# MALARIA AND BACTERIAL CO-INFECTIONS: A STUDY AMONG CHILDREN PRESENTING WITH FEBRILE ILLNESSES IN ACCRA.

**BY**

**RAYMOND BEDU AFFRIM**

**(10397193)**

**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL MICROBIOLOGY DEGREE**

**JUNE 2015**

# DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other institution. I have duly acknowledged references made to other authors' work in the reference section of the thesis.

**Student:**

Signature:………………………………………………Date………/.………/…...……

Mr. Raymond Bedu Affrim

**Supervisors:**

Signature:………………………………………………… Date………/.………/…...……

Rev. Professor. Patrick Ferdinand Ayeh-Kumi

Department of Microbiology, University of Ghana School of Biomedical and Allied Health Sciences, College of Health Sciences.

Signature:………………………………………………… Date………/.………/…...……

Professor Ben Gyan

Department of Immunology, Noguchi Memorial Institute of Medical Research, University of Ghana.

## DEDICATION

To the memory of my mother Miss Florence Akua Kofituo. Thank you for the gift of education, advice and care which has brought me this far.

To all children in deprived settings burdened with malaria and bacterial co-infections who are unable to seek quality healthcare. You are the inspiration for such studies; there is definitely light at the end of the tunnel.
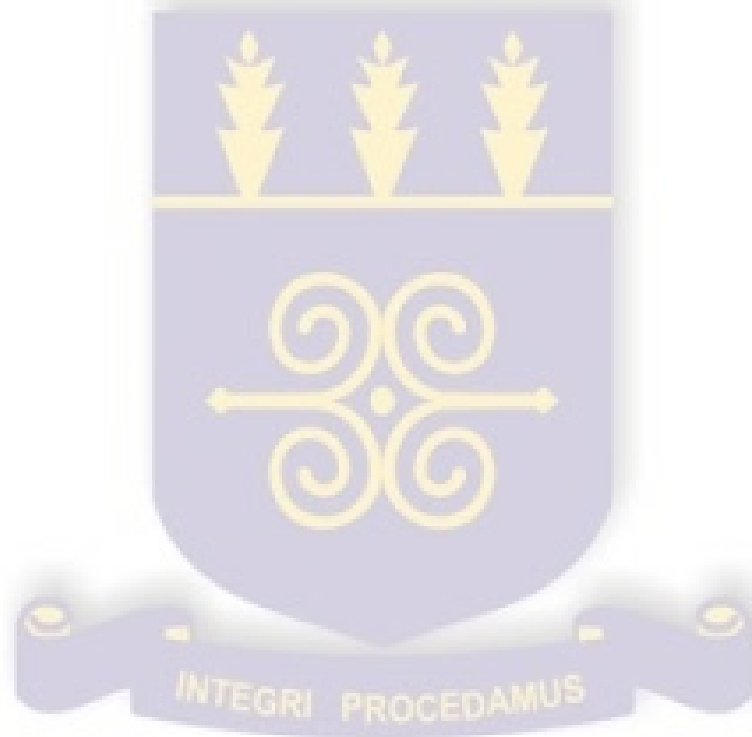
## ACKNOWLEDGEMENT

I give thanks unto the Almighty God for giving me strength and wisdom to carry out this project.

My profound appreciation goes to my supervisory team, Professor Ben Gyan, Rev. Professor Patrick Ayeh-Kumi, Dr. Simon Attah and Dr. Patience Tetteh-Quacoo, I say a big thank you for your constructive criticisms and encouragements without which this work would not have been a success. I am grateful to Professor Dorothy Yeboah Manu, Head of Microbiology Department of NMIMR for giving me the permission to use laboratory facilities in the Department for my bench work. To the Head of Microbiology Department - SBAHS, Dr Theophilus Adiku for your constructive guidance and advice for the successful completion of this work.  Many thanks go to Professor Eric Sampane-Donkor, Dr. Japhet Opintan, Dr. Korang Larbi, Rita Ofosu Agyemang and all the staff and students of the Medical Microbiology Department, School of Biomedical and Allied Health Sciences for their moral support and encouragement.

I acknowledge with deep appreciation, the indispensable help from my nephew Mr. Francis Dzidefo Krampa. Special thanks to Mr. Lorenzo Arkyeh, Christian Bonsu, Emelia Danso, Elias Asuming-Brempong and all staff of the Microbiology Department, NMIMR for being accommodative and lending their hands of support.

I wish to express my heartfelt gratitude to all my siblings, and especially my children for their immense contribution towards my education. I am indebted to Mr. Thomas Dankwah, Richael Mills, Dominic Edu and John Nyarko of the Central Laboratory, KBTH for their assistance in lab analysis.
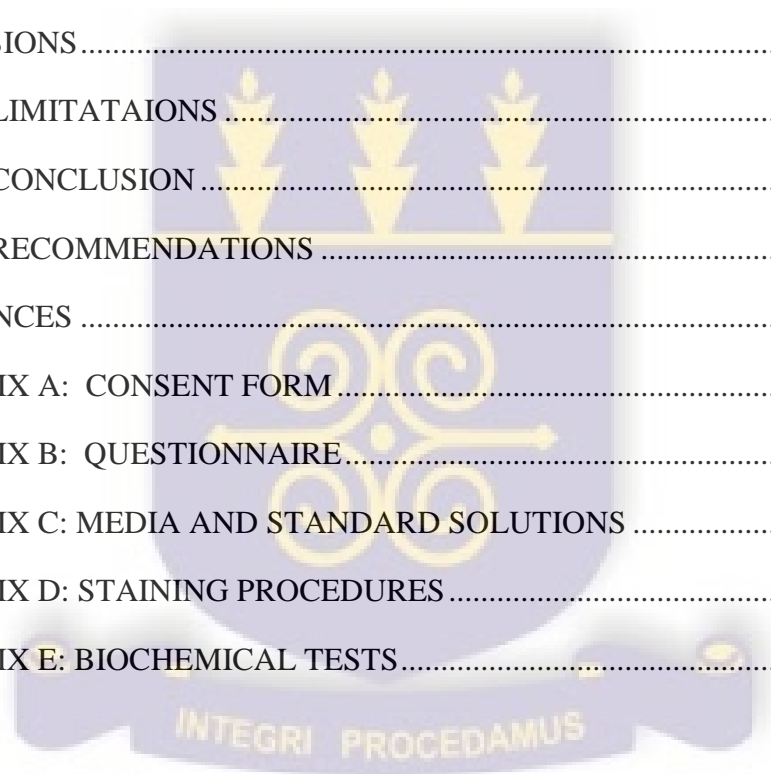
Finally, am grateful to the NMIMR for allowing me to access the Postgraduate Fund in order to research further into the immunologic aspects of this study.

**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **API** | Analytical Profile Index |
| **BA** | Blood Agar |
| **BD** | Becton and Dickinson |
| **BF** | Blood Film |
| **BHI** | Brain Heart Infusion |
| **CA** | Chocolate Agar |
| **CDC** | Centre for Disease Control |
| **EDTA** | Ethylene Diamine Tetraacetic Acid |
| **GHS** | Ghana Health Service |
| **GSS** | Ghana Statistical Service |
| **NTS** | Non-Typhoidal Salmonella |
| **iNTS** | Invasive Non-Typhoidal Salmonella |
| **Mac** | Mackonkey |
| **NMCP** | National Malaria Control Programme |
| **PMI** | President's Malaria Initiative |
| **PML** | Princess Marie Louis |
| **RDT** | Rapid Diagnostic Test |
| **SS** | Salmonella-Shigella |
| **UNICEF** | United Nations International Children's Fund |
| **WHO** | World Health Organization |

# ABSTRACT

**Background:** Malaria predisposes children in areas where malaria is endemic to concurrent bacteraemia. In the tropics, co-infections of both diseases are prevalent and are the leading causes of paediatric hospital admissions, morbidity and mortality.

**Methods:** A cross-sectional study was conducted to investigate the prevalence of co-infection of malaria and bacterial bloodstream infections among 232 children under 13 years who reported to three healthcare facilities in Accra and Dodowa with conditions of febrile illnesses suspected to be malaria. The study was conducted between the months of May and December 2014.

**Results**: Out of 1187 eligible febrile children, only 232 (19.55%) who tested positive for malaria were included in the study. They comprised 121 males and 111 females. Blood and stool specimens were taken for haematological analysis and culture for the identification of pathogenic bacteria after malaria diagnosis. Descriptive data were summarised and chi-square analysis was used in testing for associations. Fever (76.72%), anaemia (69.39%) and vomiting (49.56%) were the commonest symptoms of clinical visits. Of the 232 children tested, blood cultures were positive in 5.6% (13/232) for bacterial agents and there were no bacteria isolated from stool cultures. Anaemia, parasitaemia and white blood cell counts were high but not associated with co-infection after chi-square analysis. Co-infection of malaria and bacteraemia was associated with children who never patronised food from outside their homes. Other risk factors were in high frequencies but were not associated with co-infections.

**Conclusion:** These results may suggest co-infection of bacteraemia and malaria, however non-typhoidal *Salmonella* may not be associated with malaria in the present study.

# CHAPTER ONE

# INTRODUCTION

## 1.1    BACKGROUND

Febrile illnesses remain the leading cause of paediatric mortality and morbidity especially in sub-Saharan Africa (Bryce *et al.,* 2005; Crill *et al.*, 2006). According to the World Health Organization (WHO), febrile illness is an acute illness characterized by a rise in body temperature. Malaria and bacteraemia are among the commonest causes of these febrile illnesses and are of major public health importance in developing countries (WHO/CDR 1995; Berkley *et al*., 2005; Bryce *et al*., 2005; Uneke, 2008).

Worldwide, an estimated 76% of the under-fives' deaths occur due to undiagnosed invasive bacterial infections (Christopher *et al.,* 2013), and in Africa, bloodstream bacterial infections are responsible for 1 out of 6 deaths in children before their fifth birthday (Blomberg *et al.,* 2007). Berkley *et al*. (2005), have documented that, in malaria endemic areas, 11% of the children admitted with fever are found to have bacteraemia and 12% of these children will die because malaria was over diagnosed at the expense of other causes of fever.

Malaria, a mosquito borne infectious disease infects both humans and primates, and is caused by parasites of the genus *Plasmodium* (Warren, 1993). Globally, it remains the most important disease in tropical and sub-tropical countries, posing a huge burden on health and economic development. It has also been a major obstacle to sustainable development by the world's poorest regions (Gallup and Sachs, 2001). Approximately 198 million cases of malaria were reported at the end of 2013 with 584,000 deaths (Bassat *et al.,* 2015).

Most bacterial infections are widespread but more prevalent in regions where sanitary conditions are poor and may invade the bloodstream after a wide variety of focal infections. Transient bacteraemia is usually non-alarming but may progress to septicaemia which can be life-threatening when immediate medical attention is not given (Meremikwu *et al.,* 2005). Septicaemia is a bloodstream infection usually caused by pathogenic bacteria and together with bacteraemia may be collectively referred to as invasive bacterial infections. Varieties of bacteria found to cause febrile illnesses in children include *Staphylococcus* spp, *Streptococcus* spp, *Enterobacter* spp, *Escherichia* coli, *Klebsiella pneumoniae*, *Pseudomonas* spp, *Enterococcus* spp, *Neisseria meningitides*, *Salmonella* spp, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Campylobacter* spp (Bandyopadhyay *et al.,* 2002; Tintinalli *et al.,* 2004; Wald and Minkowski, 1980). Among the commonly reported bacterial etiologic agents isolated from African children with bacteremia are; *Salmonella* species, *Streptococcus pneumonia* and other Gram-negative bacteria (Bronzan *et al.,* 2007; Were *et al.,* 2011; Shaw, 2008; Graham, 2000; Walsh *et al*., 2000; Enwere *et al*., 2006; Roca *et al*., 2006; Sigauque *et al*., 2009).

Several studies have associated invasive bacterial infections with high mortality in children with severe malaria in sub-Saharan Africa (Bronzan *et al.,* 2007; Berkley *et al.,* 2009; Were *et al.,* 2011). Both malaria and bacteraemia mainly affect young children in the sub region and represent the principal cause of hospital admission, hence a massive burden for the under-resourced health facilities. Together, they account for more than half of all paediatric cases on admission to hospitals (Berkley *et al*., 2005). Comparing all co-infections of malaria and bacterial agents, non-typhoidal *Salmonella* (NTS), a species of *Salmonella* is consistently reported as the main bloodstream bacterial infections seen in African children with severe *P. falciparum* malaria (Gordon *et al.*,

2

2008, Bronzan *et al.,* 2007; Oundo *et al.,* 2002; Mackenzie *et al.,* 2010; Mtove *et al.,* 2011). Malaria has long been alleged to increase the risk of bacterial infections and may contribute especially to the seasonality of NTS disease (Smith, 1982; Kariuki *et al.,* 2006).

The clinical presentations of children with bloodstream infection are generally; fever, difficult breathing, tachycardia, malaise, inability to feed or lethargy, although those with asymptomatic bacteraemia are not likely to show any signs of illness (Meremikwu *et al.,* 2005). Since antiquity, clinicians have had difficulty in differentiating invasive bacteraemia from malaria based on clinical presentations alone due to these overlapping clinical features especially in the early stages (Cox *et al.,* 1996; Nsutebu *et al* 2002). Their differentiation thus requires appropriate laboratory investigations for confirmations as fever or changes in body temperature is frequently associated with malaria in many endemic areas and therefore treated accordingly. This may have led to considerable overestimation of the incidence of malaria whereas bacteraemia remains unsuspected and the causative agent unrecognised (Evans *et al.,* 2004).

## 1.2 PROBLEM STATEMENT

About 20-50% of all hospital admissions are a consequence of malaria with high case-fatality rates due to late presentation and inadequate management (WHO/UNICEF, 2003). Malaria and NTS results in substantial burden of illness and death (Morpeth, 2009). In malaria endemic regions, the presence of bacterial etiologic agents in addition to favourable environmental factors such as improper sewage disposal, poor personal hygiene, poverty, and rapidly increasing urbanization may facilitate co-infection of these diseases (Morpeth, 2009; Keong and Sulaiman, 2006).

Ghana records nearly 25-40% of all outpatient clinic visits for malaria, most of which is diagnosed clinically (WHO/UNIFEC, 2005). The presentations of malaria which include fever and general weakness are nonspecific and may well be due to other bacterial infections (Luxemburger *et al.,* 1998). It is difficult clinically to differentiate malaria from bacterial infections such as NTS or typhoid without appropriate laboratory investigations. Moreover, many facilities lack the laboratory capacity to undertake bacterial culture in routine investigations of these bacterial infections. In Africa, including Ghana, most cases of malaria and bacterial co-infections are diagnosed on the basis of clinical symptoms and treatment is presumptive without laboratory confirmation.

## 1.3 JUSTIFICATION / RATIONALE

Predictors of positive blood culture is crucial for clinicians to ensure a timely and appropriate management response. The present study will provide an epidemiological data on co-infection of malaria with bacterial agents in Accra. Data from this study may aid development of preventive strategies including active surveillance systems to control and manage co-infections as well as contribute to implementation of the guided empirical treatment of common bacteria isolates causing febrile illnesses at the study sites.

**1.4 AIM**

To investigate the prevalence of malaria and bacterial co-infections in children.

**1.4a Specific Objectives**

- ✓ To determine the prevalence of malaria and bacterial bloodstream infections in the study population.
- ✓ To determine the haematological indicators of single and co-infections of malaria and bacteria bloodstream infections
- ✓ To determine the risk factors associated with malaria and bacterial co-infections.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 MALARIA

### 2.1.1 Historical notes

Malaria derived its name from the Italian word "Mal'aria" which means "bad air", as the disease was associated with marshy areas. Malaria is an ancient disease and was previously described in Chinese medical writing (CDC, 2012). Some other earlier references to the disease include the accounts of the Hippocrates who described the symptoms of Malaria (Boyd, 1949). In 1880 Charles Louis Alphonse Laveran, a French Army surgeon in Algeria discovered Malaria parasites in the blood of a patient and 18 years later, Dr Ronald Ross, a British medical officer in India discovered that the causative agent of malaria was transmitted by mosquitoes. Subsequently, Giovanni Battista Grassi, an Italian Professor confirmed the vector to be Anopheles mosquitoes (CDC, 2012).

### 2.1.2 Etiology

Malaria is caused by intraerythrocytic protozoan parasites belonging to *Plasmodium* species (phylum Apicomplexa). Human malaria is caused by five different species of *Plasmodium: P. falciparum, P. malariae, P. ovale, P. vivax* and *P. knowlesi* (Alam, 2014). The species differ in their geographical distribution, morphology, immune response, relapse patterns and drug response. *P. falciparum* causes tropical malaria, *P. vivax and P. ovale* cause tertian malaria whilst *P. malariae* causes quartan malaria (Harinasuta *et al.,* 1988). Relapses are characteristic in *P. vivax and P. ovale* infections.

6

The most widespread species are *P. vivax* and *P. falciparum*, the latter is attributable to the severest forms of malaria whilst infections of other species are rarely life-threatening (Carpenter *et al.,* 1991; Breman, 2004). *P. ovale* is restricted to West Africa sub-region whereas *P. malariae* is found worldwide at low prevalence (Carter and Mendis, 2002). Occasionally, humans become infected with a zoonotic species, *P. knowlesi* (Daneshvar *et al.,* 2009; Chin, *et al.,* 1968; Cox-Singh *et al.,* 2008).

### 2.1.3 Life cycle

The malaria parasite has a complex life cycle divided into three stages; the exo-erythr ocytic or pre-erythrocytic stage which usually occurs in the liver, the erythrocytic stage which occurs in the erythrocytes, and the sexual stage (sporogony) which occurs in the mosquito. The exo-erythrocytic and the erythrocytic stages constitute the asexual cycle (schizogony) (Figure 2.1).
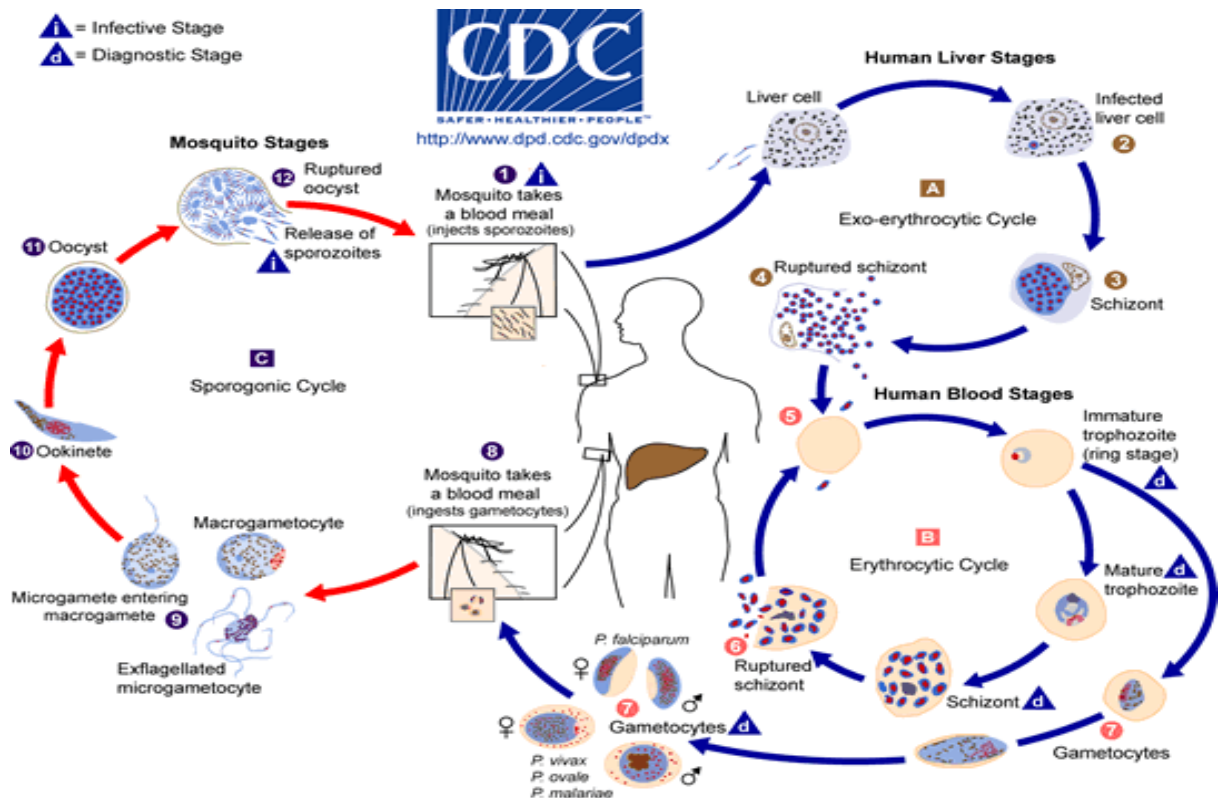


Figure 2.1 Overview of Plasmodium's life cycle. (CDC, 2006).

***2.1.3a Schizogony (Asexual stage)***

2.1.3a1 Exo-erythrocytic cycle (Human Liver stages)

Infective sporozoites from the salivary gland of the female Anopheles mosquito are introduced into the human bloodstream during a blood meal. From the bloodstream, the sporozoites invade hepatocytes where they remain for one or two weeks (prepatent period) and undergo asexual replication known as exo-erythrocytic schizogony to develop into schizonts (NIH, 2007; Khan and Lai 1999). The schizonts contain thousands of merozoites which are released into the bloodstream. It is estimated that each *Plasmodium falciparum* sporozoite can give rise to up to 40,000 merozoites. In *P. vivax and P. ovale,* some injected sporozoites may differentiate into stages called hypnozoites which may remain dormant in the liver cells for some time only to undergo schizogony causing relapse of disease when the red cells are invaded. This period of maturation is usually not accompanied by any clinical illness (Khan andLai, 1999).

2.1.3a2 Erythrocytic cycle (Human Blood stages)

Merozoites released from schizonts invade erythrocytes in the bloodstream, within 1-2 minutes. According to Miller *et al*. (2002), merozoites enter erythrocytes by a complex invasion process divided into four phases: (a) initial recognition and reversible attachment of the merozoite to the erythrocyte membrane; (b) reorientation and junction formation between the apical end of the merozoite (irreversible attachment) and the release of substances from the rhoptry and microneme organelles, leading to formation of the parasitophorous vacuole; (c) movement of the junction and invagination of the erythrocyte membrane around the merozoite accompanied by removal of the merozoite's surface coat; and (d) resealing of the parasitophorous vacuole and erythrocyte membranes after completion of merozoite invasion (Miller *et al.,* 2002; Tuteja, 2007).

8

Upon entry, the merozoites use hemoglobin as source of energy and this point are transformed into trophozoites. The early trophozoite is often referred to as the 'ring form', because of its characteristic morphology (Figure 2.1). The trophozoite enlarges and is accompanied by highly active metabolism including glycolysis of large amounts of imported glucose, the ingestion of host cytoplasm and the proteolysis of hemoglobin into constituent amino acids. Malaria parasites cannot degrade the heme by-product and free heme is potentially toxic to the parasite. Therefore, during hemoglobin degradation, most of the liberated heme is polymerized into hemozoin. It then undergoes multiple rounds of nuclear division without cytokinesis resulting in the formation of schizonts (Miller *et al.,* 2002).

Each mature schizont produces up to about 36 merozoites and these are released after lysis of the RBC to invade other uninfected RBCs (NIH, 2007). The release of erythrocytic merozoites coincides with the sharp increases in body temperature during the progression of the disease, and the repetitive intra-erythrocytic cycle of invasion–multiplication–release–invasion continues, taking about 48h in P. *falciparum, P. ovale and P. vivax* infections and 72h in *P. malariae* infection (Tuteja, 2007). This occurs somewhat synchronously and the merozoites are released at approximately the same time of the day and continue until it is brought under control by the immune system or by antimalarial drugs. The contents of the infected RBC that are released upon its lysis stimulate the production of tumor necrosis factor (TNF) and other cytokines, which are responsible for the characteristic clinical manifestations of the disease (Tuteja, 2007). Merozoites of some Plasmodium species show a distinct preference for erythrocytes of certain age. For instance, merozoites of *P. vivax* attack young immature RBCs called reticulocytes, those of *P. malariae* attack the older erythrocytes while those of *P. falciparum* indiscriminately enter into any available erythrocyte (Aikawa *et al.,* 1980).

9

A small proportion of the merozoites in the red blood cells eventually differentiate to produce micro- and macrogametocytes after a variable number of cycles. The gametocytes have no further activity within the human host but are essential for transmitting the infection to new hosts through female Anopheles mosquitoes (Figure 2.1).

### *2.1.3b  Sporogony (sexual stage) - Mosquito stages*

A mosquito taking a blood meal from an infected individual may ingest gametocytes into its midgut. These gametes fuse and undergo fertilization which occurs in the mosquito's stomach, producing zygotes.  The zygotes develop into motile, elongated ookinetes, which penetrate the mosquito's mid-gut wall and develop into oocysts.  The oocysts grow, divide, and rupture, releasing sporozoites that travel to the mosquito's salivary glands for onward transmission into another host (Figure 2-1).

The sporzoites are found in the salivary glands after 10–18 days and thereafter the mosquito remains infective for 1–2 months. Thus the infectious cycle can repeat once the mosquito feeds on another human host (Tuteja, 2007).

### 2.1.4   Transmission

Malaria is transmitted through; the injection of sporozoites during mosquito bites, blood transfusion and vertical transfer of the parasites from infected mothers to their children before or during birth (congenital malaria) (Hoffman, 1996). However, the main mode of transmission responsible for majority of the cases seen worldwide is through the bite of an infected female Anopheles mosquito.

### 2.1.5    The Vector

Out of 400 species of Anopheles mosquitoes, about 60 are capable of transmitting malaria under natural conditions, 30 of which are of major importance. The major vectors are *Anopheles gambiae* species complex and *A. funestus*. These species generally bite late in the night, are indoor resting, and are most common in the rural and peri-urban areas. Transmission is proportional to the density of the vector, number of times of bites each day, the survival of the vector after feeding and a human host (reservoir).  *A. gambiae* is the most infective vector, they are tough, long lived, naturally occurring in high densities and bite humans frequently.

### 2.1.6    Clinical manifestation

Malaria presents with symptoms such as fever, headache, muscle pain, vomiting, rapid breathing, coughs and convulsions (Warrell *et al.,* 1990). Symptoms may begin with indefinite malaise, a slow rising fever lasting several days, chills, headache, nausea, and ends with profuse sweating. After a period free of fever, the cycle of chills, fever and sweating is repeated every one to three days (Cheesbrough, 1987). Malaria can cause anaemia which may be severe particularly in young children. Severe malaria can cause black water fever, cerebral malaria, pulmonary oedema (rare but often fatal), and hypoglycaemia which is being increasingly reported in patients with severe malaria, especially children and pregnant women (Waller *et al.,* 1995; Murphy and Breman, 2001).

### 2.1.7    Epidemiology

#### *2.1.7a Global Distribution of Malaria*

Malaria is the 3rd leading cause of death for children under five years worldwide, after pneumonia and diarrheal diseases (WHO, 2013). Malaria affects a wide number of countries and has a broad distribution in both the subtropics and the tropics. Sub-Saharan Africa remains most heavily burdened, other areas of high endemicity include; India, Brazil and Sri Lanka as shown in figure 2. In Africa and some part of India, the disease occurs both in urban and rural areas and is most prevalent during rainy seasons.

An estimated 3.3 billion people are at risk with nearly 90% of all malaria deaths occurring among children in Africa. Out of about 198 million cases of malaria recorded worldwide in 2013, 584 000 died (WHO, 2014).
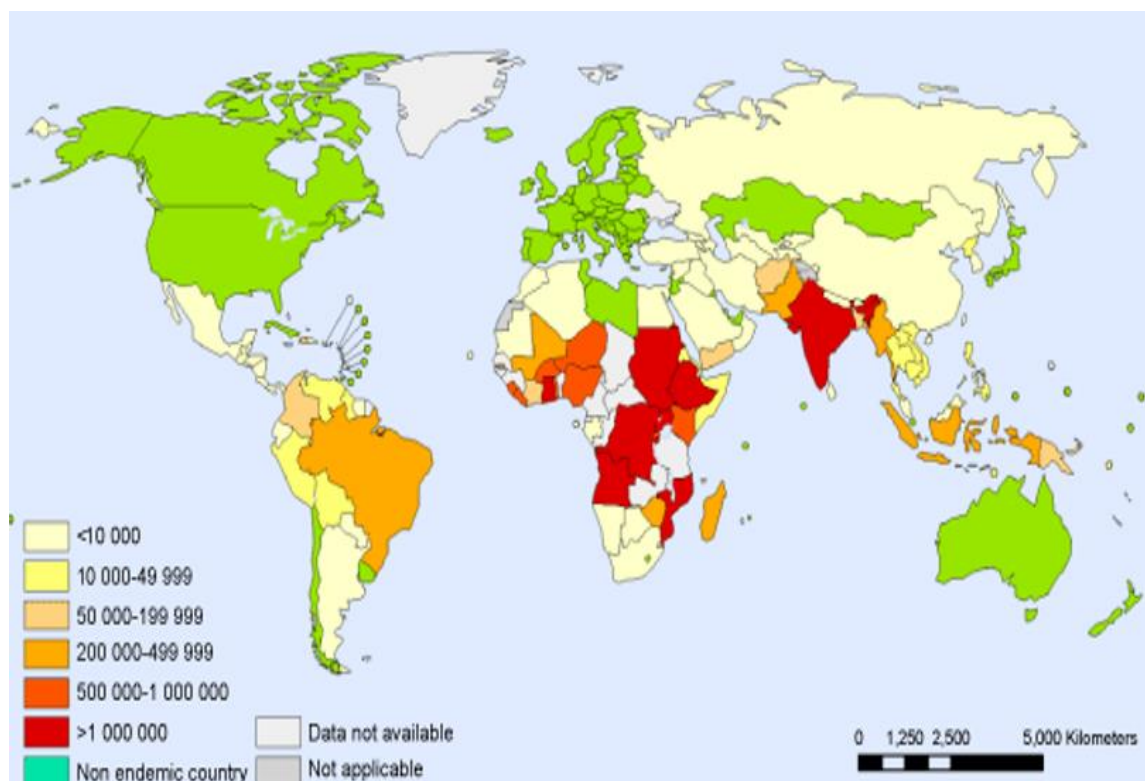


Figure 2.2: Global distribution of Malaria. SOURCE: WHO, 2012.

### *2.1.7b  Malaria situation in Ghana*

In Ghana, malaria is perennial in all parts of the country, with seasonal variations that are more pronounced in the Northern sector. Ghana's entire population of 24.2 million (2010 Census) is at risk of malaria infection (PMI, 2012), but children under five years of age and pregnant women are at higher risk of severe illness due to lowered immunity. Between 3.1 and 3.5 million cases of clinical malaria are reported in public health facilities each year, of which 900,000 cases are in children under five years (USAID, 2009). The WHO recently estimated total malaria-attributable child deaths at 14,000 per year in Ghana (WHO, 2008). The intensity of malaria transmission ranges from May-October in the Northern part of the country but may be longer in the forest zones. Peak levels of malaria infection in the population may persist for two-three months into the dry season (Ahmed, 1989).

### 2.1.8   Diagnosis

Malaria is diagnosed clinically based on a patient's signs and symptoms upon physical examination. However, the non-specific nature of symptoms, which overlap with other common infections remain a major challenge to clinical diagnosis. This can impair diagnostic specificity leading to the promotion of indiscriminate use of antimalarial agents (Mwangi *et al.,* 2005; McMorrow *et al.,* 2008; Bhandari *et al.,* 2008b). It is therefore necessary to confirm clinical findings with appropriate laboratory tests. Patients diagnosed with malaria are generally categorized as having either uncomplicated (<250000 parasites/µl) or severe malaria (>250000 parasites/µl).

*2.1.8a Diagnostic Assays*

Malaria is diagnosed in the laboratory via microscopy, rapid diagnostic tests (RDT) (Holland *et al.,* 2005) and quantitative buffy coat (QBC) method (Bhandari *et al.,* 2008b).

Conventional microscopic diagnosis requires staining thin and thick peripheral blood smears with Giemsa to give the parasites a distinctive appearance. This technique remains the gold standard for laboratory confirmation of malaria. Serological methods such as indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA) do not detect current infection but rather measures past exposure.

Molecular diagnostic methods such as polymerase chain reaction (PCR) is most useful for confirming the species of malarial parasite after the diagnosis but are not used routinely due to cost and technicality.

## 2.1.9   Treatment

Uncomplicated malaria may be treated with oral antimalarial agents; severe malaria requires parenteral therapy. The WHO recommends artemisinin combination therapy (ACT) as first line therapy for uncomplicated malaria (USAID, 2009) and parenteral artesunate (a derivative of arthemisinin) is the most potent agent for the treatment of severe malaria (Dondorp *et al*., 2005a; Dondorp *et al*., 2010). Artemisinin is a sesquiterpene lactone extracted from the leaves of *Artemisia annua* (sweet wormwood), which has been used for centuries in China for the treatment of fever. It is an effective and rapidly acting agent for elimination of blood stage parasites, with a broad spectrum of activity against asexual forms from young rings to mature schizonts, as well as gametocytes of *P. falciparum*. The drug seems to inhibit an essential calcium adenosine

triphosphatase, PfATP6, outside the food vacuole of the parasite (Eckstein-Ludwig *et al.,* 2003).

## 2.2    BACTERAEMIA

Bacteraemia is the invasion and circulation of bacteria through the vascular system. A more severe form, septicaemia results when circulating bacteria multiply at a rate that exceeds their removal by phagocytes (Berger, 1983). It is characterized by fever, chills, malaise and toxicity (Parrillo, 1993). Bacteraemia may progress to other infections such as meningitis and endocarditis (Parrillo, 1993) if left untreated.

Earlier study in Ghana found that, invasive bacterial infections were associated with a mortality of about 40%, with NTS and *Staphylococcus aureus* being among the most common organisms isolated (Evans *et al.,* 2004). *Streptococcus pneumoniae* and *Haemophilus influenzae* are also responsible for deaths in children with bacterial bloodstream infections (Berkley *et al.,* 2005). These two organisms have also been associated with occult bacteraemia (unsuspected bacteraemia) in apparently healthy children younger than 2 years of age with positive blood cultures (Berger, 1983).

### 2.2.1   Bacteraemia episodes

Bacteraemia may be described as transient, intermittent or continuous depending on their entry into the circulatory system (Mahon *et al.,* 2000). Transient bacteraemia occurs when normal flora are displaced from their usual sites into the blood (LeFrock *et al.,* 1973). Intermittent bacteraemia involves the periodic passing of bacteria from an infected part of the body into the blood. Continuous bacteraemia generally occurs through intravascular infections such as endocarditis or through catheterization and indwelling cannulas (Musher *et al.,* 2000).

### 2.2.2   Common etiologic agents of bacteraemia

A wide range of bacteria are responsible for bloodstream infections. These organisms however differ from one locality to the other with varying antimicrobial susceptibility patterns (Meremikwu *et al.,* 2005). Contrary to some studies that have named Gram positive organisms as the commonest isolates in neonates (Phiri *et al.,* 2005), Ayoola *et al* have established that Gram negative organisms are more common than the Gram positive organisms (Ayoola *et al.,* 2003).   In Ghana, NTS and *S. aureus* are the predominant aetiological agents of bloodstream infections (Nielsen *et al.,* 2012). Some bacteria responsible for bloodstream infections in children are as follows:

### *2.2.2a  Non typhoidal Salmonellae (NTS)*

Non typhoidal *Salmonellae* is a common cause of bloodstream infection among African children according to several studies conducted in Africa (Graham *et al.,* 2000; Ikumapayi *et al.,* 2007). The important strains include *Salmonella* Enteritidis, *Salmonella* Choleraesuis, and *Salmonella* Typhimurium (Brooks *et al.,* 2007). NTS is one of the three major causes of invasive disease in children below the age of three (Ikumapayi *et al.,* 2007) resulting in high morbidity and eventual death (Oundo *et al.,* 2002; Vaagland *et al.,* 2004). NTS infections occur worldwide and their mode of transmission is oro-faecal. NTS causes self-limiting gastroenteritis in healthy individuals in developed countries (Kariuki *et al.,* 2006) whilst in sub- Saharan Africa, it causes bloodstream infection in children and adults and may lead to death if prompt and appropriate antimicrobial therapy is not given (Graham *et al.,* 2000; Gordon *et al.,* 2002). A study conducted by Feasey *et al*. (2012) suggests that fatality from NTS infections ranged from 20 to 25%. Kariuki *et al* (2006) found out that NTS was responsible for 51.2% of bloodstream infection in Kenya.  In Ghana, Nielson *et al* (2012) and Evans *et al.,* (2004) reported 53.3% and 43% respectively.

### *2.2.2b  Salmonella Enterica serova Typhi (Salmonella Typhi)*

Typhoid fever also known as enteric fever is found worldwide and accounts for several cases of morbidities and mortalities. They are common in developing countries where sanitary conditions are very poor (Evans *et al.,* 2004). Typhoid infections may be mild or severe but can sometimes be life threatening if proper attention is not given. Transmission of enteric fever is oro-faecal through the ingestion of contaminated food or water. In Ghana, typhoid fever is predominant in areas with poor sanitary conditions (Nielsen *et al.,* 2012; Acquah *et al.,* 2013).

### *2.2.2c  Other Enterobacteriaceae*

*Enterobacteriaceae* is the general group of bacteria that colonize the gastrointestinal tract. They are the most significant contributors to intestinal infections, which are among the most frequent diseases in the developing world. Examples include *Escherichia coli, Klebsiella spp, Enterobacter spp, Salmonella and Shigella* (Kayser *et al.,* 2005).

*Enterobacteriaceae* have important pathogenicity factors namely; endotoxins, exotoxins, invasins and colonizing factors which aid in their adaptation in the gastrointestinal tract.

When a host is immunosuppressed, *Escherichia coli* may reach into blood stream and cause sepsis (Mahon *et al.,* 2000). *E. coli* is among the leading causes of meningitis in infants (Brooks *et al.,* 2007).

*Klebsiella pneumoniae* causes a small proportion (about 1%) of bacterial pneumonias but may also occasionally result in urinary tract infections, bacteraemia and other extra-pulmonary infections.

### 2.2.2d  *Staphylococcus aureus*

*Staphylococcus aureus* is a major pathogen causing pyogenic and toxin mediated infections in humans. It is a part of the normal microbiota of the nose, skin, mouth, and other parts of the body. It is ubiquitous and a common cause of most superficial and invasive infections notably, skin and soft-tissue infections, endovascular infections, septicaemia, endocarditis and wound infections (Del Rio *et al.,* 2009; Hakeem *et al.,* 2013).

Bloodstream infections caused by *S. aureus* are often difficult to treat, and therefore associated with relatively high morbidity and mortality (Shinefield *et al.,* 2002; Naber, 2009).  Findings from a study conducted in Ghana by Evans *et al*. (2004) have reported *S. aureus* (29%) as one of the major etiologic agents. This agrees with studies conducted by Meremikwu (2005) and Awoniyi *et al*., (2009) in Nigeria that have also reported *S. aureus* as a major organism isolated representing 48.7% and 28% respectively. In Mozambique *S. aureus* (39%) was found as one of the major pathogens isolated from neonates with bloodstream infections followed by group B *Streptococcus* (20%) (Sigaúque *et al.,* 2009). Another study conducted by Tsering *et al* (2011) in India also revealed that 97% cases of septicaemia in children was caused by *S. aureus*.

### 2.2.2e  *Streptococcus pneumoniae*

*Streptococcus pneumoniae* is an important pathogen that causes diseases ranging from upper respiratory tract infections to severe invasive diseases such as pneumonia, septicaemia and meningitis (Bogaert *et al.,* 2004; Donkor *et al.,* 2013). Transmission of pneumococcus is usually by direct contact with contaminated respiratory secretions and is highest in young children.

*Streptococcus pneumoniae* is the leading cause of death in children less than five years (Isaacman *et al.,* 2010) with about 1.2 million new cases of pneumococci infections emerging annually (Bogaert *et al.,* 2004; Donkor *et al.,* 2013). Nielsen *et al.* in 2012 conducted a study in Ghana and concluded that *S. pneumoniae* (9.1%) was among the frequent isolates in blood amongst Ghanaian children.

### 2.2.2f  *Haemophilus influenzae*

*H. influenzae* belongs to normal bacterial flora of the respiratory tract and a major cause of several invasive and non -invasive infections (García-Cobos *et al.,* 2008). Diseases caused by *H. influenzae* include childhood pneumonia, meningitis, septicaemia, acute otitis media and epiglottitis (Tristram *et al.,* 2007; Resman *et al.,* 2011). Hib is commonly found in the nose and throat of healthy individuals living in areas where vaccination is not carried out. Almost all children who are not vaccinated are exposed to Hib by the age five.

### 2.2.2g  *Neisseria meningitidis*

N*eisseria meningitidis,* commonly called meningococcus is found in the mucosa of the oropharynx of humans and a natural colonizer of the upper respiratory tract (Stephens *et al.,* 2007; Caugant and Maiden, 2009). Humans are the only natural reservoir and therefore infection is spread from man to man, the nasopharynx is the site from which meningococci are transmitted through droplet secretions or via aerosols from an infected person to a susceptible individual (Rosenstein *et al.,* 2001). Meningococcal diseases have repeatedly caused epidemics (Greenwood, 2007) and are still a global health problem affecting all ages, and a leading cause of bacterial meningitis and septicaemia (Thompson *et al.,* 2006; Antignac *et al.,* 2003).

### 2.2.3    Diagnosis of bacteremia

Prompt diagnosis and effective treatment is often required for bloodstream infection to prevent complications (Meremikwu *et al.,* 2005; Prabhu *et al.,* 2010). Bacteriological examination is therefore very essential for diagnosis of bacteraemia. Although blood cultures remain the gold standard for diagnosis, its' limitation is that most diagnostic procedures may take up to a week to complete and may cause some clinicians to depend on empirical treatment (Omoregie *et al.,* 2009).

### 2.2.3a  *Blood Cultures*

Blood cultures are mainly employed in the diagnosis of bacteraemia (Prabhu *et al.,* 2010). Physical signs and symptoms may be useful in identifying patients but have limited specificity (Kamga *et al.,* 2011). Culture and isolation of specific pathogen in bacteriological cultures is definitive diagnosis for suspected cases of bacteraemia (Meremikwu *et al.,* 2005). Positive blood cultures, though the gold standard for diagnosing bacteraemia, may give false negative results in neonates even when there are strong clinical suggestions of infection. This is attributable to the fact that antibiotics administered to mothers during pregnancy may suppress the growth of bacteria in culture, yet the neonate may have clinical symptoms and laboratory findings may not indicate bacteraemia or septicaemia (Kaufman and Fairchild, 2004).

### 2.2.4    Management of bacteremia

Antibiotics are used to treat bacterial bloodstream infections worldwide. A wide range of antimicrobials including cephalosporins, aminoglycosides, flouroquinolones and cabarpenems have been successfully used in treatment. Results of bacteriological cultures and antimicrobial susceptibility tests may take about five days, necessitating

initial empirical treatment of the infection. This practice however can add up to the already existing problems of antimicrobial resistance. Antimicrobial resistance may occur when selected antimicrobial agents are over prescribed. In order to develop effective guidelines for empirical antimicrobial treatment, knowledge of the type of etiologic agents and the pattern of antibiotic resistance are fundamental (Berkley, 2005).

## 2.3    MALARIA AND BACTERIAL CO-INFECTION

An association between malaria and susceptibility to invasive bacterial infection has been known for almost a century (Dondorp *et al.,* 2005a), and has been repeatedly documented in different settings across Sub-Saharan Africa (Cook and Zumla, 2009; Medana *et al*. 2011). This association was first described for malaria and non-Typhoid Salmonella (NTS) bacteraemia (Dondorp *et al.,* 2005b), which remains the most frequent cause of malaria associated bacteraemia in many studies, but also includes susceptibility to other Gram negative bacteria. (Cook *et al.,* 2009; Haldar *et al.,* 2007). The linking of NTS to malaria has been documented from studies in Africa (Berkley *et al.,* 1999; Oundo *et al.,* 2002). Oundo *et al.* (2002) realized in their study in Kenya that, septicaemia infection caused by some species of *Salmonella* were mostly common and severe at peak season of malaria than any other time.

It has been observed that malaria infection was often associated with NTS bacteraemia even in countries where NTS infection was very rare in healthy individuals (Brown *et al*. 2001). Supporting the concept that the malaria was the cause of the susceptibility to NTS infection, observations in British Guyana demonstrated that once malaria was cured with quinine, co-infected individuals were often able to spontaneously clear NTS infection without additional treatment.

Studies of the epidemiology of malaria-NTS co-infection have clearly shown that the incidence of malaria and NTS bacteraemia are strongly correlated (Cook *et al.,* 2009; Medana *et al*., 2011; Maneerat *et al.,* 2000) whereas stool carriage of NTS is not as closely related to the incidence of NTS bacteraemia. Where malaria transmission has declined over time, similar trends have been observed in NTS bacteremia (Cook *et al.,* 2009; Maneerat *et al.,* 2000).

In Kenyan children, nearly two-thirds of cases of bacteraemia were attributable to the effect of malaria when malaria transmission was at its highest levels (Cook *et al.,* 2009). NTS has been reported as one of the most common causes of community acquired bacteremia in children presenting to hospital in Kenya, second only to *S. pneumoniae.* However, the association between malaria and bacteremia extends only to NTS and some other common Gram negative organisms. Gram positive bacteria (Cook *et al.,* 2009) have not been implicated. High case fatality rates have been reported for patients hospitalised with malaria and bacterial co-infections, suggesting that mortality may be increased, (Haldar *et al.,* 2007; Medana *et al.,* 2002; Garcia *et al.,* 1999)

Clinical observations have prompted speculation that malaria may cause susceptibility to bacteraemia through immunoparesis (Haldar *et al.,* 2007) impairment of phagocytic cell function (Maude *et al.,* 2009; Essuman *et al.,* 2010) complement consumption (Maude *et al.,* 2009) or increased gut permeability (Haldar *et al.,* 2007). Several subsequent studies have suggested that increased susceptibility to NTS bacteraemia may persist after clearance of microscopically detectable malaria infection (Brown *et al.,* 2001; Haldar *et al.,* 2007; White *et al.,* 2009) or that susceptibility is greater at moderate than high parasite density (White *et al.,* 2009; Maude *et al.,* 2009). Other studies have suggested that the association is particularly strong in the case of severe malarial anemia (Medana *et al*. 2011; Dorovini *et al.,* 2011; Garcia *et al*, 1999; Maude *et al.,* 2009).

# CHAPTER THREE

# METHODOLOGY

## 3.1 STUDY DESIGN

The study was a cross-sectional investigation conducted between the months of May and December 2014 among children who reported to various healthcare facilities in and around Accra with conditions of febrile illnesses and were suspected to have malaria. Prior to commencement of the study, ethical clearance was sought from the College of Health Sciences, University of Ghana and the Ghana Health Service.

## 3.2 STUDY SITES

The study was conducted in three hospitals within the Greater Accra region, Ghana. The region is divided into 16 districts with a population of 4.01 million. Two of the study sites, the Maamobi and the Princess Marie Louis Children's (PML) Hospitals are located within the Accra metropolitan area whilst the Shai Osu-Doku District Hospital in Dodowa is located in the Tema metropolis. The sites combined have a total bed capacity of about 300 and a doctor to patient ratio of 1:5,000 (Ministry of Health, Ghana - unpublished reports). All the three facilities provide healthcare services to in-patients and out-patients under various specialties including medicine and surgery. The Princess Marie Louis Children's (PML) Hospital serves as one of the main paediatric referral centres in the country. It sees approximately 200 out-patient cases daily in all specialties.
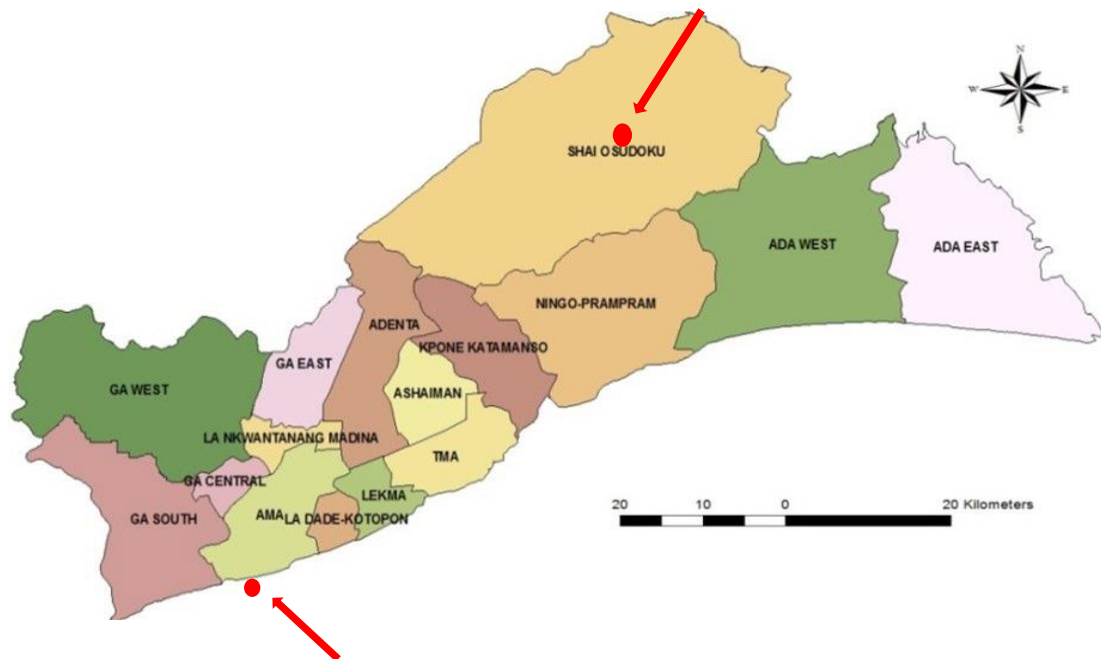
Figure 3.1: Map of Greater Accra Region showing the 16 districts (Source: Dodowa Malaria Research Center).

### 3.3 STUDY POPULATION

The population investigated were febrile children below 13 years of age seeking medical care at both inpatient and outpatient departments. The participants were selected according to the following criteria:

### 3.3.1 Inclusion Criterion

Children below 13 years presenting with malaria or symptoms of fever whose parents consented.

### 3.3.2 Exclusion Criteria

The study excluded children above 13 years, children on antibiotics and anti-malarial therapy, children of parents who did not consent, and those in critical conditions as severe anaemia.

## 3.4 SAMPLE SIZE DETERMINATION

Sample size was calculated using $n = z^2 p (1-p) / e^2$

Where n = the minimum sample size, z = the standard score, p = the known prevalence of co-infection in children and e = the allowable error margin. Using the prevalence of 11% (Berkeley *et al*, 2005) at 95% Confidence level (z = 1.96, e = 5%), the minimum number of study participants that were enrolled for the study was 100.

## 3.5 SAMPLING METHODOLOGY

Health personnel at the three study sites assisted in educating parents of all eligible participants and addressed questions and concerns. Participating children were then stratified into malarial and non-malarial cases after screening with RDT. All participants who have sufficient data for inclusion criteria were recruited into the study.

## 3.6 CONSENT AND QUESTIONNAIRE

Participation in the study was on a voluntary basis. Written informed consent was obtained from parents and guardians who were willing to include their wards. The children with their guardian were assured of confidentiality of the information they provided. A structured assessment form was used to obtain the clinical history regarding febrile illness, demographic data and risk factors (Appendix A and B).

## 3.7 SAMPLE COLLECTION AND PROCESSING

Three (3) mL of venous blood was collected from each patient aseptically with a needle and syringe. Out of this, 1mL was transferred into a sterile EDTA tube for

haematological analysis, malaria diagnosis and serological analysis (widal test). The remaining 2mL of blood was directly inoculated into 18 mL of Thioglycollate broth (Oxoid, UK) for blood culture.

Fresh stool samples were collected from the patients into Selenite F broth (10 mL/tube), and the tubes containing samples were transported to the laboratory for bacteriological culture.

## 3.8 LABORATORY ANALYSIS

### 3.8.1 Haematology

The haematological parameters for each patient were measured using automated haematology analyzer (sysmex 21N, Germany). These included haemoglobin level, total white blood cell (WBC) counts, total red blood cells (RBCs) counts, mean cell volume (MVC), platelet counts, neutrophil counts, lymphocyte counts and eosinophil counts.

### 3.8.2 Parasitological processing

#### *3.8.2a Rapid diagnostic test*

The First Response (Premier Medical Corp., India) rapid diagnostic test (RDT) kit was used in screening all blood samples for malaria. In performing this test, 20µL of whole blood and 10µL of a buffer were added to the sample well of the test kit. It was incubated for 15 minutes and the results were read immediately. Positive tests had a purple band on both the test and control regions. The negatives had no bands on the test regions.

### *3.8.2b Thick and thin blood smears*

Thick and thin films of peripheral blood were prepared to examine malaria parasites. For the thick film, a small drop (5-8uL) of blood was placed at one end of a microscope slide, evenly smeared and dried to identify malaria parasites. Thin blood films were prepared similarly, however, the small drop of blood at one end of the microscope slide was evenly spread out to cover almost the entire length of the slide using a spreader. The blood films were thoroughly air-dried and the thin film was fixed with absolute methanol for species identification (Appendix D).

The blood films were then stained with freshly prepared 20% Giemsa solution (in phosphate buffer) left to stain for 20 minutes, followed by rinsing carefully under slow running tap water. The slides were air-dried and examined with immersion oil under light microscope (X100 objective –Primo-Star Zeiss, Germany) for the presence of malaria parasites and species identification. Malaria parasites were counted against 200 White Blood Cells (WBCs) to obtain the parasite density expressed as parasite per microliters (µL) of blood.

### 3.8.3 Widal test

The widal agglutination test was performed on all blood samples by the rapid slide titration method (Lynch and Raphael, 1983) using commercial antigen suspension (Cypress Diagnostic, Belgium) for the somatic (O) and flagella (H) antigens.  To perform this test, 50µL of test serum was placed in 2 circles on a glass slide and equal volumes each of positive controls and normal saline also added in different circles.  A drop each of O or H antigens were added to the test serum in each circle and then to the negative and positive controls. The content of each circle was mixed and spread to the

27

entire circle after which it was rocked gently for 1 minute and observed for agglutination.

### 3.8.4 Blood culture

Blood culture was done manually by inoculation into Thioglycollate broth and incubated aerobically at 35°C for 7 days and examined visually daily for evidence of bacterial growth. Indicators of bacterial growth that were used included; turbidity of blood-broth mixture, growth of microcolonies, haemolysis, colour changes and gas production.

After 24hours of incubation, all cultures showing growth or no growth were sub-cultured onto solid media plates of MacConkey agar, and Blood agar and incubated at 37°C aerobically for 24 hours. In the case where Thioglycollate broth showed no growth up to day 7, subcultures were repeated from the broth on day 7 before it was discarded. All isolates from the subcultures were Gram stained and identified.

### 3.8.5 Stool culture

Stool samples were cultured on Salmonella-Shigella (SS) agar after pre-enrichment in Selenite F broth as described by Brooks *et al.* (2006). The cultures were incubated for 24 hours at 37ºC and observed for the growth of non-lactose fermenters.

### 3.8.6 Biochemical identification

Colonies from solid agar plates were subjected to biochemical tests and further identification by the API 20E (bio-Mérieux, Inc. France). TSI agar was used to determine the ability of bacteria to ferment glucose and/or lactose and their ability to produce hydrogen sulphide or other gases. Presumptive colonies have alkaline (red) slants and acid (yellow) butts, with or without $H_2S$ production (blackened agar). For

urease test, the production of ammonia from urea was shown by a change in the phenol red indicator from yellow to pink. *Salmonella* species are typically urease-negative. Oxidase test was used to determine the presence of an enzyme cytochrome oxidase, which catalyses the oxidation of reduced cytochrome by molecular oxygen. In Simmon's citrate agar, the use of citrate as a sole carbon source was indicated by the production of ammonia and a change in the colour of the medium from green to blue. Testing for indole production is important in the identification of Enterobacteria. Indole production was tested by Kovac's reagent and a positive test was indicated by red coloured compound. Motility was indicated by turbidity extending out from the line of stab inoculation. Non-motile organisms grew only in the inoculated area.

Gram positive bacteria were identified using the coagulase and catalase test. Catalase activities were detected with BD catalase reagent droppers (BD, Maryland, USA), according to the manufacturer's instruction (Appendix D).

### 3.8.7 Biochemical characterization (API 20E)

Further confirmation of the isolates was carried out with a commercial bacterial identification kit such as the Analytical Profile Index, API 20E strip kit (bio-Mérieux, Inc., France). The API is a miniaturized panel version of the conventional procedures used for the identification of Enterobacteria and other Gram-negative bacteria. The reagents used included API NaCl 0.85% medium, API 20E reagent kit, Zn reagent, oxidase and mineral oil. To prepare the strips, an incubation box (tray and lid) was used and 5 ml of distilled water were distributed into microcupules of the tray to create humid atmosphere. To prepare the inoculums, single well isolated colony was removed from an isolation plate and emulsified in 5 ml of AI 0.85% NaCl in order to achieve a homogeneous bacterial suspension. Anaerobiosis in the tests arginine dihydrolase

(ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), $H_2S$ and urea(URE) was maintained by overlaying with mineral oil. The incubation box was closed and incubated at $36\pm2°C$ for 24 h as described by the manufacturer. The strains were identified and the number codes generated were interpreted on the results obtained with the API 20E kit using the identification chart supplied by the manufacturer.

For quality Control, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and methicillin resistant Staphylococcus aureus ATCC 9213 were set up together with the test organism to control media, biochemical tests, and potency of antibiotic discs.

### 3.8.8    Antimicrobial Susceptibility Testing (AST)

Susceptibilities to various antimicrobial testing was carried out on Mueller Hinton agar as described by the Kirby–Bauer disc diffusion method (Bauer *et al.,* 1966) and interpreted by the Clinical Laboratory Standards Institute guidelines (CLSI, 2013).

### *3.8.8a    Inoculum preparation for AST*

Plates and antibiotic discs were brought to room temperature before use. Four to five colonies were touched with a straight wire loop and emulsified in peptone until the turbidity was similar to that of 0.5% McFarland standard.

### *3.8.8b    Inoculation and Application of Antibiotic discs*

A sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube to remove excess volume of the inoculum. The entire surface of the agar plate was swabbed evenly in three directions. The inoculated plates were air-dried, and antibiotic discs (Oxoid, UK) were placed on the agar using flamed forceps and were gently pressed

down to ensure contact. Once applied, the discs were not removed. The antibiotic discs used are as shown in table 3.1.

### 3.8.8c   *Incubation and Reading*

Plates were incubated aerobically at 37°C and diameters of zones of inhibitions were measured with a caliper after the 24-hour incubation period. Measured zones of inhibitions were compared with zone diameter interpretative chart (CLSI, 2013).

Table 3.1   Antibiotic Disc Concentrations Used for the isolated organisms

| Gram Positive Bacteria | | Gram Negative Bacteria | |
|---|---|---|---|
| **Antibiotic** | **Concentration (µg/disc)** | **Antibiotic** | **Concentration (µg/disc)** |
| *Cefoxitin* | *30* | *Ampicillin* | *25* |
| *Penicillin* | *10* | *Tetracycline* | *50* |
| *Erythromycin* | *15* | *Cotrimoxazole* | *25* |
| *Cefuroxime* | *30* | *Gentamicin* | *10* |
| *Gentamicin* | *10* | *Cefuroxime* | *30* |
| *Ciprofloxacin* | *5* | *Chloramphenicol* | *30* |
| | | *Ceftriaxone* | *30* |
| | | *Meropenem* | *10* |

## 3.9 DATA HANDLING AND STATISTICAL ANALYSIS

All data were handled confidentially. Clinical and laboratory data sheets were completed by the investigator only. Data was stored in bound folders and put under lock until data entry. During data entry, database files were protected under password. All data was cross-checked for correction of errors that might arise during the course of data entry. Data was entered into a database and analysed descriptively using MS excel and MS access. Measures of central tendency, frequency tables and bar charts were used in data summary.

# CHAPTER FOUR

# RESULTS

## 4.1    ENROLMENT

In total, 1187 children presenting with fever, convulsion, anaemia, diarrhoea and vomiting reported to health centres at the three sites during the span of the study. An initial 246 children were recruited but excluding incomplete questionnaires and inadequate specimens provided, 232 valid participants were enrolled. All enrolled participants were malaria positive cases diagnosed through preliminary RDT screening and then confirmed through microscopy. Consenting parents or guardians completed questionnaires while participants provided blood and stool for cultures. The participation for the study at the three sites ranged from 15.52% (Maamobi) to 58.19% (Dodowa) as shown in table 4.1. One hundred and ninety-seven participants (84.91%) were from urban communities whilst 35(15.09%) came from peri-urban communities few kilometres from the study sites.

Table 4.1: Distribution of participants from the three sites studied.

| SITE | Number consented | Percentage of study participants |
|------|------------------|----------------------------------|
| Dodowa | 135 | 58.19% |
| P.M.L | 61 | 26.29% |
| Maamobi | 36 | 15.52% |

**4.2    STUDY PARTICIPANTS**

Of the participants, 52.2% were males. The most occurring age group for males was 9-13 years whilst that of females was 0- 2 years. All participants of school going age (3-5 years for pre-school and 6-13years for basic school) were in school and came from various locations within the environs of the three study sites. The demographic characteristics of the participating children are shown in table 4.2.

Table 4.2 Demographic characteristics of the participants.

| SITE | Number | Percentage (%) |
|---|---|---|
| **Sex** | | |
| Male | 121 | 52.16 |
| Female | 111 | 47.84 |
| **Age group** | | |
| 0-2 | 60 | 25.86 |
| 3-5 | 53 | 22.84 |
| 6-8 | 54 | 23.28 |
| 9-13 | 65 | 28.02 |
| **Education** | | |
| Schooling | 172 | 74.14 |
| Not schooling | 60 | 25.86 |

**4.3     CLINICAL CHARACTERISTICS**

The most common malaria associated symptoms leading to hospital visit among the study participants were fever, anaemia and vomiting. There were no significant differences between the clinical presentation of patients and single or co-infection as shown in table 4.3

Table 4.3: Associations between clinical features and co-infection in participants.

| | Co-infections | | $x^2$ | P-value |
|---|---|---|---|---|
| | Yes | No | | |
| **Severity of parasitemia** | | | | |
| Mild | 2 | 75 | * | |
| Moderate | 7 | 93 | 1.75 | 0.30 |
| Severe | 4 | 51 | 1.62 | 0.23 |
| **Fever** | | | | |
| Yes | 11 | 168 | 0.46 | 0.73 |
| No | 2 | 51 | * | |
| **Vomiting** | | | | |
| Yes | 7 | 108 | 0.10 | 0.78 |
| No | 6 | 111 | * | |
| **Anaemia** | | | | |
| Yes | 9 | 152 | 0.00 | 1.00 |
| No | 4 | 67 | * | |
| **Convulsion** | | | | |
| Yes | 0 | 25 | 1.66 | 0.37 |
| No | 13 | 194 | * | |
| **Diarrhoea** | | | | |
| Yes | 5 | 60 | 0.75 | 0.36 |
| No | 8 | 159 | * | |
| **Sickling** | | | | |
| Yes | 0 | 24 | 1.42 | 0.62 |
| No | 13 | 219 | * | |

## 4.4    LABORATORY FINDINGS

Of the 232 participants who tested positive for *P. falciparum* after RDT and microscopy,

the median level of parasiteamia was 88,000/µL (range: 6000/µL-380,000/µL).

Children aged 9-13 years had higher levels of parasiteamia compared to all other age

groups. There were no associations between parasiteamia and clinical presentation. The

haematological features of the participants are shown in table 3.

Table 4.4: Haematological features of single and co-infections among participants

|  | Parasitemia (<250000parasite/µl) n=196 | Hyperparasitemia (>250000 parasite/µl)) n=36 | Co-infection (malaria + bacteraemia) n=13 |
|---|---|---|---|
| **Haemoglobin** | | | |
| Non-anaemic | 35 | 34 | 4 |
| Anaemic | 145 | 2 | 9 |
| Severe anaemia | 16 | 0 | 0 |
| **WBC counts** | | | |
| Low | 0 | 0 | 0 |
| Normal | 122 | 15 | 5 |
| High | 74 | 21 | 8 |
| **Neutrophil %** | | | |
| Low | 15 | 3 | 2 |
| Normal | 133 | 26 | 10 |
| High | 50 | 5 | 1 |
| **Lymphocyte %** | | | |
| Low | 59 | 22 | 3 |
| Normal | 126 | 12 | 8 |
| High | 11 | 2 | 2 |

Shown in table 4.4 are the isolates from positive blood cultures. The proportion of blood cultures yielding a clinically significant positive result was 5.6% (13/232) after identification using appropriate biochemical tests and API 20E. Enterobacteria were isolated in 8 positive while the remaining 5 were *Staphylococcus aureus.* Blood and stool cultures from the participants were negative for salmonella species.

All the *S. aureus* isolates showed complete resistance to penicillin (100%) whiles 80% resistance was for Co-trimoxazole. The Enterobacteriacae were completely resistant to ampicillin. Tetracycline and ceftriaxone also shows high resistance. The etiologic agents of bacteraemia are shown in table 4.4 and their corresponding antimicrobial susceptibility patterns are shown in tables 4.5a and 4.5b.

Table 4.5: Isolates from positive blood culture

| Organisms | Total isolates | Age groups |
|---|---|---|
| **Gram Negative Bacteria** | | |
| *Citrobacter freundii* | 1 | 0-2 |
| *Providencia stuartii* | 2 | 0-2, 6-8 |
| *Providencia alcalifaciens* | 1 | 0-2 |
| *Enterobacter amnigenus* | 1 | 3-5 |
| *Proteus mirabilis* | 1 | 6-8 |
| *Pseudomonas aucimobilis* | 1 | 9-13 |
| *Enterobacter amnigenus* | 1 | 9-13 |
| | | |
| **Gram positive Organisms** | | |
| *Staphylococcus aureus* | 5 | 3-5, 6-8, 9-13 |

36

Table 4.6a: Antimicrobial susceptibility profiles of the *Staphylococcus aureus* isolates.

| Antibiotics | Staphylococcus aureus |
|---|---|
| Cefoxitin | 100% S |
| Penicillin | 100% R |
| COX | 100% S |
| Erythromycin | 100% S |
| Co-trimoxazole | 80%R |
| Cefuroxime | 100%S |
| Gentamicin | 100%S |
| Ciprofloxacin | 100%S |

*S - Sensitive; R - Resistance.*

Table 4.6b: Antimicrobial susceptibility profiles of the Enterobactereacae isolates.

| | *P stuartii* | *P stuartii* | *P alcalifaciens* | *P aucimobilis* | *E amnigenus* | *E amnigenus* | *P mirabilis* | *C. freundii* | |
|---|---|---|---|---|---|---|---|---|---|
| Ampicillin | R | R | R | R | R | R | R | R | 100%R |
| Tetracycline | S | R | R | R | R | R | R | R | 88%R |
| Cotrimoxazole | S | R | R | S | S | S | R | S | 63%S |
| Gentamicin | S | S | S | R | R | R | R | S | 50%S |
| Cefuroxime | S | R | S | S | R | R | S | R | 50%S |
| Chloramphenicol | S | S | S | - | S | S | R | S | 88%S |
| Ceftriaxone | S | R | R | - | S | S | R | S | 57%S |
| Cefotaxine | S | R | S | R | R | R | R | R | 85%R |
| Ciprofloxacin | S | S | S | S | S | S | R | R | 85%S |
| Amoxicillin | S | S | S | S | R | S | S | S | 88%S |
| Meropenem | R | S | S | S | S | S | S | R | 85%S |

## 4.5    RISK FACTORS

Table 4.6 shows differences in proportions of the risk factors usually associated with invasive bacterial infections.  More than half of the children ate outside their homes frequently, and of those who had toilet facilities at home, only 24.8% (31 out of 125) had flush toilets (water closet). Recent antibiotic use within four weeks prior to the study was also minimal (14.65%).

Table 4.7.   Risk factors for invasive bacterial infections.

| | Co-infections | | $x^2$ | P-value |
|---|---|---|---|---|
| | Yes | No | | |
| **Recent antibiotic use** | | | | |
| Yes | 1 | 33 | * | |
| No | 12 | 186 | 0.53 | 0.70 |
| **Sickling** | | | | |
| Yes | 0 | 24 | 1.59 | 0.37 |
| No | 13 | 195 | * | |
| **Toilet facility at home** | | | | |
| Yes | 7 | 6 | 0.00 | 1.00 |
| No | 118 | 101 | * | |
| **Type of toilet facility** | | | | |
| Bush/polytene | 1 | 14 | 0.00 | 1.00 |
| KVIP | 10 | 175 | 0.37 | 0.69 |
| WC | 2 | 30 | * | |
| **Eating out** | | | | |
| Never | 7 | 42 | 10.28 | <0.05 |
| Occasionally | 3 | 44 | 1.90 | 0.18 |
| Regularly | 3 | 133 | * | |
| **Water source** | | | | |
| Packaged | 9 | 105 | 0.60 | 1.00 |
| Borehole/tap | 4 | 107 | 0.26 | 1.00 |
| Well/Rain | 0 | 7 | * | |

*reference*

38

# CHAPTER FIVE

# DISCUSSIONS

The purpose of this study was to determine the prevalence of co-infection of malaria and bacteraemia and also to assess the risk factors for bacterial infections within selected communities in the Accra sub-metro. Malaria coupled with bacterial infections are a major public health concern as the tendency of misdiagnosis is eminent and therefore tends to increase morbidity and mortality.

More children reported to the clinics with febrile illnesses suspected to be malaria than adults. They lived within the environs of the study sites and those who were of school going age were enrolled in various schools. The finding of more children especially from Dodowa and Maamobi adds to anecdotal reports indicating that children are more at risk of febrile illnesses suspected to be malaria compared to adults. Age is an influential factor in malaria infection and may contrast Svenson *et al.* (1995) who found that the severity of malaria was not age dependent. Health survey from the PML Children's hospital also indicate that the causes of frequent visits to clinics was febrile related illnesses all of which was suspected malaria. Their increased risk of malaria could be attributable to; immature immunity especially in children under the age of 2 years (WHO, 1996; Rijkers *et al.,* 1998; Klein Klouwenberg and Blont, 2008) and multiple exposures to the vector bites since children may not take necessary precautions about the vector when they engage in outdoors activities.

Both diseases have similar symptoms characterized by fever, weakness, body weight loss, anaemia and in some cases gastrointestinal disturbances (Niikura *et al.,* 2008; Samal and Sahu, 1991). The similarity in symptoms is one of the causes of difficulties that arises in the preliminary diagnosis of the diseases and there is no report from the Ministry of Health, Ghana, on the status of co-infections in the country. Bacterial co-infections in malaria positive children was evaluated based on culture and identification with API20E. Thirteen (13) out of the 232 participants had dual malaria and bloodstream bacterial infections, representing 5.6% co-infection. Surprisingly, none of the cultured samples were positive for NTS despite being an important co-infection with malaria and where many of the participants did not have potable water and toilet facilities in their homes. The finding of no positive cultures for NTS is in huge contrast to other studies in the country and elsewhere in Africa that have associated the susceptibility of NTS to malaria infection, while others have related the two as most common in the tropics and subject to misdiagnosis (Evans *et al.,* 2004; Nielson *et al.,* 2012; Bronzan *et al.,* 2007; Lepage *et al.,* 1987; Maitland *et al.,* 2006; Nesbitt *et al.,* 1989).

*Staphylococcus aureus* was the only Gram positive organism among the six different organisms isolated from blood cultures, all others were Gram negative. S. aureus may not necessarily be attributed to malaria, instead, it confirms its implications in community-acquired bacteraemia among children in rural sub-Saharan Africa. Recent investigations elsewhere in Africa show that *S. aureus* was the most frequent cause of bacteraemia caused by Gram-positive organisms in infants and young children presenting at a hospital in Nigeria (Johnson *et al*. 2008) and Mozambique (Sigauque *et al*. 2009). These infections were single-infections and not co-infection with malaria. It also agrees to findings from other developing countries that suggest *S. aureus* to

be a major aetiological agent of septicaemia (Evans *et al.,* 2004; Meremikwu *et al.,* 2005; Hill *et al.,* 2007; Kizito *et al.,* 2007; Komolafe and Adegoke, 2008) which constitute a significant threat to child survival in these areas. The reason why *S. aureus* was predominant cannot be explained from this study, however, it's implications in community-acquired bacteraemia among children at a rural sub-Saharan are highlighted. All of the *S. aureus* were resistant to penicillin (Table 4.5a). Usage of penicillin is therefore not appropriate for the treatment of Staphylococcal systemic infections as it may lead to treatment failure. Cefoxitin, Erythromycin, Co-trimoxazole, Gentamicin and Ciprofloxacin are more effective drugs of choice as the organisms showed no resistance to these groups of antibiotics.

Several published reports have also demonstrated that Gram-negative organisms either exceed or rival Gram-positive organisms in bloodstream infections in both adults and children from African countries (Gordon *et al.,* 2002; Ayoola *et al.,* 2003; Archibald *et al.,* 2003) as observed in this study. It is unknown why Gram negative organisms were mostly implicated in bloodstream infections but a likely reason that could explain this occurrence is that the Gram Negative isolates belong to the group of *Enterobacteriacae* that usually colonize the gut and progress to cause systemic invasion. It is noteworthy that, all the laboratory requisition forms of the participants specified for malaria tests and there were no requests for blood associated bacterial infections. Nevertheless, the isolation of some bacteria in the blood may indicate missed diagnosis which would consequently lead to mistreatment. This may add to growing evidence that, much attention is not paid in cases of malaria related co-infections in Ghana as their clinical symptoms may present in the same way. Systemic bacteraemia reported herein in children with

*Plasmodium falciparum* malaria makes the identification of concurrent infections a prerequisite for adequate treatment given their overlap in clinical signs.

With the abundance of affordable antimalarial medicines, self-diagnosis of malaria is often the main diagnostic approach used by many Ghanaians for febrile illnesses. The second clinical diagnosis is often sought after the self-diagnosis and medication has failed (Nyamongo, 2002). While prompt and accurate diagnosis of malaria is part of effective disease management, many other etiologic agents could be responsible for febrile presentations. If these presentations are accurately diagnosed, it could help to reduce indiscriminate use of antimalarial especially in young children and improve differential diagnosis of febrile illness. It is hence important to supplement febrile conditions with blood cultures to ensure accurate diagnosis despite their confounding clinical symptoms.

The data presented support accumulating evidence that invasive bacterial infections are important concurrent infections in paediatric populations with malaria. Studies from the Gambia have found community acquired invasive bacteraemia infections to be higher in children aged 2-29 months compared to adults (Enwere *et al.,* 2006). A similar study at a rural hospital in Mozambique showed an increased risk of community acquired bacteraemia in children <3 years of age compared to adults (Sigauque *et al.*, 2009). These finding of higher bacteraemia in children may not be comparable to results from this study as adults were not included and the few isolates obtained from blood cultures were not enough to draw definitive conclusions. The absence of NTS isolates in positive blood cultures also imply that, results from this study show no significance in associating NTS co-infections with malaria as other authors (Bronzan *et al.,* 2007; Evans *et al.,* 2004) have previously demonstrated.

On the other hand, the frequent diagnosis of malaria without routine bacterial cultures to diagnose infections in most primary and secondary health facilities, some clinicians may be tempted to give alternative remedies to other infections that mimic malaria. This may lead to unguided empirical treatment especially with antibiotics, which, in turn, may result into antimicrobial resistance and can affect a patient's rate of recovery. There is therefore further emphasizes the need for supplementary tests in ensuring accurate diagnosis before commencement of therapy.

Anaemia has been shown to be prevalent in areas where malaria is endemic (Le Hung *et al.,* 2005). The association between malaria and anaemia has been well documented (Adam *et al.,* 2005; Huddle *et al.,* 1999; Kagu *et al.,* 2007; Mayor *et al.,* 2007; Muhangi *et al.,* 2007; Ouma *et al.,* 2007; Tarimo, 2007). Malaria is characterized by a drop in the level of haemoglobin, resulting from the destruction of erythrocytes and as expected, the results from this study shows that anaemia was predominant among the participants. In Sub-Saharan Africa where malaria is endemic, the association between anaemia and malaria is so strong that anaemia is often taken as a proxy indicator of the malaria control programmes (Le Hung *et al.,* 2005).

Although no mortality was recorded during the span of the study, the prevalence of anaemia was high and may be disadvantageous in malaria positive patients, potentially leading to severe anaemia as more RBCs are invaded and lysed when infection progresses without treatment. It is worth mentioning that the 6.9% severe anaemia observed in of children from this study is a frequent complication of *Plasmodium falciparum* infections that occurs in young children (Breman, 2001) with a case-fatality rate reaching 23% in malaria holoendemic areas (Obonyo *et al.,* 2007). This study could not establish any associations between malaria and anaemia despite the high proportions of anaemic children. A reason that accounted for this was the lack of a

malaria negative control group. However, some studies have reported no association (Le Hung *et al.,* 2005; Stoltzfus *et al.,* 2000; Stoltzfus *et al.,* 1997) between malaria and anaemia. A study by Mato (1998) among Yanomami Amerindian population from the Southern Venezuelan Amazon also found no association between malaria and anaemia.

WBC counts are essential in predicting the health status or immunocompetence of a person and are lower to the normal counts during malaria. While increased counts indicate infections, reduced WBC counts, on the other hand, suggest an increased susceptibility to infections. Interestingly, this study found high WBC counts in co-infected children compared to children with only malaria and showed that, the WBC counts of the children did not fall below the standard value as generally anticipated. It contrasts studies that suggest that children who test positive for *P. falciparum* and those with high parasite densities are associated with low WBC counts (Omalu *et al* 2008). The reasons for this disparity cannot be discerned from the study, however, a risk factor for NTS infection in malaria is attributable to the reduced WBC counts which impair the defense of the body and subsequently, immunity to diseases. Since no lower counts of WBCs were recorded, it may be a likely reason why NTS was not isolated after culture as it is arguable that the immune system of the children was not impaired to an extent of predisposing them to NTS bacteraemia. This conclusion is however based on thin evidence.

Among the risk factors used to access possible transmission of bacterial of NTS among the participants were; parasitaemia, source of drinking water, sickle cell disease, younger age, lack of in-house toilet facilities, and lack of potable water. Surprisingly, participants who never patronized food from outside their homes were associated with bacteraemia (p<0.01). All the other factors showed no association.

## 5.1    LIMITATAIONS

This study is subject to several limitations. To begin with, the study was conducted in urban areas which are known to have lesser prevalence of bacterial infections compared to rural centres. Choosing study sites from rural areas would have been preferable for prevalence studies involving NTS.

Secondly, molecular techniques involving PCR are more sensitive and would have been more accurate in diagnosis compared to blood cultures since some studies have argued that the sensitivity of culture from 1mL of blood is minimal thus giving only 1CFU/mL for organisms such as NTS. Furthermore, failure to collect other parameters such as temperature, BMI, blood sugar and the comparatively lower sample size are other limitations that prevent the study from drawing a definitive conclusion.

## 2.5    CONCLUSION

In conclusion, malaria has gained more recognition through research compared to bacterial etiologic agents. Similarities in the clinical presentations of both diseases may influence clinicians to pay particular attention to the management of malaria in patients while co-infections may be missed out on the first diagnosis. This study predominantly found *S. aureus* and Enterobacteria to be responsible for co-infections in malaria patients.  NTS which has been previously reported by various authors may not be associated with malaria in the population studied.  Although none of the stool cultures was positive for bacteria, there is the need to provide safe drinking water, good toilet facilities and practice adequate personal hygiene in order to

prevent the transmission of bacterial infections, especially in areas where malaria is endemic areas.

## 5.3    RECOMMENDATIONS

Knowledge of the prevalence, risk factors of NTS and malaria co-infections in Ghana, particularly in high risk areas is critical in misdiagnosis of malaria regionally and globally. There is the need for clinicians, public health officials GHS/Policy makers to include invasive bacterial infection such as NTS in routine diagnosis of persons presenting with febrile illnesses.

Malaria still remains a public health concern especially in children. In the environs of the study sites where drainage is poor and mosquito breeding habitats have an extensive distribution, parents and caregivers should protect their children with long lasting insecticide treated bed nets. Furthermore, preventive strategies among the general public should include; promotion of inbuilt toilet facilities at home to maximise personal hygiene. Provision of potable water for communities without access to running tap water.

Traditional microbiological culture methods employed to detect systemic bacteraemia are often time consuming and have modest sensitivity. Therefore, molecular methods may be useful in accurately diagnosing bacteraemia.

# REFERENCES

Acquah, S. E., Quaye, L., Sagoe, K., Ziem, J. B., Bromberger, P. I. and Amponsem, A. A. (2013). Susceptibility of bacterial etiological agents to commonly-used antimicrobial agents in children with sepsis at the Tamale Teaching Hospital. BMC Infect Dis, 13**,** 89.

Adam, I., Khamis, A.H. and Elbashir, M.I. (2005) Prevalence and risk factors for anaemia in pregnant women of eastern Sudan. Transactions of the Royal Society of Tropical Medicineand Hygiene 99, 739-743

Ahmed, K. (1989). Epidemiology of malaria in Ghana. Ghana Med. J. **23,** 190-196.

Aikawa, M., Suzuki, M., and Gutierrez, Y. (1980). Pathology of malaria. Malaria, 2, 47-102.

Alam, A. (2014). Serine proteases of malaria parasite Plasmodium falciparum: potential as antimalarial drug targets. Interdisciplinary perspectives on infectious diseases, 2014.

Antignac, A., Ducos-Galand, M., Guiyoule, A., Pirès, R., Alonso, J. M. and Taha, M.-K. (2003). Neisseria meningitidis strains isolated from invasive infections in France (1999–2002): phenotypes and antibiotic susceptibility patterns. Clin Infect Dis, 37, 912-920.

Archibald, L. K., Kazembe, P. N., Nwanyanwu, O., Mwansambo, C., Reller, L. B., & Jarvis, W. R. (2003). Epidemiology of bloodstream infections in a bacille Calmette-Guerin-vaccinated pediatric population in Malawi. Journal of Infectious Diseases, 188(2), 202-208.

Awoniyi, D., Udo, S. and Oguntibeju, O. (2009). An epidemiological survey of neonatal sepsis in a hospital in Western Nigeria. Afr J Microbiol Research, 3**,** 385-389.

Ayoola, O. O., Adeyemo, A. A. and Osinisu, K. (2003). Aetiological agents,clinical features and outcome of septicaemia in infants in Ibadan. WAJM, 22.

Bandyopadhyay, S, Bergholte J, Blackwell CD, Friedlander JR, Hennes H. (2002). Risk of serious bacterial infection in children with fever without a source in the post–haemophilus influenzae Era when antibiotics Are reserved for culture-proven bacteremia. Arch Pediatr Adolesc Med, 156:512–517.

Bassat, Q., Ogutu, B., Djimde, A., Stricker, K., and Hamed, K. (2015). Tailoring a Pediatric Formulation of Artemether-Lumefantrine for Treatment of Plasmodium falciparum Malaria. Antimicrobial Agents and Chemotherapy, 59(8), 4366-4374.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. (1966): Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

Berger, S. A (1983) Pseudobacteremia due to contaminated alcohol swabs. J Clin Microbiol 18(4): 974-975.

Berkley, J, Mwarumba S, Bramham K, Lowe B, Marsh K. (1999). Bacteremia complicating severe malaria in children. Trans. R. Soc. Trop. Med. Hyg. 93:283–286.

Berkley, J. A., Bejon, P., Mwangi, T., Gwer, S., Maitland, K., Williams, T. N., and Newton, C. R. (2009). HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria. Clinical infectious diseases, 49(3), 336-343.

Berkley, J. A., Maitland, K., Mwangi, I., Ngetsa, C., Mwarumba, S., Lowe, B. S., Newton, C. R. J. C., Marsh, K., Scott, J. A. G., English, M., (2005). Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area. BMJ, 10:1136.

Bhandari, A., Dratler, S., Raube, K., and Thulasiraj, R. D. (2008a). Specialty care systems: A pioneering vision for global health. Health Affairs, 27(4), 964-976.

Bhandari, P. L., Raghuveer, C. V., Rajeev, A, and Bhandari, P. D. (2008b). Comparative study of peripheral blood smear, Quantitative buffy coat and Modified centrifuged blood smear in malaria diagnosis. Indian J Pathol Microbiol; 51(1): 108-12

Blomberg, B., Manji, K. P., Urassa, W. K., Tamim, B. S., Mwakagile, D. S., Jureen, R., and Maselle, S. Y. (2007). Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study.BMC infectious diseases, 7(1), 1.

Bogaert, D., De Groot, R. and Hermans, P. (2004). Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis, 4, 144-154.

Boyd, M. F. (Ed.). (1949). Malariology: A comprehensive survey of all aspects of this group of diseases from a global standpoint (Vol. 2). Saunders.

Breman, J. G. (2001). The Ears of the Hippopotamus: Manifestations, Determinants, and Estimates of the Malaria Burden. American Journal of Tropical Medicine and Hygiene.;64(Suppl. 12):1–11.

Breman, J. G., Alilio, M. S., and Mills, A. (2004). Conquering the intolerable burden of malaria: what's new, what's needed: a summary. The American journal of tropical medicine and hygiene, 71(2 suppl), 1-15.

Bronzan, R. N., Taylor, T. E., Mwenechanya, J., Tembo, M., Kayira, K., Bwanaisa, L., and Phiri, A. (2007). Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. Journal of Infectious Diseases, 195(6), 895-904.

Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A. (2007) Medical Microbiology. 24th Edition. McGraw-Hill Companies, Inc. San Francisco. Pp. 145-150.

Brown, H., Rogerson, S., Taylor, T., Tembo, M., Mwenechanya, J., Molyneux, M., & Turner, G. (2001). Blood-brain barrier function in cerebral malaria in Malawian children. The American journal of tropical medicine and hygiene,64(3), 207-213.

Bryce, J., Boschi-Pinto, C., Shibuya, K., Black, R. E., & WHO Child Health Epidemiology Reference Group. (2005). WHO estimates of the causes of death in children. The Lancet, 365(9465), 1147-1152.

Carpenter, C. C., Pearson, G. W., Mitchell, V. S., & Oaks Jr, S. C. (Eds.). (1991). Malaria: Obstacles and Opportunities. National Academies Press.

Carter, R., and Mendis, K. N. (2002). Evolutionary and historical aspects of the burden of malaria. Clinical microbiology reviews, 15(4), 564-594.

Caugant, D. A., and Maiden, M. C. (2009). Meningococcal carriage and disease—population biology and evolution. Vaccine, 27**,** B64-B70.

50

CDC. (2012). CDC Health Information for International Travel 2012. Oxford University Press, New York, USA

Cheesbrough, M. (1987). Medical Laboratory Manual for Tropical Countries. Volume 1 (No. Ed. 2). Tropical Health Technology.

Chin, W., Contacos, P. G., Collins, W. E., Jeter, M. H., and Alpert, E. (1968). Experimental mosquito-transmission of Plasmodium knowlesi to man and monkey. The American Journal of Tropical Medicine and Hygiene, 17(3), 355-358.

Christopher, A., Mshana, S. E., Kidenya, B. R., Hokororo, A., and Morona, D. (2013). Bacteremia and resistant gram-negative pathogens among under-fives in Tanzania. Ital J Pediatr, 39(1), 27.

Clinical and Laboratory Standards Institute (CLSI). (2013).  M100-S23. Performance standards for antimicrobial susceptibility testing: 23rd informational supplement. CLSI, Wayne, PA.

Cook, G.C. and Zumla, A.I. (eds.) Manson's Tropical Diseases (Saunders Elsevier, 2009).

Cox-Singh, J., Davis, T. M., Lee, K. S., Shamsul, S. S., Matusop, A., Ratnam, S., ... and Singh, B. (2008). Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. Clinical infectious diseases, 46(2), 165-171.

Daneshvar, C., Davis, T. M., Cox-Singh, J., Rafa'ee, M. Z., Zakaria, S. K., Divis, P. C., and Singh, B. (2009). Clinical and laboratory features of human Plasmodium knowlesi infection. Clinical infectious diseases, 49(6), 852-860.

Del Rio, A., Cervera, C., Moreno, A., Moreillon, P. and Miró, J. M. (2009). Patients at risk of complications of Staphylococcus aureus bloodstream infection. Clin Infect Dis, 48**,** S246-S253.

Dondorp, A, Nosten, F., Stepniewska, K., Day, N., White, N., S. E. A. Q. A. M. A. T. (2005a). Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet; 366:717–25.

Dondorp, A. M., Desakorn, V., Pongtavornpinyo, W., Sahassananda, D., Silamut, K., Chotivanich, K., Newton, P. N., Pitisuttithum, P., Smithyman, A. M., White, N. J., Day, N. P. (2005b). Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. PLoS Med, 2(8), e204.

Dondorp, A. M., Fanello, C. I., Hendriksen, I. C., Gomes, E., Seni, A., Chhaganlal, K. D., ... and AQUAMAT group. (2010). Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. The Lancet, 376(9753), 1647-1657.

Donkor, E. S., Adegbola, R. A., Wren, B. W. and Antonio, M. (2013). Population biology of Streptococcus pneumoniae in West Africa: multilocus sequence typing of serotypes that exhibit different predisposition to invasive disease and carriage. PloS one, 8**,** e53925.

Dorovini-Zis, K., Schmidt, K., Huynh, H., Fu, W., Whitten, R. O., Milner, D., ... & Taylor, T. E. (2011). The neuropathology of fatal cerebral malaria in malawian children. The American journal of pathology, 178(5), 2146-2158.

Eckstein-Ludwig, U., Webb, R. J., Van Goethem, I. D. A., East, J. M., Lee, A. G., Kimura, M., and Krishna, S. (2003). Artemisinins target the SERCA of Plasmodium falciparum. Nature, 424(6951), 957-961.

Enwere, G., Biney, E., Cheung, Y., Zaman, S. M., Okoko, B., Oluwalana, C., and Cutts, F. T. (2006). Epidemiologic and clinical characteristics of community-acquired invasive bacterial infections in children aged 2–29 months in The Gambia. The Pediatric infectious disease journal, 25(8), 700-705.

Essuman, V. A., Ntim-Amponsah, C. T., Astrup, B. S., Adjei, G. O., Kurtzhals, J. A., Ndanu, T. A., & Goka, B. (2010). Retinopathy in severe malaria in Ghanaian children–overlap between fundus changes in cerebral and non-cerebral malaria. Malar J, 9, 232.

Evans, J. A., Adusei, A., Timmann, C., May, J., Mack, D., Agbenyega, T., ... & Frimpong, E. (2004). High mortality of infant bacteraemia clinically indistinguishable from severe malaria. Qjm, 97(9), 591-597.

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S., and Gordon, M. A. (2012). Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. The Lancet, 379(9835), 2489-2499.

Gallup, J. L., and Sachs, J. D. (2001). The economic burden of malaria. The American journal of tropical medicine and hygiene, 64(1 suppl), 85-96.

García, F., Cebrián, M., Dgedge, M., Casademont, J., Bedini, J. L., Neves, O., Grau, J. M. (1999). Endothelial cell activation in muscle biopsy samples is related to clinical severity in human cerebral malaria. Journal of Infectious Diseases, 179(2), 475-483.

García-Cobos, S., Campos, J., Cercenado, E., Román, F., Lázaro, E., Pérez-Vázquez, M., De Abajo, F. and Oteo, J. 2008. Antibiotic resistance in Haemophilus influenzae decreased, except for β-lactamase-negative amoxicillin-resistant 67 isolates, in parallel with community antibiotic consumption in Spain from 1997 to 2007. Antimicrob Agents Chemother, 52**,** 2760-2766.

Gill, J., Kumar, R., Todd, J., & Wiskin, C. (2006). Methicillin-resistant Staphylococcus aureus: awareness and perceptions. Journal of hospital infection, 62(3), 333-337.

Gordon, M. A., Banda, H. T., Gondwe, M., Gordon, S. B., Boeree, M. J., Walsh, A. L., ... and Molyneux, M. E. (2002). Non-typhoidal salmonella bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. Aids, 16(12), 1633-1641.

Gordon, M. A., Graham, S. M., Walsh, A. L., Wilson, L., Phiri, A., Molyneux, E., & Molyneux, M. E. (2008). Epidemics of invasive Salmonella enterica serovar enteritidis and S. enterica Serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. Clinical Infectious Diseases, 46(7), 963-969.

Graham, S. M., Molyneux, E. M., Walsh, A. L., Cheesbrough, J. S., Molyneux, M. E. and Hart, C. A. (2000). Nontyphoidal Salmonella infections of children in tropical Africa. Pediatr Infect Dis J, 19**,** 1189-1196.

Greenwood, B. (2007). The changing face of meningococcal disease in West Africa. Epidemiol Infect, 135, 703-705.

Hakeem, L., Laing, R. B., Tonna, I., Douglas, J. G. and Mackenzie, A. R. (2013). Invasive Staphylococcus aureus infections in diabetes mellitus. Br J Diabetes Vasc Dis, 13, 164-177.

Haldar, K., Murphy, S. C., Milner Jr, D. A., & Taylor, T. E. (2007). Malaria: mechanisms of erythrocytic infection and pathological correlates of severe disease. Annu. Rev. Pathol. Mech. Dis., 2, 217-249.

Harinasuta, T., Bunnag, D., Wernsdorfer, W. H., & McGregor, I. (1988). The clinical features of malaria. Malaria: principles and practice of malariology. Volume 1., 709-734.

Hill, P. C., Onyeama, C. O., Ikumapayi, U. N., Secka, O., Ameyaw, S., Simmonds, N., Donkor, S. A., Howie, S. R., Tapgun, M. and Corrah, T. (2007). Bacteraemia in patients admitted to an urban hospital in West Africa. BMC Infect Dis, 7, 2.

Hoffman, S. L. (1996). Malaria vaccine development: a multi-immune response approach. American Society for Microbiology (ASM).

Holland, C. A., & Kiechle, F. L. (2005). Point-of-care molecular diagnostic systems—past, present and future. Current opinion in microbiology, 8(5), 504-509.

Huddle, J. M., Gibson, R. S., & Cullinan, T. R. (1999). The impact of malarial infection and diet on the anaemia status of rural pregnant Malawian women. European journal of clinical nutrition, 53(10), 792-801.

Ikumapayi, U. N., Antonio, M., Sonne-Hansen, J., Biney, E., Enwere, G., Okoko, B., Oluwalana, C., Vaughan, A., Zaman, S. M. and Greenwood, B. M. (2007). Molecular epidemiology of community-acquired invasive non-typhoidal Salmonella among children aged 2–29 months in rural Gambia and discovery of a new serovar, Salmonella enterica Dingiri. J Med Microbiol, 56**,** 1479-1484.

Isaacman, D. J., Mcintosh, E. D. and Reinert, R. R. (2010). Burden of invasive pneumococcal disease and serotype distribution among Streptococcus pneumoniae isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. Int J Infect Dis, 14**,** e197-e209.

Johnson, A. W. B., Osinusi, K., Aderele, W. I., Gbadero, D. A., Olaleye, O. D., & Adeyemi-Doro, F. A. (2008). Etiologic agents and outcome determinants of community-acquired pneumonia in urban children: a hospital-based study. Journal of the National Medical Association, 100(4), 370-385.

Kagu, M. B., Kawuwa, M. B., & Gadzama, G. B. (2007). Anaemia in pregnancy: a cross-sectional study of pregnant women in a Sahelian tertiary hospital in Northeastern Nigeria. Journal of Obstetrics and Gynaecology, 27(7), 676-679.

Kamga, H. L. F., Njunda, P. F., Nde, A. L., Assob, J. C. N., Nsagha, D. S. and Weledji, P. (2011). Prevalence of septicaemia and antibiotic sensitivity pattern of bacteria isolate at the University Teaching Hospital, Yaounde. Afr J Clin Exp Microbiol, 12**,** 2 - 8.

Kariuki, S., Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J. and Hart, C. A. (2006). Characterisation of community acquired non-typhoidal Salmonella from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. BMC Microbiol, 6**,** 101.

Kaufman, D. and Fairchild, K. D. (2004). Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev, 17**,** 638-680.

Kayser, F. H., Bienz, K. A., Eckert, J., & Zinkernagel, R. M. (2005). General bacteriology. Medical microbiology. New York: Kayser Microbiology, 146-309.

Keong, B. C. M., & Sulaiman, W. (2006). Typhoid and malaria co-infection-an interesting finding in the investigation of a tropical fever. Malaysian J Med Sci, 13, 74-5.

Khan, N. and Lai. A. (1999). "The malaria website: pathology/immune response." . Biology Department, Brown University.

Kizito, M., Mworozi, E., Ndugwa, C. and Serjeant, G. R. (2007). Bacteraemia in homozygous sickle cell disease in Africa: is pneumococcal prophylaxis justified? Arch Dis Child, 92, 21-23.

Klein Klouwenberg, P., & Bont, L. (2008). Neonatal and infantile immune responses to encapsulated bacteria and conjugate vaccines. Clinical and Developmental Immunology, 2008.

Komolafe, A. O. and Adegoke, A. A. (2008). Incidence of bacterial Septicaemia in Ile-Ife Metropolis, Nigeria. Malays J Microbiol, 4(2), 51- 61.

Komolafe, A. O., & Adegoke, A. A. (2008). Incidence of bacterial septicaemia in Ile-Ife metropolis, Nigeria. Malaysian Journal of Microbiology, 4(2), 51-61.

Le Hung, Q., de Vries, P. J., Giao, P. T., Binh, T. Q., Nam, N. V., and Kager, P. A. (2005). Anemia, malaria and hookworm infections in a Vietnamese ethnic minority.

LeFrock, JL, Ellis, CA, Turchik, JB, Weinstein, L (1973) Transient bacteremia associated with sigmoidoscopy. N Engl J Med **289**(9)**:** 467-469.

Lepage, P., Bogaerts, J., Van Goethem, C., Ntahorutaba, M., Nsengumuremyi, F., Hitimana, D., & Levy, J. (1987). Community-acquired bacteraemia in African children. The Lancet, 329(8548), 1458-1461.

Luxemburger, C., Nosten, F., Kyle, D., Kiricharoen, L, Chongsuphajasiddhi, T. and White, N. (1998). Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low transmission. Trans R Soc Trop Med Hyg; 92:45-49.

Lynch, M.J. and S.S. Raphael, 1983. Immunology and Serology. Lynch's Medical Laboratory Technology, Saunders, Philadelphia.

Mackenzie, G., Ceesay, S. J., Hill, P. C., Walther, M., Bojang, K. A., Satoguina, J., and Greenwood, B. M. (2010). A decline in the incidence of invasive non-typhoidal Salmonella infection in The Gambia temporally associated with a decline in malaria infection.

Mahon, CR, Manuselis, G. (2000). Textbook of Diagnostic Microbiology. 2nd Edition. Elsevier's Health Sciences, Philadelphia. Pp 997-1009.

Maitland, K., Berkley, J. A., Shebbe, M., Peshu, N., English, M., & Newton, C. R. C. (2006). Children with severe malnutrition: can those at highest risk of death be identified with the WHO protocol?. PLoS Med, 3(12), e500.

Maneerat, Y., Viriyavejakul, P., Punpoowong, B., Jones, M., Wilairatana, P., Pongponratn, E., & Udomsangpetch, R. (2000). Inducible nitric oxide synthase expression is increased in the brain in fatal cerebral malaria. Histopathology, 37(3), 269-277.

Mato, S. P. (1998). Anemia and malaria in a Yanomami Amerindian population from the southern Venezuelan Amazon. *The American journal of tropical medicine and hygiene* **59**(6)**:** 998-1001.

Maude, R. J., Dondorp, A. M., Sayeed, A. A., Day, N. P., White, N. J., & Beare, N. A. (2009). The eye in cerebral malaria: what can it teach us?. Transactions of the Royal Society of Tropical Medicine and Hygiene, 103(7), 661-664.

Mayor, A., Aponte, J. J., Fogg, C., Saúte, F., Greenwood, B., Dgedge, M., and Alonso, P. L. (2007). The epidemiology of malaria in adults in a rural area of southern Mozambique. Malaria journal, 6(1), 3.

McMorrow, M. L., Masanja, M. I., Abdulla, S. M., Kahigwa, E., and Kachur, S. P. (2008). Challenges in routine implementation and quality control of rapid diagnostic tests for malaria–Rufiji District, Tanzania. The American journal of tropical medicine and hygiene, 79(3), 385-390.

Medana, I. M., Day, N. P., Sachanonta, N., Mai, N. T., Dondorp, A. M., Pongponratn, E., ... & Turner, G. D. (2011). Coma in fatal adult human malaria is not caused by cerebral oedema. Malar J, 10, 267.

Meremikwu, M. M., Nwachukwu, C. E., Asuquo, A. E., Okebe, J. U., & Utsalo, S. J. (2005). Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. BMC infectious diseases, 5(1), 1.

Miller, L. H., Baruch, D. I., Marsh, K., & Doumbo, O. K. (2002). The pathogenic basis of malaria. Nature, 415(6872), 673-679.

Morpeth, S. C., Ramadhani, H. O., & Crump, J. A. (2009). Invasive non-typhi Salmonella disease in Africa. Clinical Infectious Diseases, 49(4), 606-611.

Mtove, G., Amos, B., Nadjm, B., Hendriksen, I. C., Dondorp, A. M., Mwambuli, A., and Deen, J. (2011). Decreasing incidence of severe malaria and community-acquired bacteraemia among hospitalized children in Muheza, north-eastern Tanzania, 2006–2010. Malar J, 10(320), 1475-2875.

Muhangi, L., Woodburn, P., Omara, M., Omoding, N., Kizito, D., Mpairwe, H., and Elliott, A. M. (2007). Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene, 101(9), 899-907.

Murphy, S. C., and Breman, J. G. (2001). Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. The American journal of tropical medicine and hygiene, 64(1 suppl), 57-67.

Musher, D. M., Alexandraki, I., Graviss, E. A., Yanbeiy, N, Eid, A., Inderias, L. A., Phan, H. M., Solomon, E. (2000). Bacteremic and nonbacteremic pneumococcal pneumonia. A prospective study. Medicine (Baltimore) **79**(4)**:** 210-221.

Mwangi, T. W., Mohammed, M., Dayo, H., Snow, R. W., and Marsh, K. (2005). Clinical algorithms for malaria diagnosis lack utility among people of different age groups. Tropical Medicine and International Health, 10(6), 530-536.

Naber, C. K. (2009). Staphylococcus aureus bacteremia: epidemiology, pathophysiology, and management strategies. Clin Infect Dis, 48, S231-S237.

National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (2007). Understanding Malaria: Fighting an Ancient Scourge. NIH Publication No. 07-7139.

National Institutes of Health (NIH). (2007). "Understanding malaria: fighting an ancient scourge." U.S. Department of Health and Human Services.

Nesbitt, A., & Mirza, N. B. (1989). Salmonella septicaemias in Kenyan children. Journal of tropical pediatrics, 35(1), 35-39.

Nielsen, M. V., Sarpong, N., Krumkamp, R., Dekker, D., Loag, W., Amemasor, S., Agyekum, A., Marks, F., Huenger, F., Krefis, A. C., Hagen, R. M., Adu-Sarkodie, Y., May, J. and Schwarz, N. G. (2012). Incidence and characteristics of bacteraemia among children in Rural Ghana. PloS one, 7**,** e44063.

Niikura, M., Kamiya, S., Kiyoshi, K., Fumie., K. (2008). Coinfection with Nonlethal Murine Malaria Parasites Suppresses Pathogenesis Caused by Plasmodium berghei NK65. J. Immunol. 180: 6877-6884.

NMCP/GHS, 2008. Strategic Plan for Malaria Control in Ghana (2008-2015).

Nsutebu, E. F., Ndumbe, P. M., & Koulla, S. (2002). The increase in occurrence of typhoid fever in Cameroon: overdiagnosis due to misuse of the Widal test?. Transactions of the Royal Society of Tropical Medicine and Hygiene, 96(1), 64-67.

Nyamongo, I. K. (2002). Health care switching behaviour of malaria patients in a Kenyan rural community. Social science and medicine. 54 377-386.

Obonyo, C. O., Vulule, J., Akhwale, W. S., & Grobbee, D. E. (2007). In-hospital morbidity and mortality due to severe malarial anemia in western Kenya. The American journal of tropical medicine and hygiene, 77(6 Suppl), 23-28.

Omalu, I. C., Oguche, S., Gyang, V. P., Akindigh, T. M., Egah, D. Z., and Gokop, B. (2008). Standard white blood cell count for malaria density estimation: A need for review? *Annals of Tropical Medicine and Public Health*, *1*(1), 29.

Omoregie, R., Egbe, C. A., Ogefere, H. O., Igbarumah, I. and Omijie, R. E. (2009). Effects of Gender and Seasonal Variation on the Prevalence of Bacterial Septicaemia Among Young Children in Benin City, Nigeria. Libyan J Med, 4**,** 153 - 157.

Ouma, P., Van Eijk, A. M., Hamel, M. J., Parise, M., Ayisi, J. G., Otieno, K., and Slutsker, L. (2007). Malaria and anaemia among pregnant women at first antenatal clinic visit in Kisumu, western Kenya. Tropical Medicine and International Health, 12(12), 1515-1523.

Oundo, J. O., Muli, F., Kariuki, S., Waiyaki, P. G., Iijima, Y., Berkley, J., and Lowe, B. (2002). Non-typhi salmonella in children with severe malaria. East African medical journal, 79(12), 633-639.

Parrillo, J. E. (1993). Pathogenetic mechanisms of septic shock. New England Journal of Medicine, 328(20), 1471-1477.

Phiri, A., Milledge, J., Calis, J. C. J., Graham, S. M., Wilson, L. K., Soko, D., ... & Molyneux, E. M. (2006). Aetiology of neonatal sepsis at QECH, Blantyre: 1996-2001. Malawi Medical Journal, 17(3), 92-96.

PMI, 2012. Malaria Operational Plan – FY 2012 (Year 5): Ghana.

Prabhu, K., Bhat, S., & Rao, S. (2010). Bacteriologic profile and antibiogram of blood culture isolates in a pediatric care unit. Journal of laboratory physicians, 2(2), 85.

Resman, F., Ristovski, M., Ahl, J., Forsgren, A., Gilsdorf, J. R., Jasir, A., ... & Riesbeck, K. (2011). Invasive disease caused by Haemophilus influenzae in Sweden 1997–2009; evidence of increasing incidence and clinical burden of non-type b strains. Clinical Microbiology and Infection, 17(11), 1638-1645.

Rijkers, G. T., Sanders, E. A. M., Breukels, M. A., & Zegers, B. J. M. (1998). Infant B cell responses to polysaccharide determinants. Vaccine, 16(14), 1396-1400.

Roca, A., Sigauque, B., Quintó, L., Mandomando, I., Valles, X., Espasa, M., ... & Levine, M. (2006). Invasive pneumococcal disease in children <5 years of age in rural Mozambique. Tropical Medicine & International Health, 11(9), 1422-1431.

Rosenstein, N. E., Perkins, B. A., Stephens, D. S., Popovic, T. and Hughes, J. M. (2001). Meningococcal disease. N Engl J Med, 344, 1378-1388.

Samal, K. K., & Sahu, C. S. (1991). Malaria and Widal reaction. The Journal of the Association of Physicians of India, 39(10), 745-747.

Shaw, A., Reddy, E. and Crump, J. (2008). Etiology of community-acquired bloodstream infections in Africa. In: 46th Annual Meeting of the Infectious Diseases Society of America. Washington, D.C., 20.

Shinefield, H., Black, S., Fattom, A., Horwith, G., Rasgon, S., Ordonez, J., Yeoh, H., Law, D., Robbins, J. B. and Schneerson, R. (2002). Use of a Staphylococcus aureus conjugate vaccine in patients receiving hemodialysis. N Engl J Med, 346, 491-496.

Sigauque, B., Roca, A., Mandomando, I. (2009). Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr Infect Dis J; 28:108–13.

Smith, D. C. (1982). The rise and fall of typhomalarial fever: I. Origins. Journal of the history of medicine and allied sciences, 37(2), 182-220.

Stephens, D. S., Greenwood, B. and Brandtzaeg, P. (2007). Epidemic meningitis, meningococcemia, and Neisseria meningitidis. Lancet, 369**,** 2196-2210.

Stoltzfus, R. J., Chwaya, H. M., Montresor, A., Albonico, M., Savioli, L., & Tielsch, J. M. (2000). Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0-to 5-y old Zanzibari children and these relationships change with age. The Journal of nutrition, 130(7), 1724-1733.

Stoltzfus, R. J., Chwaya, H. M., Tielsch, J. M., Schulze, K. J., Albonico, M. and Savioli, L. (1997) Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. Am. J. Clin. Nutr. 65:153-159.

Svenson, J. E., MacLean, J. D., Gyorkos, T. W., & Keystone, J. (1995). Imported malaria: clinical presentation and examination of symptomatic travelers. Archives of Internal Medicine, 155(8), 861-868.

Tarimo, D. S. (2007). Appraisal on the prevalence of malaria and anaemia in pregnancy and factors influencing uptake of intermittent preventive therapy with sulfadoxine-pyrimethamine in Kibaha district, Tanzania.

Thompson, M. J., Ninis, N., Perera, R., Mayon-White, R., Phillips, C., Bailey, L., Harnden, A., Mant, D. and Levin, M. (2006). Clinical recognition of meningococcal disease in children and adolescents. Lancet, 367**,** 397-403.

Tintinalli, J. E., Kellen, G. D., Stapczynski, J. S. (2004). Causes of bacteremia in children have been reported as P. Bacteremia, sepsis, and meningitis in children. In Emergency Medicine: A Comprehensive Study Guide. 6th edition.:735–742.

Tristram, S., Jacobs, M. R. and Appelbaum, P. C. (2007). Antimicrobial resistance in Haemophilus influenzae. Clin Microbiol Rev, 20, 368-389.

Tsering, D. C., Chanchal, L., Pal, R. and Kar, S. (2011). Bacteriological profile of septicemia and the risk factors in neonates and infants in sikkim. J Glob Infect Dis, 3, 42-5.

Tuteja, R. (2007). Malaria− an overview. FEBS Journal, 274(18), 4670-4679.

Uneke, C. J. (2008). Concurrent malaria and typhoid fever in the tropics: the diagnostic challenges and public health implications. Journal of Vector Borne Diseases, 45(2), 133.

USAID (2009). Presidential Malaria Initiative. Malaria Operational Plan – Year Three (FY 2010), Ghana (Draft Report). Pp 6-14.

Vaagland, H., Blomberg, B., Krüger, C., Naman, N., Jureen, R. and Langeland, N. (2004). Nosocomial outbreak of neonatal Salmonella enterica serotype Enteritidis meningitis in a rural hospital in northern Tanzania. BMC Infect Dis, 4, 35.

Wald, E. R., and Minkowski, J. M. (1980). Bacteremia in childhood. Southern medical journal, 73(7), 904-905.

Waller, D., Krishna, S., Crawley, J., Miller, K., Nosten, F., Chapman, D., and White, N. J. (1995). Clinical features and outcome of severe malaria in Gambian children. Clinical infectious diseases, 21(3), 577-587.

Walsh, A. L., Phiri, A. J., Graham, S. M., Molyneux, E. M., and Molyneux, M. E. (2000). Bacteremia in febrile Malawian children: clinical and microbiologic features. The Pediatric infectious disease journal, 19(4), 312-319.

Warrell, D. A. (1993). Clinical features of malaria. Bruce-Chwatt's Essential malariology. London, Boston, Melbourne, Auckland: Edward Arnold, 35-49.

Warrell, D. A., Molyneux, M. E., & Beales, P. F. (1990). Severe and complicated malaria. World Health Organization Division of Control of Tropical Diseases. Transactions of the Royal Society of tropical medicine and hygiene, 84(Supplement 2).

Were, T., Davenport, G. C., Hittner, J. B., Ouma, C., Vulule, J. M., Ong'echa, J. M., and Perkins, D. J. (2011). Bacteremia in Kenyan children presenting with malaria. Journal of clinical microbiology, 49(2), 671-676.

White, V. A., Lewallen, S., Beare, N. A., Molyneux, M. E. and Taylor, T. E. (2009). Retinal pathology of paediatric cerebral malaria in Malawi. PL05 One 4, e4317

WHO. (2008). World malaria report 2008. World Health Organization.

WHO. (2014). World malaria report 2013. World Health Organization.

WHO/CDR (1995). Management of Childhood Illness: Assess and Classify the Sick Child Age 2 Months Up to 5 Years. Atlanta, Georgia: WHO Division of Diarrheal and Acute Respiratory Disease Control and UNICEF.

WHO/UNICEF. (2005). World malaria report 2005: Roll Back Malaria. World Health Organization and UNICEF.

WHO/UNICEF. Africa Malaria Report 2003

World Health Organization. (1996). Perinatal mortality: a listing of available information.

World Health Organization. (2013). Children: reducing mortality. Geneva (Fact Sheet No. 178).

## APPENDIX A:  CONSENT FORM

UNIVERSITY OF GHANA MEDICAL SCHOOLCOLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MICROBIOLOGY, P.O. BOX 4236

KORLE-BU, ACCRA, GHANA

**Participant's Name:** _____

**Participant's ID No:** _____

MALARIA AND BACTERIA CO-INFECTIONS: A STUDY AMONG CHILDREN PRESENTING WITH FEBRILE ILLNESSES IN ACCRA.

**Background**

Malaria afflicts about 90 countries and territories in the tropical and subtropical regions and almost one half of them are in Africa. WHO estimates 300 to 500 million malaria cases worldwide annually, with about 90% from Africa. In addition, the estimated annual mortality attributed to malaria ranges from 700,000 to 2.7 million globally and 75% of them are African children and expectant mothers.

Non-typhi *Salmonella* (NTS) is among the three most common pathogens causing bacteremia in children and adults in sub-Sahara Africa (Shaw, 2008). NTS bacteremia in Africa is highly associated with other diseases (particularly HIV, malaria and sickle cell) and malnutrition. Malaria has long been suspected to increase the risk of NTS infection in children. Thus it is very common to see patients undergoing both malaria and typhoid treatment even if the diagnosis has not been confirmed.

In malaria endemic region, there exists an association between falciparum malaria and salmonella bacteremia and this often confuses diagnosis and delays appropriate management. Both malaria and typhoid fever share social circumstances which are imperative to their transmission. The clinical presentation of NTS bacteremia is non-specific and in the absence of blood culture, may be confused with other febrile illness such as malaria. Co-infection with NTS has been associated with increased malaria mortality.

69

**Purpose of study**

The purpose of this study is to determine the prevalence of the co-infection of malaria and non-typhoidal salmonella infection in children.

**What is required of you**

You will be asked to provide some information on your child such as age, locality, educational level, duration of febrile condition, sources of water for domestic usage, etc.

Peripheral blood from finger prick will be taken then; 3mL of venous blood may also be taken from your child if your child meets that criterion. After this your child will be asked to provide stool into the container that will be given you.

All participants are to provide both specimen types assigned to the group except under special conditions where either urethral swab or urine specimen may be accepted from male participants.

**Risk**

The clinical records of your child for the past six (6) months will be reviewed. However, information obtained about you will be confidential and will not be by any means discussed publicly against your name. You will be assigned an ID number which will be used in public discussion and not your name. Collection of the vaginal and urethral swab specimens is invasive which may be painful and uncomfortable. You are assured that these specimens will be taken by a specialist and so will not be very much painful or affect you negatively. You may spend some more time than normal check-up times but you are fully assured that there will not be any unnecessary time wasting.

**Benefits**

The possible benefit for you participating will be getting tested for the infection of *Malaria* and non-Typhoidal Salmonella free of charge. This will aid your doctor's decision and subsequently appropriate treatment. Also, the information obtained from the study will reveal the state of co-infection in the community which will help select appropriate measures to diagnose, prevent and control its occurrence.

**Terms of participation**

- Participation is voluntary and so you are not under any obligation to participate.
- You may decide to withdraw from the study at any time without having to give any explanation/reason.
- Your refusal to take part in the study will not influence the study or your subsequent health care delivery.
- All information obtained from you will be confidential.
- You will not receive any financial compensation for participation.

**Signing**

A: TO BE COMPLETED BY RESEARCHER

I have fully explained to _____as

a participant; the procedure, risk and benefits of the above described study.  I have also addressed

all his/her questions and concerns.

_____          _____      _____

  Name                                                                    Sign/thumbprint          Date

B: TO BE COMPLETED BY PARTICIPANT

I _____ have been fully informed

of the above described study in a language that I fully understand.  By signing this form, I give

my consent to participate in the study.

_____          _____

  Date                                              Sign/thumbprint

71

## APPENDIX B:  QUESTIONNAIRE

**Target Number: ……………….**

**A:  Personal data:**

**Name of Patient:** …………………………………………………………………

Location of residence……………………………………………………………….

**Age:** [    ] **(0- 2) years**        [    ] **(3- 5) years**        [    ] **(6- 8) years**    [    ] **(9- 13) years**

**Sex:** [       ]      **1**: Male        **2**: Female

**B:  Clinical data:**

**Fever: [      ]      1:** Yes      **2:** No      **Vomittng:** [      ]      **1**: yes      **2**: No

**Diarrhoea:** [      ]    **1**: Yes    **2**: No      **Sickling status:**     [      ]   **1**: Positive      **2**: Negative

**Anaemia:** [      ]    **1**: Yes   2: No      **Convulsion:  [      ]      1:** Yes    **2:** No

**Antibiotic use** [      ]      1:Yes      2:No

  1.  **Educational background:**  (a) None   (b) Primary    (c) J.S.S.

        Name them _____

     **Environmental**

1.  Number of times you have eaten outside in the  past weeks : ……………………

2.  Number of persons in households …………………………..

3.  Do you have toilet facility in house…….if yes….state which one……………………….

4.   What is the sources of household water    [      ]tapwater   [      ]borehole   [      ]river
            [      ]packaged water  Others………………

        Date _____

        Signature/thumbprint _____

# APPENDIX C: MEDIA AND STANDARD SOLUTIONS

***Preparation of agar media and standard solutions for culture, identification and antimicrobial susceptibility testing of bacteria isolates***

The following media and standard solutions were aseptically prepared according to manufacturer`s instructions using sterile distilled water. Where necessary the media and solutions were autoclaved at 121°C and 15 psi pressure for 15 min.

With the agar plates, dehydrated powders were dissolved in appropriate volumes of distilled water according to manufacturer`s instructions. Substances were mixed thoroughly and gently heated to completely dissolved and autoclaved. When cooled to about 50-55°C, approximately 25ml volumes were dispensed into 90 cm sterile Petri dishes, left to set and agar surfaces dried. For the agar slopes, dehydrated powders were dissolved in appropriate volumes of distilled water according to manufacturer`s instructions. Substances were mixed thoroughly and gently heated to completely dissolve. Appropriate volumes were then dispensed into appropriate tubes before autoclaving. Autoclaved tubes were slanted at appropriate gradients during setting for the agar slopes.

Quality and sterility of prepared media were ascertained by inoculating randomly selected media with *Pseudomonas* and *Escherichia coli* positive control strains.

### a) Mueller Hinton Agar

| *Composition* | *gm/Ltr* |
|---|---|
| Beef infusion solids | 2.0 |
| Acid Hydrolysed Casein | 17.5 |
| Starch | 1.5 |
| Agar No.1 | 17.0 |
| pH   7.3 ± 0.1 | |

*Preparation*

Prepared according to the manufacturer`s (BIOTEC) instructions. When cooled to about 55 °C, approximately 25 ml volumes were dispensed into 90 cm sterile Petri dishes, allowed to set and agar surfaces dried.

**b) MacConkey Agar**

| *Composition* | *gm/Ltr* |
|---|---|
| Peptic digest animal tissue | 20.0 |
| Lactose | 10.0 |
| Bile salts | 5.0 |
| NaCl | 5.0 |
| Neutral red | 0.075 |
| Agar | 12.0 |

pH   7.4 ± 0.2

*Preparation*

Prepared according to the manufacturer`s (OXOID) instructions. When cooled to about 55 °C, approximately 25 ml volumes were dispensed into 90 cm sterile Petri dishes, allowed to set and agar surfaces dried.

**c) Blood Agar**
   *Composition*

| i)     Blood Agar Base | *gm/Ltr* |
|---|---|
| Beef Extract | 10.0 |
| Tryptose | 10.0 |
| NaCl | 5.0 |
| Agar | 15.5 |

ii)    5-10 % Sheep Blood                50 ml/Ltr
         pH 7.3± 0.2

*Preparation*

Prepared according to the manufacturer`s instructions. When cooled to about 55 °C, approximately 25 ml volumes were dispensed into 90 cm sterile Petri dishes, allowed to set and agar surfaces dried.

**d) Nutrient Agar**

| *Composition* | *gm/Ltr* |
|---|---|
| Peptone | 5.0 |
| Beef/Yeast Extract | 3.0 |
| NaCl | 5.0 |
| Agar | 15.0 |

pH 7.0 ± 0.2

*Preparation*Prepared according to the manufacturer`s instructions. When cooled to about 55 °C, approximately 25 ml volumes were dispensed into 90 cm sterile Petri dishes, allowed to set and agar surfaces dried.

### e) Triple Sugar Iron (TSI)

| *Composition* | *gm/Ltr* |
|---|---|
| Peptone | 20.0 |
| Yeast extract | 3.0 |
| `Lab-Lemco powder` | 3.0 |
| NaCl | 5.0 |
| Lactose | 10.0 |
| Glucose | 1.0 |
| Ferric citrate | 0.03 |
| Sodium thiosulphate | 0.3 |
| Phenol red | *q.s* |
| Agar | 12.0 |

pH 7.4± 0.2

### *Preparation*

Prepared according to the manufacturer`s (OXOID) instructions. Completely dissolved mixtures were dispensed into appropriate tubes before they were autoclaved. The tubes were slanted at appropriate gradient before setting.

### f) Urea slope

| *Composition* | *gm/100ml* |
|---|---|
| Glucose | 1.0 |
| Peptone | 1.0 |
| NaCl | 5.0 |
| Di-sodium phosphate | 1.2 |
| Potassium dihydrogen phosphate | 0.8 |
| Phenol red | 0.012 |
| Agar | 15.0 |

pH 6.8 ± 0.2

### *Preparation*

Prepared according to the manufacturer`s (OXOID) instructions. The completely dissolved mixture was autoclaved and allowed to cool. 5ml of filtered sterilized 40% urea solution was then added aseptically to the autoclaved mixture before dispensed into sterile tubes and sloped.

### g) Citrate Agar Slant

| *Composition* | *gm/Ltr* |
|---|---|
| Magnesium sulfate (heptahydrate) | 0.2 |
| Ammonium di-hydrogen phosphate | 1.0 |
| Di-potassium phosphate | 1.0 |
| Sodium citrate (dehydrate) | 2.0 |
| NaCl | 5.0 |
| Bromothymol blue | 0.08 |
| Agar | 15.0 |

pH 6.9

*Preparation*

Prepared according to the manufacturer`s (OXOID) instructions. Completely dissolved mixtures were dispensed into appropriate tubes before they were autoclaved. The tubes were slanted at appropriate gradient before setting.

### h) Peptone water

| *Composition* | *gm/Ltr* |
|---|---|
| Peptone | 10.0 |
| NaCl | 5.0 |

pH 7.1± 0.2

*Preparation*

Prepared according to the manufacturer`s (BIOTEC) instructions. Completely dissolved mixtures were dispensed into appropriate tubes before they were autoclaved.

### i) Kovac`s indole reagent

*Composition*

| | |
|---|---|
| *p*-dimethylaminobenzaldehyde | 5.0g |
| Amyl alcohol | 75ml |
| Conc. HCl | 25ml |

*Preparation*

The aldehyde was first dissolved in the alcohol by warming the mixture gently in a water bath. The mixture was allowed to cool and the acid carefully added and then kept in a brown bottle to protect it from sun light.

**j) McFarland 0.5 Turbidity Standard (per 100ml)**

*Composition*

1ml Conc. $H_2SO_4$

0.5g Dihydrate barium chloride ($BaCl_2.2H_2O$)

*Preparation*

1ml of Conc. $H_2SO_4$ was added to 99 ml of distilled water and thoroughly mixed for 1% v/v solution of $H_2SO_4$ . 0.5g of Dihydrate barium chloride ($BaCl_2.2H_2O$) was dissolved in 50ml distilled water for 1% v/v solution of barium chloride. 0.6ml of the prepared 1% v/v barium chloride solution was then added to 99.4ml of the prepared 1% v/v $H_2SO_4$ solution. Solutions thoroughly mixed and dispensed into capped tubes.

# APPENDIX D: STAINING PROCEDURES

### a) Gram stain

The dried smear was fixed and covered with crystal violet stain for 30 seconds. The stain was quickly washed off with clean water and all the water was tipped off. The smear was then covered with Lugol's iodine for 30 seconds, after which it was washed with clean water. It was then decolourized rapidly with acetone-alcohol and washed immediately with clean water. The smear was then covered with neutral red stain for 2 minutes and then washed. Then the back of the slide was wiped clean and placed in a draining rack for the smear to air-dry. The smear was then examined microscopically first with 40X objective to check the staining and to see the distribution of the material. Then it was examined with the oil immersion objective to look for bacteria and cells.

**Results**

Gram positive bacteria……………………………………………………Dark purple

Yeast cells…………………………………………………………………...Dark purple

Gram negative bacteria……………..……………………………………….Pale to dark red

Nuclei of pus cells…………………………………………………………...Red

Epithelial cells…………………………..…………………………………...Pale red

### b) Giemsa stain

Giemsa stain is used to differentiate nuclear and/or cytoplasmic morphology of platelets, RBCs, WBCs and parasites. It is the most dependable stain for blood parasites particularly in thick films is the Giemsa stain containing azure B. The stain must be diluted for use with water buffered to PH 7.2.

**Procedure**

Allow the blood film to air dry thoroughly for one hour. Stain with diluted Giemsa stain 1:20 for 20 minutes .Wash by placing film in buffered water for 2 to 5minnutes. Let air dry in a vertical position and examine using x40 then oil immersion.

**Results**:

Erythrocytes stain pink, platelets show a light pale pink, lymphocyte cytoplasm appear sky blue and leucocyte nuclear chromatin stain magenta.

# APPENDIX E: BIOCHEMICAL TESTS

## a)    CATALASE TEST

Three millilitres of hydrogen peroxide solution was poured into a test tube. A sterile glass rod was then used to remove a good growth of the test organism and immersed into the hydrogen peroxide solution. Immediate bubbling was looked out for.

## b)    COAGULASE TEST

The plasma was diluted 1 in 10 in physiological saline. Three small test tubes were taken and labeled as follows:

T = test organism

Pos = positive control

Neg = negative control

Then 0.5 ml of the diluted plasma was pipetted into each tube. Five drops of the test organism culture was added to the tube labeled 'T', 5 drops of the positive control was added to the tube labeled 'Pos' and 5 drops of sterile broth was added to the tube labeled 'Neg'. After mixing gently, the 3 tubes were incubated at 35ºC and examined for clotting after an hour.

## c)    OXIDASE TEST

**Principle:**

Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen, the final electron acceptor in some electron transport chains. Thus, the enzyme oxidizes reduced cytochrome c to make this transfer of energy.

The cytochrome oxidase test uses dyes such as p- phenylenediamine dihydrochloride that substitute for oxygen as artificial electron acceptors. In the reduced state the dye is colorless however in the presence of cytochrome oxidase and atmospheric oxygen p- phenylenediamine is oxidized, forming indophenol blue.

**Quality Control Used:**

Positive Control: *Pseudomonas aeruginosa* (ATCC 27853).

Negative Control: *E. coli* (ATCC 25922).

**Procedure:**

1. Two to three drops of the reagent was dropped onto a filter paper strip.

2. Using a sterile plastic loop, a pure colony of the test organism was smeared onto the area on the filter paper containing the reagent.

3. Bacterial colonies observed to have developed a deep blue color at the inoculation site within 10 seconds were considered to have a cytochrome oxidase activity. The test organism was therefore positive for oxidase test.

**d) INDOLE TEST**

**Principle:**

Indole, a benzyl pyrrole, is one of the metabolic degradation products of the amino acid tryptophan. Indole production is an important characteristic in the identification of many species of microorganisms being particularly useful in separating Escherichia coli (positive) from members of the Klebsiella- enterobacter-Hafnia-Serratia group (mostly negative). The indole test is based on the formation of a red complex when indole reacts with the aldehyde group of p-dimethylaminobenzaldehyde.

**Quality Control Used:**

Positive Control: *Escherichia coli* (ATCC *25922*)

Negative Control: *Klebsiella pneumoniae* (ATCC 700603)

**Procedure:**

The test organism was inoculated into MIO medium and incubated at 35ºC for 18-24hrs

Two to three drops of Kovac's reagent using indole reagent droppers were added to the medium after incubation.

The development of a bright fuchsia red colour at the interface of the reagent and the MIO medium within seconds after adding the reagent is indicative of the presence of indole and was interpreted as a positive test

### e)        CITRATE TEST

**Principle:**

This test is commonly used as part of a group of tests that distinguish between members of the Enterobacteriaceae based on their metabolic by-products. The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. A subsequent increase in the pH of the medium is demonstrated by the color change of a bromothymol blue pH indicator.

In most common formulation, citrate is the sole source of carbon in the Simmons citrate medium while inorganic ammonium salt ($NH_4H_2PO4$) is the sole fixed nitrogen source. When an organic acid such as citrate is used as a carbon and energy source, alkaline carbonates and bicarbonates are produced.  The visible presence of growth on the medium and the change in pH indicator color due to the increased pH are the signs that an organism can import citrate and use it as a sole carbon and energy source; such organisms are considered to be citrate positive.

Citrate, a Krebs cycle intermediate, is generated by many bacteria; however, utilization of exogenous citrate requires the presence of citrate transport proteins. Upon uptake by the cell, citrate is cleaved by citrate lyase to oxaloacetate and acetate. The oxaloacetate is then metabolized to pyruvate and $CO_2$. Further metabolic breakdown is dependent upon the pH of the medium.  Under alkaline conditions, pyruvate is metabolized to acetate and formate. The carbon dioxide that is released will subsequently react with water and the sodium ion in the medium to produce sodium carbonate, an alkaline compound that will raise the pH.  In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source.

**Procedure:**

Simmons citrate medium was prepared according to the manufacturer. Tubes were allowed to attain room temperature prior to inoculation. Fresh pure cultures were for inoculation. A single isolated colony was stabbed to the bottom of the tube and lightly streaked the surface of the slant. The cap was placed loosely on the tube since citrate utilization requires oxygen. Inoculated tubes were incubated for 18 to 24hrs at 35°C in an ambient condition.

### f)       TRIPLE SUGAR IRON (TSI) AGAR FERMENTATION

**Principle:**

TSI Agar is used for the determination of carbohydrate fermentation and hydrogen sulfide production in the identification of Gram-negative bacilli. TSI Agar contains three sugars (dextrose, lactose and sucrose), phenol red for the detection of carbohydrate fermentation and ferrous ammonium sulfate for the detection of hydrogen sulfide production (indicated by blackening in the butt of the tube).Carbohydrate fermentation is detected by the presence of gas and a visible color change (from red to yellow) of the pH indicator, phenol red. The production of hydrogen sulfide is also indicated by the presence of a precipitate that blackens the medium in the butt of the tube.

**Procedure:**

The medium was prepared based on the manufacturer's instructions. Tubes were allowed to attain room temperature before inoculation. Fresh pure cultures were used for inoculation. A selected single isolated colony was stabbed to the bottom of the tube after which the surface of the slant was lightly streaked. The cap of tube was placed loosely and inoculated tubes were incubated for 18 to 24hrs at 35°C in an ambient condition

### G). THE A.P.I.20E SYSTEM

### Principle

The API20E contains microamounts of prepared media, which when inoculated will give results similar to the macromolecular testing. The biochemical tests carried out by this system are as follows:

1. Orthonitrophenolgalactosidase
2. Arginine dehydrolase
3. Lysine decarboxylase
4. Ornithine decarboxylase
5. Citrate
6. Hydrogen sulfide
7. Urease
8. Tryptophan deaminase
9. Indole
10. Voges-Proskauer
11. Gelatin
12. Glucose
13. Mannitol
14. Inositol
15. Sorbitol
16. Rhamnose
17. Saccharose/sucrose
18. Melibiose
19. Amygdaline
20. Arabinose

### Setting up and reading an API strip

Underneath each strip is an incubation chamber consisting of a series of wells. These were filled with distilled water at the start of the test.

Using aseptic technique, a colony of the unknown isolate was transferred to a small volume of sterile distilled water, emulsified and vertex for 3 minutes.

The suspension was used to inoculate each of the chambers of the API strip.

Most wells were filled only to the opening of the cup.

Some reactions occur in the absence of air. A drop of mineral oil was placed over these cups.

The test was incubated overnight at thirty-seven degrees Celsius.

It is important to check the purity of the suspension. This was done by inoculating a loopful onto a non-selective medium. If more than one bacterium was present, positive results from each member of the mixture will accumulate leading to an incorrect identification. The purity plate was streaked for single colonies.

It was incubated along with the API test strip.

Following incubation numerous colonies have developed on the plate. They were all of the same type, so we may assume the suspension was pure.

Each reaction on the developed API strip was observed and scored using the API interpretation table. This revealed if a test was negative or positive.

Having established which results were negative and which were positive, the reactions were placed in groups of three and each group was given a score - called an octal score - from zero to seven. The final group included the oxidase test which was performed separately.

If all three reactions were negative the score is zero. If the first reaction in the group was positive the test scored one point. The second test, if positive, scored two points and a positive in the third test scored four points. It infers that a single positive in the third test can be differentiated from a positive in each of the first two tests, which scored one plus two points. By using this system of numbering, every possible combination of results has a unique score from zero to seven.