ASSESSMENT OF MALARIA AND BACTERAEMIA IN FEBRILE PAEDIATRIC PATIENTS FROM PRINCESS MARIE LOUISE CHILDREN HOSPITAL IN ACCRA

BY

DAVID DELALI MENSAH (BSc. Hons)

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text**.**

David Delali Mensah (20251873) …… …….
Student name and ID Signature Date

Certified by:

Prof. E. H. Frimpong …… …….
(Supervisor) Signature Date
Dept. of Clinical Microbiology,
SMS (KNUST)

Prof. E. H. Frimpong …… ……
(Head of Dept.) Signature Date
Dept. of Clinical Microbiology,
SMS (KNUST)

DEDICATION

This thesis is dedicated to God Almighty and to my wonderful family, Evans my Dad, Enyonam my Mum and my two Brothers Edem and Edudzi.

If I ever have the chance of choosing a family again, I will surely choose you all over again. Love you.

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ABSTRACT

Clinical differentiation between severe malaria and invasive bacterial infection is difficult because of the overlap in disease symptoms.

This study compares the number of cases of bacteraemia with malaria infections among febrile paediatric patients. The study also looked at the causative agent of bacteraemia and the antibiotic profile of the isolated bacteria. It also compared clinical diagnosis with laboratory diagnosis.

The study was conducted at Princess Marie Louis Children's Hospital in Accra. Venous blood samples were collected from paediatric patients aged between 5 months to 5 years who were admitted with fever of 38 ^{0}C and above, tested for malaria parasitaemia, and cultivated for possible bacterial growth and their antibiotic profile identified. In a total of 182 patients recruited for the study, bacteraemia was 10.2 % and malaria infection was 21.6 %. Malaria and bacteraemia co-infection was 2.8%. There was a weak linear correlation between clinical diagnosis and laboratory diagnosis of malaria and bacteraemia using Student T-test analysis, thus, 0.215 with $P < 0.01$ and 0.184 with $P < 0.05$ respectively which implies a very little evidence to infer that clinical diagnosis and laboratory diagnosis of patients with fever provided similar results.

The most occurring organisms were Coagulase Negative *Staphylococcus*, *Staphylococcus aureus* and *Salmonella paratyphi* B.

The antibiotic profile indicated that *Staphylococcus aureus* and Coagulase Negative *Staphylococcus* (CNS) were all resistant to Penicillin class antibiotics. *Pseudomonas* spp. had 100% resistance to chloramphenicol, cotrimoxazole and gentamycin. *Salmonella paratyphi* B also showed 100% resistance to chloramphenicol and tetracycline and 40% resistance to cotrimoxazole and gentamicin.

The presence of antibiotic resistant pathogens in our environments has also been highlighted in this study. A continuation of this study involving other hospitals and a larger sample size will provide more data on these resistant pathogens and their distribution which will contribute to health care policy planning in Ghana and the sub-region at large.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria is the most important parasitic diseases of man which is caused by the protozoan *Plasmodium* parasite that is transmitted by infected female Anopheles mosquitoes which often bites at night and leads to infection of the red blood cells. It is a major cause of morbidity and remains the biggest cause of loss of number of days of healthy life. Malaria remains the single largest cause of death in Africa, where it kills one child in every 30 seconds (GFHR, 2006) this translates to the deaths of approximately 3000–6000 children a day. Malaria kills children in three ways; infection in pregnancy (low birth weight and preterm delivery), acute febrile illness (cerebral malaria, respiratory distress and hypoglycemia) and chronic respiratory infection (severe anaemia). Low birth weight is frequently the consequence of malaria infection in pregnant women and has become the major risk factor for death in the first month of life. In areas of stable malaria transmission most adult women have developed enough natural immunity that infection does not usually result in symptoms, even during pregnancy. In areas of unstable malaria transmission women have acquired little immunity and are thus at risk of severe malaria (Ofori *et al*., 2009; Snow *et al.,* 1999). Complication with malaria includes cerebral malaria, renal failure, impaired vision, ataxic gait, hyper reactive malaria syndrome (HMS) and Burkitt's lymphoma. Malaria typically results in flu-like symptoms that appear 9 to 14 days after an infectious mosquito bite. Initial symptoms can include headache, fatigue and aches in the muscles and joints, fever, chills, vomiting and diarrhea which can quickly progress into severe disease and death. Among young children fever is the most common symptom of malaria.

Four *Plasmodium* species have been well known to cause human malaria, namely, *Plasmodium falciparum, plasmodium vivax, Plasmodium ovale, and plasmodium malariae.* A fifth one, *Plasmodium knowlesi,* has been recently documented to cause human infections in many countries of Southeast Asia (Daneshvar *et al.*, 2009). Of the four malaria parasites that affect humans, *Plasmodium falciparum* is the most common in Africa and the most deadly.

Co infections with Malaria

Malaria infection has long been suspected to predispose an individual to bacterial infection, especially those with Gram-negative bacteria, such as non-typhoidal salmonella infections, (Mabey *et al*., 1987, Green *et al*., 1993) which are a major cause of morbidity and mortality in young children in many parts of Africa.

Bacterial infections have been documented to complicate severe forms of malaria (Berkley *et al*., 1999). However, it remains unclear whether such infections are attributable to malaria, other risk factors, or are coincidental. Laboratory evidence has showed that there is impaired immune function in *Plasmodium falciparum* malaria. Opsonization and phagocyte killing by macrophages becomes impaired after ingestion of parasite-derived haemozoin (Scorza *et al*., 1999). Deficient cell mediated cytotoxicity has been demonstrated even in patients with low-level parasitaemia. Patients with malaria have reduced circulating T-Iymphocytes, impaired proliferative T-cell response, and have plasma anti-lymphocyte antibodies. Humoral immunity has also been shown to be impaired (Gilbreath *et al*., 1983, Greenwood *et al*., 1972). The close resemblance of clinical features of malaria to those of other febrile illnesses, such as septicaemia and urinary tract infections is

major issue because failure to treat concurrent bacterial infection in a child with malaria may lead to severe morbidity and mortality.

1.2 Statement of problem

The contribution of malaria to morbidity and mortality among people in Africa has been a subject of academic, political (national and international), and global health interest. While the health importance of malaria is generally acknowledged worldwide, there is a growing concerned that the rate of morbidity and mortality figures may not be accurate. Other diseases with similar symptoms to malaria may be contributing to the rising figures of morbidity and mortality figures being reported for malaria. This is because patients reporting to a hospital or health facility in Sub-Saharan Africa with clinical symptoms including fever, headache, vomiting, diarrhea, lethargy and anaemia are often regarded as having malaria (Font *et al*., 2001). The clinical diagnosis of malaria has always remains challenging because malaria features mimic those of many other infectious diseases but are not usually recognized in most cases (Luxemburger *et al*., 1998). Differentiation of the clinical presentation of malaria from bacterial sepsis may be difficult leading to a wide underestimation of the role of bacteraemia in causing morbidity and mortality among children in Sub-Saharan Africa. Failure to treat concurrent bacterial infection in a child with malaria may lead to severe illness.

Putting a stop to blind treatment of malaria has therefore become critical to avoid mismanagement in malaria treatment. In Ghana, people now have cheap access to the most potent malaria drugs, thus, Artemisinin-based combination therapies (ACTs). If care is not taken the medicine could become ineffective. Most a times, when a bacterial infection is assumed, due to inadequate laboratory capacity to

conduct culture and sensitivity, treatment is often given in the form of broad spectrum antibiotics. The selection of antibiotic resistance is also greatly enhanced leading to increase in prevalence of cases that are resistant to the first line anti-biotic drugs. The need for a simple point of care diagnosis for all fever related illness cannot be overemphasized and laboratory evidence remains the only acceptable means of confirming infection. Until this is achieved, it will be difficult to tell the true incidence of malaria infection from other diseases.

Data on the prevalence of invasive bacterial infection in malaria among young children are limited. Such information is important to health workers on how frequently antibacterial therapy may be indicated in children presenting with non-focal febrile illness and malaria parasitaemia. However, there are growing body of evidence that bacterial bloodstream infections may account for hospital admissions with fever and a higher proportion of child deaths despite the widespread perception that bacterial infections have only marginal significance in public health. In Kenya, Berkley and colleagues found in a study they conducted in Killifi District Hospital, that deaths in children with bacteraemia were rapid: 103 of 308 deaths occurred on the day of admission (33.4 %), 79 occurred the next day (25.6 %), and 35 occurred on day 3 (11.4 %). Among children under the age of 5 years, the incidence of admission to the hospital with a diagnosis of malaria was 3893 admissions per 100,000 per year, and the incidence of admission with a diagnosis of gastroenteritis was 2057 per 100,000 per year. The stimulus for their study was the observation that patient deaths in Kilifi usually occurred after a febrile illness. Although the area has a high rate of malaria, the disease was associated with less than a quarter of the deaths and there were few data on other causes of death. They discovered that

community-acquired bacteraemia was responsible for at least one third of deaths in infants and one quarter of deaths in children under one year of age (Berkley *et al*., 2005). Recent studies also revealed co-morbidity of bacteraemia with HIV/AIDS, malnutrition, or sickle-cell anaemia. William *et al* detected that out of 2157 episodes of bacteraemia in a coastal district of Kenya, 1749 of these children (81%) were typed for sickle-cell anaemia (William *et al.,* 2009). More importantly, bacterial sepsis was shown to be responsible for about 26% of child deaths in a hospital in Kenya, compared to a fatality rate of 22% in children suffering from malaria in the same hospital (Berkley *et. al*., 2009).

In Ghana, a study conducted in 2004 at Kumasi by Evans *et. al*., indicated infant bacteraemia to be substantial, with a high fatality rate of 39% (Evans *et al*., 2004). Studies done by the Department of Pediatrics, University of Ghana Medical School, among newborns revealed neonatal bacteraemia of 22.2% with high fatality rate (Anyebuno and Newman 1995).

1.3 Justification

While public interest tends to focus on malaria, tuberculosis and HIV, the morbidity and mortality burden of systemic bloodstream infections are still insufficiently investigated. Recently published data from Tanzania reveals clinical overestimation of malaria, whereas invasive bacterial disease was underestimated (Crump *et al*., 2011). Clinical differentiation between severe malaria and invasive bacterial infection is difficult because of the overlap in disease symptoms (Evans *et al.,* 2004). A further factor complicating the diagnosis and therapy is self-treatment with antimicrobial drugs prior to professional health care, which may impede the diagnosis and increases the risk of emergence and spread of antibiotic resistance (Ilic *et al.,* 2012). Apart from a few well-equipped hospitals, health facilities in most

malaria endemic countries in sub-Saharan Africa lack microbiological diagnostic capacities necessary to diagnose bacteraemia and to isolate bacterial pathogens in order to allow targeted treatment (Petti *et al*., 2006). Clinical diagnosis of malaria is common in many areas where laboratory infrastructure is lacking. In much of the malaria-endemic world, resources and trained health personnel are so scarce that presumptive clinical diagnosis is the only realistic option. Improved diagnostic accuracy is essential in the treatment of all fever related diseases. Appropriate diagnosis and treatment require better knowledge of the spectrum of infective agents in malaria-endemic countries. Accordingly, the aim of the study was to diagnose all febrile paediatric patients in order to assess the incidence of bacteraemia as compared to malaria infections and to identify bacterial pathogens leading to sepsis as well as the spectrum of antibiotic resistances.

1.4 Objectives

To assess the number of bacterial bloodstream infections as compared to malaria infections among febrile paediatric patients, and to determine the causative agent and its antibiogram.

1.5 Specific objectives:

1. To assess all febrile paediatric patients admitted to Princess Marie Louis Children's Hospital for malaria parasite.
2. To assess all febrile paediatric patients admitted to Princess Marie Louis Children's Hospital for invasive bacterial infection by blood culture.
3. To determine the causative bacterial agents involved and their antibiogram.
4. Compare clinical diagnosis of malaria and bacteraemia with laboratory diagnosis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mortality rate and disease burden of malaria

Malaria, which has been described as a disease of poverty and underdevelopment, remains a complex and overwhelming health problem with an estimated 655 000 persons dying of malaria in 2010. 86% of the victims were children under 5 years of age, and 91% of malaria deaths occurred in the WHO African Region (WHO 2010). Malaria is endemic in 107 countries and territories in tropical and subtropical regions, with sub-Saharan Africa being the most affected. An estimated 3 billion people, almost half the world's population, live in areas where malaria transmission occurs. About 50 million pregnant women, worldwide, are exposed to malaria a year of which 60% of them occur in Africa (Crawley *et al*., 2007). Pregnant women are twice as likely to become infected with *P. falciparum* malaria as non-pregnant women living under the same conditions (Lindsay *et al* 2000). Studies in sub-Saharan Africa indicated that 25% of deliveries in areas of stable transmission show evidence of *Plasmodium falciparum* malaria infection in the placenta (Desai *et al*., 2007). A study done in Ghana by Ofori *et al* also indicated that the prevalence of malaria parasites in the placenta was 35.9% (61/170) as the incidence of Low Birth Weight (LBW) infants was 17.7% (30/170), most common among pregnant women with placental *P. falciparum* infection in the Dangme-West district in Greater Accra Region (Ofori *et al*., 2009).

About 90 % of all deaths attributable to malaria occur in sub-Saharan Africa. This is attributable to two main causes. First, the majority of infections in this region are

caused by *Plasmodium falciparum*, the most dangerous of the four human malaria parasites; and second, the most effective malaria vector the mosquito, *Anopheles gambiae*, is mostly widespread in the region and the most difficult to control (Brabin 1983).

2.1.2 Geographical Distribution and Population at Risk of malaria

Malaria transmission occurs primarily in tropical and subtropical regions in sub-Saharan Africa, Central and South America, the Caribbean island of Hispaniola, the Middle East, the Indian subcontinent, South-East Asia, and Oceania. In areas where malaria occurs there is variation in the intensity of transmission and risk of malaria infection. Highlands above 1500 m and arid areas of 1000 mm rainfall per year have less malaria. Although urban areas have been at lower risk, population growth has contributed to urban malaria transmission (Knudsen *et al*., 1992).

East Asia and the Pacific countries have the highest rates of drug resistance, which has contributed to the resurgence of malaria in many areas, particularly along international borders. Central Asia has seen a recent resurgence in *Plasmodium vivax* malaria. Malaria transmission in Latin America and the Caribbean occurs mainly in countries that share the Amazon rainforest, and population movements associated with gold mining and forestry have led to isolated malaria epidemics in these areas. In recent years, four countries have been certified by the WHO Director-General as having eliminated malaria: United Arab Emirates (2007), Morocco (2010), Turkmenistan (2010), and Armenia (2011). Not one case of *Plasmodium falciparum* was reported in Europe in 2009 this is the first time the whole continent has had a malaria-free year (WHO, 2011).

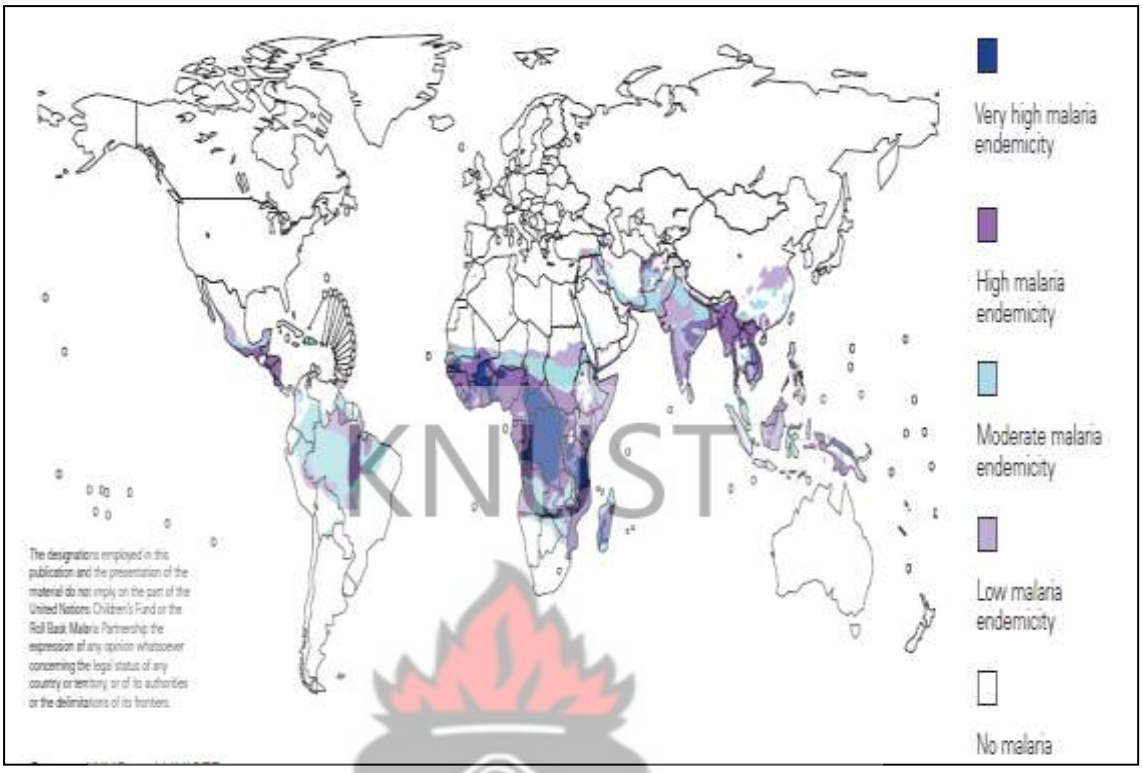

Figure 2.0: Global malaria Distribution and Endemicity, 2003

(Source: WHO and UNICEF, 2005c)

2.1.3 Clinical over estimation of malaria cases.

Malaria is one of the most common causes of illness and death among children in sub-Saharan Africa. The Roll Back Malaria (RBM) partnership proposes to reduce by 75% the 2005 malaria burden by 2015. However, establishing the role of malaria in causing disease or death is not straightfor-ward. A study conducted in children by Okwara *et al* at the University of Nairobi teaching hospital Kenyatta National Hospital, in Nairobi, Kenya suggest that up to two-thirds of children hospitalised with fever without obvious infective focus have malaria, and that up to one-fifth have either bacteraemia or significant bacteriuria. Bacterial co-infection

appears to be similarly present among children with and without malaria parasitaemia.

At 10 Tanzanian hospitals, 39% of "positive" malaria slides were false positives and at 17 Kenyan outpatient clinics, the positive predictive value of a "positive" slide was only 22% (negative predictive value 93%) when compared with expert microscopy. In a recent Tanzanian study of 4,474 severely ill patients at 10 hospitals, 54% had a negative malaria slide. Two thirds of slide-negative patients were not treated with antibiotics, and a greater proportion of these individuals died (12%) compared with those with a positive slide (7%). The research group further reported that with the exception of children under 5 years of age in the highest transmission regions, most patients treated for ma-laria in North Eastern Tanzania had no evidence of para-sitemia (Reyburn et al 2006)

Zurovac *et al*., 2006 carried out a study in which out of 359 consultations performed by 31 clinicians at 17 facilities. Clinical assessment was suboptimal. Blood slide microscopy was performed for 72.7% of patients, who represented 78.5% of febrile patients and 51.3% of afebrile patients. About 95.5% of patients with a positive malaria microscopy result and 79.3% of patients with a negative result received antimalarial treatment. The prevalence of confirmed malaria was low in both high (10.0%) and low-(16.3%) transmission settings. Combining data from both settings, the sensitivity of routine microscopy was 68.6%; its specificity, 61.5%; its positive predictive value, 21.6% and its negative predictive value, 92.7%. (Zurovac *et al*., 2006)

A study in Nigeria also highlighted over diagnosis and over treatment of malaria in Lagos. Out of a total of 1,211 children aged between 0-12 years only 16.9% of them

had malaria positive slides (Oladipo *et al*., 2012). Presumptive treatment of all childhood fevers or suspected malaria cases as Malaria results in malaria over diagnosis, which means other Causes of febrile illnesses, such as pneumonia and meningitis, are missed at initial presentation at health facilities until a much later time when the child's condition does not improve.

2.1.4 Epidemiology of Malaria in Ghana

Malaria is a major public health concern in Ghana with the entire population of 24.2 million at risk. According to the Ghana Health Service (GHS) health facility data, malaria is the number one cause of morbidity, accounting for about 38% of all outpatient illnesses, 36% of all admissions, and 33% of all deaths in children under age five. 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. An estimated 14,000 deaths in children under five were attributable to malaria (Ghana statistical service 2003).

Malaria is more than a health issue as the activities of other sectors may increase or decrease the malaria disease burden. In addition, malaria impact adversely on productivity of all sectors of the economy.

2.1.4.1 Distribution and Transmission of Malaria in Ghana

The malaria status of Ghana is hyper endemic with transmission being high throughout the year. The entire population of Ghana of 24.2 million is at risk of malaria, although transmission rates are lower in some urban areas. Transmission occurs year-round with seasonal variations. Ghana can be stratified into three malaria epidemiologic zones: the northern savannah; the tropical rainforest; and the coastal savannah/mangrove swamps. The major vectors are *Anopheles gambiae* and

An. funestus. These species generally bite late in the night, are indoor resting, and are commonly found in the rural and peri-urban areas where socio-economic activities lead to the creation of breeding sites. President Malaria Initiative (PMI), in collaboration with the Noguchi Memorial Institute for Medical Research (NMIMR), has documented high rates of outdoor biting (typically > 50 % out biting pre-indoor residual spray) in the Northern Savannah (Ghana statistical service 2003). *Anopheles melas* is found in the mangrove swamps of the Southwest and *An. arabiensisin* Savannah areas of Northern Ghana. Northern Ghana experiences pronounced seasonal variations with a prolonged dry season from September to April. The normal duration of the intense malaria transmission season in the northern part of the country is about seven months beginning in April/May and lasting until September (Adams *et al.*, 2004).

2.2 The Malaria Vector

Mosquitoes belong to the order Diptera, sub-order Nematocerca of the class Insecta which contain three subfamilies *Toxorhynchitinae, Culicinae* and *Anophelinae*. Mosquitoes belong to the genus *Anopheles,* subfamily *Anophelinae* and are the exclusive vectors of human malaria because of their behaviour, physiology and the close relationship with humans. In sub-Saharan Africa, where 90% of the world's malaria cases occur, *Anopheles gambiae* Giles, *An. arabiensis*Patton of the *An. gambiae* complex and *An. funestuts*Giles from the *Anopheles funestus* group are the most efficient malaria vectors (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987).

2.2.1 The sibling species of *Anopheles gambiae* complex

The members of the complex (*Anopheles bwambae*, *An.quadriannulatus* A and B, *An.melas*, *An. Merus*, *An. Arabiensis* and *An. gambiae s.s.*) show differences in seasonality of breeding, adaptation to natural or human-disturbed habitat, host preference, susceptibility to parasite infection and habitat selection such as indoor/outdoor resting (Coluzzi *et al*., 1979).

2.2.1.1 *Anopheles bwambae*

This specie is found only in Bwamba hot spring in Uganda (White, 1985). Larvae of *An. bwambae* are mainly found in spring water habitat having a comparatively higher conductivity, temperature and pH than normal fresh water sites habituated by larvae *of An. gambiae s.s.* Adults of *An. bwambae* are found not more than 10 km away from hot springs. During daytime, adults rest mainly on the buttressed bases of large tress, on fallen logs and sticks and under loose dry leaves (White 1985). They feed on both domestic animals and humans (Lumsden, 1952). Due to its restricted distribution it does not play an important role in disease distribution. However some *An. bwambae* adults have been found with both sporozoites and filariae (White, 1985).

2.2.1.2 *Anopheles quadriannulatus*

Anopheles quadriannulatus is tolerant of cold conditions and found mainly in Eastern and Southern Africa. The adult exploit sun lit residual puddles or temporary or semi-permanent ponds for egg laying (White, 1974). *Anopheles quadriannulatus* also bites mainly during the first half of the night. Female were considered to be strictly zoophilic. Even though malaria parasite has not been found in wild *Anopheles quadriannulatus*, it has been shown to be susceptible to *P. falciparum* in

the laboratory. It is also susceptible to the human filarial worm *Wuchereria bancrofti* (Hunt and Gunders, 1990).

2.2.1.3 *Anopheles melas*

Anopheles melas is limited to the cost of Western Africa (Coluzzi, 1984). *Anopheles melas* larvae usually develop in water such as estuaries, lagoons and mangrove swamps which have salinity around or lower than seawater (Coluzzzi, 1984; Diop *et al*., 2002). This species can occur up to 150 km inland. They are occasionally found in fresh water ponds in small members. Adult *Anopheles melas* are influenced by both rain and tides hence their densities vary throughout the year (Fonseca *et al*., 1996; Diop *et al*., 2002). Its preferred host is man but it feeds on animas when man is absent (Akogbeto, 2000; Diop *et al*, 2002). Under natural conditions *Anopheles melas* does not seem to live more than15 days. It is an efficient vector of *Wuchereria bancrofti*.

2.2.1.4 *Anopheles merus*

Anopheles merus is found in coastal regions where larvae are found in salty water such as lagoons, swamps, ponds formed by seawater and diluted by rain. They are found exclusively in East Africa, South Africa and some island in the Indian Ocean (Coluzzi, 1984). *Anopheles merus* is zoophilic but it is anthropohilic in the absence of animals. *Anopheles merus* transmit malaria and bancroftian in the laboratory. However, under natural condition it is not a good vector of bancroftian filariasis due to its short life span and feeding preference (Southgate and Bryan, 1992).

2.2.1.5 *Anopheles arabiensis*

Anopheles arabiensis is better adapted to humid environment with annual rainfall less than 1000 mm. It is the main predominant member of the complex found in arid regions of West Africa, Ethiopia and Sudan (Coluzzi *et al*., 1979). They lay their eggs in temporary waters bodies which are usually small, shallow and exposed to the sun (Gillies and DeMeillon, 1968; Gilles and Coetzee 1987). They also exploit permanent, artificial water bodies such as rice fields and the garden wells (White *et al*., 1972).

This species prefares feeding on humans but in areas where cattle abound they prefer to feed on the cattle (Coluzzi *et al*., 1979). *Anopheles arabiensis* is one of the main malaria and bancroftian filariasis vectors in Afro-tropical region (White, 1969).

2.2.1.6 *Anopheles gambiae* sensu stricto

Anopheles gambiae sensu stricto is the main malaria vector in sub- Saharan Africa. The species has 5 cytological forms known as Forest, Savanna, Barmako, Mopti and Bissau (Coluzzi *et al*., 1979). There are also 2 molecular forms M and S which differ in both transcribed and nontranscribed species in the rDNA repeat unit (Favia *et al*., 2001; Gentile *et al*., 2001). Interbreeding between M and S form produce fertile progeny under laboratory conditions however M-S hybrids are rarely observed in nature.

A study of microsatellite throughout the genome failed to find significant and consistent differences among the chromosomal forms except for loci on 2R (Lanzaro *et al*., 1998). In a similar microsatellite study, Wang *et al.* (2000) confirmed little differences between M and S molecular forms except for microsatellite tightly linked to, but distinct from, the rDNA region of the X chromosome. There is evidence for

further genetic differentiation between M and S rDNA types for genes not on the X chromosome. This is for an insecticide resistant gene *Knockdown resistance (Kdr)* (Weill *et al*., 2000) located on the left arm of the second chromosome, 2L, location 20C. In the case of the *Kdr,* the S type has a much higher frequency of the resistance allele compared to M type captured at the same time. Hence this is not a case of a fixed difference; rather, there are extreme frequency differences between sympatric M and S forms (Ranson *et al*., 2000). In West Africa (Mali and Burkina Faso) the M molecular forms correspond to the Mopti chromosomal form and the S molecular forms always corresponds to the Savanna or Bamako form (Favia *et al*., 1997). In other areas this clear correspondence breakdown (della Torre *et al*., 2001).

Anopheles gambiae s.s is wide spread in Africa through better adaptation to wet region than to Savanna areas (Lindsay *et al*., 1998). The Forest and Bissau chromosomal forms are found in humid and coastal areas of West Africa. The Bamako, Mopti and Savanna chromosomal forms are more adapted to dry environment (Favia *et al*., 1997). The distributions of the Savanna chromosomal forms overlap that of Bamako and Mopti ranges. The Savanna chromosomal form is generally absent from sahelian zones and from areas of large irrigation schemes where they are displaced by mopti. The M molecular form is generally found in domestic environment and larval habitats created by human activities, where as the S form is generally found in rain-dependent temporary breeding sites (Favia *et al*., 1997). *Anopheles gambiae s.s* is an effective vector of malaria, bancroftian filariasis and arboviruses.

2.2.2 Life Cycle of *Anopheles* Mosquito

The *Anopheles* mosquitoes are found in areas that harbour an abundance of water in order to breed. The larvae of these mosquitoes can be found in ponds, marches, swamps, ditches, rain pools, and on the shores of streams and rivers (Gillies and DeMeillon, 1968). Some breed in shady areas such as forests, while others breed in open fields where there is plenty of sunlight.

Even though the *Anopheles* mosquito can live 10 to 14 weeks under tropical climate, the average life span is about 3 to 4 weeks (Service, 1993). Gillies (1988) stated that environmental factors such as temperature, humidity and availability of natural enemies determine the life span of the adult mosquito. The *Anopheles* mosquitoes have similar life cycle that is completed in 4 distinct developmental stages (complete metamorphosis) thus is egg, larva, pupa and imago (adult) as shown in figure 2.4 below. Once the male has mated with the female, she must find a blood meal for sustenance before laying her eggs. Thereafter, the female will deposit its eggs on the surface of water. In about 2 to 3 days, the eggs hatch(Service, 1993) and the *Anopheles* larvae remain under water, feeding on plankton and micro-organisms by filtering the water with their mouth brushes for approximately 7 to 14 days before turning into pupae (Service, 1996). The adult mosquito emerges from the pupae after 2 to 3 days, and is able to fly in a matter of minutes. The entire duration from egg to adult mosquito is dependent on temperature and may be as short as 2 weeks (Service 1993, 1996).

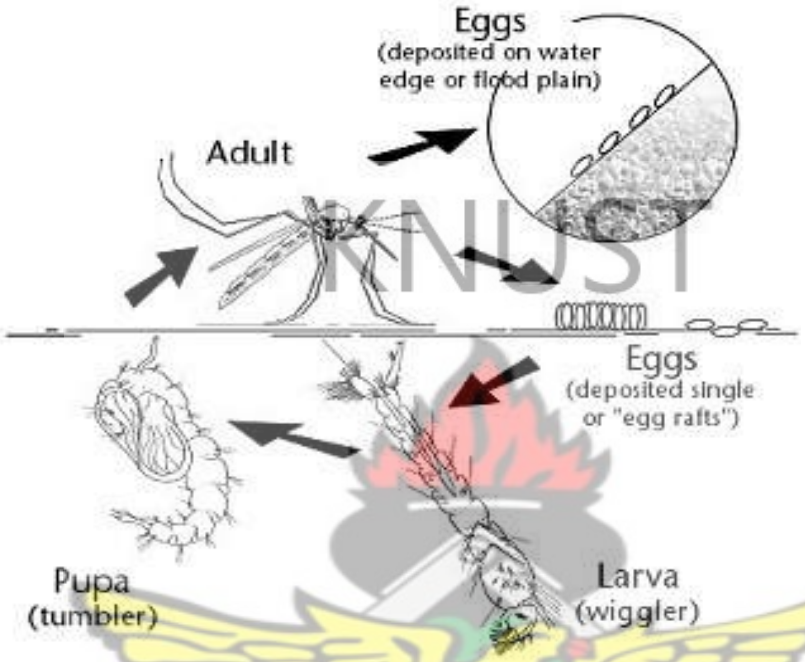

Figure 2.1: Life cycle of mosquito

Source: http://whatcom.wsu.edu/commun/wnvhomeowners.htm

2.3 The Malaria Parasites

The parasites that cause malarial disease are protozoan organisms of the *Plasmodium* genus that also infect many animal species including primates, lizards and birds. Five species of *Plasmodium* (*Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi*) cause malaria in humans. *P. falciparum* is the most virulent parasite, and is responsible for the majority of malaria-related mortality. It is found in all malaria endemic regions of the world and is the most common human malaria parasite in Africa (WHO, 2005).

2.3.1 *Plasmodium falciparum*

Malaria caused by this species also called malignant (Rich *et al.,* 2009) or falciparum malaria (Perkins *et al*., 2011) is the most dangerous form of malaria (Perlmann *et al*.,2000), with the highest rates of complications and mortality. Roughly 50% of all malarial infections are caused by *P. falciparum.*

2.3.2 *Plasmodium malariae*

It is closely related to *Plasmodium falciparum* and *Plasmodium vivax* which are responsible for most malarial infection. It is found worldwide and also called "benign malaria" Its infection is not as dangerous as that produced by *P. falciparum* or *P. vivax*. *P. malariae* causes fevers that recur at approximately three-day intervals (a quartan fever) longer than the two-day (tertian) intervals of the other malarial parasites, hence its alternate names quartan fever and quartan malaria.

P. malariae can be maintained at very low infection rates among a sparse and mobile population because unlike the other *Plasmodium* parasites, it can remain in a human

host for an extended period of time and still remain infectious to mosquitoes (Mohapatra *et al*., 2008).

2.3.3 *Plasmodium vivax*

Plasmodium vivax is the most frequent and widely distributed cause of recurring Benign tertian malaria. *P. vivax* is one of the species of malaria parasites that commonly infect humans. It is less virulent than *Plasmodium falciparum*, and is seldom fatal (WHO, 2005). *P. vivax* is found mainly in the United States, Latin America and in some parts of Africa (Carter and Mendis, 2002). *P. vivax* can cause death due to splenomegaly (a pathologically enlarged spleen), but more often it causes debilitating, but nonfatal, symptoms (Lindsay and Hutchinson 2006). Overall it accounts for 65% of malaria cases in Asia and South America (Gething *et al*., 2012).

2.3.4 *Plasmodium ovale*

Plasmodium ovale causes tertian malaria in humans. It is closely related to *Plasmodium falciparum* and *Plasmodium vivax*, which are responsible for most malaria. It is rare compared to these two parasites, and substantially less dangerous than *P. falciparum*. *P. ovale* has recently been shown by genetic methods to consist of two subspecies, *P. ovale curtisi* and *P. ovale wallikeri* (Sutherland *et al*., 2010).

While it is frequently said that *P. ovale* is very limited in its range being limited to West Africa, the Philippines, eastern Indonesia, and Papua New Guinea (Faye *et al*., 1898).

2.3.5 *P. knowlesi*

P. knowlesi was reported to be the most common cause of hospitalization for malaria in the Kapit Division of Sarawak in Malaysian Borneo (Singh *et al*., 2004). There have also been reports of locally acquired *P. knowlesi* infections from Southern Thailand, the Myanmar-China border, the Philippines, and Singapore (Daneshvar *et al* 2009) indicating that transmission occurs in many Southeast Asian countries. *P. knowlesi* is primarily a chronic infection of the long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques (Daneshvar *et al*., 2009). It is easily confused with *Plasmodium malariaeon* blood film microscopy in cases of human infection, because the morphologic appearances are almost identical (Daneshvar *et al*., 2009). However, *P. knowlesi* is unique amongst the primate and human malarias in that it has a 24-h erythrocytic cycle (Daneshvar *et al*., 2009), which is a characteristic that is likely to accelerate the development of complications (Cox-Singh *et al*., 2008).

2.4. Pathophysiology of malaria

Malaria parasite exhibits a complex life cycle involving an insect vector (mosquito) and a vertebrate host (human). The four main *Plasmodium* species that infect humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* all exhibit a similar life cycle with only minor variations. The life cycle of the *Plasmodium* parasite in humans consist of two stages: first in the liver cells (Tissue Schizogony), then in the blood cells (Erythrocytic Schizogony).

2.4.1. Tissue Schizogony

When the female *Anopheles* mosquito vector takes a blood meal, sporozoites contained in the saliva of the mosquito are inoculated into the blood of a human host. Malaria infection can also occur by transfusion of infected donor blood, by injection through the use of needles and syringes which are contaminated with infected blood. Sporozoites are carried by the circulatory system to the liver and invade hepatocytes. The intracellular parasite undergoes an asexual replication known as exoerythrocytic schizogony within the hepatocyte. Exoerythrocytic schizogony culminates in the production of merozoites which are released into the bloodstream. One parasite cell in the liver stage produces 10 000 (in *P. vivax*) to 30 000 (in *P. falciparum*) merozoites. These then enter the blood system where they start multiplying further. Some sporozoites grow into trophozoites and remain dormant (hypnozoites), and re-emerge after 1-18 months (in *P. vivax* or *P. ovale*). Recurrence in *P. falciparum* (in less than a year) and *P. malariae* (up to 50 years) is due to re-emergence of parasites living at very low levels in the blood.

2.4.2. Erythrocytic Schizogony

Merozoites invade erythrocytes and undergo a trophic period in which the parasite enlarges. The merozoite then develops into a trophozoite stage which is also the feeding stage. It finally breaks free, thereby, destroying the cells. Trophozoite enlargement is accompanied by an active metabolism including the ingestion of host cytoplasm and the proteolysis of hemoglobin into amino acids. The end of the trophic period is manifested by multiple rounds of nuclear division without cytokinesis resulting in a schizont. Merozoites bud from the mature schizont, also called a segmenter, and the merozoites are released following rupture of the infected erythrocyte. Invasion of erythrocytes reinitiates another round of the blood-stage

replicative cycle. This multiplication can quickly result in severe disease and death. The blood stage is responsible for the pathology associated with malaria. *P. malariae* exhibits a 72 hour periodicity, whereas the other three species exhibit 48 hour cycles. Fever is caused by release of waste material when infected cells rupture in the blood. Cerebral malaria is caused by clotting of red blood cells in the brain blood capillaries as a result of the malaria infection. Severe anaemia is caused by destruction of both infected and uninfected cells by the parasite or by the body itself. The failure of other organs, like kidneys, liver and spleen, is caused by the flood of waste materials and the clotting of blood capillaries, to the point where the body can no longer cope. As an alternative to the asexual replication cycle, the parasite can differentiate into sexual forms known as macro and micro gametocytes.

2.4.3 Sexual phase (Sporogony)

The formation of gametocyte and development of gamete takes place in the mosquito vector. Soon after infection of the human host, some parasites, instead of dividing, start forming sexual cells, called gametocytes. Micro gametes, formed by a process known as exflagellation, are flagellated forms which will fertilize the macrogamete leading to a zygote. The zygote develops into a motile ookinete which penetrates the gut epithelial cells and develops into an oocyst. The oocyst undergoes multiple rounds of asexual replication resulting in the production 8-10 000 sporozoites. After 7-20 days depending on the external conditions, there is rupture of the mature oocyst releasing the sporozoites into the hemocoel (i.e. body cavity) of the mosquito. The sporozoites migrate to and invade the salivary glands, thus completing the life cycle. From here they are injected into another human when the mosquito takes its blood meal.

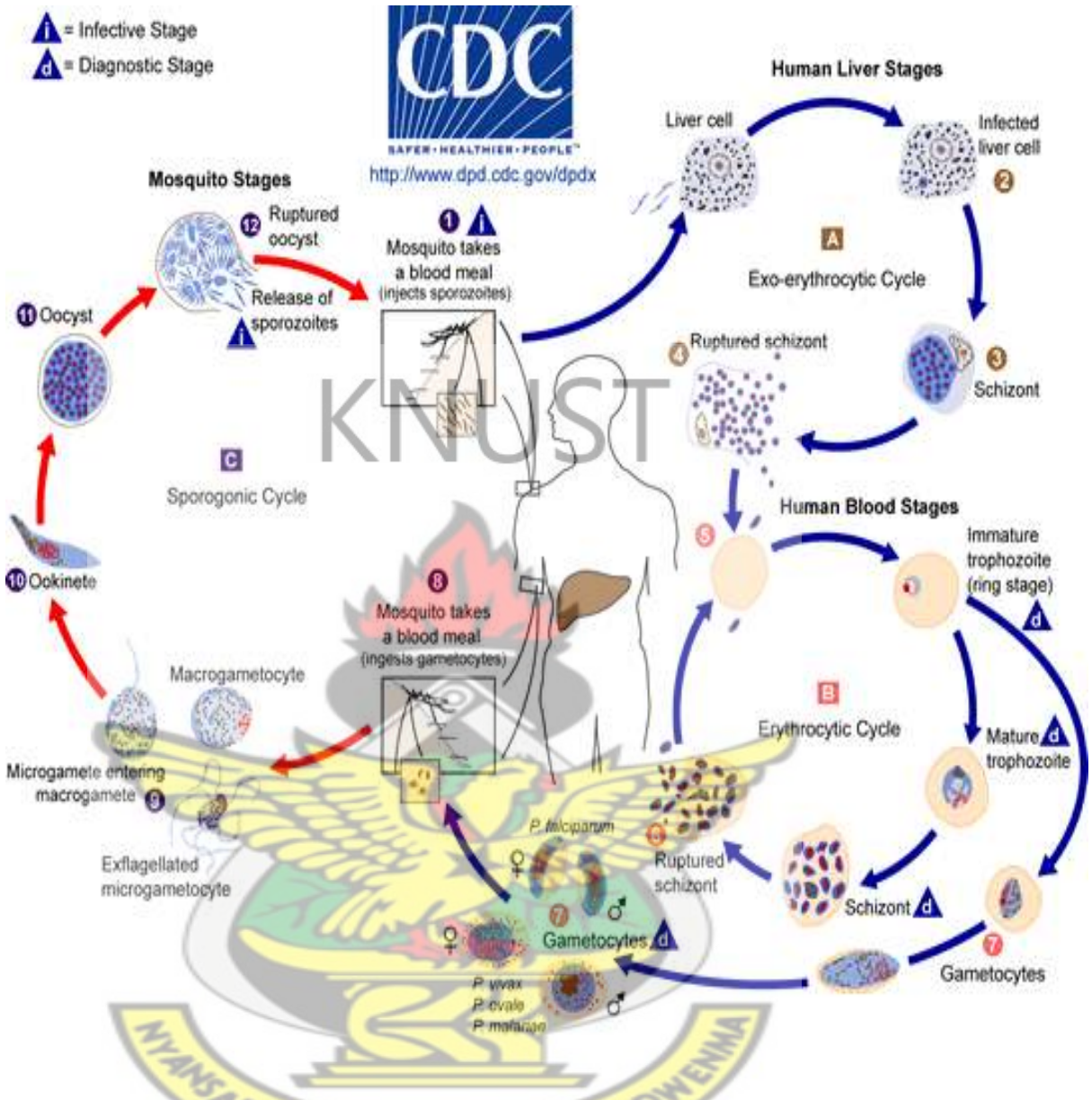

Figure 2.2: Life cycle of Plasmodium spp.

Source: Centers for Disease Control (CDC) www.dpd.cdc.gov/dpdx

2.4.4 Clinical features of malaria

Malaria infection could be either asymptomatic i.e. no record of illness, clinical (characterized by febrile episodes) or severe (complicated). Clinical symptoms that are associated with uncomplicated malaria include fever, chills, sweats, headaches, cough, nausea and vomiting progressing to severe complications (1-2 days). Severe or complicated malaria leads to symptoms such as severe anaemia, kidney failure, coma, hypoglycemia, respiratory distress and death. These symptoms are primarily due to the asexual multiplication of the blood stage of the parasite. The rupture of the erythrocytic schizonts together with toxic metabolites characterizes the febrile episodes that are common to uncomplicated malaria. *Plasmodium falciparum* known to be the most deadly of all the *Plasmodium* species that cause disease in humans causes severe complications such as cerebral malaria, glomeronelophritis, nephrotic syndrome, hypoglycemia, jaundice, pulmonary oedema and renal failure. *Plasmodium vivax* is also known to cause hepatosplenomegaly while *Plasmodium malariae* is known to cause nephritic syndrome. Persistent parasite may cause recurrent fever when infection recrudesces even decades following primary infection. Fever decreases in frequency and severity overtime. Anaemia and splenomegaly are possible. Persistence of low level parasitaemia in blood may lead to chronic malaria. Recurrent attacks of malaria anaemia, hepatosplenomegaly, diarrhea and weight loss, increases other factors especially bacteria gastro enteritis. Partial immunity may result with onset of progresive falciparum malaria associated with increased incidence of Burkitt's 2^0 to imperial T-cell immunity.

2.4.5 Diagnosis of Malaria

A definitive diagnosis of malaria is by the identification of the parasite in blood. The presence of asexual forms must be established. The discovery of gametocytes of *Plasmodium falciparum* only is not sufficient to confirm the diagnosis of active malaria since they may be found in the peripheral blood for weeks after the over attack has subsided or been cured. Clinical judgment is needed in the diagnosis of malaria. The detection of parasite in blood film does not prove that the patient is suffering from malaria because parasitaemia may be entirely asymptomatic in semi immune indigenous children. For the diagnosis of the parasite, examination of a thick blood film is sufficient. The species of the parasite can usually be identified in the thick blood film but should be confirmed by examination of a thin film which shows the undistorted parasites within the red cells. Direct microscopic examination of intracellular parasites on stained blood films is the current standard diagnosis for malaria. However, several other approaches exist such as Antigen detection tests also known as rapid or "dipstick" tests (Fortier *et al*., 1987). Simple light microscopic examination of Giemsa stained blood films is the most widely practiced and useful method for definitive malaria diagnosis.

2.4.6 Treatment and management of malaria

Malaria is preventable, treatable and curable. People at risk can prevent malaria if they sleep under insecticide treated mosquito nets. Pregnant women, especially those who are pregnant for the first time, can take medicines to prevent and treat malaria. People can treat malaria with a wide variety of effective medicines, and they can cure malaria if they seek and receive early treatment from the hospital. Since there is presently no effective vaccine for malaria, development of a vaccine that protects

against the development of malaria is an important step in preventing morbidity and mortality associated with the disease. The primary treatment interventions for the management of severe malarial anemia are the use of antimalarial drugs, transfusion, and fluid replacement. The most widely used are quinine and its derivatives and antifolate combination drugs. Tetracycline and derivatives such as doxycycline are very potent antimalarials and are used for both treatment and prophylaxis. In areas where response to quinine has deteriorated, tetracyclines are often used in combination with quinine to improve cure rates. Clindamycin has been used but offers only limited advantage when compared to other available antimalarial drugs. Parasitological response is slow to clindamycin (Kremsner *et al*., 1994).

2.5 Bacteraemia

Bacteraemia is the presence of viable bacteria in the circulating blood (Spraycar, 1995). This may or may not have any clinical significance because harmless, transient bacteraemia may occur following dental work or other minor medical procedures; however, this bacteraemia is generally clinically benign and self-resolving in children who do not have an underlying illness or immune deficiency or a turbulent cardiac blood flow. The concern with occult bacteraemia is that it could progress to a more severe local or systemic infection if left untreated. Most episodes of occult bacteraemia spontaneously resolve, and serious sequelae are increasingly uncommon. However, serious bacterial infections occur, including pneumonia, septic arthritis, osteomyelitis, cellulitis, meningitis, and sepsis, possibly resulting in death (Kuppermann, 1999).

Patients with occult bacteraemia do not have clinical evidence other than fever (Harper, 1993). First described in the 1960s in young febrile children with

unsuspected pneumococcal infection, bacteraemia is defined as the presence of bacteria in the bloodstream of a febrile child who was previously healthy; the child does not clinically appear to be ill and has no apparent focus of infection (Swindell, 1993). Occult bacteraemia has been defined as bacteraemia not associated with clinical evidence of sepsis (shock or purpura) or toxic appearance, underlying significant chronic medical conditions, or clear foci of infection (other than acute otitis media) upon examination in a patient who is discharged and sent home after an outpatient evaluation (Kuppermann, 1999).

Often, the only manifestation of occult bacteraemia is fever or a minor infection (Harper, 1993). Therefore, in a busy clinic or emergency department, infants and young children with occult bacteraemia are difficult to distinguish from others.

Many septic episodes are nosocomial and in some hospitals, septic episodes are due to microorganisms with increased antimicrobial resistance and are associated with greater mortality than are community-acquired episodes (Anton *et. al*., 2010). Pathogens such as viruses, fungi, mycobacteria, mycoplasmas, and fastidious bacteria, are all likely to invade the blood. Common blood borne pathogens that frequently causes bacteraemia includes *staphylococcus aureus*, *Escherichia coli* and other members of the *Enterobacteriacae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Candida albicans* (Towns *et al*., 1993, Weinstein *et. al*., 1997 Anton *et. al*., 2010). Other microorganisms such as *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* rarely (5%) represent true bacteraemia (Towns *et al*., 1993, Weinstein *et al*., 1997). More so, Viridans group *Streptococci*, *Enterococci* and Coagulase negative *Staphylococcus* (CNS) also represents true bacteraemia (Towns *et al*., 1993, Weinstein *et al*., 1997). Anaerobic bacteria are

present in vast numbers as members of the normal bacterial flora in a variety of non-sterile body locations. The oropharynx, gastrointestinal tract, female genital tract and skin contain high numbers of anaerobic organisms that dwarf the number of aerobic bacteria. Most infectious processes that involve anaerobic bacteria occur when the integrity of these normally colonized surfaces are interrupted.

2.5.1 Pathophysiology of bacteraemia

Much of the pathophysiology of occult bacteraemia is not fully understood. The presumed mechanism begins with bacterial colonization of the respiratory passages or other mucosal surface; bacteria may egress into the bloodstream of some children because of host-specific and organism-specific factors. Once viable bacteria have gained access to the bloodstream, they may be spontaneously cleared, they may establish a focal infection, or the infection may progress to septicaemia; the possible sequelae of septicaemia include shock, disseminated intravascular coagulation, multiple organ failure, and death (Harper, 1993). Often, fever is the only presenting sign in patients with occult bacteraemia and is defined as increased temperature caused by resetting the thermoregulatory centre in the hypothalamus by action of cytokines (McCarthy, 1998). The cytokines may be produced in response to viral or bacterial pathogens or by immune complexes. An increased temperature does not always represent a fever. Hyperthermia may also be due to increased heat production as occurs in exercise or decreased heat loss as occurs in over bundling, neither of which involves resetting of the hypothalamic thermostat.

Chase et al found that a number of clinical variables (respiratory failure, vasopressor use, neutrophilia, bandemia, thrombocytopenia, indwelling venous catheter, abnormal temperature, suspected line or urinary infection, or endocarditis) were

predictive of bacteraemia in a sample of 5630 emergency department patients with suspected infection (Chase, 2012).

A child's immune system helps determine which bacteria gain initial access to the bloodstream, whether bacteremia spontaneously resolves or progresses to serious bacterial illness, and whether cytokines are produced to mount a fever response. The risk of life-threatening bacterial disease is greatest in young infants when their immune system is least mature; they have poor immunoglobulin G (IgG) antibody response to encapsulated bacteria and decreased opsonin activity, macrophage function, and neutrophil activity (Baker, 1999). Clearly, some children are more susceptible to bacterial infection, which may initially be uncomplicated bacteraemia but could rapidly lead to more serious complications. Immunosuppression due to neoplastic disease or its treatment or defects in antibody responses or neutrophil responses predispose certain children to invasive infection. Bacteraemia should be considered, with a low threshold for evaluation and treatment, in patients with impaired immunity or invasive medical devices such as indwelling central venous lines.

2.5.2 Etiologic agents of bacteraemia

Many infectious disease process that cause systemic illnesses results from a variety of organisms that have entered the bloodstream. Bacteraemia occurs during the course of many infections and surgery. Diseases such as typhoid fever, brucellosis, leptospirosis and endocarditis also cause bacteraemia. It usually occurs when pathogens enter the bloodstream from abscesses, infected wounds or burns, or from areas of localized disease as in pneumococcal pneumonia, meningitis, pyelonephritis,

osteomyelitis, cholangitis, peritonitis, enterocolitis and puerperal sepsis (Cheesbrough, 2006). Many septic episodes are nosocomial and in some hospitals, septic episodes are due to microorganisms with increased antimicrobial resistance and are associated with greater mortality than are community-acquired episodes (Anton *et. al*., 2010). Pathogens such as viruses, fungi, mycobacteria, mycoplasmas, and fastidious bacteria, are all likely to invade the blood. Common blood borne pathogens that frequently causes bloodstream infections includes ***staphylococcus aureus***, *Escherichia coli* and other members of the *Enterobacteriacae*, ***Pseudomonas aeruginosa***, *Streptococcus pneumoniae*, and *Candida albicans* (Towns *et al*., 1993, Weinstein *et. al*., 1997 Anton *et. al*., 2010). Other microorganisms such as *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* rarely (5%) represent true bacteraemia. More so, viridans group streptococci, enterococci and Coagulase negative *Staphylococcus* (CNS) also represents true bacteraemia (Towns *et al*., 1993).

Anaerobic bacteria are present in vast numbers as members of the normal bacterial flora in a variety of non-sterile body locations. The oropharynx, gastrointestinal tract, female genital tract and skin contain high numbers of anaerobic organisms that dwarf the number of aerobic bacteria. Most infectious processes that involve anaerobic bacteria occur when the integrity of these normally colonized surfaces are interrupted. Bacteraemia with anaerobic bacteria was first well documented in the 1970s, when blood culture media were improved to make their isolation routine. Anaerobic organisms typically accounted for 10 to 15% of the organisms isolated and, in one study, represented 26% of the total infection (Ryon *et al*., 1994; Wilson *et al*., 1972).

2.5.3 Epidemiology of bacteraemia

Bacteraemia with anaerobic bacteria was first well documented in the 1970s, when blood culture media were improved to make their isolation routine. Anaerobic organisms typically accounted for 10 to 15% of organisms' isolated (Ryon *et al*., 1994) and in one study, represented 26% of the total infection (Wilson *et al*., 1972). Data from the US and Europe show an increase in the incidence of bacteraemia (Peters *et al*., 2004; Haug *et al*., 1994). In addition, the spectrum of organisms causing bacteraemia has also changed over the years. Between 1960s and 1970s, Gram-negative organisms were most frequently isolated from patients with nosocomial bacteraemia of 24,179 cases from a prospective nationwide surveillance study in US hospitals (Wisplinghoff *et al*., 2004). From 1980 onwards, infections due to Gram-positive organisms have become increasingly frequent (Stainberg *et al*., 1996). Nevertheless, in the last few years, reemergence of Gram- negative bacteria as well as the evolution of bacterial resistance to a variety of antimicrobials have been reported in many studies conducted in hospital settings (Karchmer 2000; Wu *et al*., 2006). These shifts may be attributed to the increased usage of invasive medical technology, the changing patterns of antimicrobial usage, the evolution of bacterial resistance, the shift in the elderly population and the increased usage of aggressive drug therapy, which results in immunodeficiency. Taken together, these findings suggest that the spectrum of organisms causing bacteraemia as well as their drug resistance trends vary geographically (and may even vary from a hospital to a hospital within a city), which necessitate conducting local studies to identify the common pathogens and their resistance trends in order to formulate local guidelines and promote more appropriate antibiotic treatment.

2.5.3.1 Bacteraemia in Ghana

According to WHO statistics of 2008, malaria accounts for 18% of deaths among children below five years in Ghana, closely followed by pneumonia (13%), diarrhea (12%) and pre-maturity at birth (12%). Neonatal sepsis is causing 9% of fatal **cases.** Apart from a few well-equipped hospitals, health facilities lack microbiological diagnostic capacities necessary to diagnose bacteraemia and to isolate bacterial pathogens in order to allow targeted treatment (Petti *et al*., 2006). A further factor complicating the diagnosis and therapy is self-treatment with antimicrobial drugs prior to professional health care, which may impede the diagnosis and increases the risk of emergence and spread of antibiotic resistance (Ilic *et al*., 2012).

2.5.4 Diagnosis of Bacteraemia

Blood culture techniques are used for the diagnosis of bacteraemia. In order to test for bacterial growth, the blood of patient is collected and inoculated aseptically into a blood culture bottle. Blood cultures are incubated aerobically at 37 ^{0}c and monitored daily for growth for 7 days or 14 days when brucellosis is suspected (Cheesbrough, 2006). Laboratory culture of organism from a clinical specimen is a very effective method of confirming bacteraemia. Blood cultures especially has a high predictive value for enteric fever, although a large number of factors such as the stage of the illness, previous infection, antibiotic use or vaccination need to be taken into account when culture is being done.

2.5.6 Specific organisms

2.5.6.1 *Staphylococci*

Staphylococci are Gram-positive cocci occurring in clusters. *Staphylococci* are small spherical cells found in grapelike clusters. Staphylococci are non-motile, catalase-producing bacteria. The genus *Staphylococcus* includes over 30 species and subspecies. They can be cultured on normal nutrient mediums both aerobically and anaerobically. The most important species from the viewpoint of human medicine is *Staphylococcus aureus*. A number of extracellular enzymes and exotoxins such as coagulase, alphatoxin, leukocidin, exfoliatins, enterotoxins, and toxic shock toxin are responsible for the clinical symptoms of infections by this pathogen. The antibiotics of choice for therapy of these infections are penicillinase-resistant penicillins. Laboratory diagnosis involves identification of the pathogen by means of microscopy and culturing. *S. aureus* is a frequent pathogen in nosocomial infections and causes outbreaks in hospitals. Hand washing by medical staff is the most important prophylactic measure in hospitals. *S. aureus* is among the most frequent causal organisms in human bacterial infections (Kayser *et al*., 2005).

2.5.6.2 Coagulase-negative *Staphylococci* (CNS)

Studies have revealed a dramatic increase in the incidence of Coagulase Negative *Staphylococcus* (CNS) as an agent of bacteraemia (Reimer *et al*., 1997). Coagulase negative *Staphylococci* (CNS) are present in the normal flora of human skin and mucosa. Coagulase-negative *Staphylococci* are classic opportunists, example, *Staphylococcus epidermidis* and other species are frequent agents in foreign body infections due to their ability to form biofilms on surfaces of inert objects. *S. saprophyticus* is responsible for between 10 and 20% of acute urinary tract infections

in young women (Kayser *et al*., 2005). CNS causes mainly foreign body infections. Examples of the foreign bodies involved are intravasal catheters, continuous ambulant peritoneal dialysis (CAPD) catheters, endoprostheses, metal plates and screws in osteosynthesis, cardiac pacemakers, artificial heart valves, and shunt valves. These infections frequently develop when foreign bodies in the macroorganism are covered by matrix proteins such as fibrinogen or fibronectin to which the *staphylococci* can bind using specific cell wall proteins. They then proliferate on the surface and produce a polymeric substance which forms the basis of developing biofilm. The *staphylococci* within the biofilm are protected from antibiotics and the immune system to a great extent. Such biofilms can become infection foci from which the CNS enter the bloodstream and cause sepsis.

2.5.6.3 ***Streptococcus and Enterococcus***

Streptococci are Gram-positive, nonmotile, catalase-negative, facultative anaerobic cocci that occur in chains or pairs. They are classified based on their haemolytic capacity, thus alpha (α), beta (β) and gamma (ɣ)-haemolysis and the antigenicity of a carbohydrate occurring in their cell walls (Lancefield antigen). β -haemolytic group A *Streptococci* (*Streptococcus pyogenes*) cause infections of the upper respiratory tract and invasive infections of the skin and subcutaneous connective tissue. Depending on the status of the immune defences and the genetic disposition, this may lead to scarlet fever and severe infections such as necrotizing fasciitis, sepsis, or septic shock. Sequelae such as acute rheumatic fever and glomerulonephritis have an autoimmune pathogenesis. The α-haemolytic *Streptococcus pneumonia* causes infections of the respiratory tract. Penicillins are the antibiotics of choice. Resistance to penicillins is known among *Pneumococci* and is increasing. Laboratory diagnosis involves pathogen detection in the appropriate material. Persons at high risk can be

protected from pneumococcal infections with an active prophylactic vaccine containing purified capsular polysaccharides. Certain oral streptococci are responsible for dental caries. Oral streptococci also cause half of all cases of endocarditis. Although enterococci show only low levels of pathogenicity, they frequently cause nosocomial infections in immunocompromised patients (Murray *et al*., 1992; Kayser *et. al*., 2005).

Streptococci are round to oval, Gram-positive, non-motile, non-sporing bacteria that form winding chains or diplococci. They do not produce catalase. Most are components of the normal flora of the mucosa and can cause infections in humans and animals (Kayser *et. al.*, 2005).

2.5.6.4 *Enterobacteriaceae*

The most important bacterial family in human medicine is the *Enterobacteriaceae*. This family includes genera and species that cause well-defined diseases with typical clinical symptoms such as typhoid fever, dysentery and plague as well as many opportunists that causes nosocomial infections (urinary tract infections, pneumonias, wound infections, sepsis). *Enterobacteriaceae* are Gram-negative, usually motile, facultative anaerobic rod bacteria. The high levels of metabolic activity observed in them are employed in biochemical identification procedures. The species are subdivided into epidemiologically significant serovars based on O, H, and K antigens. The most important pathogenicity factors of *Enterobacteriaceae* are colonizing factors, invasins, endotoxin, and various exotoxins. *Enterobacteriaceae* are the most significant contributors to intestinal infections, which are among the most frequent diseases of all among the developing world populace. Their natural habitat is the intestinal tract of humans and animals. Some species cause

characteristic diseases. While others are facultative pathogenic, they are still among the bacteria most frequently isolated as pathogens and are often responsible for nosocomial infections. Examples include *Escherichia coli, Klebsiella, Enterobacter, Salmonella and Shigella* (Kayser *et al.,* 2005).

2.5.6.5 *Salmonella* (Gastroenteritis, Typhoid Fever, Paratyphoid Fever)

All *Salmonellae* are classified in the species *Salmonella enterica* with seven subspecies. Nearly all human pathogen *Salmonellae* are grouped under *Salmonella. enterica*, subsp. *enterica. Salmonella*e are further subclassified in over 2000 serovars based on their O and H antigens. Typhoid salmonelloses are caused by the serovars typhi and paratyphi A, B, and C. The *Salmonellae* are taken up orally and the invasion pathway is through the intestinal tract, from where they enter lymphatic tissue, first spreading lymphogenously, then hematogenously resulting in a generalized septic clinical symptom. Human carriers are the only source of infection. Transmission is either direct by smear infection or indirect via food and drinking water. Anti-infective agents are required for therapy (ampicillin, cotrimoxazole, 4-quinolones). An active vaccine is available to protect against typhoid fever. Enteric salmonelloses develop when pathogens are taken up with food. The primary infection source is usually livestock. These relatively frequent infections remain restricted to the gastrointestinal tract.

2.5.6.7 *Mycobacteria*

The true incidence of *Mycobacteria tuberculosis* bacteraemia is, however, poorly described since blood cultures for *Mycobacteria* are obtained routinely from HIV-infected patients with less than 200 CD41 lymphocytes per ml (200) but rarely from HIV-positive patients with less advanced disease or from HIV-negative individuals. Bouza *et al*., described 50 patients with positive *Mycobacterial* blood cultures, 81% of whom were HIV positive (Bouza *et. al*., 1993). Occasional patients with a variety of immunosuppressive conditions including leukemias, severe combined immunodeficiency syndrome, steroid therapy, cytotoxic chemotherapy, multiple myeloma, other nonhematologic malignancies, diabetes mellitus, anthrasilicosis, asthma, and other infections appear to be at increased risk for *M. avium* complex bacteremia (Haylik *et al*., 1992).

2.5.6.8 Fungi

With the increasing number of patients with complex clinical conditions and undergoing complex medical therapy, more unusual infectious disease processes have emerged. Fungal pathogens as agents of a variety of infections, including fungaemia, are part of this trend. Fungi are now found much more frequently as the cause of both community- and hospital-acquired infections, and efforts to culture them have become increasingly sophisticated (Pfaller *et al*., 1992). Major predisposing factors leading to these infections include immunosuppression, use of broadspectrum antibiotics, use of central venous catheters especially in association with the administration of hyperalimentation solutions, and more aggressive attempts to prolong the survival of patients with complicated, serious disease (Pfaller *et. al*.,

1992). As the culture methodology has improved, more fungal pathogens are now detected. Whereas in the past the organisms associated with invasive human infection included *Histoplasma capsulatum*, *Coccidioides immitis*, *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus* spp., *Blastomyces dermatitidis*, and rarely others, there are now frequent reports of illnesses associated with a much longer list of saprophytic moulds and yeasts. Among these are multiple species of *Candida*, *Trichosporon beigelii*, *Fusarium* spp., *Malessezia* spp., and zygomycetes (Pfaller *et. al*., 1992). Both because of the serious illnesses in patients who acquire these organisms and the possible resistance of the pathogens to antifungal agents, patients with systemic fungal infections often have poor clinical outcomes.

2.6 Drug resistance to antimicrobial drugs

Antimicrobial resistance has become an important public health problem associated with serious consequences for the treatment of infection. This ultimately affects both economic and social development. The problem has been attributed to the misuse of antimicrobial drugs, which provide selective pressure, favoring the emergence of resistant strains. Drug resistance has played a significant role in the occurrence and severity of epidemics of diseases in some parts of the world. Population movement has also introduced resistant organisms to areas previously free of drug resistance. The World Health Organization has provided some interventions to contain the problem of antimicrobial resistance. These include creating a national task force, developing indicators to monitor and evaluate the impact of antimicrobial resistance, and designing reference microbiological facilities that would coordinate effective surveillance of antimicrobial resistance among common pathogens (WHO 2001).

While these interventions seem to have been well implemented in the developed world, lack of resources has constrained implementation of these interventions in many developing countries. Thus, although a global problem, antimicrobial resistance tends to be more significant in developing countries than in the developed world (Newman *et al.*, 2011).

Many factors contribute to treatment failure, for instance, incorrect dosing, noncompliance with duration of dosing regimen, poor quality of drug, drug interactions, poor absorption, and misdiagnosis in the individual. These factors may also contribute to the development of drug resistance in micro organisms resulting from the exposure of the micro organism to suboptimal drug levels.

2.6.1 Antimicrobial drug resistance in Ghana

In Ghana, antimicrobial therapy constitutes a major form of treatment. It is mainly empirical due to a relative lack of appropriate laboratory facilities for culture and susceptibility testing of bacteria in several health facilities. Even where laboratory facilities are available, culture and susceptibility tests may not be requested due to the fact that this is an extra cost to be paid by the patient. In Ghana, drug resistance has been reported in Korle-Bu teaching hospitals (KBTH) in Accra (Newman 1990, 1996: Opintan *et al*,. 2007) and Komfo Anokye teaching hospitals (KATH) Kumasi (Ohene, 1997). These reports show bacterial resistance to commonly utilized and relatively cheap drugs like Ampicillin, Tetracycline, and Cotrimoxazole, which have been the mainstay of antimicrobial treatment for decades. A study carried out in eleven hospitals covering nine of the ten regions in Ghana by Newman et al in 2004 observed high percentage of resistance for Tetracycline 82%, Cotrimoxazole 73%, Ampicillin 76%, and Chloramphenicol 75%. Multidrug resistance was observed to a

combination of Ampicillin, Tetracycline, Chloramphenicol, and Cotrimoxazole. On the other hand, a lower percentage of resistance was observed for Ceftriaxone 6.3%, Ciprofloxacin 11%, and Amikacin 9.9% (Newman *et al*., 2011).

2.6.2 Mechanisms of antimicrobial resistance

In general, resistance to micro organisms occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance, while for other drugs, multiple mutations appear to be required. Single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying drug response characteristics, from highly resistant to completely sensitive (Thaithong, 1983; WHO, 2010).

There are four major mechanisms that mediate bacterial resistance to drugs. Bacteria produce enzymes that inactivate the drug; example, beta-lactamases can inactivate penicillins and cephalosporins by cleaving the beta lactam ring of the drug. Secondly, bacteria synthesize modified targets against which the drug has no effect; example, a mutant protein in the 30S ribosomal subunit can result in resistance to streptomycin, and a methylated 23S rRNA can result in resistance to erythromycin. Bacteria can also decrease their permeability such that an effective intracellular concentration of the drug is not achieved; example, changes in porins can reduce the amount of penicillin entering the bacterium. Bacteria can actively export drugs using a multidrug resistance pump (MDR pump, or efflux pump). By this mechanism, the bacteria uses the efflux pump to imports protons and, in an exchange-type reaction, exports a variety of foreign molecules including certain antibiotics, such as quinolones. Most of these drug resistant mechanisms are due to a genetic change in

the organism, either a chromosomal mutation or the acquisition of a plasmid or transposon. There are other mechanisms which constitute non genetic bases of drug resistance. For instance, organisms that would ordinarily be killed by penicillin can lose their cell walls, survive as protoplasts, and are insensitive to cell wall active drugs. Later, if such organisms re-synthesize their cell walls, they are fully susceptible to these drugs. Bacteria can be in a resting state that is, not growing. They are therefore insensitive to cell wall inhibitors such as penicillins and cephalosporins. Similarly, *Mycobacterium tuberculosis* can remain dormant in tissues for many years, during which time it is insensitive to drugs. If host defenses are lowered and the bacteria begin to multiply, they become susceptible to the drugs.

2.6.3 Drug resistance in antibiotics

Antibiotic resistant organisms are common in the hospital setting because wide spread antibiotic use in hospitals selects for these organisms (Raveh *et al*., 2001). More so, hospital strains are often resistant to multiple antibiotics. This resistance is usually due to the acquisition of plasmids carrying several genes that encode for the enzymes that mediate resistance. Hospital acquired infections are caused by antibiotic resistant organisms such as *Staphylococcus aureus* and enteric gram-negative rods such as *Escherichia coli* and *Pseudomonas aeruginosa* (Hassan *et al*., 2011).

2.6.4 Drug resistance in antimalarial drugs

Drug resistance has been documented for almost all antimalarials in current use, including old drugs like Quinine and the newly introduced artemisinins (Roche *et al* 1993). Artemisinin based combination therapys (ACTs) have considerably

contributed to reduce malaria burden. However, the recent emergence of artemisinin-resistant *P. falciparum* parasites in Western Cambodia-Thailand border is alarming (WHO, 2010). Malaria drugresistance has led to increase mortality and morbidity, increased economic cost in terms of new drugs, and changes of disease management policies.

2.6.4.1 Prevention of drug resistance in malaria

The strategies that can be adopted to reduce drug resistance in malaria include the use of insecticide-treated bed nets, indoor residual insecticide spraying, environmental control or controlling mosquito breeding sites and other personal protection measures including the use of repellent soap or screening windows and chemoprophylaxis. These strategies are aimed specifically at preventing malaria infection and reduction of overall malaria infection rates. This will not necessarily reduce the likelihood of development of drug resistance. The interventions aimed at preventing drug resistance generally focus on reducing overall drug pressure through more selective use of drugs, improving the way drugs are used through improving prescriptions, follow-up practices, and patient compliance or using drugs or drug combinations which are less likely to foster resistance or have properties that do not facilitate development or spread of resistant organisms. Other disease control programmes, such as TB and HIV, rely on directly observed therapy (DOT) as a way to ensure patients comply with the drug prescription. While this has not yet received serious consideration for malaria, the use of drugs with single-dose regimens (Sulfadoxine-Pyrimethamine SP, mefloquine) could potentially make DOT possible.

Combination therapy of antimalarial drugs, such as mefloquine, or amodiaquine, with an artemisinin derivative has received much attention recently (White *et al*., 1999). Artemisinin drugs are highly efficacious, rapidly active, and have action against a broader range of parasite developmental stages. This action apparently yields two notable results. First, artemisinin compounds, used in combination with a longer acting antimalarial drug, can rapidly reduce parasite densities to very low levels at a time when drug levels of the longer acting antimalarial drug are still maximal. This greatly reduces both the likelihood of parasites surviving initial treatment and the likelihood that parasites will be exposed to suboptimal levels of the longer acting drug (White *et al*., 1999). Second, the use of artemisinins has been shown to reduce gametocytogenesis by 8- to 18-fold (White *et al*., 1999). This reduces the likelihood that gametocytes carrying resistance genes are passed onwards and potentially may reduce malaria transmission rates. Use of combination therapy has been linked to slowing the development of mefloquine resistance and reductions in overall malaria transmission rates in some parts of Thailand and has been recommended for widespread use in sub- Saharan Africa (White *et al*., 1999).

2.7 Antimicrobial Assay Methods

These assays determine the in-vitro susceptibility of an isolate to a range of chemotherapeutic agents. Antimicrobial susceptibility methods include Agar Diffusion Method and Broth Dilution Methods.

2.7.1 Agar diffusion methods

The antibiotic diffuses from a paper disc or small cylinder into an agar medium that contain test organisms. A common application of these methods is the Kirby-Bauer

test; where paper discs containing known concentrations of antibiotics are applied to the surface of seeded Mueller-Hinton agar and the plate incubated. After overnight incubation, zone of inhibition sizes are measured in millimeters (Cheesbrough, 2000). Zone of inhibition is observed as a failure of the organism to grow in the region of the antibiotic (Baron *et al.*, 1994).

2.7.2 Broth Dilution Method

This method depends upon inoculation of broth containing antibiotics at varying levels; usually, doubling dilutions are used. This method is used to determine Inhibitory Concentration (MIC) or breakpoint of an antimicrobial agent required to inhibit the growth of a bacterial isolate (Mahon *et al*, 2007). It can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria. A dilution test is carried out by adding dilutions of an antimicrobial to a broth or agar medium. Standardized inoculums of the test organism are then added. After overnight incubation, the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth (Cheesbrough, 2006). To determine the MBC, a 0.01ml aliquot of each clear tube or well from the MIC determination is subcultured to an agar medium and incubated at 37 0C for 24 hours. After overnight incubation, the numbers of colonies that grow on subculture are compared with the actual number or organisms inoculated into the MIC test tubes (Mahon *et al*., 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design and Study site

The study was performed at the Princess Marie Louise Children's Hospital (PML), Accra involving all consecutive consenting paediatric patients who reported to the hospital with fever or symptoms leading to primary diagnosis of malaria. The blood cultures were done at the Department of Bacteriology, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Accra. Due to the distance between Legon and Princess Marie Louise Children's Hospital and time factor, the malaria tests were done in collaboration with Lancet laboratory Korle-Bu, (nearer to PML) and the results sent immediately to the hospital for the patients benefit.

3.2 Patient recruitment and Sample size determinations

The population group most susceptible to both malaria and bacteraemia are children below the ages of 5 hence the primary focus group for the study was paediatric admissions with clinical presentation of fever above 38 ^{0}C and above. Participants for the study were recruited from the emergency ward from January 2011 to December 2012.

Student T test was used, with significance level set at $p< 0.05$. Sample were taken from 182 patients for the study, based on a minimum sample size of 100 and 95% confidence level using the formula: $n = (Z^2 \times SD^2) / E^2$

Where:

n = sample size

Z = standard score for the 95% confidence limit = 1.96

SD = standard deviation = 0.5

E = maximum allowable error = 9.8%

$n = [(1.96)^2 \times (0.5)^2] / (0.098)^2$

n=100

3.2.1 Informed consent, possible risks and benefits

All patients and patient's guardians were informed about the study and asked to give consent (on behalf of their children). Consent was sought by the physician from eligible participants at the time of admission and examination before blood was collected. Since this study involved sick children, guardians were informed and care was taken to ensure their decision was not under pressure. No patient was obliged to participate and patients were allowed to step out of the study at any time (see Appendix I: informed consent). Collection of blood was considered a low-risk procedure and was conducted by qualified physicians. All procedures of clinical assessment, sampling, treatment, and laboratory investigations were done by a physician along established standard operating procedures. All patients enrolled in the study received a free-of-charge diagnostic test on malaria, sickle cell phenotype, hematological, biochemical and blood culture and sensitivity.

3.2.2 Inclusion and exclusion criteria

Patients with fever (38^oC and above), presenting to the health facility, were included in the study. Patients with an obvious septic focus even when no malaria is suspected were also included in order to determine the nature of the causative agents leading to bacterial sepsis (see Appendix I).

3.3 Sampling

Blood collection was performed by a physician under good clinical practice conditions after disinfection of the skin. Blood was taken before antibiotic therapy. One collection of a total of 5 ml of venous blood was taken from each paediatric patient. In order to test for bacterial growth, a minimum of 2.5 ml of blood was inoculated into a BD BACTEC blood culture bottle (BD BACTEC Lot no. 7108604) and samples were transported within 24 hours of collection to NMIMR-Legon. The remaining 2.5 ml of blood samples were stored in EDTA vacutainers and transported to Lancet laboratory thick and thin blood films was prepared, stained with Giemsa and examined microscopically for malaria parasites.

3.4 Procedures and Transportation

Blood cultures were transported within 24 hours and incubated at 37^{o}C at NMIMR. The dates and times of sample collection, blood culture inoculation and arrival at the NMIMR laboratory were all documented. At the Department of Bacteriology of Noguchi memorial Institute for Medical Research (NMIMR), cultivation and microbiological analysis of blood cultures, including species determination and antibiotic profiling were performed. The blood samples (stored in EDTA vacutainers) were transported to the Lancert laboratory, Korle-Bu, for hematological, biochemical and malaria tests. All laboratory results were immediately reported to the treating physician without delay for the benefit of the patients.

3.4.1 Malaria diagnosis

Patients were examined for malaria; thick and thin blood films were prepared, stained with Giemsa and examined microscopically for malaria parasites.

3.5. Bacterial isolation

The blood culture bottles were incubated manually at aerobic conditions at 37 ^{0}C for 7 to 14 days (Cheesbrough, 2006). Blind subcultures were made after the first 24 hours of incubation on blood agar and MacConkey agar and incubated at 37 ^{0}C overnight and a preliminary report given to the hospital. After 48 hours of incubation, a second blind subculture was made. Antibiotic sensitivity was carried out on any growth obtained after the subculture and results were reported immediately to the treating physician for the patients benefit. If there was no bacterial growth a final subculture was made after 7 days of incubation and a report given to the hospital. Figure 3.5 shows a subculture of bacterial colonies on blood ager plate from primary growth after 24 hours incubation under aerobic condition.

3.5.1. Identification of Bacterial Growth

The following steps were followed for the identification of isolated bacteria;Colonial morphology and Gram stain reaction; Biochemical test [Analytical profile index (API)]; Serology [Gram positive cocci Catalase test (*Staph. spp*.); Coagulase test (*Staph. aureus*); sliding antisera for *Salmonella spp*].

The sub-cultures on selective media (MacConkey agar) were used for colonial morphology determination thus, differentiating lactose fermentors from non-lactose fermentors. The obtained isolate identity was confirmed by Gram staining and subsequently biochemical tests such as the Analytical Profile Index for gram negative rods, catalase activity and Coagulase test for the Gram positive cocci were done.

Plate 3.0: BD BACTEC blood culture bottle inoculated with 2.5 ml of blood

Plate 3.1: Blood agar plate showing growth of *Staphylococcus aureus*.

3.5.2 Gram staining procedure

The slides were placed on a solid holder and the fixed smear covered with crystal violet stain for 60s. The stain was then washed off with clean water. After tipping off the water the smears were covered with Lugol's iodine for 60s after which the iodine was washed off with water. Decolourization was done rapidly (for few seconds) with acetone-alcohol and immediately washed off with water. The smears were covered with neutral red stain for 2 minutes. The stain was washed off with water and the slides placed in a rack for the smear to air-dry.

The smear was then examined microscopically, first with the 40x objective to check the staining and distribution of the smear, and then with oil immersion objective to look for bacterial and cells. The figure bellow shows a specimen prepared on a slide for Gram staining.

Plate 3.2: Preparation of smear for Gram staining

3.5.3 Catalase and latex Agglutination (Coagulase) test

Catalase test was performed on the Gram positive cocci to differentiate between *Staphylococcus sp* and *Streptococcus* sp. This test was used to identify the presence of the catalase enzyme produced by some organisms to decompose the hydrogen

peroxide produced as an end product of the aerobic breakdown of sugars. The test was performed by placing a drop of hydrogen peroxide on a glass slide and with the aid of a plastic inoculating loop, touching the bacterial colonies and immersing the loop in the reagent. Vigorous bubbling indicated the presence of the catalase enzyme as the hydrogen peroxide was converted into water and oxygen.

Further identification of the catalase positive Gram positive cocci, *Staphylococcus* was done using a Staphylase kit ProlexTM Latex Agglutination System (Pro-Lab Diagnostics) to differentiate between *Staphylococcus aureus* and other Staphylococcus species.

3.5.4 Oxidase test for identification of Gram negative bacteria

The oxidase test was performed on all Gram negative rods to determine if they possessed the cytochrome oxidase enzyme. A small strip of filter paper was impregnated with the oxidase reagent N, N, N', N'-tetra-methyl-p-phenylenediamine dihydrochloride. Using a straight plastic inoculating loop, the suspected bacterial colonies were touched and then the loop was rubbed on the filter paper. Positive results were based on the observance of a blue colouration on the filter paper indicating the presence of the cytochrome oxidase enzyme production by the organism. (Cheesbrough, 2006).

Further confirmatory test was done using the Analytical Profile Index (API 20E) strips (bioMerieux) test kit shown in Plate 4 and figure 3.0 in the appendix. This test is designed for the identification of members of the *Enterobacteriaceae* family and other associated organisms.

The *Salmonella typhi* isolates were further confirmed by serology. Colonies were picked off the ager plate with an inoculation loop and emulsified on a clean slide

with a drop of saline solution. Drops (approximately 10ul) of antigen suspension representative of each antigen type were mixed with an equal volume of the fresh emulsion. In *S.typhi* positive isolates, an agglutination reaction was observed with antigen composing nine, twelve and Vi in the somatic (0) antigen group and antigen composing d in phase II of the (H) or flagella antigen group (Cheesbrough, 2006).

3.5.5 Antimicrobial Sensitivity test

Antibiotic sensitivity was done using the standard Kirby-Bauer method with antibiotic discs on Müller Hinton agar plates. The antibiotics that were used includes; Penicillin, Flucloxacillin, Erythromycin, Cotrimoxazole, Gentamycin, Ceftriaxone, Cefotaxime, Cefuroxime, Cefuroxime, Ampicillin and Tetracyclin (Bauer *et al*., 1966).

The inoculating loop was used to pick two or three colonies from the plate into a 5 ml Muller-Hinton broth and mix thoroughly by overtaxing. The turbidity of the mixture was compared to Macfarlane standard. A sterile swab stick was dipped into the suspension and rotated against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid to ensure that the swab was not dripping wet. The dried surface of a Muller-Hinton agar plate was inoculated by streaking the swab three times over the entire agar surface by rotating the plate approximately 60 degrees each time to ensure an even distribution of the inoculums (CLSI, 2006; Cheesbrough, 2006). Discs prepared with different antibiotics (Abteck Biological Lot: KI17/P) were placed firmly on the inoculated medium and incubated for 24hours. Holding the plate a few inches above a black, non-reflective surface illuminated with reflected light, a ruler or caliper was used to measure each zone

with the unaided eye while viewing the back of the Petri dish (Cheesbrough, 2006; Bauer *et al*., 1966). The results were compared with the zone diameter interpretive standards of the Clinical Laboratory Standards Institute NCCLS, 2006. The plate bellow shows the diameter of a zone being measured on a Muller-Hinton agar (NCCLS, 2006; Bauer et al., 1966)

Plate 3.4: Measuring of zone of inhibition on a Muller-Hinton agar

3.6 Clinical assessment

Study subjects were children aged between three months and 5 years admitted to the study with an acute febrile illness(temp > 37.5° C) or clinical symptoms suggestive of severe malaria according to WHO guidelines (WHO, 2009) including children with the following symptoms and signs: a Blantyre coma score of 2 or less; severe anaemia, with a haemoglobin level of <5g/dl or a haematocrit of <15%; severe respiratory distress, being the presence of either marked in-drawing of the bony structure of the lower chest wall or deep (acidotic) breathing; prostration, being the inability to sit upright in a child normally able to do so, or to drink in the case of children too young to sit. Through interview, information on the use of antimalarial or antibiotic drugs during the preceding week was obtained. All patients enrolled in the study were given counseling and received treatment according to the guidelines of the hospital and the national guidelines by the physician.

3.7 Data management and statistical analysis

The hospital identification number and the study identification number of all patients screened were recorded in a patient screening log sheet. All other documents only carry the study identification number in order to ensure anonymity of the patients. The patient screening log sheet was kept in the hospital. Data were entered into Microsoft Office Excel, and results analyzed using Statistical Package for Social Sciences (SPSS) Version16.

CHAPTER FOUR

RESULTS

4.1. Characteristics of study participants

The study population involved182 children aged between 5 months to 5 years who reported to the hospital with fever ranging from 38 ^{0}C to 40 ^{0}C. A total of 92, (50.5 %) were males and 90, (49.5 %) were females. The average age of all the patients is 1.83 and the median age is one year. The median age of the male and the female patients is one year respectively. The average age of the males is 1.75 and that of the female is 1.91. Table 4.1 shows the age distributions of the participants.

Table 4.0: Age group of the patients

Factor		Number involved	Percentage (%)
Age	< 1 year	9	5.1
	1-2years	124	68.1
	3-5years	70	38.5
Sex of Patients	Male	92	50.5
	Female	90	49.5

4.2 Laboratory diagnosis.

Table 4.1: Summary of laboratory results

Lab test	**Positive cases**	**Percentage (%)**
BF (Malaria)	38	21.6
Blood culture (positive)	40	22.9
BF Negative (Blood culture +ve)	25	13.74
BF Positive (Blood culture –ve)	26	14.29
Bacteraimia	18	10.2
Malaria & bacteraemia	5	2.8

4.3. Standard Microbiological investigation: Culture and sensitivity

4.3.1. Culture

A total of 182 blood samples were cultured out of which 40 organisms were isolated from 40 blood samples. The isolates include 33 Gram positive organisms and 7 Gram negative organisms. The 33 Gram positive organisms include 4 rods, 19 Coagulase Negative Staphylococcus (CNS), 5 *Staphylococcus aureus,* 3 beta haemolytic streptococcus spp. and two *Micrococcus leteus*.

Table 4.5.1.1 shows the organisms isolated. See Appendix 2 for Plate1 shows API strip identifying *Erwinia nigrifluens* and Plate 2 shows API strip identifying *Acinetobacter spp*.

Table 4.2: Organisms Isolated

Organisms	Number of isolates	Percentage %
Erwinia nigrifluens	1	2.5
Acinetobacter spp.	1	2.5
Pseudomonas spp	1	2.5
Micrococcus luteus	2	5.0
Salmonella paratyphi B	2	5.0
Gram negative cocci	2	5.0
Beta Haemolytic *Streptococci*	3	7.5
Gram positive rod / Contaminants	4	10.0
Staphylococcus aureus	5	12.5
Coagulase negative staphylococcus	19	47.5
Total	40	

4.3.2. Antibiotic susceptibility Testing

Table 4.3: Antibiotic profile of the organisms isolated

Gram Positives	**Total**	**Beta- Haemolytic *Streptococcus***	***Staphylococcus aurues***	**CNS**
Variables	**n=27**	**n=3**	**n=5**	**n=19**
Penicillin	21(72.4)	3(100.0)	5(100.0)	16(64.0)
Ampicillin	23(79.3)	3(100.0)	4(80.0)	19(100)
Flucloxacillin	20(62.1)	2(66.7)	3(60.0)	15(79.0)
Cefuroxime	16(55.1)	1(100.0)	4(80.0)	10(53.0)
Cotrimoxazole	14(48.3)	1(100.0)	2(40.0)	9(49.0)
Erythromycin	17(58.6)	1(100.0)	3(60)	10(53)
Gentamycin	10(34.5)	1(100.0)	3(60.0)	4(21.0)
Tetracycline	17(58.6)	1(100.0)	3(60.0)	10(53.0)
Gram Negatives	**Total**	***Pseudononas* spp**	***Salmonella paratyphi* B**	***Acinetobacter spp.***
Varables	**n =4**	**n =1**	**n= 3**	**n= 1**
Cefuroxime	3(60.0)	0(00.0)	2(40.0)	1(100.0)
Cefotaxime	1(20.0)	0(000.0)	0(00.0)	0(00.0)
Ceftrixime	3(60.0)	0(000.0)	2(40.0)	0(00.0)
Chloramphenicol	2(40.0)	1(100.0)	1(100.0)	1(100.0)
Cotrimoxazole	3(60.0)	0(000.0)	2(40.0)	1(100.0)
Gentamycin	4(100.0)	1(100.0)	2(40.0)	1(100.0)
Tetracycline	4(100.0)	1(100.0)	3(100.0)	1(100.0)

Plate 4.0: Antibiotic profile of a *Staphylococcus aureus* on an agar plate

Sensitive - Gentamycin, Ampicillin, Erythromycin and Cefuroxime.

Resistance - Cotrimoxazole, Penicillin and flucloxacillin.

4.4 Clinical assessment (Patients on antibiotic before reporting to hospital)

Only 152 of the 182 febrile paediatric patients were available for this analysis due to missing patient records for 30 patients. The clinical data obtained indicated that out of the 152 patients, 14.5 % (n=22) were on antimalaria drugs like artemether lumifantrin or artesunate amodiaquine before reporting to the hospital. Fourteen of these 152 patients, 9.2 %, were also on antibiotic like syrup cefuroxime, amoxicillin syrup or clavolanic acid, before reporting to the health facility and 25 % (n=38) of them were on both antibiotic and antimalaria drugs while 78 did not take any drug before reporting to the hospital.

4.4.1 Comparing Clinical Diagnosis with Convention blood culture and Blood Film (BF) Analysis

The results of provisional diagnosis and confirmatory results of the laboratory diagnosis are shown in table 4.4 below.

Table 4.4: Clinical diagnosis compared with laboratory diagnosis

Disease	Clinical Diagnosis	Laboratory Diagnosis
Malaria	103 (67.76 %)	34 (22.37 %)
Bacteraemia	53 (34.87 %)	14 (9.21 %)

Table 4.5: Correlation between Clinical diagnosis and Laboratory diagnosis

Diagnosis	Number Positive	Number Negative	Correlation by T test
Clinical Diagnosis for Malaria	103	49	0.215
Laboratory Diagnosis for Malaria	34	118	
Correlation is significant at the 0.01 level (2-tailed).			
Clinical Diagnosis for Bacteraemia	53	99	0.184
Laboratory Diagnosis for Bacteraemia	33	119	

Correlation is significant at the 0.05 level (2-tailed).

CHAPTER FIVE

5.0 Discussion conclusion and recommendation

5.1 Discussion

The objectives of this study were to assess the number of cases of bacterial bloodstream infections as compared to malaria infections among febrile paediatric patients, and to determine the causative agent and their antibiotic profile. The results indicated in table 4.3 showed that of the total 182 patients investigated, 38 (21 %) and 18 (10.2 %), positive blood cultures. However, while 5 (2.86 %) were positive for both malaria and blood culture. With the development and widespread use of effective vaccines to the common serious bacterial infections of infancy (*Haemophilus influenzae* type B and *Streptococcus pneumoniae*), the rate of infectious caused by these pathogens has dramatically declined. Many of the studies in children with bacteraemia were done prior to the introduction of one or both of these vaccines and, as such, may overestimate the likelihood of bacteraemia.

The presence of malaria parasites does not appear to show association with invasive bacterial infection in this population. This finding is unexpected given that malaria infection is known to depress immunity (Mabey *et al.,* 1987; Green *et al.,* 1993) and needs further validation; however, there was limited power to examine such association due to small numbers of patients. Evans *et at* suggested that infants with symptoms and signs of severe malaria but negative malaria film require immediate antibiotic treatment since it was impossible to distinguish patients found to be malaria-film-positive from those with a positive blood culture on the basis of clinical signs or white blood cell counts (Evans *et al*., 2004). One may argue that some film-negative cases could become positive on repeated examinations, or were malaria infections that had been treated. It was not possible to consistently include in the

study repeat malaria films or to provide more sensitive assessments of ongoing parasitaemia and recent parasitaemia, respectively.

The correlation between the clinical diagnosis of malaria and the laboratory diagnosis of malaria is 0.215. It implies a low significant with *P-value* less than 0.01. The correlation between the clinical diagnosis of bacteraemia and the blood culture for bacteraemia is 0.184 which also implies a low significant with *P-value* less than 0.05 indicating a weak positive linear correlation between them. The results of correlation between Clinical diagnosis and the laboratory diagnosis suggests that relying on only clinical diagnosis for treatment of patients with fever for malaria or bacteraemia may not be accurate. Most of the patients diagnosed clinically by the physician as malaria (103 out of 182) tested negative. This may be due to some factors such as false negative or interference of laboratory results by drugs taken by the patient prior to the laboratory test. However, confirmation of all fever cases by laboratory analysis is essential to avoid giving the wrong treatment to patients. A study done by Evans *et al*, on paediatric patients shows that, on the basis of clinical sings alone malaria film positive (n=182) and negative (n= 69), patients were indistinguishable as 40% of the film negative patients were bacteraemic (Evans *et al*., 2004).

Clinical assessment of the patients revealed that most of the children were given drugs by their parents before seeking medical assistance at the hospital. The results showed that 14.5 % (n=22) were on antimalarial drugs, 14 patients, 9.2 %, were also on antibiotic and 25 % (n=38) of them were on both antibiotic and antimalarial drugs

before reporting to the hospital. The situation where parents give self medication to their wards may have serious health implications, example, interfering with the laboratory results. Passerini and his colleagues stated in a research they conducted that, prior antibiotic therapy before blood sampling can influence blood culture performance (Passerini *et al*., 2009). Also, blind treatment of infection posses the danger of drug resistance as most patients do not take the required dosage since they are not under any supervision by a clinician. The resulting effect is that the micro organisms causing the infection are exposed to suboptimal levels of the drug thereby developing resistance to the drugs.

The study also identified that out of 182 paediatric patients, 40 positive blood cultures were obtained. The organisms isolated include 33 Gram positive organisms and 7 Gram negative organisms. The 33 Gram positive includes 4 Gram positive rods, 19 Coagulase Negative *Staphylococcus* (CNS), 5 *Staphylococcus aureus*, 3 Beta haemolytic *Streptococcus spp* and two Gram positive cocci (*Micrococcus leteus*). The 7 Gram negative organisms isolated includes 2 *Salmonella paratyphi* B, one *Acinectobacter spp* and one *Erwinia nigrifluens* 2 Gram negative cocci (which could not be identified) and one *Pseudomonas* spp. The 4 gram positive rods were considered as contaminants. It was however difficult to conclude that all the 19 Coagulase negative staphylococcus were contaminants since other studies have revealed a dramatic increase in the incidence of Coagulase Negative Staphylococcus (CNS) as an agent of bacteraemia (Reimer *et al*., 1997). However Coagulase Negative Staphylococci (CNS) are present in the normal flora of human skin and mucosa and are classic opportunists and can contaminate blood culture if the skin is not properly disinfected.

This study also sought to investigate the antibiogram of the bacteria isolated. From table 4.5.2, CNS showed 64% resistance to Penicillin, 100% resistance to Ampicillin, and 79% resistance to Flucloxacillin. *Staphylococcus aureus* on the other hand also showed 100% resistance to penicillin, 80% resistance to Ampicillin and 60% resistance to flucloxacillin. It was realized that *Stahpylococcus aureus* and the other CNSs organisms showed high resistance to gentamicin, tetracycline and cotrimoxazole and had varied resistance to the other drugs used for the study. This confirms the resistance of *Staphylococcus* spp. to antibiotics in the penicillin class which had been reported since the 1940's (Chambers, 2009). Resistance to the beta lactam antibiotics has been reported to be an indication of extended spectrum beta lactamase (ESBL) production (Todar, 2011). *Pseudomonas* spp. had 100% resistance to chloramphenicol, cotrimoxazole and gentamycin. *Salmonella paratyphi* B also showed 100% resistance to chloramphenicol and tetracycline, and 40% resistance to cotrimoxazole and gentamycin. Although the 2001 National Guidelines of Ghana listed chloramphenicol as first choice for treating typhoid fever, >80% of all bacteria identified were resistant to this drug. Resistance to the quinolone antibiotic tetracycline was also high in all the organisms isolated. A research conducted by Newman *et al.*, 2004 found that over 70% of the isolates were resistant to tetracycline, trimethoprim-sulphamethoxazole, Ampicillin and chloramphenicol and reported that 11% of the isolates were ciprofloxacin resistant in Ghana. A high percentage of chloramphenicol resistance was reported in Ghana by Groß *et al*., 2011. This drug was still considered the first choice treatment for typhoid fever in 2001 in Ghana. Similarly, 91.7% of all S. *enterica* were resistant to chloramphenicol in 2009 (Groß et al 2011).

5.2 Conclusion

In febrile paediatric patients at Princess Marie Louise Children Hospital in Accra, the rate of bacteraemia is 10.2 % as compared to the rate of malaria infection 21.64 % and the rate of malaria and bacteraemia co-infection is 2.8%. The correlation between clinical diagnosis by the physician and laboratory diagnosis for malaria and bacteraemia are 0.215 and 0.184 with low significant *P-value* less than 0.01 and 0.05 respectively suggesting a weak correlation. This study has shown that clinically diagnosed of Children with fever should be supported by laboratory diagnosis, including blood culture and blood film analysis, to identify the common causes of childhood fevers and ensure that patients are given the right treatment.

This study has also highlighted the presence of antibiotic resistant pathogens in our environments and further study including especially other hospitals and a larger sample size will provide data on these resistant pathogens which will be useful in health care policy planning in Ghana and the sub-region at large.

5.3 Recommendations

1. The high resistance of organisms to antimicrobial drugs is a major health problem. It is therefore recommended that this study is done on a bigger and more diverse sample size to provide data on the distribution of these resistant pathogens for health care policy planning in Ghana and the sub-region at large.
2. The implementation of bacteriologic diagnosis should be considered even in smaller hospitals in a rural setting to monitor pathogen distribution and resistance rates.
3. More antibiotics currently administered in our hospitals should be included in the study to determine the level of resistance of microorganisms to them.

5.4 Limitation

In Africa over 70% of malaria cases do not present initially to health facilities but diagnosed and managed at home with traditional remedies or drugs bought from local shops (Amexo *et al.*, 2004). Patients only attend health centers after self-treatment fails (Chandramohan *et al.*, 2002). This might have affected the laboratory results.

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Appendix 1

Consent Form

NMIMR-IRB CONSENT FORM

Title: ***Assessment of bacteria versus malaria infections in hospital admissions in Ghana***

Principal Investigator: Dr. Michael Kaser

Address: School of Public Health, College of Health Sciences, Ghanaian-German Centre Health Research, University of Ghana, PO Box LG13, Legon, Accra, Ghana, +233-271013867

General Information about Research

Fever, vomiting, diarrhea, headache, lethargy, unconsciousness and anemia may be caused by malaria, infection with bacteria, or both. The aim of our research is to improve the diagnosis and treatment of malaria, bacteremia and the combination of both. You will be asked to take part in a study which will contribute to this aim. Your part in research will take the time of the clinical examination and blood collection. If you agree to participate, you will be clinically examined and a blood sample of 5/10 ml (children/adults) will be taken. We will test the blood sample for the presence of malaria parasites, bacteria, hematological and biochemical parameters. In case we are able to isolate bacteria we will test which antibiotics work best to treat your bacterial infection. According to your needs you will be referred to the respective hospital unit.

Possible Risks and Discomforts

Collection of blood is considered a low-risk procedure and will be conducted by experienced physicians.

Possible Benefits

The individual patient will receive a malaria test and an investigation for bacteria in the blood, as well as hematological and biochemical tests (where indicated) for free (covered by project funds). The community will benefit from an improvement of the diagnosis and treatment of malaria and/or bacteremia.

Alternatives to Participation

You are free to decide if you want to participate in the study. Your decision will not affect the health care you would normally receive.

Confidentiality

All information collected will be kept confidential. You will not be named in any reports.

Voluntary Participation and Right to Leave the Research

Your participation is voluntary, and you can leave the study at any time without any disadvantage concerning your medical care.

Contacts for Additional Information

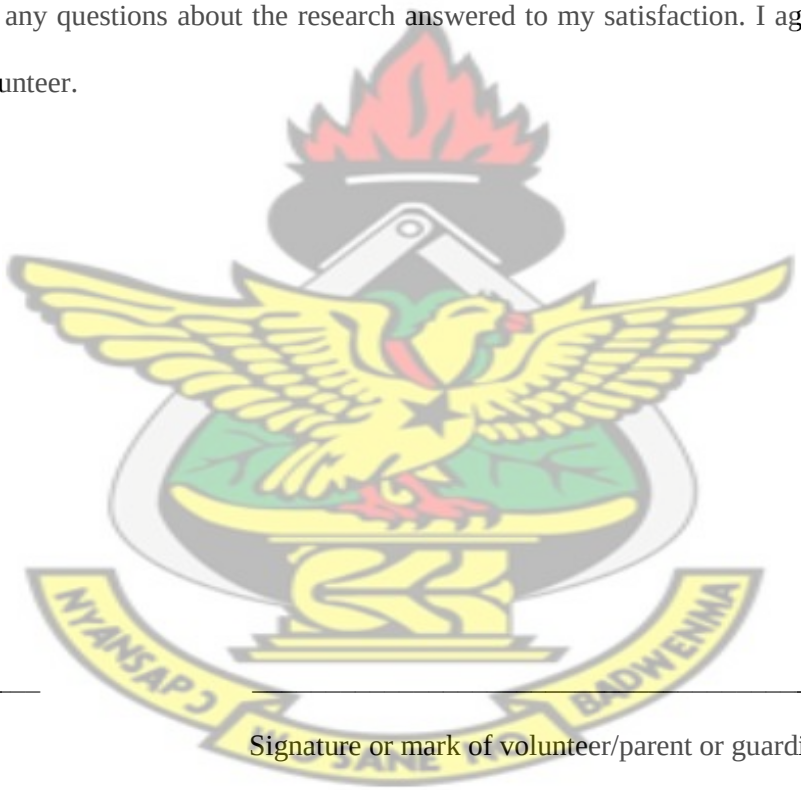
For questions about the research and in case of research-related health concerns, please call Dr. P. Puplampu (0244263582).

Your rights as a Participant

This research has been reviewed and approved by the IRB of Noguchi Memorial Institute for Medical Research. If you have any questions about your rights as a research participant you may also contact Rev. Dr. Ayete-Nyampong, Chairperson, NMIMR-IRB, mobile 0208152360

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the study with the research title ***Assessment of bacterial bloodstream versus malaria infections in hospital admissions in Ghana*** has been read and explained to me. I have been given the opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.



_____ _____

Date Signature or mark of volunteer/parent or guardian

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Signature of Witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Signature of Person Who Obtained Consent

Appendix 2

Procedure for Analytical Profile Index (API).

A large colony (2-3mm diameter) of the bacterium (pure culture) was inoculated into the sterile distilled water. A McFarland standard was used to check the suspension. Holding the strip at a slight angle up from the table top, the bacterial suspension was inoculated into each well with the sterile pipette. Touching the end of the pipette to the side of the cupule and allowing capillary action to draw the fluid into the well, the bulb was slowly squeezed. This was to ensure that any bubbles forming in the wells were eliminated. Each well was filled up to the neck. LDC, ODC, ADH, H2S, and URE were filled as described above, but they were then filled up to the top with sterile mineral oil. CIT, VP, and GEL have boxes around their names. These test wells were filled up to the top with the bacterial suspension as shown in figure 3.0 below.

The bottom of the incubation chamber has small indented wells in the bottom: These were filled with water just enough to fill these indentations making sure the water is not too much to slop onto the API strip. The strip was placed into this bottom. The top of the incubation chamber was placed over the bottom, and labelled. It was incubated at 37° C for 18-24 hours after which it was examined and the color changes in each strip read. The appropriate reagents were added to their respective compartments: one drop of Kovac's to the IND (read within a couple of minutes), one drop of Barritt's A and B to VP (reaction may take up to 10 minutes) and one drop of $FeCl_3$ to TDA. Three test reactions are added together at a time to give a 7-digit number in the codebook. Plate 3.3 shows an API strip after an overnight incubation.

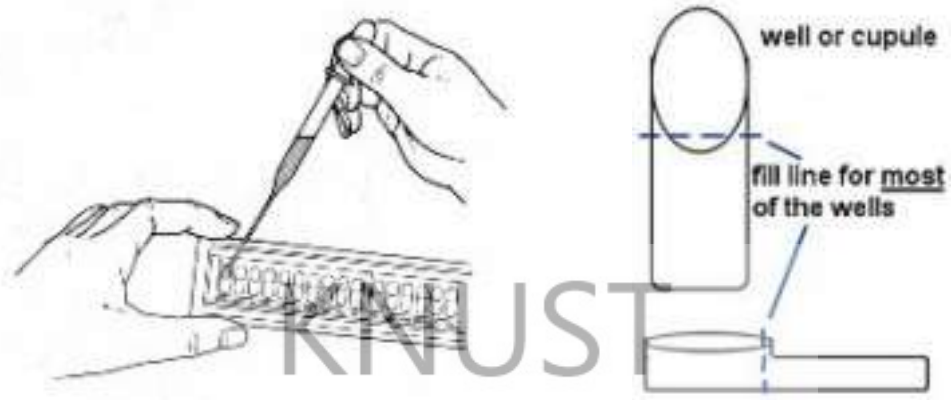


Figure 3.0: A diagram showing inoculation of bacterial suspension in API

(Source: Alderson *et al.*, 1991)

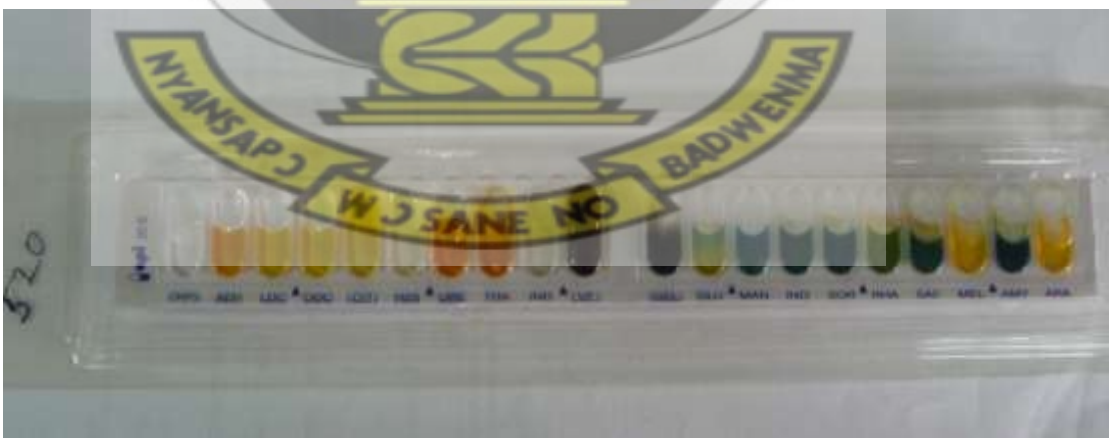


Plate 3.3: API 20E confirmatory test

***API 20E (bioMerieux) test after inoculation and overnight incubation and the addition of reagents to the cupules containing indole (IND), Voges-Proskauer (VP) and tryptophan deaminase (TDA)**

API strip identifying *Erwinia nigrifluens* and *Acinetobacter* spp.

Plate 1: API strip identifying *Erwinia nigrifluens* showing positive reaction to Voges-Proskauer

Plate 2: API strip identifying *Acinetobacter* spp. showing positive reaction to indole and hydrogen peroxide production.