18-22 months), showed a proportion of (25.5 & 25.8) respectively, had a higher percentage of bacteraemia, whereas extreme age categories of (3-5 month & 33-37 months) had lower proportion of bacteraemia which tied at (6.5%).

Table 4.1. Demographic and Clinical Characteristics of the Study Population

Variable	Category	Frequency	Results for indicated group	
			Malaria Positive/Bacteraemia Negative,n (%)	**Malaria Positive/Bacteraemia Positive, n (%)**
No.Participants	All	384	353 (91.9)	31 (8.1)
Gender	Female	178 (46.4)	165 (46.7)	13 (41.9)
	Male	206 (53.6)	188 (53.3)	18 (58.1)
Age (months)	3-5	62	60	2 (6.5)
	8-12	85	78	7 (25.5)
	13-17	91	83	8 (25.8)
	18-22	56	51	5 (16.1)
	23-27	38	36	2 (6.5)
	28-32	32	27	5 (16.1)
	33-37	20	18	2 (6.5)

Data presented as numbers and proportions among two groups of the study population (Malaria positive/bacteraemia negative and Malaria positive/bacteraemia positive).

4.2 Cellular Component Changes Associated with Malaria and Bacteraemia Co-infection

Blood cellular components analyzed and compared included; total white blood cells count (WBC), lymphocytes, monocytes, granulocytes, platelets and red blood cells count was based on automated Coulter Act Diff3 haematology analyzer. Cellular level changes were illustrated upon comparison study children population median against established normal levels in the established by previous studies in the same region (Ouma *et al*., 2021). Differences in cellular component levels among study population categories when compared to normal ranges of other studies populations (Oumal *et al*., 2021) may be credited to factors such as food, hereditary differences, contact to infectious diseases, ecological factors and socio-income status.

Table 4.2. Blood Cellular Component levels among the Study Population

Variable	Mal +ve/ Bact-ve	Mal +ve / Bact+ve	Normal range	P.value
Participant No	353	31		N/A
WBC($\times 10^3$/µl)	12.0 (8.8- 15.5)	12.3 (8.8- 15.8)	9.7 (5.4-19.2)	0.783
Lymph (%)	40.5 (31.2- 51.9)	40.3 (31.2- 51.7)	69.9 (51.1- 82.6)	0.140
Mono (%)	6.6 (4.4- 9.6)	6.5 (4.3- 9.6)	7.3 (3.7-13.2)	0.658
Gran (%)	51.9 (39.1- 63.6)	60.1 (39.1- 63.8)	22.3 (10.3- 40.7)	0.186
Plts($\times 10^3$/µl)	152.0 (100.5- 235.0)	151.7 (100.2- 235.0)	393 (147-734)	0.980
Rbc($\times 10^6$/µl)	4.3 (3.9- 4.8)	4.1 (3.6- 4.9)	4.26(2.91-5.58)	0.131

Data indicated as median and interquartile range (Q1 and Q3), and Pvalues.

White blood cells count in the study population children had a higher median values when compared to control levels (12.0, 12.3 versus 9.7) showing lower levels against normal levels. Lymphocytes in the study category with children with malaria and bacteraemia negative and bacteraemia positive had 40.5,40.3 against 69.9, indicating lower levels of lymphocytes in the study children, reflecting lymphocytopenia in the study categories. The median levels of granulocytes were much higher in both study categories against normal levels range established in this region (Ouma *et al*., 2021). The results showed elements of granulocytosis in study population. Median values and interquartile ranges for monocytes, red blood cells (Rbc) and platelets were all reduced in both study categories compared to normal established ranges from (Ouma *et al*., 2021).

Table 4.3: Occurrence of Hb Genotypes Among Study Population

Variable	**Category**	**Hb genotypes**		
		Hb AA, n (%)	Hb AS, n (%)	Hb SS, n (%)
Age (months)	3-36	307 (79.9)	73 (19.0)	4 (1.1)
Gender	Male	163 (53.1)	40 (54.8)	3 (75)
	Female	144 (46.9)	33 (45.2)	1 (25)
Bacteraemia	Yes	0	31(42.5)	0
	No	307	42(57.5)	4
	Male	0	18 (58.1)	0
	Female	0	13 (41.9)	0
Bacterial isolate	*Staphylococcus aureus*	0	12 (39)	0
	Streptococcus. Pyogenes	0	1 (3)	0
	Viridans streptococci	0	2 (6.5)	0
	Listeria monocytogenes	0	3 (10)	0
	Enterococcus cloacae	0	2 (6.5)	0
	Escherichia coli	0	1 (3)	0
	Salmonella species.	0	10 (32)	0

Haemoglobin genotype HbAA was the most predominant with 79.9% (307 of 384). Haemoglobin genotype HbAS distribution amongst study participants was 19.0% (73 of 384) and Hb SS had 1.1% (4 of 384). Most bacterial pathogens isolated were from children population group with haemoglobin genotype HbAS with malaria and bacteraemia co-infection, had prevalence of 8.1 % (31 of 384). Children with haemoglobin genotype SS occurrence was at 1.1% (4 of 384).

In relation to gender, Hb AA in male had 53.1%9163 OF 307) and female had 46.9% (144 of 307). Hb AS, Male had 54.8% (40 of 73) and Female with 45.2% (33 of 73), whereas HbSS, Male had 75% (3 of 4) and Female with 25% (1 of 4). Children population with haemoglobin genotype AS with malaria and bacteraemia co-infection was 42.5% (31 of 73), of this male were at 58.1% (18 of 31) and Female were at

41.9% (13 of 31). This result shows that, Male were the majority of children with Hb AS genotype with malaria and bacteraemia co-infection (58.1% (18 of 31). All children population with Hb AA and Hb SS had mono infection of malaria and no bacteraemia.

Table 4.4. Bacterial Isolates among Children with Malaria and Bacteraemia Co-infection

Bacterial isolate	**Frequency**	**Percentage(%)**
Gram positive		
Staphylococcus aureus	12	39
Streptococcus pyogenes	1	3
Viridans streptococci	2	6.5
Listreria monocytogenes	3	10
Gram negative		
Enterococcus cloacae	2	6.5
Escherichia coli	1	3
Salmonella species	10	32
Total	31	100

All bacterial isolates were isolated from study population with haemoglobin genotype Hb AS children with malaria and bacteraemia co-infection, n= 31. Bacterial isolates species identified included *Non-typhi Salmonella* (32%, n=10), *Escherichia coli* (3%, n=1), *Enterobacter cloacae* (6.5%, n=2), *Staphylococcus aureus* (39%, n=12), *Listeria monocytogenes* (10%, n=3), *Streptococcus pyogenes* (3%, n=1) and, *Viridans streptococci*

(6.5%, n=2).

Male children with malaria and bacteraemia co-infection were 4.7% and Female children 3.9% (18 of 384,13 of 384), respectively of study population. Percentage gram positive bacteria isolated was 4.7% (18 of 31) and bacteraemia of Gram-negative bacteria was 3.4% (13 of 384). Gram negative bacteria isolates identified all

belonged to the family of *Enterobacteriaceae*. Among gram negative bacteria, *Salmonella spp*. was the most important isolates among *Enterobacteriaceae spp*. (77%; 10 of 13) and these results concurs with previous studies done in other parts of Africa (Williams *et al.*, 2009; Were *et al.*, 2011). Other gram-negative isolates identified were; *Enterobacter cloacae* (15%; 2 of 13), *Escherichia coli* (8%; 1 of 13).

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

In the current study, blood cellular components level changes in haemoglobin genotypes AA, AS and SS were characterized in study children aged between 3- 36 months and residing in this malaria holoendemic region of Siaya County, infected with malaria and coinfected with either gram positive (+ve) and gram negative (-ve) bacteremia.

Findings from this study indicate that blood cellular level range changes in children with malaria and bacterial positive compared to children with malaria and bacteraemia negative was not statistically significant as shown table 4.2, P-values . Malaria and bacteraemia among children is common co-infection in the study area, predominantly due to climatic conditions such as high humidity, closeness of large water bodies, social status of the communities and poor access to health facilities that provides ambient environment for malaria and bacteria survival.

5.2 Cellular Component Changes Associated with Bacteraemia and Malaria Infection

Being the first study describing blood cellular component level changes in children with haemoglobin genotype AA and AS presenting with Malaria and bacteraemia co-infection in Kenya, the study has enumerated important certain changes that occurs in malaria and bacteraemia co-infection in children.

Finding of full blood counts from automated cell counter analysis, shows minimal changes in granulocytes in children with malaria and bacteraemia co-infection. Granulocyte changes in children is not altered by haemoglobin genotype in children

with malaria and bacteraemia co-infection. Granulocyte cells which includes of neutrophils, eosinophils and basophils are important cells that indicates presence of bacteria and parasites in the blood. In situation where individual granulocyte cell parameters are not enumerated on the automated blood count results, it is imperative to perform peripheral blood film examination and to find out which granulocytic cell lineage is elevated or deranged. Previous studies done earlier in Malawi and Kenya have shown that bacteraemia is associated with increased blood cellular elements alteration and increased severity of anaemia in children presenting at the hospitals with *plasmodium* parasites (malaria) (Bronzan *et al*., 2007).

Thrombocytopenia is largely attributed to the increased peripheral destruction of platelets in circulation and in support to previous studies done in Kenya (Maina *et al*., 2010; Were *et al*., 2011). Immunologically, malaria infection significantly elevates the rate of sequestration and eventual destruction of platelets and this also compounded with effect of immune complexes formed as result of plasmodium parasitaemia. Results presented in this study showed that children with malaraia and either with or without bacteraemia had reduced thrombocytes (platelets) levels when compared to normal reference range established for the region (Ouma *et al*., 2021). Other previous studies in the region showed malaria as a trigger of thrombocytopenia (Were *et al*., 2011).

Erythrocytopenia in malaria and bacteraemia, primarily occurs because of increased red cell destruction by plasmodium parasites in children with Hb genotype AA and AS respectively. Malaria and bacteraemia co-infection in children with haemoglobin genotype Hb AA and AS, resulted in the reduced median and interquartile range compared tonregionestablished levels (Ouma *et al*., 2021), resulting to slight erythrocytopenia and this sequentially may have been noted as mild malaria anaemia.

Monocytosis which is normally a prominent feature in children with Hb genotype AS with malaria and bacteraemia co-infection, though not noted in this study analysis. Immunologically, monocytosis is usually likenked to increased levels of plasmodium pigments that are said to aggravate monocytes (Were *et al*., 2011).

5.3 Occurrence of Haemoglobin Genotypes among Study Population

Haemoglobin genotype HbAA was the most predominant with 79.9% (307 of 384), followed by haemoglobin genotype HbAS, with distribution amongst study participants at 19.0% (73 of 384) and, while Hb SS had 1.1% (4 of 384). Most bacterial pathogens isolated were from children population group with haemoglobin genotype HbAS with malaria and bacteraemia co-infection, with isolation percentage of 8.1 % (31 of 384). Children with haemoglobin genotype SS had malaria and bactereamia co-infection 1.1% (4 of 384).

5.4 Bacterial isolates among Children with Malaria and Bacteraemia Co-infection

Blood culture results obtained from the study showed that both gram positive and negative bacteria were both associated with bacteraemia in children with malaria. *Staphylococcus aureus* (39% ,12 of 31) was the most common gram-positive isolates from blood culture (Table 4.4), and this result is in harmoniousness with results from other previous studies on paediatrics subjects (Sigauque *et al*., 2009). Similarly, other research in Africa on malaria and bacterial diseases, showed that, children with bacteraemia and malaria co-infection with haemoglobin genotype AS are normally associated with high prevalence of *staphylococcus aureus* and non-typhi *salmonella (*Were *et al*., 2011; Graham *et al*., 2000; Walsh *et al*., 2000; Williams *et al*., 2009), and this is in support to this current study. All bacterial isolates were from children with haemoglobin genotype HbAS, this illustrate and confirms that haemoglobin

genotype HbAS with malaria infection predisposes children to invasive bacterial infections. Other recent studies (Scott *et al*., 2011), have shown that, HbAS protection against malaria itself is a risk factor for invasive bacterial diseases and this could easily explain prevalence of malaria and bacteraemia co-infection in the study children population as illustrated in result (table 4.3).

Accumulated evidence from other studies done in Africa Shows that malaria and bacteraemia co-infection is a common finding in children (Berkley *et al*., 2005; Bronzan *et al*., 2007). Non typhi *salmonella* (NTS) was the most common gram-negative isolates with 10% (10 of 13) and overally at 32% (10 of 31), in children with haemoglobin genotype HbAS with malaria and bacteraemia co-infection. This finding is not agreement with close similar study done in Kilifi district hospital, where they highlighted that HbAS correlated with improved protection from both gram positive and gram-negative bacterial infections (Scott *et al*., 2011). All cases of invasive bacteraemia infection were in Hb AS genotype that is normally thought to correlate with lower carriage of malaria parasites and these results are contrary to other previous studies (Scott *et al*., 2011). *Streptococcus pneumonia* has been the most prevalent gram-positive isolates from previous studies of malaria and bacteraemia, but in this study there was no isolate of this species in blood culture. And this could be explained by increased vaccination streptococcal pneumonia vaccine among population and that could be due to herd immunity produced in study population. This could also be majorly due to exclusion criteria; clinical evidence of acute respiratory infection, where any children that presented with respiratory distress were excluded from the study. It may also be attributed to fastidiousness of these organisms; and unauthorised antimicrobial treatment ensuing in reduced pathogen salvage, particularly in individuals with low bioburdens. With a higher rate of *S. aureus*

isolates, the results is similar with studies elsewhere in Africa (Sigauque *et al*., 2009) and the spectrum of isolates reported in this study aslo tracked those of similar studies in the neighboring regions (Berkley *et al*., 2005). Finding shows increased cases of bacteraemia in haemoglobin genotype HbAS with malaria infection, is partly due to the fact that malaria infection causes increased red cell haemolysis resulting to haemolytic anaemia, mechanism believed to potentiate the vulnerability to salmonellosis (Bronzan *et al*., 2007)**.** Findings on bacterial isolates of gram negative in nature have also been confirmed previously in study done in Kenya (Were *et al*., 2011) and from other parts of sub-Saharan Africa that non-typhoidal *salmonella* (NTS) is an significant source of serious illness in young children (Mtove *et al*., 2010). However, few informaion is known about the epidemiology of this bacteria, for example how the bacteria conveyed in and to young children.

The importance of sepsis caused by non-typhoidal *salmonella* (NTS), is still not yet widely recognized and this is largely because microbiological blood culture diagnosis of septicaemia has not yet taken place into routine testing of febrile, sick child admitted in hospitals. Clinical finding of invasive bacterial infections in children is problematic without well-equipped and properly mentored microbiology personnel's and because clinical features overlap with other conditions. Noted, in previous study that the common risk factors for non-typhoidal *Salmonella* infection contrast from developed countries, with high incidence of starvation, HIV, malaria and anemia (Brent *et a*l., 2006). Previous study done in Kilifi, Kenya (Williams e*t al*., 2009) established that *Streptococus pneumoniae* was the leading gram positive cocci isolate is contrary to results from this current study, which has identified *staphylococcus aureus* bacteria as most prevalent isolate from children with bacteria and malaria co-

morbidity with haemoglobin genotype HbAS, with percentage isolation of (38.7%, n=12 of 31).

5.5 Conclusions

1. Lymphocytopenia was the common cellular changes occuring both in malaria and bacteraemia positive and negative children with haemoglobin genotypes AA and AS.
2. *Salmonella* spp and *Staphylococcus aureus* bacteria are the most prevalent gram negative and gram positive isolates respectively from blood culture of children with haemoglobin AS genotype presenting malaria.
3. Haemoglobin AS genotypes children were most infected by malaria and bacteraemia comorbidity.

5.6 Recommendations

1. The study recommends that the Ministry of Health (MOH) should incorporate haemoglobin electrophoresis test for all paediatric patients admitted with malaria and bacteraemia co-morbidity and also for children with severe malaria infection with deranged blood cellular elements. This will be important in ensuring that children with haemoglobin genotypes AS children are tested for bacteraemia by performing blood culture and in this way the actual cause of fever will be answered without subjecting them to unnecessary treatment with antibiotics and also reduce their hospital stay once the underlying cause is treated.
2. Blood culture should be performed for all paediatric patients with haemoglobin AS genotype with malaria infection as seen in the study, that average number of them is vulnerable to bacteraemia co-morbidity. Blood culture and bacteriological diagnosis of bacteria needs technical proficiency

and venture in set-up, consumables, and quality control, altogether must be reinforced at the Country/ County levels.

3. Complete blood count analysis should be done as a routine test in the management of children with malaria and bacteraemia co-infection. This will ensure common cells changes associated with certain haemoglobin genotypes are considered in their differential clinical diagnosis.

5.7 Limitation of the Study

The present study had some limitation. Blood culture may not have yielded expected results outcome due to varied sample collection in some more severe children and which may have affected probability of bacterial pathogens isolation. Non-disclosure on the prior antibiotic use by the guardian, may also have contributed overall isolation percentage.

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APPENDICES

Appendix 1.1: Informed consent form

My name is Mr. Godfrey Ogulla; I am a MSC student from Kenyatta University. I am conducting a study on" **To determine blood cellular component changes and haemoglobin genotypes in children with malaria and bacteraemia co-infection in Siaya County, western Kenya".** The information will be used by the Ministry of Health to improve access and quality for screening of children in this hospital as well as in other regions of Kenya.

Procedures

Participation in this study will require that I ask you some questions and also examine you in order to screen you for malaria. Some specimen will be taken from you for further tests. I will record the information from you in a questionnaire.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time.

Please remember that participation in the study is voluntary. You may ask questions related to the study at any other time.

You may refuse to respond to any questions and you may stop interview at any time. You may also stop being in the study at any time without any consequences to the service you receive from this clinic or any other organization now or in the future.

Discomfort and risks

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview at any time. The

interview may add approximately half an hour to the time you wait before you receive your routine services.

Benefits

If you participate in this study, you will help us learn how to provide effective screening services that can improve the health of children and reduce the risk malaria.

Rewards

If you agree to participate in this study, lunch will be provided and transport expenses will be reimbursed.

Confidentiality

The interview and examination will be conducted in a private setting within the clinic. Your name will not be recorded on the questionnaire. Everything will be kept private.

Contact information

If you have any question you may contact Dr. Margaret Muturi on 0722758523 or Prof. Collins Ouma on 0722381214 or the Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke,Secretary.kuerc@ku.ac.ke,ercku2008@gmail.com.

Appendix 1.2: Questionnaire

1. Study No: _____

2. Date of birth: _____

3.Sex:_____

3. Ethnic group: Luo ☐ Luhya ☐ Others ☐

4. Primary care taker level of education (Tick appropriate)

☐ ☐ ☐ ☐ ☐

5. Is the child currently breastfeeding_____

6. Type of the house constructed? _____

7. Which of the following type of malaria prevention program are used in the home?

Mosquitoes net ☐ Mosquitoes repellant ☐ Mosquitoes coils/spray ☐

9. Presenting signs and symptoms

Fever ☐ diarrhea ☐ edema ☐ pallor ☐ jaundice ☐ headache ☐

10. Has the child received any of the medications in the past week? ☐

12.Which medication did the child take

13. Has the child been hospitalized before? Yes ☐ No ☐

Photo 6.1.Culture Media Preparation

Photo 6.2 Media Quality Control

Photo 6.3 Biochemical Testing and Identification Using TSI Slants

Photo 6.4 Pure Plating of Isolate

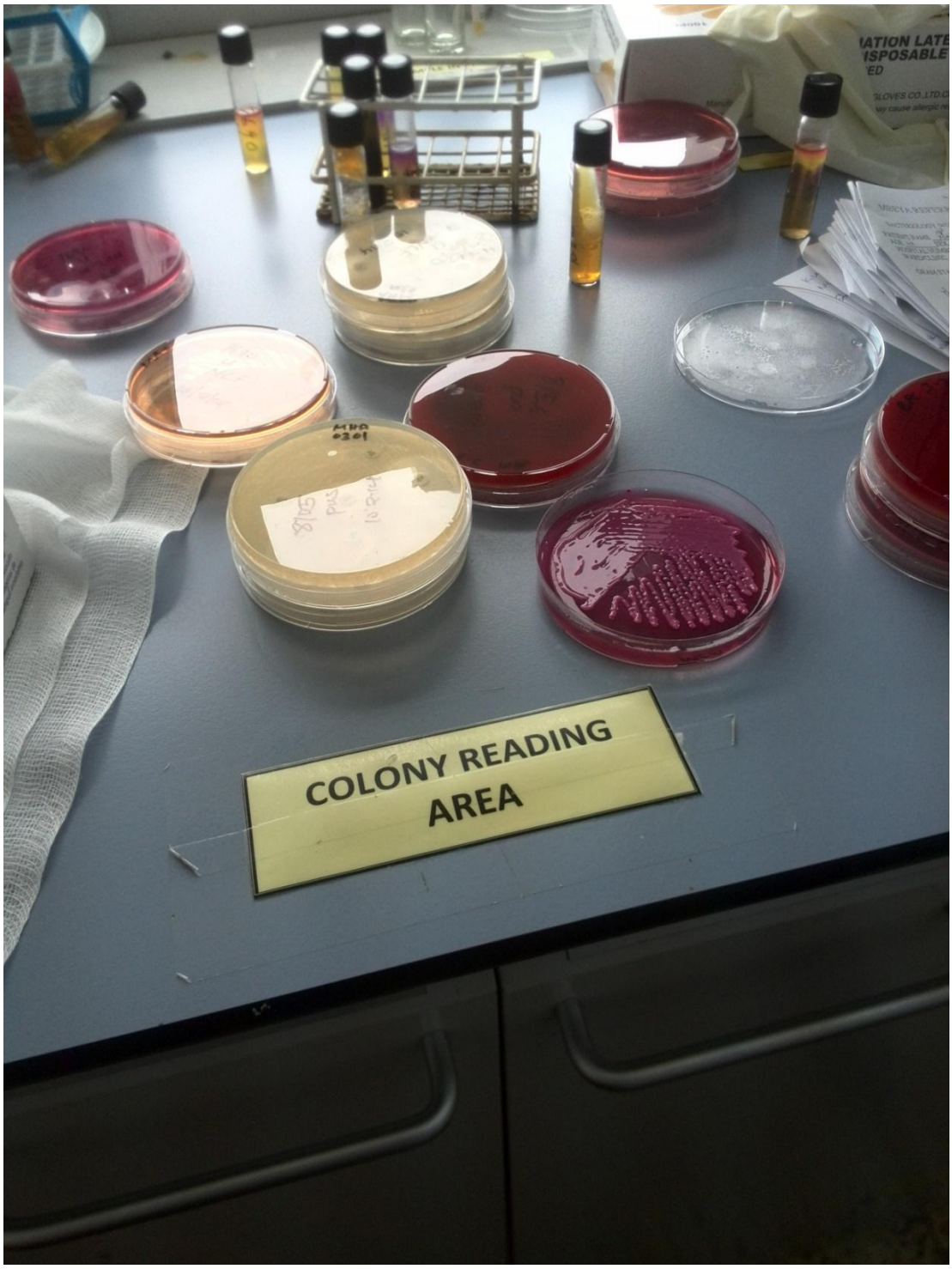


Photo 6.5 Culture Media Storage

Appendix 1.3: Kenyatta University Letter of Ethical Approval

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
ercku2008@gmail.com
Website: www.ku.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 8710901/12
Fax: 8711242/8711375

Our Ref: KU/R/COMM/51/624

Date: 19th February, 2016

Godfrey Adongo
Kenyatta University,
P.O Box 43844,
Nairobi

Dear Adongo

APPLICATION NUMBER PKU/384/1354– "TO DETERMINE BLOOD CELLULAR COMPONENTS OF HAEMOGLOBIN GENOTYPES IN CHILDREN WITH MALARIA AND BACTEREMIA CO-MORBIDITY IN WESTERN KENYA".

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic "To determine blood cellular components of haemoglobin genotypes in children with malaria and bacteremia Co-morbidity in Western Kenya".

2. APPLICANT
Godfrey Adongo

3. STUDY SITE
Siaya County Referral Hospital, Kenya.

4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 19th February,2016.

5. ADVICE/CONDITIONS
   i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
   ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
   iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
   iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

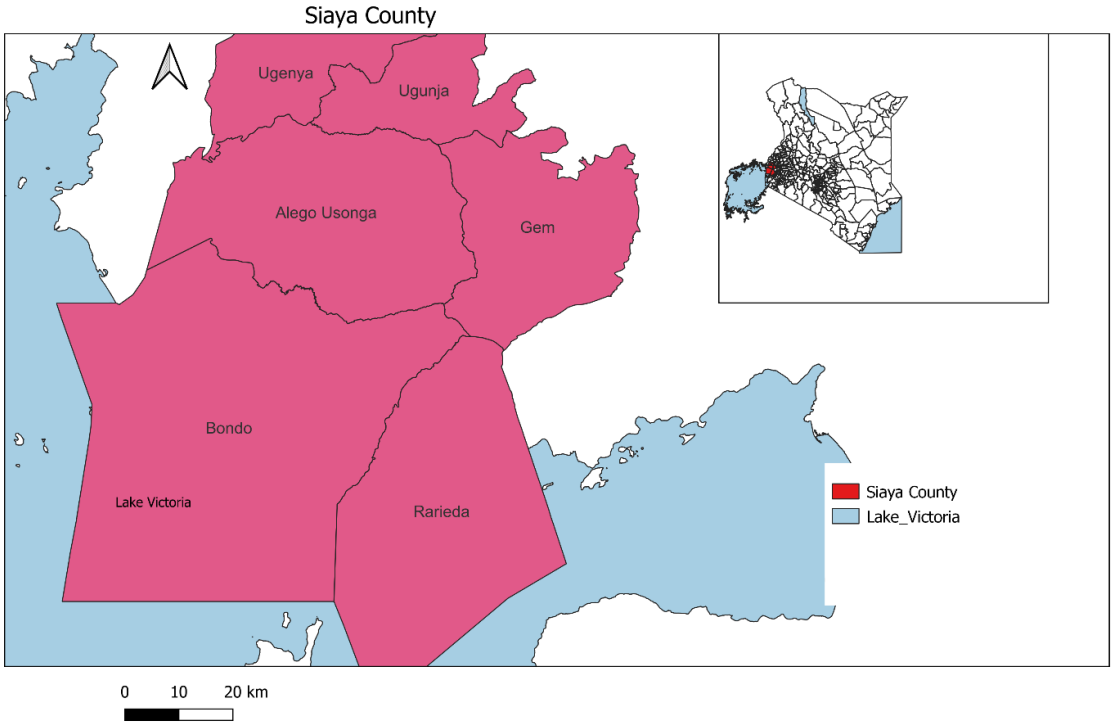
I ..... accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of..... 18th March 2016.

cc. Vice-Chancellor
DVC-Research Innovation and outreach

Appendix 1.4: Map of the study site

A map of Siaya County in western Kenya



Location of Siaya County and the study area Siaya County Referral Hospital (Ong'echa JM, Keller CC et al. 2006).