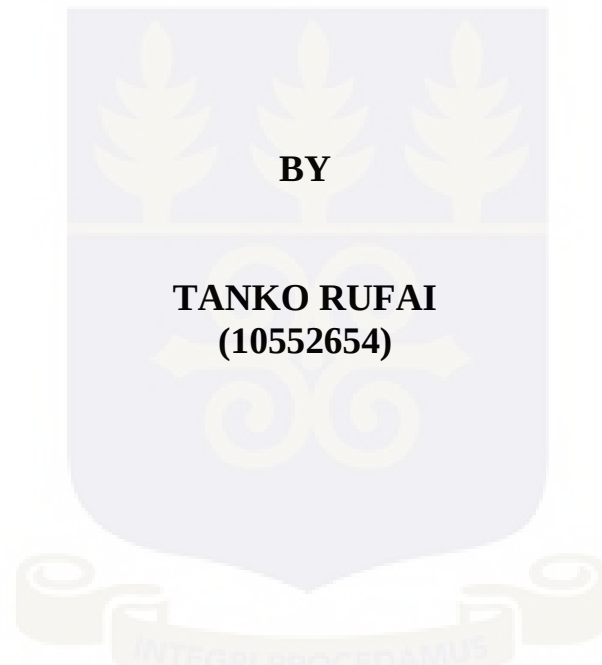


**SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA**

**MALARIA AND TYPHOID FEVER CO-INFECTION: A STUDY AMONG
PATIENTS PRESENTING WITH FEBRILE ILLNESSES IN THE GA
WEST MUNICIPAL HOSPITAL, AMASAMAN**



**THIS DESSERTATION IS SUBMITTED TO THE UNIVERSITY OF
GHANA, LEGON IN PARTIAL FULFILLMENT OF THE
REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY
DEGREE IN APPLIED EPIDEMIOLOGY AND DISEASE CONTROL**

JULY, 2017

DECLARATION

I, Tanko Rufai, hereby declare that with the exception of cited references to other people's work which has been duly acknowledged, this work is the result of my own research work done under supervision and has neither been presented elsewhere either in part or whole for another degree.

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DEDICATION

This work is dedicated to my mother Hajia Fulera and to the memory of my father Rufai Fuseini. I will forever be grateful for your advice, prayers and support. Finally to my wife Saudat Tanko, my children Amir Zaki Tanko and Rabab Tanko for their love and encouragement.



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Glory be to Almighty God for making the impossible possible. Father Your loyal servant am grateful.

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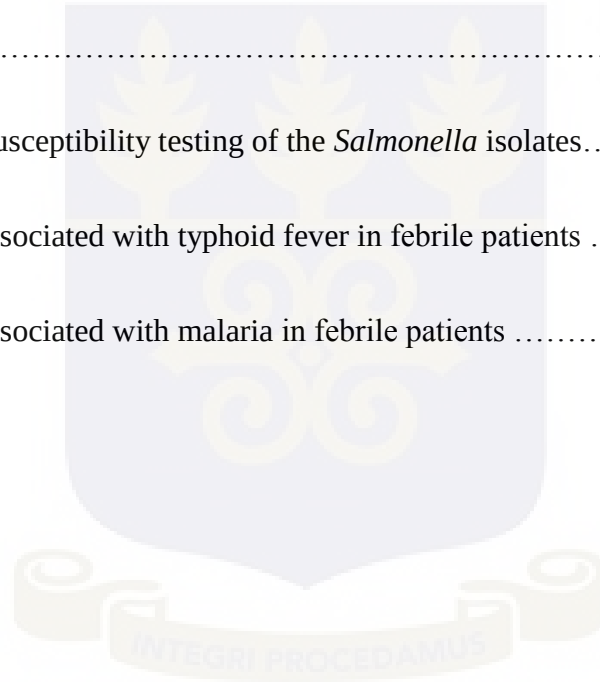
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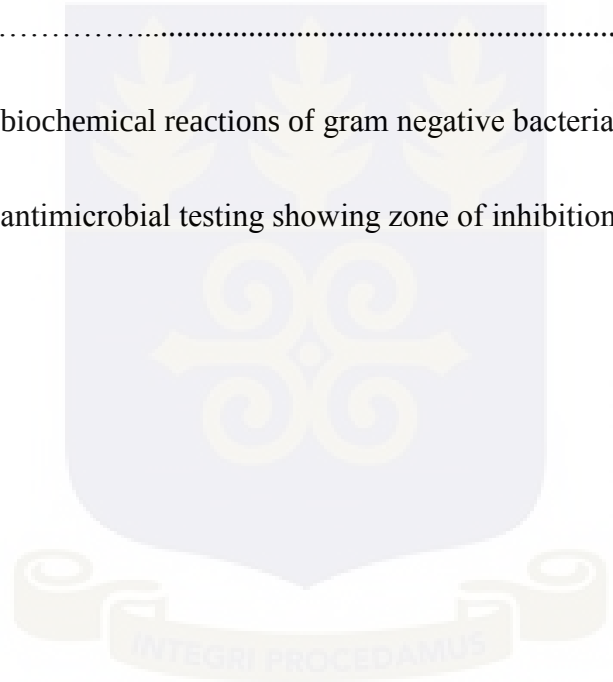
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LIST OF ABBREVIATIONS

μL	—	Microliter
AFI	—	Acute febrile illness
SSA	—	Sub-Saharan Africa
BA	—	Blood Agar
BF	—	Blood Film
BHI	—	Brain Heart Infusion
CA	—	Chocolate Agar
CDC	—	Centre for Disease Control and Prevention
EDTA	—	Ethylene Diamine Tetra Acetic Acid
GHS	—	Ghana Health Service
GSS	—	Ghana Statistical Service
GWMH	—	Ga West Municipal Hospital
MAC	—	MacConkey
RDT	—	Rapid Diagnostic Test
WHO	—	World Health Organization
PCR	—	Polymerase Chain Reaction
DHIMS	—	District Health Information Management System
IQR	—	Interquartile Range

ABSTRACT

Introduction: Malaria and typhoid fever cause major health problems especially in low and middle income countries. People in endemic areas are at risk of developing both infections concomitantly. These study was conducted to provide an epidemiological data on co-infection of malaria and typhoid fever in Ga West Municipality Hospital.

Methods: A cross-sectional study involving one hundred and fifty seven (157) febrile patients attending Ga West Municipal Hospital, Amasaman from February to May, 2017. Blood samples were collected for blood culture, Widal test, and blood film preparation for microscopy. Data were analyzed using Stata version 13 statistical software.

Results: The study population involved 157 febrile patients aged between 2years to 37years who reported to the hospital with fever (temperature 37.6 °C to 42 °C). A total of 82 (52.2%) of the study participants were females. The median age of all the patients was 6years (IQR=3-11/years).

Out of the 157 febrile patients, 57/157(36.31%) had malaria, 23/157 (14.64%) had typhoid fever using Widal test and 10/157 (6.37) by blood culture. Comparing patients with only malaria, the geometric mean parasite density was 174485 (45782-665000) for those with co-infection (p-value=0.009). Malaria for male 31/57(54.4%) and typhoid 6/10(60%). With age ≤ 10 ; malaria 42/57 (73.7%) and typhoid 8/10 (80%).

The co-infection of malaria and typhoid fever using Widal test and blood culture was 5.73% and 1.91% respectively. The isolates exhibited high resistance ranging from 60% - 100% against ampicillin, tetracycline, co-trimozazole, gentamicin, cefuroxime, chloramphenicol, and meropenem. The sensitivity also ranged from 66.7% - 100% against cefotaxime, ceftrizone, ciprofloxacin and amikacin. No isolate of *Salmonella typhi* were susceptible to gentamicin,

cefuroxime and co-trimoxazole. Other species of *Salmonella* were also not susceptible to tetracycline, ampicillin, co-trimoxazole and cefuroxime. All of the *Salmonella* isolates were susceptible to ciprofloxacin and amikacin.

Conclusion: These result of malaria and typhoid fever co-infection for blood culture and Widal test is 1.9% and 5.73%. All of the *Salmonella* isolates were susceptible to ciprofloxacin and amikacin.



CHAPTER ONE

INTRODUCTION

1.1 ACUTE FEBRILE ILLNESS

Fevers can be arbitrarily classified as acute, subacute and chronic fevers based on duration. Acute fevers that last less than 7 days are typical of diseases such as malaria and viral-related upper respiratory tract infection while those that last more than 2 weeks in duration are categorized as sub-acute fevers, usually seen in cases of typhoid fever and intra-abdominal abscess, among others (Ogoina, 2011).. Acute febrile illness (AFI) is characterised by a rise in body temperature above the normal range of 36.5–37.5 °C (Hutchison et al., 2008). Chronic or persistent fevers which last more than 2 weeks in duration are typical of chronic bacterial infections such as tuberculosis, viral infections , cancers and connective tissue diseases (Ogoina, 2011). Any acute fever which is left untreated can become chronic fever.

1.2 MALARIA FEVER

Malaria is one of the febrile illness and the most common fatal disease in the world caused by one or more species of *plasmodium*. These are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. Malariae*, and *P. Knowlesi* (Singh & Daneshvar, 2013; Samatha et., 2015). The most virulent species, *P. falciparum* is also the most prevalent in Africa, while *P. vivax* is the most widely distributed parasite outside of Africa (Gething et al., 2012).

1.2.1 Global Distribution of Malaria

Globally, about 214 million new cases of malaria was diagnosed in 2015 of which Africa accounted for 88%, South-East Asia (10%) and the Eastern Mediterranean region (2%) (WHO, 2015). Within the same period, a total of 438,000 malaria deaths was recorded worldwide of which

90 % (394,200 deaths) occurred in Africa (WHO, 2015). The remaining deaths were recorded in South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). Of the 306,000 deaths recorded globally among children under-fives, 95% (292,000 deaths) was from the African Region.

In Ghana malaria occurs throughout the year and affects people of all ages. The demographic health survey conducted in 2014 showed that incidence of malaria infection in children under five years ranged from 11.2% to 40% with the rural areas mostly having the highest prevalence (37.7%) (GDHS, 2014).

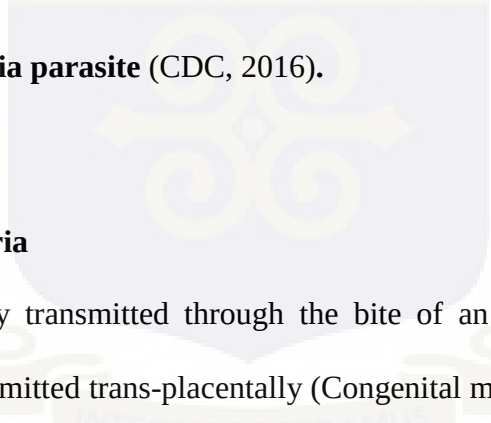
1.2.2 Etiology and life cycle

Malaria is caused by *Plasmodium* and belongs to the phylum Apicomplexa. It is transmitted when one is bitten by the female Anopheles mosquitoes and in humans it is caused by *Plasmodium falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi* (Daneshvar et al., 2009). The species differ in their geographical distribution, type of disease they cause and drug response. The most widespread species are *Plasmodium vivax* and *Plasmodium falciparum*, the latter is attributable to the severest forms of malaria whilst infections of other species are rarely life-threatening (Sutherland et al., 2010). *Plasmodium ovale* is restricted to West Africa sub-region where as *Plasmodium malariae* is found worldwide at low prevalence (Carter & Mendis, 2002). *Plasmodium falciparum* and *Plasmodium malariae* causes tropical and quartan malaria (Harinasuta & Bunnag, 1988). *Plasmodium vivax* is found in South-East Asia, Central and South America and just like *Plasmodium ovale*, causes tertian malaria (Harinasuta & Bunnag, 1988). Drug response and relapse patterns also differ between species. For example, relapses are

characteristic in *Plasmodium vivax* and *Plasmodium ovale* infections. Occasionally, humans become infected with a zoonotic species, *Plasmodium knowlesi* found in Asia (Singh et al., 2004; Daneshvar et al., 2009).

The most common species that causes malaria in Ghana is the *Plasmodium falciparum*. Others like *Plasmodium malariae*, and *Plasmodium ovale* (MOH, 2015) are also available. *Plasmodium vivax* has not been identified in Ghana (MOH, 2015). The principal vectors are *Anopheles gambiae* and *Anopheles funestus*.

The life cycle of the malaria parasite takes place in humans and the female *Anopheles* mosquito. Malaria parasites are transmitted through the bite of an infective female *Anopheles* mosquito. The mosquito is the definitive host and man is the intermediate host. In humans, parasites multiply asexually in the liver (exo-erythrocytic) (Vaughan, Aly, & Kappe, 2008) and in the red blood cells (erythrocytic) schizogony. After several cycles, gametocytes (sexual forms) develop in the red blood cells of humans (Greenwood et al., 2008; Pukrittayakamee et al., 2008; Miller et al., 2002) and are released into the blood which are ingested by female *Anopheles* mosquitoes during a blood meal. Development in the mosquito is known as sporogony. Male and female gametocytes fuse into zygotes which undergo further development in the stomach of the mosquito into ookinetes (Barillas-Mury & Kumar, 2005). The ookinetes migrate to the mid gut to form oocysts which subsequently develop into sporozoites, found in the salivary glands of the mosquito. Injection of sporozoites into a new host during feeding continues the life cycle (Barillas-Mury & Kumar, 2005).



Leishmania parasite (CDC, 2016).

transmitted through the bite of an infected mosquito.
transmitted trans-placentally (Congenital malaria)

transmitted through the bite of an infected mosquito.
transmitted trans-placentally (Congenital malaria)

a fever

a fever

with severe anaemia, kidney failure, coma, hypoglycemia, respiratory distress and death (Caraballo & King, 2014). Malaria tends to be particularly severe in infants, children < 5 years, pregnant women, non-immune persons and adults with compromised immunity.

1.2.5 Diagnosis of malaria

Malaria is diagnose by the identification of the parasite in blood. The gold standard is by examination of blood films using microscopy. Rapid diagnostic tests (RDT) detect the antigen of the parasite (Holland & Kiechle, 2005) and quantitative buffy coat method (Bhandari et al., 2008). Serological methods such as immunofluorescence or enzyme immuno assay can be used to detect malarial antibodies which can give indication of recent infection. Malaria can also be diagnose using polymerase chain reaction (PCR). Confirming clinical diagnosis with appropriate laboratory test is very vital.

1.2.6 Treatment and management

Malaria is a preventable and curable disease. Artemisinin-based combination therapy (ACTs) for uncomplicated malaria are highly effective against *Plasmodium falciparum* (WHO, 2016). In Ghana, artermether lumefantrine, artesunate-amodiaquine, or dihydroartemisinin piperaquine is also recommended (MOH, 2015). For preventive measures it is recommended that people sleep under insecticide treated nets. The interventions such as antimalarial drugs (quinine and its derivatives), transfusion, and fluid replacement are mostly used in severe malaria.

1.3 TYPHOID FEVER

Typhoid fever is a systemic protracted febrile illness commonly caused by *Salmonella typhi*. Similar but mild form, Paratyphoid fever is caused by *S. paratyphi* A, *S. paratyphi* B and *S. paratyphi* C (Andualem et al., 2014). Typhoid fever causes serious morbidity in many regions of the world, accounting for 21 million cases and 222,000 deaths annually (WHO, 2015). Typhoid fever is common in malaria endemic settings, usually leading to mix-infection. South and Central Asia, Africa and South and Central America are considered endemic with rates exceeding 100 per 100,000 population per year (Bhan et al., 2005)

In Africa, due to scarce resource and limited laboratory capacity to diagnose the disease accurately, most data on typhoid fever are not credible. A survey conducted in Egypt found an incidence of 59 cases per 100,000 persons per year for typhoid fever (Srikantiah et al., 2006).

1.3.1 Causative agent

Salmonella typhi belongs to the *Enterobacteriaceae* family and genus *Salmonella*. It affects only human and causes typhoid fever (Bhan et al., 2005) . *Salmonella typhi* is a gram-negative aerobic and facultative anaerobic rod-shaped bacterium (Todar, 2008). They are non-sporing and with the exception of *Salmonella typhi*, non-capsulate (Cheesbrough, 2006). It grows optimally at 35-37°C, rod-shaped with a length of 2-3 µm and a diameter of 0.4-0.6 µm (Cheesbrough, 2006). It moves with the aid of its flagella (H-d antigen). *Salmonella typhi* contains somatic or O, antigens associated with toxin, H-d associated with flagella; and Vi-antigen for virulence. It interferes with the complement (C3b) mediated opsonization of *Salmonella typhi*. This prevents it from binding with the phagocytes and subsequently inhibits phagocytosis.

1.3.2 Transmission

Typhoid fever is spread through ingestion of contaminated food and water. Humans excrete the bacteria in their faeces during the infection and usually persist if they become carriers. Other medium of transmission is by eating raw fruits and vegetables contaminated with human feces, milk products and shellfish. The bacterium can be viable for a long duration on food surface, seawater and sewage water. It can survive in freezing temperature for 3 months. . Foods are usually contaminated by flies which act as mechanical carriers and the bacteria can proliferate to cause typhoid in human (Guzman et al., 2006). It usually enters the bloodstream to the intestinal mucosa and subsequently multiplying in the lymph nodes. Most often the incubation period varies between 7 to 21 days.

1.3.3 Clinical manifestation

The main clinical symptoms related to typhoid fever are prolonged fever, malaise, anorexia, vomiting, severe headache, bradycardia, splenomegaly (Heymann et al., 2008). Initially the fever is minimal but rises gradually until second week where it can be high and persistent (39–40 °C). Other symptoms include abdominal discomfort, dry cough, myalgia and constipation is more common in adults than diarrhoea seen in children. A transient, macular rash of rose-colored spots can occasionally be seen on the trunk (Holmberg, 2012). Physical manifestations that are normally seen are coated tongue, tender abdomen, hepatomegaly or splenomegaly. Most often nausea and vomiting are not common but can be present in severe cases. Complications occur in about 15% of all cases, and include intestinal haemorrhage or perforation, psychosis, meningitis, and hepatosplenomegaly.

Prior to the discovery of antibiotics case fatality rates were high in untreated cases (10–20%). With the advent of antibiotics, case fatality rate is only about 1% although relapse may occur in 15–20% of patients while 10% of untreated patients would be infectious within 3 months and 2–5% would turn out to be chronic carriers (Heymann et al., 2008). Generally, reinfection of typhoid is rare because of the development lifelong immunity after primary infection.

1.3.4 Diagnosis

The presence of clinical symptoms characterised by fever is indicative of typhoid fever but needs laboratory confirmation (WHO, 2003). The *Salmonella typhi* can be isolated from blood within a week and in urine and faeces after first week. Even though blood culture is mostly used for diagnosis, bone marrow culture can also be used to isolate *Salmonella typhi*. The sensitivity and specificity of the conventional Widal test are low due to cross-reactivity with other microorganisms.

1.3.5 Treatment

Antibiotic treatment is used to resolve typhoid fever infection symptoms (Bhan, Bahl, & Bhatnagar, 2005). Sensitivity patterns of *Salmonella* isolates in the area determine choice of antibiotics (Bhan et al., 2005). With increasing multidrug resistance (MDR), previously effective drugs such as ampicillin, chloramphenicol are no longer recommended (Gupta et al., 2008; Lutterloh et al., 2012). Fluoroquinolones (ofloxacin, ciprofloxacin) are used for treating adults but should be guided by appropriate antimicrobial susceptibility testing (Heymann et al., 2008 ; Bhan et al., 2005; WHO, 2003).

Third-generation cephalosporin's such as cefotaxime and ceftriaxone can be used in cases with isolates resistant to nalidixic acid. Ceftriaxone used for children and azithromycin for treating uncomplicated typhoid fever (Effa & Bukirwa, 2008). When there is intestinal perforation, surgery is commonly recommended.

1.4 CO-INFECTION OF MALARIA AND TYPHOID FEVER

Malaria and typhoid fever co-infection was first described during the American civil war by Woodward in 1862 among young soldiers presenting with intermittent pyrexia. It was suggested that it could be a mix infections (Smith, 1982a). Subsequently, many studies have long established this association (Ammah et al., 1999; Gopinath et al., 1995). People with poor hygiene can contracting both diseases. Patients with co-infection mostly associated with nausea, vomiting, abdominal pain, diarrhea and continuous fever (Khan et al., 2005). Anaemia due to massive haemolysis or dyserythropoiesis can occur during malaria infection which can lead to increase iron in the liver and which support the growth of *Salmonella* (Bashyam, 2007). Some studies have shown that Complement C1q and C4B deficiency can make a person susceptible to typhoid fever infection (Warren et al., 2002; Bishof et al., 1990).

1.5 PROBLEM STATEMENT

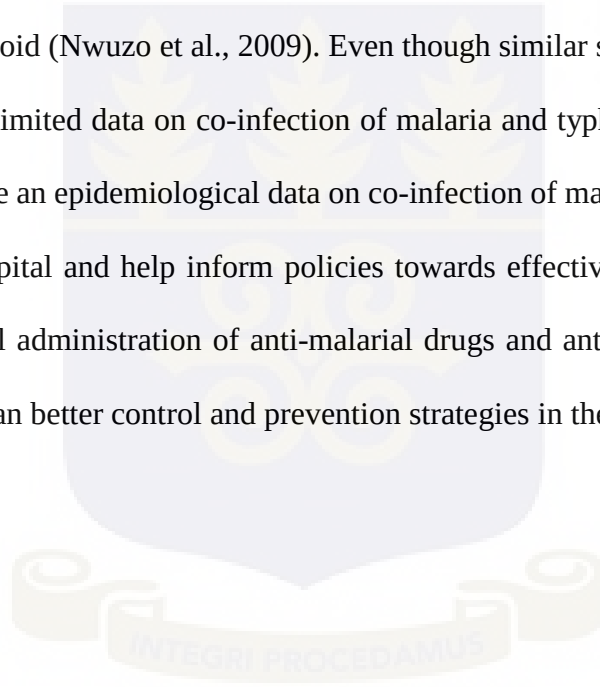
Malaria and typhoid fever are well known undifferentiated febrile illnesses which may be responsible for varying degrees of morbidity and mortality in developing and middle income countries including Ghana. Due to lack of availability of diagnostics in low and middle income countries, most cases of acute febrile illnesses are diagnosed as malaria (Stoler & Awandare, 2016). In Ga West Municipal Hospital (GWMH), the number of malaria cases increased from 3934

in 2015 to 4603 in 2016 (DHIMS 2, 2017). Typhoid fever also increased from 1369 to 2033 during the same period (DHIMS 2, 2017). Meanwhile, people in endemic areas are at risk of contracting both infections concurrently (Uneke, 2008 ; Nsutebu et al.,2003). Predisposition to co-infection is usually influenced by their similar epidemiological factors such as dense population, poor hygiene, and sanitation practices (Iheukwumere et al., 2013; Sharma et al., 2016). Due to their similar clinical presentations and the likelihood of a misdiagnosis and mistreatment of febrile patients, it has been suggested that malaria and typhoid fever should be treated concurrently in endemic communities (Uneke, 2008; Iheukwumere et al., 2013). However, concurrent treatment may have some public health implications in the sense that, irrational use of antibiotic or anti-malarial may result in increasing surge of drug resistance, unnecessary cost and exposure of patients to side effects of antibiotic (Sharma et al., 2016).

Febrile patients reporting to the GWMH are usually tested for malaria using mRDT at the outpatient department and subsequently treated separately or concomitantly without investigation of other possible causes. These tests lack sensitivity at low levels of parasitaemia and persistently positive tests (for some antigens). In severe cases, patients are requested to do Widal test for typhoid at the laboratory which is prone to error (Mbuh, Galadima, & Ogbadu, 2003). Meanwhile reliable diagnosis is by microscopic examination of blood film for malaria or blood, stool or bone marrow culture for *Salmonella*. It is therefore advisable to perform both tests on individuals presenting with fever of malaria- typhoid signs and symptoms using accurate diagnostic methods to ascertain true co-infection followed by appropriate treatment (Mbuh et al., 2003). Due to drug resistance, it is usually necessary to carry out sensitivity tests before making an informed choice of an antibiotic for treatment.

1.6 JUSTIFICATION / RATIONALE

Treatment of both diseases is common among patients especially in the tropics even if their diagnosis has not been confirmed. This may interfere with diagnosis and also means of increasing antibiotic resistance. Studies have shown that there are more typhoid cases in areas of drug resistant malaria and a cross reaction between malaria parasites and *Salmonella* antigens may cause false positive Widal agglutination test. An accurate diagnosis such as positive blood culture is crucial for effective management of patients. A number of studies have shown that malaria could be co-infecting with typhoid (Nwuzo et al., 2009). Even though similar studies have been done in other countries, there is limited data on co-infection of malaria and typhoid fever in Ghana. The present study will provide an epidemiological data on co-infection of malaria and typhoid fever in Ga West Municipal Hospital and help inform policies towards effective management of febrile patients against irrational administration of anti-malarial drugs and antibiotics. Data from these study will also help to plan better control and prevention strategies in the municipality.



1.7 CONCEPTUAL FRAMEWORK OF MALARIA AND TYPHOID FEVER INFECTION

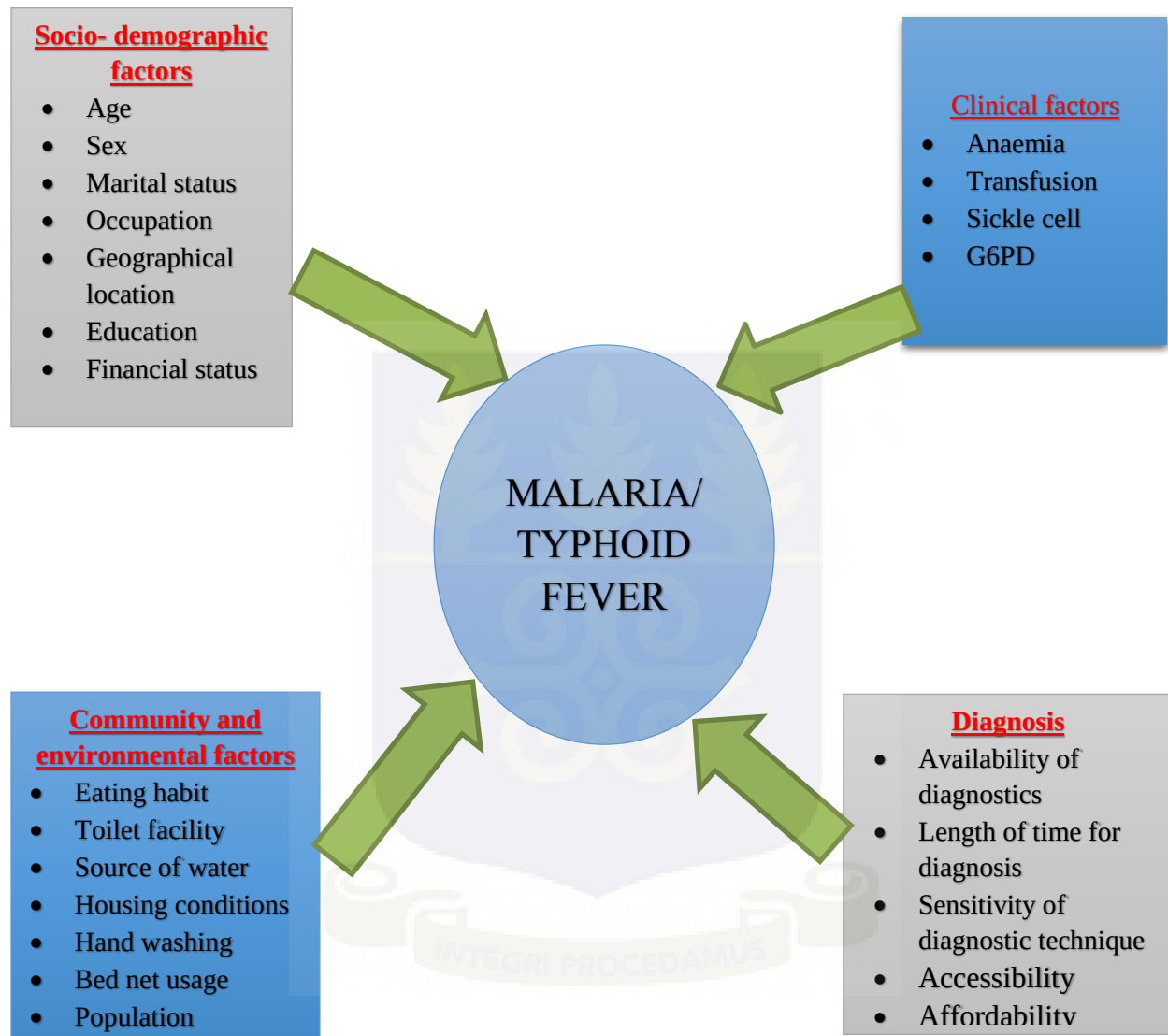


Figure 2: Conceptual Framework

1.7.1 Narration of conceptual framework

People are at risk of acquiring malaria and typhoid fever infection due to factors related to environment, community, demographic and socio-economic status. Usually these are among

contributing factors to disease occurrence in a given community. Malaria and typhoid fever are common in areas with high poverty, unrestrained urbanization and poor infrastructure impact on contamination of water supplies. Dense population which may result in illegal infrastructure development, poor sewage system, poor hygiene and sanitation practices leading to malaria and typhoid infection. According to Parry, (2006), eating food outside, eating vegetables and salad and water contaminated with human feces pose as risk of typhoid infection.

Other reported risk factors include contact with carriers, poor hands washing and housing (Bhan et al., 2005). Factors such as indiscriminate disposal of waste, sewage spillage, lack of good toilet facilities, inadequate sanitary facilities in homes and general poor sanitation culture lead to malaria and typhoid fever (WHO, 2003). Improperly cooked and hygienically handled food and food stuff are being consumed indiscriminately due to deteriorating socioeconomic statuses coupled with lack of health education. Typhoid fever occurs at any age but occurs more commonly in children and young adults. Using insecticide treated nets (ITNs) help reduce malaria (Klinkenberg et al., 2010)

Clinical factor such as Blood transfusion use in the management anaemia can cause malaria. Malaria also causes breakage of red blood cells which increases iron content in the body. Meanwhile *Salmonella* thrive in the presence of iron. Anaemic patients are highly susceptible to *Salmonella* infection. Lack of diagnostic equipment and reagents contribute to poor diagnosis early stage leading to the spread of infections and under estimation of the disease burden. Abuse of antibiotics may eventually result in drug resistance, which would contribute to having typhoid fever carriers who may spread the infection. Availability of diagnostics, length of time for

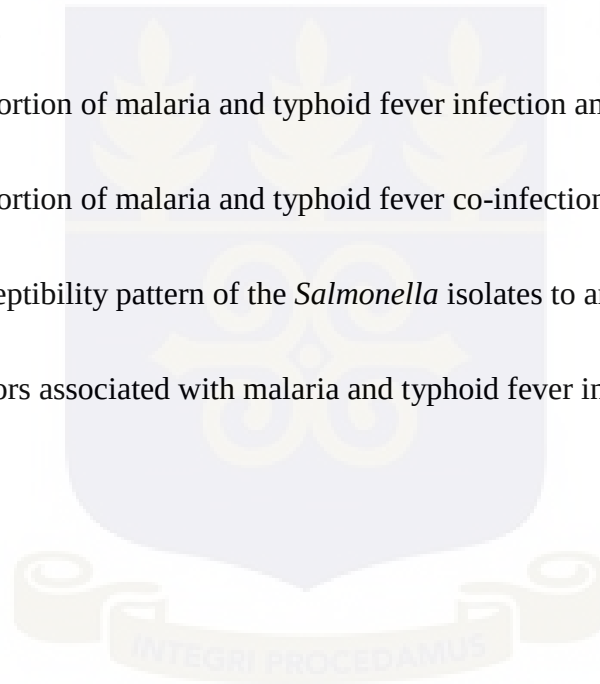
diagnosis, sensitivity of diagnostic technique which can lead to misdiagnosis and mismanagement of patients.

1.8 GENERAL OBJECTIVE

To determine the proportion of malaria and typhoid fever co-infection, assess their risk factors and susceptibility patterns of the *Salmonella* isolates to antimicrobial agents among febrile patients attending the Ga West Municipal Hospital

1.8.1 Specific objectives

1. To determine the proportion of malaria and typhoid fever infection among febrile patients
2. To determine the proportion of malaria and typhoid fever co-infection among febrile patients
3. To determine the susceptibility pattern of the *Salmonella* isolates to antimicrobial agents
4. To assess the risk factors associated with malaria and typhoid fever infection



CHAPTER TWO

LITERATURE REVIEW

2.1 MALARIA AND TYPHOID FEVER AS A CAUSE OF FEBRILE ILLNESSES

Malaria and typhoid are common causes of febrile illnesses globally but more endemic in Africa and Asia. Diagnosing febrile patients based on clinical signs and symptoms is difficult to differentiate between malaria and typhoid fever (Moses et al., 2016).

In one study conducted in Kumasi and Sunyani metropolis using mRDT for malaria and Widal test for typhoid fever, out of the 129 patients, 22(17%) tested positive for typhoid fever, 24 (18.6%) tested positive for falciparum malaria (Afoakwah et al., 2011).

Several studies in Nigeria have reported on malaria and typhoid fever among febrile patients. In Abakaliki, Ebonyi state, 33/250 (13.2%) tested positive for malaria, 53/250 (21.2%) typhoid fever by Widal test and 2 (0.8%) by blood culture (Nwuzo et al., 2009). In Calabar, 202/250 (80.8%) tested positive for malaria, 117 (46.8%) for typhoid by the Widal test and 2 (0.8%) with blood culture. The study further found that males 97 (85.8%) were more infected with malaria than females 105 (76.6%) but this was not statistically significant while the prevalence of typhoid fever was higher in females 77 (56.2%) than males 40 (35.4%) and was statistically significant (Archibong et al., 2016). A study amongst one hundred (100) patients with signs and symptoms of malaria and typhoid in Uyo, Akwa Ibom State, Nigeria, found that 41/100 (41%) tested positive for malaria, 64/100 (64%) for typhoid using widal and 11/64 (17%) using blood cultures (Edet et al., 2016). A Similar study at the University of Uyo Teaching Hospital among 145 patients, reported 51(35.2%) had malaria and 10(7.0%) typhoid/paratyphoid. The study further found females were more infected than males (Moses et al., 2016). A study by Ukaegbu et al., (2014),

reported 162/300 (54%) for malaria, 68/162 (42%) for typhoid fever by Widal test and 9/162 (5.6%) by stool culture test (Ukaegbu et al., 2014). A study at Nnewi, Anambra state, out of 256 patients, 202(78.90 %) had malaria, 147(57.42 %) by widal and 38(14.84 %) by culture had typhoid fever. The study further found that, the age group (11-30) years were mostly infected with malaria and typhoid fever (Ekesiobi et al., 2008). An investigation carried out in Ekwulumili Community, Anambra State, reported 40/200 (20%) for malaria, 11 (5.5%) typhoid fever (Onyido et al., 2014). In a research carried out by Mbuh et al., (2003), 60/218(27.5%) tested positive for malaria, 22/218 (10.1%) typhoid by the Widal test and 1/250 (0.5%) by the culture method.

Studies from Ethiopia have also reported different prevalence rate for malaria and typhoid fever among febrile patients. A study conducted by Birhanie et al., (2014) among 200 febrile patients recorded 73/200 (36.5%) for malaria, 38/200 (19%) using widal test and 1/200 (0.5%) with blood culture for typhoid fever (Birhanie et al., 2014). The study also showed that malaria was higher in males while typhoid fever was greater in females. Another study involving 502 who were being diagnosed with typhoid fever, of which 343 (68.3%) of the febrile patients showed positive slide Widal test while only 8(1.6%) patients were culture-proved to have typhoid fever (Wasihun et al., 2015).

In Cameroon, 315 children aged 6months and 15years were studied. Malaria prevalence was 43.4% and 70.2% for microscopy and PCR respectively. The prevalence of typhoid fever was 4.4% using rapid diagnostic test kit (Achonduh-Atijegbe et al., 2016). Another study in Kumba, Cameroon recorded malaria prevalence of 90.3% (186/206) with parasite density 866 (range: 40 – 64880) parasites/ μ L of blood, 7.9% (14/178) for typhoid (Ndip et al., 2015).

A Study by Sharma et al., (2016) in India, reported 60/3010 (1.99%) cases of typhoid by blood culture, 48/60 (80%) were also positive for malaria parasite by peripheral smear examination. In Guntur, a prevalence of 340/582 (58.4%) for malaria, 132/582 (22.6%) typhoid by Widal and 10/582 (1.8%) blood culture (Samatha et al., 2015). The study also found that 235/582 (40.3%) had no malaria or typhoid fever. Another study had revealed that typhoid 35/108 (32.40%) is more prevalent than malaria 11/108 (10.80%) in acute fever (Sandhya et al., 2015).

2.2 CO-INFECTION OF MALARIA AND TYPHOID FEVER

In the last two decades, concurrent infection of typhoid fever and malaria have been confirmed by studies from Africa and Asia (Ohanu et al., 2003; Sur et al., 2006; Kanjilal et al., 2006). The prevalence using Widal test ranged from 4.4% to 70% (Tanyigna et al., 2000); (Ibadin & Ogbimi, 2004), while cultural technique alone ranged from 11.1% to 26.6% (Smith et al., 2004; Khan et al., 2005; Akinyemi et al., 2007). In Ghana, prevalence of malaria- typhoid fever co-infection of 4.65% have been reported using mRDT and Widal test (Afoakwah et al., 2011).

Association between typhoid fever and malaria have been reported in many studies. Several in Nigeria. In one study, co-infection obtained when typhoid was diagnosed by Widal test (10.1%) was significantly higher than by blood culture method (0.5%) (Mbuh et al., 2003). The study further observed that overestimation of co-infection with malaria and typhoid fever will greatly reduce if the diagnosis is based on blood culture. A Study by Nwuzo et al., (2009), reported a co-infection of 2/250 (0.8%) by culture method and 14/250 (5.6%) by the Widal test. Another study recorded (28.0%) by Widal test and (0.8%) by blood culture method (Archibong et al., 2016). Other studies had also reported various prevalence malaria and typhoid fever co-infection in different

states; Uyo, 16% (n = 100) and 17% (n = 64) for widal and blood culture respectively (Edet et al., 2016), Nnewi 29/256(14.36%) by culture method while 147/256 (57.42%) for widal test (Ekesiobi et al., 2008), Ekwulumili Community, Nnewi South, co-infection was (5.0%) 10/200 using the widal test. More co-infection was observed among females (5.41%) (Onyido et al., 2014). In Lagos, co-infection of malaria and typhoid was observed among patients with complications (Akinyemi et al., 2007).

A study conducted in New Delhi, India reported 1.59% (48/3010) using culture method for typhoid and blood smear for malaria parasite however Widal test for typhoid and rapid diagnostic test for malaria parasite showed co-infection rate of 3.38% (105/3010) (Sharma et al., 2016). Samatha et al., (2015), found co-infection of 38/582 (6.5%) using widal and 4/582 (0.7%) using blood culture.

In Ethiopia, Birhanie et al., (2014) showed in their study that, co-infection 13 (6.5%) and 1 (0.5%) for widal and blood culture while in Kumba, Cameroon had 6.74% co-infection rate (Ndip et al., 2015). In Pakistan, dual infection of malaria-typhoid fever was reported comprising of *Salmonella typhi* and *Salmonella para typhi* A or B (Khan et al., 2005).

2.3 ANTIBIOTIC SUSCEPTIBILITY OF SALMONELLA

Antimicrobial resistance have been attributed to the irrational use of drugs causing the emergence of resistant strains. Antimicrobial resistance leads to ineffective chemotherapy, which usually leads to treatment failure. It can also increase morbidity, cost and ultimately increased risk of death. Due to the difficulty in diagnosing the cause of a fever based on clinical features alone, overtreatment with antibacterial drugs is also a common occurrence. The choice of antibiotic

should be based on sensitivity patterns of *Salmonella* isolates (Bhan et al., 2005). Ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole, are not effective for treatment of typhoid fever (Lutterloh et al., 2012 ; Gupta et al., 2008).

Antimicrobial resistance tends to be more pronounced especially in Africa and Asia (Newman et al., 2011). Some factors that contribute to treatment failure or drug resistant are incorrect dosing, poor quality of drug, and misdiagnosis in the individual. Rates of multi-drug resistant (MDR) among *Salmonella typhi* was found to be up to 80% in southeast Asia (Wain et al., 1999), 70% in east Africa (Mengo et al., 2010), and also non-*Salmonella typhi* (Folster et al., 2012; Miriagou et al., 2004). This could be due to the unnecessary overuse of antimicrobial drugs and further complicated by the substandard medicines in circulation in several LMICs, which risks patient safety and may promote antimicrobial resistance (Bate et al., 2008; Bate et al., 2009; Caudron et al., 2008). A study on MDR in Delhi in 1993, by Daga et al., (1994), highlight the importance of ciprofloxacin in the treatment of typhoid fever. A study involving clinical profile among 100 children with positive blood culture for *Salmonella typhi*, reported that, the isolates were not susceptible to amoxicillin, chloramphenicol and co-trimoxazole, but sensitive to ciprofloxacin and ceftriaxone (Kalra et al., 2003). In another study from Rourkela in 2000, the number of MDR strains of *Salmonella typhi* constituted 16.1% of the total isolates and were susceptible to chloramphenicol, ceftriaxone and ciprofloxacin (Das & Bhattacharya, 2000).

In Laos, of the 1095 patients with data for hospital antimicrobial use, 56% received an antimicrobial drug, and 12% received more than 1 (Mayxay et al., 2013). On the basis of final diagnosis, only 7% of these patients were regarded as having been treated appropriately (Mayxay

et al., 2013). Although malaria was the admission diagnosis for 148 adults in Tanzania, 44.8% of them were still prescribed empiric antibacterial agents (Nadjm et al., 2012). A Rwandan study on malaria hospitalization after implementation of a malaria-control program documented a decrease in the risk of high parasitaemia after the intervention (Sievers et al., 2008). In one study in Cameroon, gentamycin and ciprofloxacin were found to be sensitive in treating typhoid fever (Ndip et al., 2015).

2.4 RISK FACTORS FOR MALARIA AND TYPHOID FEVER

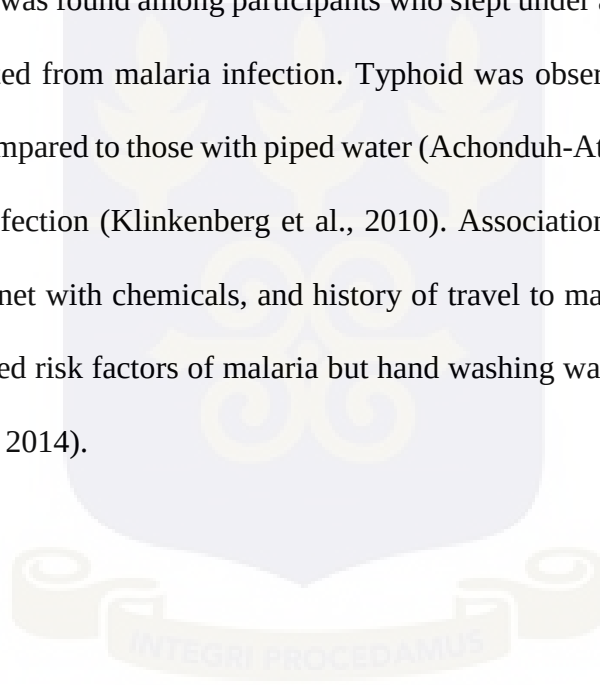
Typhoid can be transmitted by chronic carriers especially where there is poor personal and food hygiene. In the USA, up to 30% of infections are due to diagnosed chronic carriers (Bhan et al., 2005). In endemic areas, transmission is high during dry and rainy seasons. Poverty, uncontrolled urbanization and inadequate infrastructure do contribute to the contamination of water supplies.

Usually, transmission is through poor hygiene and sewage contamination of water (Bhutta, 2006). Pipe-borne water remained the only source of drinking water which is likely to be contaminated due to rusted and leakage of pipes. Parry (2006), noted that eating food outside, eating vegetables and salad and water contaminated with human faeces pose a risk of typhoid infection. Other risk factors include contact with other patients, poor hand washing and housing (Bhan et al., 2005).

The socioeconomic factors, affecting the prevalence and distribution of typhoid/paratyphoid bacilli include indiscriminate disposal of waste, sewage spillage, lack of good toilet facilities, inadequate sanitary facilities in homes and generally poor sanitation culture (WHO, 2003). Typhoid fever occurs at any age but occurs more commonly in children and young adults.

Recently, contrary to these popular findings the disease was found to affect even children aged 1-5 years in Delhi, India (Walia et al., 2006). In France, children between 1 to 5 years were more affected (Desenclos et al., 1996). The age group at greatest risk is 5 to 25 years (Saha et al., 2001). A study in Pakistan, showed that children are usually burdened with typhoid fever (Graham, 2002; Brooks et al., 2005; Siddiqui et al., 2006). Another study has also revealed that incidence of typhoid fever is highest in children less than 5 years, with higher complications (Bhutta, 2006).

A significant association was found among participants who slept under an insecticide treated nets (ITN) were more protected from malaria infection. Typhoid was observed for participants who had a borehole or well compared to those with piped water (Achonduh-Atijegbe et al., 2016). ITNs usage reduces malaria infection (Klinkenberg et al., 2010). Association between bed net usage, impregnation of the bed net with chemicals, and history of travel to malaria endemic areas were not significantly associated risk factors of malaria but hand washing was associated with typhoid infection (Birhanie et al., 2014).



CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

A cross- sectional study was conducted to collect both qualitative and quantitative data on patients age two years and above with temperature reading $>37.5^{\circ}\text{C}$ at the outpatient department between the months of February and May 2017 in Ga West Municipal Hospital. Blood sample was taken from these febrile patients suspected to have malaria or typhoid fever to determine the proportion of malaria and typhoid fever co-infection and susceptibility patterns of the *Salmonella* isolates to antimicrobial agents.

3.2 STUDY SITE

Ga West Municipality (GWM) is one of the 16 districts in the Greater Accra Region. The district is 60% rural and 40% peri-urban and urban, and is made up of about 150 communities with Amasaman as the district capital.

The GWM shares boundaries with Ga East and Accra Metropolitan Area to the East, Akwapim South to the North, Ga South to the South and Ga Central to the North-South. It occupies a total land surface area of 299.578 square kilometers. The projected population of the Municipality for 2015 was 256026 (GSS). Currently the municipality is divided into three (3) sub-municipal areas for the purpose of planning and delivery of services namely: Amasaman, Ofankor and Pokuase. The Municipality has a district hospital, four health centres, three clinics, four community based health planning services (CHPS) zones compounds, nine urban CHPS, ten private hospital/clinic and four maternity homes. The Ga West Municipal Hospital is the nearest referral hospital that serves both Amasaman municipality and its environs.

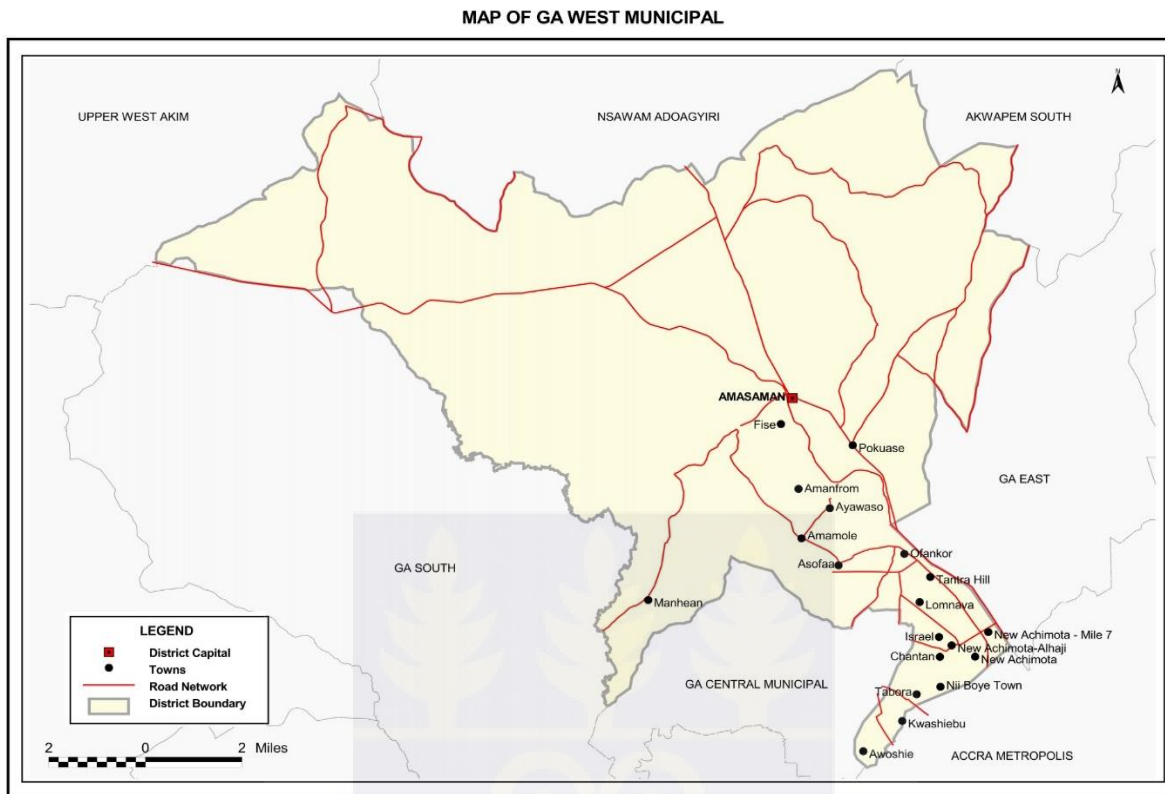


Figure 3: Map of Ga West Municipal

Source: Ghana statistical service.

3.3 VARIABLES

The variables to be measured are:

Dependent variables: Malaria and typhoid fever

Independent variables:

- **Socio-demographic factors:** Age, sex, marital status, educational level, occupation and location
- **Environmental factors:** Source of water, availability of toilet facility, bed net usage, hand washing habit and eating habit

- **Clinical factors:** Fever, vomiting, diarrhoea, abdominal pain, joint pain, fatigue , headache, history of transfusion and antibiotic used

In the laboratory, data on the malaria parasites and isolated *Salmonella* species was collected after blood film examination, culture and the antibiotic susceptibility pattern of the isolates as follows.

1. Causative agents for malaria and typhoid fever

- malaria parasite and type of species
- Gram negative organisms (*Salmonella* species)

2. Susceptibility of *Salmonella* isolates

- Resistance pattern of the isolates
- Sensitivity pattern of the isolates

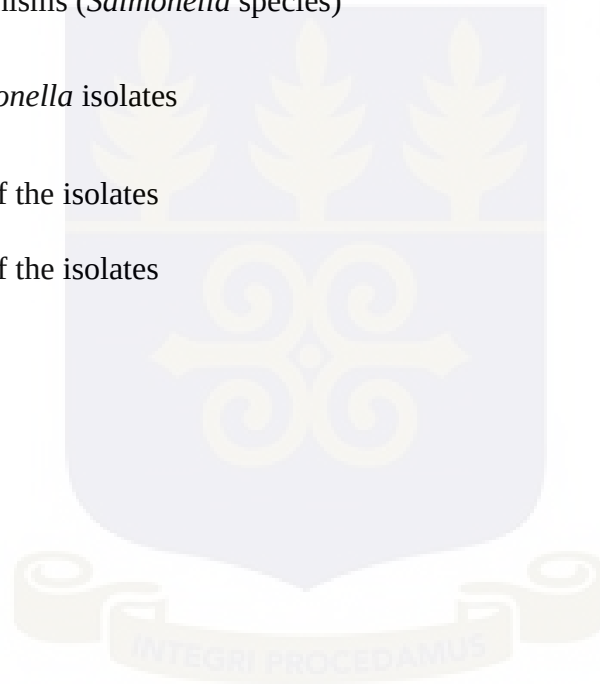


Table 1a: Definition and scale of measurement for variables

Variables	Operational Definition	Scale of Measurement	Data collection technique
Malaria	Presence of malaria parasites in the blood of patients	Categorical a. Malaria parasite present b. No malaria parasite seen	Laboratory result
Typhoid fever	Isolation of Salmonella typhi in the blood of patients	Categorical a. Salmonella isolated b. No Salmonella isolated	Laboratory result
Gender	Sex of patients	Categorical a. Male b. Female	Structured questionnaire/interview
Marital status	Description of patients relation status	Categorical a. Married b. Single c. N/A	Structured questionnaire/interview
Educational level	Highest level of education	Categorical (ordinal) a. Primary b. JSS c. SSS d. Tertiary e. Others f. N/A	Structured questionnaire/interview
Occupation	Occupation of patients	Categorical a. Civil servant b. Merchant c. Unemployed d. N/A	Structured questionnaire/interview
Location/residence	Place of habitation of patients	Categorical	Structured questionnaire/interview
Age	Age of patients	Continuous	Structured questionnaire/interview
Source of water	Source of household water used by patients to be interviewed	Categorical a. Tap water b. Borehole c. River d. Packaged water e. Others	Structured questionnaire/interview

Table 1b: Definition and scale of measurement for variables

Availability of toilet facility	Presence of toilet facility in the house	Categorical / Binary a. Yes b. No	Structured questionnaire/interview
Bed net usage	Possession and usage of insecticide treated net	Categorical / Binary a. Yes b. No	Structured questionnaire/interview
Hand washing habit	Washing of hands by patients before eating using soap	Categorical a. Good b. Poor	Structured questionnaire/interview
Eating habit	How often patients patronize and eat foods outside home	Categorical a. Frequent b. Infrequent c. Never	Structured questionnaire/interview
Fever	Patients body temperature $> 37.5^{\circ}\text{C}$ using thermometer	Continuous	Review of case notes /folder
Clinical presentation	Patient complain for visiting the hospital	Categorical a. Abdominal pain b. Diarrhea/vomiting c. Joint pain/Fatigue d. Headache e. Antibiotic used f. Others	Review of case notes /folder

3.4 SAMPLING

3.4.1 Study population

The study population were patients' aged ≥ 2 years who reported at the outpatient department, Ga West Municipal Hospital between February and May 2017. After taken the body's temperature using the infrared thermometer, those whose reading was $>37.5^{\circ}\text{C}$, suspected of malaria and typhoid and have met the inclusion criteria were recruited.

3.4.1.1 Inclusion criteria

The inclusion criteria involved;

1. Patients with fever $>37.5^{\circ}\text{C}$, presenting to the health facility at the outpatients department
2. Patients who have consented to the study.

3.4.1.2 Exclusion criteria

The study excluded;

1. Patients who were on antibiotics and anti-malarial therapy
2. Patients below < 2 years
3. Those who did not consent to the study
4. Patients in critical conditions such as convulsion.

3.4.2 Sample size determination

Sample size was calculated from Cochran's formula $n = z^2p(1-p) / e^2$

Where N = the minimum sample size, Z = standard score for the 95% confidence limit = 1.96

P = the known prevalence of 11.2% for malaria in Greater Accra (GDHS, 2014), e = the allowable error margin=5%, the minimum number of study participants that were enrolled for the study was 153.

3.4.3 Sampling method

The study was a cross-sectional study. Patients age ≥ 2 years presenting with febrile illnesses at the outpatient department between the months of February and May 2017 in Ga West Municipal Hospital and were suspected to have malaria or typhoid fever. After taken the body's temperature using infrared thermometer, those whose temperature reading was $>37.5^{\circ}\text{C}$ and have consented were randomly selected.

3.5.1 Ethical considerations

Before the commencement of the study, ethical clearance was obtained from the Ghana Health Service Ethics Review Committee. Permission was obtained from the Ga West Municipal Hospital. Consent was obtained from each patients or guardians of patients age ≥ 2 years presenting with febrile illnesses at the outpatient department of Ga West Municipal Hospital. Privacy and confidentiality of the study participants were ensured by conducting interview in private and conducive environment as far as possible and also information collected was secured and accessible only to the principal investigator and his supervisor. No patient was obliged to participate and patients were allowed to step out of the study at any time (see Appendix: informed consent).

3.5.2 Informed consent, possible risks and benefits

All patients or patient's guardians were informed about the study in English or translated in to the local language. Written informed consent form was given to patients or guardians. Parental consent was sought on behalves of children. Translation in to the local language was done where the need arises. No patient was obliged to participate and patients were allowed to step out of the study at any time (see Appendix: informed consent). Collection of blood was done by qualified medical laboratory scientists using standard operating procedures. All patients enrolled in the study received a free-of-charge diagnostic test on malaria, sickle cell phenotype, full blood count (FBC), blood culture and sensitivity.

3.5.3 Sample collection and processing

Data on the socio-demographic and clinical characteristics of the study participants were collected using an android technology software kobo-collect by interview. After interviewing each patient, 10mL of venous blood sample was collected from adult patients aseptically with a needle and

syringe. Out of this, 7mL was inoculated immediately to 45mL brain heart infusion broth and the remaining 3ml was transferred into a sterile EDTA tube for malaria diagnosis and serological analysis (Widal test). Similarly, 3-4mL blood was collected from children and 1.5–2mL blood was inoculated to 9mL broth to isolate *Salmonella typhi* and other *Salmonella species*. The collection tubes were labelled with unique identification numbers.

3.6 LABORATORY ANALYSIS

3.6.1 Haematology

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

3.6.2 Rapid diagnostic test

The first response rapid diagnostic test (RDT) (Standard Diagnostic Inc.) was used in screening all blood samples for malaria. In performing this test, 20µL of whole blood and 10µL of a buffer was added to the sample well of the test kit. It was incubated for 15 minutes and the results was read immediately. Positive tests would have a purple band on both the test and control regions. The negatives would have no bands on the test regions.

3.6.3 Determination of malaria parasitaemia

Thick and thin films were prepared to examine malaria parasites. For the thick film, 6µl of blood was placed at one end of a microscope slide and was quickly spread (circular movement) using Spreader. For thin blood films, 2µl of blood was put on the same slide. Using the same spreader, the blood was touched and allowed to run along the edge of the slide. Keeping the spreader at an angle of 45 degrees, and ensuring that the spreader is in even contact with the surface of the slide,

the spreader was pushed forward to make a thin film. The blood films were thoroughly air-dried and the thin film was fixed with absolute methanol for species identification.

The blood films were stained with 10% Giemsa solution for 20 minutes for the detection of *Plasmodium* parasites and speciation respectively. The slides were observed microscopically under x100 (oil immersion) objective. Parasite density was done by counting the number of parasites against 200 white blood cells on thick films and multiply by the total leucocyte count of the patient. At least 100 high power microscopic fields was examined before declaring a slide negative.

3.6.4 Widal test

The Widal agglutination test was performed on all blood samples by the rapid slide titration method using commercial antigen suspension for the somatic (O) and flagella (H) antigens (Medsorce Ozone Biomedicals, India). In performing this test, 50µL of test serum was placed in two circles on a glass slide and equal volumes each of positive control and normal saline in each of the last two circles respectively. A drop each of O or H antigens was added to the test serum in each circle and then to the negative and positive controls. The content of each circle was mixed using disposal mixing sticks provided and spread to the entire circle after which it was rock gently for 1 minute and observed for agglutination. Antibody titration was performed for slide reactive samples using the tube technique. Antibody titer of >1: 80, was considered to be significant and usually suggestive of infection according to the manufacturer instruction.

3.6.5 Blood culture

Blood culture was done manually by inoculation into thioglycollate broth and incubated aerobically at 35- 37°C for 7 days and examined visually daily for evidence of bacterial growth. Indicators of bacterial growth that was used include; turbidity of blood-broth mixture, growth of

micro- colonies, haemolysis, colour changes and gas production. After 24hours of incubation, all cultures showing growth or no growth was sub-cultured onto solid media plates of MacConkey agar (MAC), and Blood agar (BA) and Chocolate agar (CA).The BA and MAC agar were incubated at 35-37°C aerobically and CA anaerobically (5-10% CO₂) for 24-48 hours. In the case where thioglycollate broth showed no growth up to day 7, subcultures were repeated from the broth on day 7 before it was discarded. On MacConkey, *Salmonellae* are non-lactose fermenters and form moist colonies blood agar. *Salmonella* measures about 2-4mm. All isolates from the subcultures were gram stained and identified through series of biochemical test.

3.6.6 Gram staining procedure`

The slides were placed on a solid holder and the fixed smear covered with crystal violet stain for 1minute. The stain was then washed off with clean water. After tipping off the water, the smears were covered with lugol's iodine for 1minute after which the iodine was washed off with water. De-colorization 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 allowed to air-dry using rack. The slides were examined microscopically, first with the X40 objective to check the staining and distribution of the smear, and then with oil immersion objective (X100) to look for bacterial and cells.

3.6.7 Identification of suspected isolates by the standard manual method

The standard manual method was used to characterize the isolates obtained. The following steps were followed for the identification of isolated bacterial, colonial morphology, gram stain reaction; biochemical test such as Oxidase, Indole, Triple Sugar Iron Agar, Citrate and Urea. A sterilized vertical wire loop was used to pick about two colonies and inoculated into the various biochemical tests (Triple Sugar Iron (TSI) Agar, Citrate, and Urea).

3.6.8 Indole test

It is based on the ability of the organism to split tryptophan into indole. Tryptone broth in a test tube was inoculated with the suspected organism and incubated for 24 hours at 35-37°C. A few drops of Kovac's reagent was added. A bright red color indicate a positive test. *Salmonella* species are negative.

3.6.9 Oxidase test

The bacteria uses the enzyme cytochrome oxidase to oxidize the substrate “tetramethyl-p-phenylenediamine dihydrochloride” to indophenol giving a dark purple color which is positive while no colour is negative. The test organism was smeared on the moist filter paper soaked with the oxidase reagent. *Salmonella* species are negative.

3.6.10 Triple sugar iron (TSI) agar test

The test is based on ability of the bacteria to ferment glucose, lactose or sucrose and forms hydrogen sulphide (H₂S). The wire loop was used to pick the test organism and inoculated in to the TSI agar and incubated for 24-48 hrs. The reaction for *Salmonella typhi* is alkaline slant and acid butt with weak hydrogen sulphide production.

3.6.11 Citrate test

This test is based on the ability of the organism to use citrate as the only source of carbon for metabolism. The wire loop was used to pick the test organism and streaked over the slant citrate agar in a tube and incubated for 24-48 hrs. Growth on the slant and change in colour to blue indicates positive result while no change in green colour is negative. *Salmonella typhi* and *Salmonella paratyphi* show negative reaction while other *Salmonella* species also indicate positive reaction.

3.6.12 Urease test

It is based on the ability of the organism to breakdown urea to ammonia using the enzyme urease. The wire loop was used to pick the test organism and inoculated in to urea agar in a tube and incubated for 24-48 hrs. A positive test gives pink-red whilst the negative test gives a yellow-orange colour. *Salmonella* species show a negative test.

3.6.13 Antimicrobial susceptibility testing (AST)

The susceptibility of the *Salmonella* isolates to antimicrobial agents was performed using the Kirby-Bauer disk diffusion method with antibiotic discs on Müller Hinton agar plates (*Bauer et al.*, 1966). The antibiotics that were used include; ampicillin, tetracycline, co-trimoxazole, gentamycin, cefotaxime, cefuroxime, chloramphenicol, ceftriaxone, ciprofloxacin, amikacin and meropenem. About 2-3 colonies of the same morphological type was picked and emulsified in a test tube containing 2.5mL of sterile peptone water to form bacterial suspension. The suspension was vortex and its turbidity compared with Barium chloride (0.5McFarland turbidity standard). A sterile swab stick was dipped into the suspension and inoculated on the surface of a Muller-Hinton by streaking the entire agar surface (Wikler, 2006;Cheesbrough, 2006). Discs prepared with different antibiotics (Biomark Laboratory, India) were placed firmly on the inoculated medium, inverted and incubated at 35- 37°C for 24 hrs. A was used ruler to measure each zone inhibition (Cheesbrough, 2006; Bauer et al., 1966). The results were compared with the zone diameter interpretive standards of the Clinical Laboratory Standards Institute NCCLS, 2006 (Wikler, 2006; Bauer et al., 1966).

3.6.14 Laboratory quality control

Standard operational procedures were followed during processing of each sample and all the instruments used for sample processing were checked every morning for proper functioning.

Stained quality control (QC) slides was used to check the quality and performance of the Giemsa stain. Malaria-positive blood was used to prepare QC thick and thin films and stained at the same time as the patient slides. Before examining the stained patient slides, the QC slides are checked for the quality of red-cell staining to control the buffer quality, and WBCs are examined for staining of nuclei and granules and of parasite chromatin and red cell inclusions, if present. If the QC slides are satisfactory, the stain was considered good quality.

All media prepared and antibiotics to be used were performed according to the clinical and laboratory standards institute (CLSI) guideline. Sterility testing was performed on all media prepared. One plate or tube from each batch was incubated at 35°C for 48 hours, one at room temperature for 24-48 hours, and a third is refrigerated for 7 days, then incubated at 35° C for 48 hours. Some known isolates were also grown on specific media that were required for their growth and observed to see if the media supported their growth. When there was significant growth, the media were considered to be of good quality and when there were no growths, or growths were not significant, the media was considered to be of poor quality. Susceptibility testing was controlled using the strain *Escherichia coli* ATTC 25922.

3.7 DATA ANALYSIS

The data were entered and cleaned in MS excel, and exported to Stata 13.0 for analysis. Descriptive statistic was performed by looking at the proportions. Continuous variables obtained from the patients are presented using means and standard deviation. Categorical variables were presented as proportions and frequencies in tables and graphs. The laboratory isolated pathogens and its antibiotic susceptibility were also presented in frequencies and proportions.

Chi-square and odds ratio (OR) by logistic regression were calculated to determine association between independent variable with the dependent variable. *P*-value <0.05 was considered statistically significant.



CHAPTER FOUR RESULTS

4.1a Characteristics of study participants

The study participants involved one hundred and fifty seven (157) febrile patients aged between 2years to 37years who reported to the hospital with fever ranging from 37.6 °C to 42 °C. A total of 82 (52.2%) of the study participants were females. The median age of all the patients was 6years (IQR=7-28/years). The median age of the males was 5years (IQR=3-10/years) and that of the female was 6years (IQR=4-11/years). Majority of the patients 78(49.7%) were within the age range 1-5years and most of them were students 114 (72.6%). Most of the patients 82 (52.2%) were resident within the Amasaman sub-district (Table 1).

Table 2: Socio-demographic characteristics of study participants

Socio-demographic variables		Frequency (N %)
Sex	Female	82 (52.2)
	Male	75 (47.8)
Age group	1-5	78 (49.7)
	6-10	38 (24.2)
	11-15	19 (12.1)
	16-20	6 (3.8)
	>20	16 (10.2)
Education	Not Schooling	28 (17.8)
	Nursery/KG	64 (40.8)
	Primary	39 (24.8)
	JHS	16 (40.8)
	SHS	7 (4.5)
	Tertiary	3 (1.9)
Occupation	Civil servant	5 (3.2)
	Merchant	12 (7.6)
	N/A	26 (16.6)
	Student	114 (72.6)
Residence by Sub-district	Amasaman	82 (52.2)
	Pokuase	43 (27.4)
	Ofankor	9 (5.7)
	Others	23 (14.6)

4.1.1b Clinical complains among patients presenting with febrile illness

During the period of investigations, all the patients reported with fever, 68.79% presented with headache and 45.86% with abdominal pain (See figure 4 below)

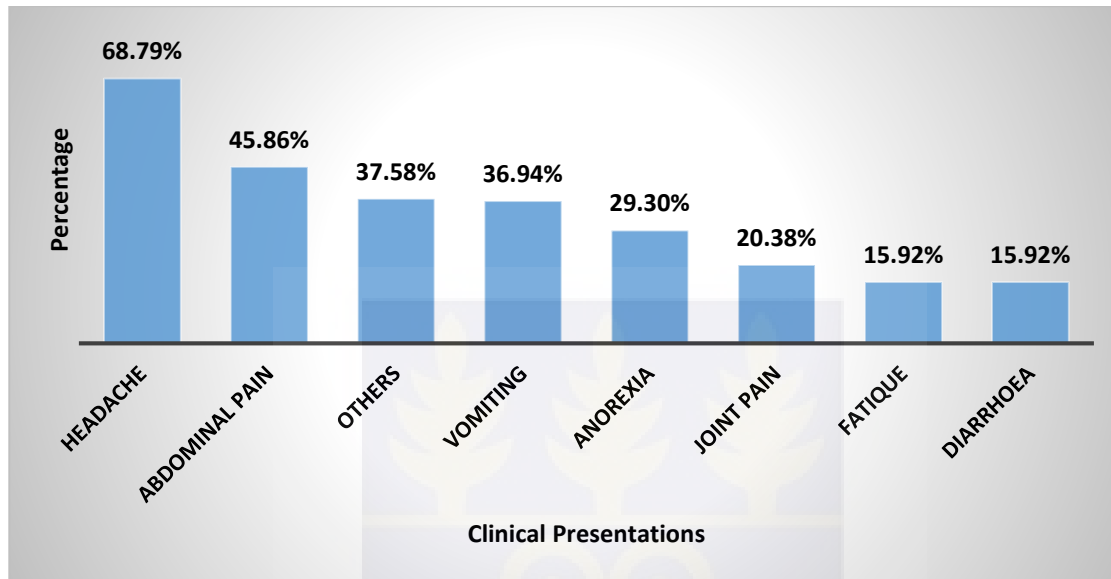


Figure 4: Clinical presentation among febrile patients

4.2 Malaria and typhoid fever as a cause of acute febrile illness

4.2.1a Proportion of febrile illness due to malaria, typhoid fever and their co-infection

Out of the 157 febrile patients tested for malaria using RDT and confirming with microscopy, 57 were positive for malaria, giving an overall positivity rate of 36.31%. For typhoid fever, using the Widal test, 23 (14.64%) were reactive while the blood culture recorded 10 (6.37%). The co-infection of malaria and typhoid fever using Widal test and Blood culture was 9 (5.73%) and 3(1.91%) respectively (See figure 5).

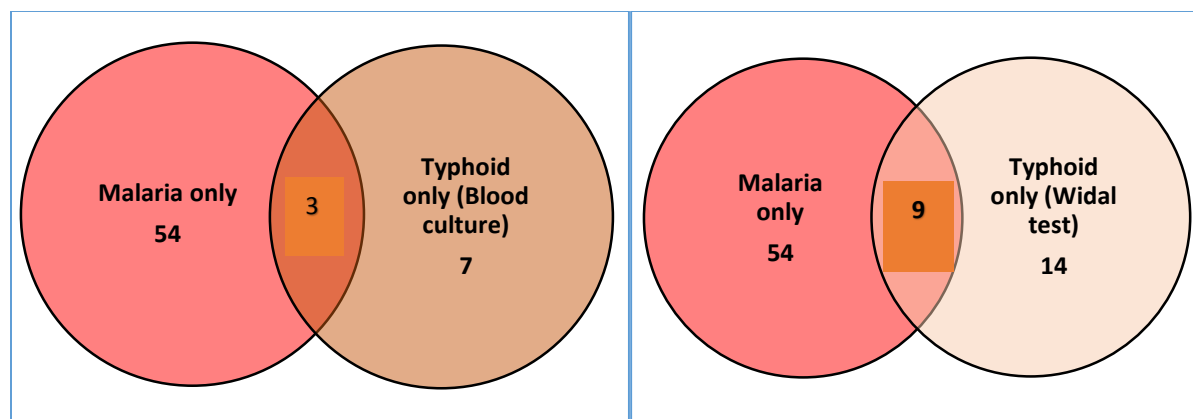


Figure 5: Prevalence of malaria, typhoid and their co-infection among febrile patients using Widal test and blood culture

4.21b Parasite density among patients with malaria and typhoid fever

The geometric mean parasite density (GMPD) was higher in patients with malaria and typhoid fever co-infection and was statistically significant ($p=0.009$)

Table 3: Parasite density in patients with malaria and co-infection

Parasitaemia in	Typhoid fever	Malaria only	Co-infection
para/μl blood (range)			
Low parasitaemia <1000	0	449 (233-680)	0
Moderate parasitaemia (1000-9999)	0	4774 (1208-9000)	9000 (9000-9000)
High parasitaemia (≥ 10000)	0	72622 (10775-712348)	174485 (45782-665000)
F (P-value)		5.085 (0.009)	5.085 (0.009)

Data have been presented as geometric mean (range). F represents F statistic

4.3 Malaria, typhoid fever and socio-demographic characteristics

Malaria 54.4% (31/57) and typhoid fever 60% (6/10) was higher among males. The patients within the age group 1-5years were the most affected for both malaria 49.1% (28/57) and typhoid fever 50% (5/10) as compared to the other age groups. Malaria and typhoid fever infection were higher among students. There was no statistical difference between the various groups (P-value= >0.05) (see table 3).



Table 4: Prevalence of typhoid fever and malaria in association with socio-demographic characteristics

Socio-demographic variables		Typhoid fever		P-value	Malaria		P-value
		Negative	Positive		Negative	Positive	
Sex	Female	78 (95.1)	4 (4.9)	0.424	56(68.3)	26 (31.7)	0.210
	Male	69 (92.0)	6 (8.0)		44(58.7)	31 (41.3)	
Age group	1-5	73 (93.6)	5 (6.4)	0.643	50(64.1)	28 (35.9)	0.975
	6-10	35 (92.1)	3 (7.9)		24(63.2)	14 (36.8)	
	11-15	19 (100.0)	0 (0.0)		11(57.9)	8 (42.1)	
	16-20	5 (83.3)	1 (16.7)		4 (66.7)	2 (33.3)	
	>20	15 (93.8)	1 (6.2)		11(68.8)	5 (31.2)	
Educational background	Not	26 (92.9)	2 (7.1)	0.794	18(64.3)	10 (35.7)	0.925
	Schooling						
	Nursery/KG	59 (92.2)	5(7.8)		41(64.1)	23 (35.9)	
	Primary	37 (94.9)	2 (5.1)		25(64.1)	14 (35.9)	
	JHS	16 (100)	0 (0.0)		10(62.5)	6 (37.5)	
	SHS	6 (85.7)	1 (14.3)		5 (71.4)	2 (28.6)	
	Tertiary	3 (100.0)	0 (0.0)		1 (33.3)	2 (66.7)	
Occupation	Civil servant	5 (100.0)	0 (0.0)	0.918	3 (60.0)	2 (40.0)	0.989
	Merchant	11 (91.7)	1(8.3)		8 (66.7)	4 (33.3)	
	N/A	24 (92.3)	2 (7.7)		17(65.4)	9 (34.6)	
	Student	107 (93.9)	7 (6.1)		72(63.2)	42 (36.8)	
Residential location	Amasaman	80 (97.6)	2 (2.4)	0.198	52(63.4)	30 (36.6)	0.684
	Ofankor	8 (88.9)	1 (11.1)		5 (55.6)	4 (44.4)	
	Others	21 (91.3)	2 (8.7)		17(73.9)	6 (26.1)	
	Pokuase	38 (88.4)	5(11.6)		26(60.5)	17(39.5)	

Data have been presented as count (percentage)

4.4 Antimicrobial test results of *Salmonellas* species isolated

All isolates exhibited high resistance ranging from 60% (9/15) to 100% (15/15) against ampicillin, tetracycline, co-trimoxazole, gentamicin, cefuroxime, chloramphenicol, and meropenem. The sensitivity also range from 66.7% (10/15) to 100 (15/15) against cefotaxime, ceftriazone, ciprofloxacin and amikacin. No isolate of *Salmonella typhi* was susceptible to gentamicin, cefuroxime and co-trimoxazole. Other species of *Salmonella* including *Paratyphi A* were also not susceptible to tetracycline, ampicillin, co-trimoxazole and cefuroxime. All of the *Salmonella* isolates were susceptible to ciprofloxacin and amikacin 100% (15/15) (see Table 4).

Table 5: Antimicrobial susceptibility profiles of the *Salmonellae* isolates

Antibiotics	<i>S. typhi</i> N=10 (%)	<i>S. paratyphi A</i> N=3 (%)	Other <i>S. species</i> N=2 (%)	Total N=15 (%)
Ampicillin	8 (80)R	3 (100)R	2 (100)R	13 (86.7)R
Tetracycline	9 (90)R	3 (100)R	2 (100)R	14 (93.3)R
Co-trimoxazole	10 (100)R	3 (100)R	2 (100)R	15 (100)R
Gentamicin	10 (100)R	2 (66.7)R	2 (100)R	14 (93.3)R
Cefotaxime	7 (70)S	2 (66.7)S	1 (50)S	10 (66.7)S
Cefuroxime	10 (100)R	3 (100)R	1 (50)R	14 (93.3)R
Chloramphenicol	6 (60)R	1 (33.3)R	2 (100)R	9 (60)R
Ceftriaxone	8 (80)S	2 (66.7)S	1 (50)S	11 (73.3)S
Ciprofloxacin	10 (100)S	3 (100)S	2 (100)S	15 (100)S
Amikacin	10 (100)S	3 (100)S	2 (100)S	15 (100)S
Meropenem	8 (80)R	2 (66.7)R	2 (100)R	12 (80)R
R- resistant	S- sensitive			

Legend

Ampicillin (AMP) – 10ug

Tetracyclin (TET) –10µg

Ciprofloxacin (CIP) – 5ug

Gentamicin (GEN) – 10ug

Cefuroxime (CRX) – 30ug

Cefotaxime (CTX)

Co-trimoxazole (COT) – 25ug

Chloramphenicol (CHL) – 10ug

Amikacin (AMK) – 30ug

Meropenem (MEM) –10µg

Ceftriaxone (CTR) –30µg

4.5 Risk factors associated with malaria and typhoid fever infection

Table 5 shows differences in proportions of the risk factors usually associated with malaria and typhoid fever infections. Majority of the participants ate outside their homes occasionally with 90% (9/10) typhoid fever infection and 30 out of the 157 who had no toilet facilities at home recorded 30% (3/10) typhoid fever. Those who use borehole/well as their source of water also recorded 80% (8/10). A total of 103 of the patients do not use bed nets with 66.7% (38/57) malaria infection. There was no statistical significance ($p>0.05$) between the risk factors and malaria and typhoid fever infection.

Table 6: Determinants associated with typhoid fever in febrile patients

Risk factors		Negative	Positive	P-value	OR	95% CI
Eating habit (outside)	Frequently	13 (100.0)	0 (0.0)	0.326	0.000	0.000
	Infrequent	124 (93.2)	9 (6.8)	0.631	0.652	0.072 – 5.917
	Never	10 (90.9)	1 (9.1)	0.590	*	*
Toilet facility	No	27 (90.0)	3 (10.0)	0.365	1.859	0.440 – 7.852
	Yes	120 (94.5)	7 (5.5)	*	*	*
Source of water	Borehole	85 (91.4)	8 (8.6)	0.167	2.412	0.489 – 11.895
	Packaged	4 (100.)	0 (0.0)	0.597	0.000	0.000
	River	5 (100.0)	0 (0.0)	0.553	0.000	0.000
	Tap water	49 (96.1)	2 (3.9)	0.704	*	*
	Others	4 (100.0)	0 (0.0)	0.597	0.000	0.000
Hand washing habits	Good	95 (94.1)	6 (5.9)	*	*	*
	Poor	52 (92.9)	4 (7.1)	0.768	0.821	0.222 – 3.042

Data have been presented as count (percentage). OR (CI) represents odds ratio (confidence interval). * represents the reference variable.

Table 7: Determinants associated with malaria fever among febrile patients

Risk factors		Negative	Positive	P-value	OR	95% CI
Bed net usage	No	65 (63.1)	38 (36.9)	0.833	1.137	0.567 – 2.278
	Yes	35 (64.8)	19 (35.2)	*	*	*

*Data have been presented as count (percentage). OR (CI) represents odds ratio (confidence interval). * represents the reference variable.*



CHAPTER FIVE

DISCUSSION

5.1 Malaria and typhoid fever as causes of acute febrile illness

Malaria and typhoid fever infections continue to be major diseases of public health concerns, especially in the tropics and are known to present clinically similar symptoms with fever being the major presentation (Igharo et al., 2012). During the period of investigation, all the patients reported with fever. Most of them also complained of headache (68.8%) and abdominal pain (45.9%).

In the current study, 36.3% of patients with febrile illness were found to have malaria infection and typhoid fever 14.6% and 6.4% using the traditional Widal test and blood culture respectively. Blood culture is the gold standard for diagnosis of typhoid fever and therefore there is overestimation using the Widal test which gives a false positive of (8.3%). This leads to exposure of patients to side effects of antibiotics and subsequently developing resistance.

Despite the fact that the two diseases are endemic in Ghana, findings from this study show that malaria was the cause of fever among the febrile patients than typhoid fever. Study conducted in Kumasi and Sunyani metropolises reported a prevalence of 18.6% for malaria and 17% for typhoid fever (Afoakwah et al., 2011). In contrast, current finding for malaria is higher than the previous study and this could be due to the fact that slide examination using microscopy was used as compared to malaria rapid diagnostic test (mRDT) used in that study. Malaria rapid diagnostic test is less sensitive and can give false negative when parasite density is low. This finding for typhoid fever using the Widal test is in agreement with the report from the metropolises. Unfortunately, there was no culture but typhoid fever has been showed to cross-react with malaria using Widal

test (Pradhan, 2011) and could lead to over-diagnosis. This finding for malaria among febrile patients is comparable with studies from Nigeria; Akoko State, 37.6% (Igharo et al., 2012), Imo State, 39% (Opara et al., 2011), and University of Uyo Teaching Hospital 51/145(35.2%) (Moses et al., 2016). In Ethiopia, Birhanie et al., (2014) reported 73/200 (36.5%), Cameroon, malaria prevalence was 43.4% and 70.2% for microscopy and PCR among 315 patients (Achonduh-Atijegbe et al., 2016).

But it is less than the reports from Calabar, 202/250 (80.8%) (Archibong et al., 2016), Nnewi, Anambra state, 202/256 (78.90 %) (Ekesiobi et al., 2008), Cameroon, malaria prevalence was 43.4% and 70.2% for microscopy and PCR among 315 patients (Achonduh-Atijegbe et al., 2016), Kumba, Cameroon ,90.3% (186/206) (Ndip et al., 2015). In India, (48/60 (80%), Sierra Leone 62.3% (Sundufu et al., 2012) and West Gojam, Ethiopia, 62% (Uneke, 2008). This finding was also higher than what was reported in Abakaliki, Ebonyi State, 33/250 (13.2%) (Nwuzo et al., 2009). The discrepancy of the results between the studies might be due to seasonal variation, the difference in geographical locations and sample size.

The reported finding for typhoid fever using the gold standard in this study is substantiated by the findings from Ghana 6.5%(Labi et al., 2014), Cameroon 7.9% (Ndip et al., 2015) but higher than what was reported in Abakaliki, Ebonyi state, 2/250 (0.8%) (Nwuzo et al., 2009), Calabar, 2/250 (0.8%) (Archibong et al., 2016), Ethiopia 0.5% (Birhanie et al., 2014) and India 1.2% (Samatha et al., 2015) and Mbuh et al., (2003), 1/250 (0.5%).

The discrepancy of the findings could be attributed to differences in the environmental conditions of the studied population. Factors such as improvement in sanitary conditions and availability of

potable water could have contributed to reasons why they observed lower rates of typhoid fever infections in the area.

The current finding is also lower than what was reported by other studies. In Uyo, Akwa Ibom State, 11/64 (17%) (Edet et al., 2016) and Nnewi, Anambra state, 38/256 (14.84 %) (Ekesiobi et al., 2008) and this could be due to indiscriminate disposal of waste, sewage spillage, lack of good toilet facilities, inadequate sanitary facilities in homes and general poor sanitation culture in the study area (WHO, 2003).

The study further found that males 97 (85.8%) were more infected with malaria than females 105 (76.6%) but this was not statistically significant while the prevalence of typhoid fever was higher in females 77 (56.2%) than males 40 (35.4%) and that was statistically significant (Archibong et al., 2016). A study amongst residents of Uyo, Akwa Ibom State, Nigeria a total of 41/100 (41%) tested positive for malaria, 64/100 (64%) using widal, 11/64 (17%) using blood cultures (Edet et al., 2016). A similar study at the University of Uyo Teaching Hospital among 145 patients, reported 51(35.2%) had malaria and 10(7.0%) typhoid/paratyphoid. A total of 20(13.8%) males and 31(21.4%) females had malaria, while 3(2.1%) males and 7(4.8%) females had typhoid (Moses et al., 2016). The study further found that age groups (11-30) years were mostly infected with malaria and typhoid fever (Ekesiobi et al., 2008).

In this study, malaria infection was higher in males 54.4% (31/57) but there was no statistically significant association ($P = 0.210$). This is similar to what was reported in Ethiopia (Birhanie et al., 2014) and in Nigeria where they found that males were more infected with malaria than females

but not statistically significant (Archibong et al., 2016) but in contrast with study from Sierra Leone, females (53.4%) were more affected (Sundufu et al., 2012). This might be due to the fact that males often refuse to sleep inside insecticide treated nets, sleeping late in the night and most often spends a lot of time outside.

The prevalence of typhoid fever was greater in males 60% (6/10) but not statistically significant ($P = 0.424$). However, another study has reported higher prevalence among females (Birhanie et al., 2014). Meanwhile, this finding is supported by a study in Nigeria, which showed that the frequency of typhoid fever was 29.4% among males and 22.9% among females (Alhassan et al., 2012). Males may acquire the infection due to the high patronage of outside foods and also females are more concern about their personal hygiene than male, thus increasing the frequency of typhoid fever.

The age group 1-5/years had the highest malaria infection 49.1% (28/57) and followed by 6-10years 24.6% (14/57) but there was no statistically significant association ($P=0.975$). This might be due to the low immune response against malaria infection, inappropriate use of bed nets, and inappropriate use of antimalarial drugs in case of children. This is consistent with the general observation that the age group is more vulnerable to the disease in areas of high transmission (Gilles, 1993). Naturally acquired immunity builds up in older adults following repeated exposure to the parasite and is manifested by lower parasite densities and fewer clinical malaria episodes than younger children (Sharma et al., 2004). About 53% (30/57) malaria cases were from Amasaman sub-district and this could be due to possible breeding sites due to many streams within

the locality. It was observed that Pokuase sub-district had the highest number of typhoid cases and could be due to the poor sanitary environment and inadequate treated water supply within the area.

5.2 Malaria and typhoid fever co-infection

In this present, study the prevalence of co-infection rate by Gold standard technique i.e. Blood culture for typhoid and peripheral blood smear examination for malaria parasite was found to be 1.9% (3/157). However serological Widal test for typhoid and microscopic examination for malaria parasite showed co-infection rate 5.73% (9/157). In Ghana, prevalence of malaria- typhoid fever co-infection of 4.65% have been reported using mRDT and Widal test (Afoakwah et al., 2011).

Generally, the Widal test is used in most hospital laboratories in the tropics because it is easily available, economical and does not need much expertise, but gives inaccurate results, non-specific, poorly standardized, confusing and of limited diagnostic value (Koeleman, Regensburg, Van Katwijk, & MacLaren, 1992). Cross-reactions usually occurs with other infectious diseases common in the tropics such as tuberculosis, rheumatoid arthritis etc. It has been observed that the incidence of co-infection will reduce if the diagnosis of typhoid fever is based on blood culture (Mbuh et al., 2003). In Lagos, *Salmonella* species were isolated in patients with severe malaria (Akinyemi et al., 2007). Typhoidal *Salmonellosis* is implicated for most of the co-infection of malaria and typhoid fever (Ohanu et al., 2003; Smith et al., 2004).

Meanwhile, the Widal test usually overestimates and therefore making it difficult to assess patient suffering from a true co-infection. In this current study, the finding of 1.9% co-infection is

substantiated by other studies from Nigeria, Ethiopia, Cameroon and India. In one study, co-infection of malaria and typhoid fever was 6.5% by Widal test and 0.7% by culture method (Samatha et al., 2015).

Association between typhoid fever and malaria have been reported in many studies especially in Nigeria. In one study, co-infection obtained when typhoid was diagnosed by Widal test (10.1%) and using blood culture (0.5%) (Mbuh et al., 2003). A Study by Nwuzo et al., (2009), reported a co-infection of 2/250 (0.8%) by culture method and 14/250 (5.6%) by the Widal test. Another study recorded (28.0%) by Widal test and (0.8%) by blood culture method (Archibong et al., 2016). Other studies have also reported higher prevalence of malaria and typhoid fever co-infection in different states; Uyo, 16% (n = 100) and 17% (n = 64) for Widal test and blood culture respectively (Edet et al., 2016), Nnewi 29/256 (14.36%) by culture method while 147/256 (57.42%) for Widal test (Ekesiobi et al., 2008) and 1.33% on comparing blood culture with peripheral smear which is similar to our findings (Alhassan et al., 2012).

In India, 1.59% (48/3010) using blood culture for typhoid and peripheral blood smear examination for malaria parasite, however Widal test for typhoid and rapid diagnostic test for malaria parasite showed co-infection rate of 3.38% (105/3010) (Sharma et al., 2016). Samatha et al., (2015), found co-infection of 38/582 (6.5%) using Widal test and 4/582 (0.7%) using blood culture, 1.6% of typhoid and malaria co infection by blood culture and peripheral smear examination while 8.5% by serological method (Verma et al., 2014).

In Ethiopia, Birhanie et al., (2014) showed in their study that, co-infection among febrile patients was 13 (6.5%) and 1 (0.5%) for Widal test and blood culture while in Kumba, Cameroon had

6.74% co-infection rate (Ndip et al., 2015). In Pakistan, dual infection of malaria-typhoid fever was seen among patients comprising of *Salmonella typhi* and *Salmonella Paratyphi* A or B (Khan et al., 2005).

Parasitaemia in patients co-infected with both diseases was significantly higher than was observed for patients with only malaria. This finding is supported by a study in Cameroon (Ndip et al., 2015). According to the WHO definition of severity, parasitemia is indicated as a possible predictor of disease severity which may influence the outcome of a malaria infection.

This study also shows that 42.7% of the febrile patients neither had malaria nor typhoid fever. This could be due to other bacteria, viral or fungi infections. A Study conducted in Ghana, found that 80% feverish patients were treated as malaria based on clinical diagnosis. However, based on clinical suspicion confirmed by microscopy, the proportion of febrile illness due to malaria was found to be 10.8%. (Keziah L. Malm et al., 2014).

5.3 Antimicrobial drug susceptibility

Antimicrobial resistance tends to be more common in low and middle income- countries (LMICs) like Ghana (Newman et al., 2011). Some factors that contribute to treatment failure or drug resistant are incorrect dosing, poor quality of drug, and misdiagnosis in the individual. The emergence of multidrug resistance can be attributed partly to the unnecessary overuse of antimicrobial drugs and further complicated by the substandard medicines in circulation in several LMICs, which risks patient safety and may promote antimicrobial resistance (Bate et al., 2008; Bate et al., 2009; Caudron et al., 2008). In this study, all isolates exhibited high resistance ranging from 60% (9/15) to 100% (15/15) against ampicillin, tetracycline, co-trimoxazole, gentamicin,

cefuroxime, chloramphenicol, and meropenem. Ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole, were not effective in treating typhoid fever (Lutterloh et al., 2012; Gupta et al., 2008).

The sensitivity also ranges from 66.7% (10/15) to 100 (15/15) against cefotaxime, ceftazidime, ciprofloxacin and amikacin. No isolate of *Salmonella typhi* was susceptible to gentamicin, cefuroxime and co-trimoxazole. Other species of *Salmonella* including *Paratyphi A* were also not susceptible to tetracycline, ampicillin, co-trimoxazole and cefuroxime. Many *Salmonella typhi* strains contain plasmids encoding resistance to chloramphenicol, ampicillin and co-trimoxazole (Daga et al., 1994). A study for a clinical profile in Ahmedabad in 2000, about 80% were resistant to amoxicillin, chloramphenicol and cotrimoxazole, but sensitive to ciprofloxacin and ceftriaxone (Kalra et al., 2003). The indiscriminate use of these antibiotics and self-mediations may account for this high resistance to these antibiotics. All of the *Salmonella* isolates were susceptible to ciprofloxacin and amikacin 100% (15/15) (see Table 4.3.1). This compares with finding in Nigeria (Ibrahim et al., 2005). The high susceptibility of *Salmonella* to ciprofloxacin and amikacin recorded in this study could be due to the relatively high cost of ciprofloxacin and amikacin, hence are not abuse.

A study conducted in Ghana, observed high resistance to ampicillin, tetracycline, chloramphenicol, and co-trimoxazole while ceftriaxone, ciprofloxacin, and amikacin were sensitive (Newman et al., 2011). In 2011, Groß et al., (2011) reported a high chloramphenicol among *Salmonella* resistance in Ghana (Groß et al., 2011).

In Laos, of the 1095 patients with data for hospital antimicrobial use, 56% received an antimicrobial drug, and 12% received more than 1 (Mayxay et al., 2013). On the basis of final diagnosis, only 7% of these patients were regarded as having been treated appropriately (Mayxay et al., 2013). Although malaria was the admission diagnosis for 148 adults in Tanzania, 44.8% of them were still prescribed empiric antibacterial agents (Nadjm et al., 2012). In one study in Cameroon, gentamycin and ciprofloxacin were found to be sensitive for the treatment of typhoid fever (Ndip et al., 2015).

5.4 Risk factors associated with malaria and typhoid fever

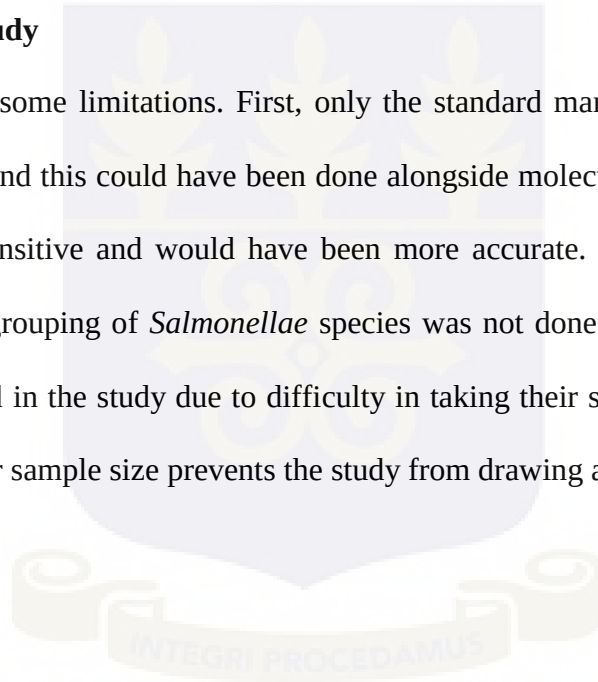
The proportions of the risk factors usually associated with malaria and typhoid fever infections such as bed net usage was not significantly associated with malaria even though about 65% of the patients do not use bed net which provides protection against malaria infection in contrast to this finding. A significant association was found where participants who slept under ITN were more protected from malaria infection. A significant association between the main source of drinking water and typhoid was observed for participants who had a borehole or well compared to those with piped water (Achonduh-Atijegbe et al., 2016). Insecticide treated nets (ITNs) have been shown to be highly efficient at reducing malaria on a community level in urban Ghana (Klinkenberg et al., 2010)

It can be inferred from (Table 5) that those who ate outside though irregularly, had more of the typhoid cases and this could be due to buying foods within an insanitary environment. Eating food outside, drinking contaminated water and eating vegetables and salad that are contaminated with human waste pose as risk factors for typhoid infection (Parry, 2006).

Eating habit, toilet facility at home, sources of water for drinking were also not significantly associated with typhoid. This finding is supported by the study in Ethiopia (Birhanie et al., 2014). In this study, children less than 10 years had more of typhoid and malaria cases. A study in India, found that children between 1-5 years were more affected with typhoid (Walia et al., 2006). It is also supported by a study in France, (Desenclos et al., 1996) and in Pakistan (Graham, 2002; Brooks et al., 2005; Siddiqui et al., 2006).

5.5 Limitation of the study

This study is subject to some limitations. First, only the standard manual method was used in isolating the organisms and this could have been done alongside molecular techniques involving PCR which are more sensitive and would have been more accurate. Secondly, serotyping for antigenic structure and grouping of *Salmonellae* species was not done. Thirdly, children below 2 years were not included in the study due to difficulty in taking their sample and ethical issues. Comparatively, the lower sample size prevents the study from drawing a definitive conclusion.



CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Even though malaria and typhoid fever are common in Ghana and have a similar characteristic, most of the patients were infected with malaria as compare to typhoid fever among the study population. In this current study, the prevalence of malaria among febrile patients is 36.3% and typhoid fever 6.4% using the blood culture. Co-infection of malaria and typhoid fever was 1.9%. However serological Widal test gave a false positive co-infection of 6/157 (3.8%). The study also revealed that parasite density is higher among those with co-infection than those with malaria alone. Children less than 10years were more affected by malaria and typhoid fever. Males were also more affected by both diseases.

The isolates exhibited high resistance from 60% - 100% against ampicillin, tetracycline, co-trimoxazole, gentamicin, cefuroxime, chloramphenicol and meropenem. The sensitivity also ranged from 66.7% - 100% against cefotaxime, ceftrizone, ciprofloxacin and amikacin. No isolate of *Salmonella typhi* was susceptible to gentamicin, cefuroxime, and cotrimoxazole. Other species of *Salmonella* were also not susceptible to tetracycline, ampicillin, co-trimoxazole and cefuroxime. All of the *Salmonella* isolates were susceptible to ciprofloxacin and amikacin.

The proportions of the risk factors usually associated with malaria and typhoid fever infections such as bed net usage were not significantly associated with malaria even though about 65% of the patients do not use the bed net and 38/57 (66.7%) had malaria. Eating habit, toilet facility at home, sources of water for drinking were also not significantly associated with typhoid.

6.2 Recommendations

6.2.1 Implications for clinical practice

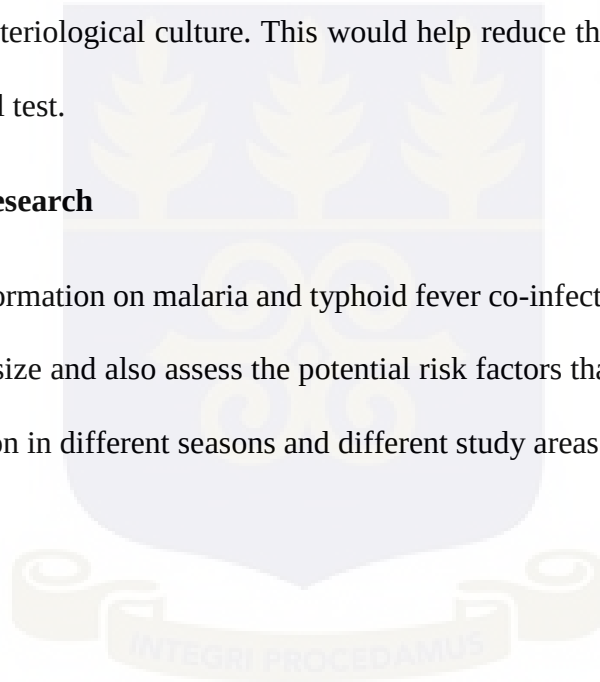
Due to the low prevalence of malaria and typhoid fever co-infection, clinicians should not treat concurrently but rather stick to differential diagnosis. This would help reduce the development of drug resistance among the population

6.2.2 Implication for policy

The district laboratories in the country must be well resource to be able to carry out microbiological investigation such as bacteriological culture. This would help reduce the use of non-specific test such as serological Widal test.

6.2.3 Implications for research

Since there is limited information on malaria and typhoid fever co-infection, similar study may be done on a larger sample size and also assess the potential risk factors that can lead to malaria and typhoid fever co- infection in different seasons and different study areas



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APPENDIX A: CONSENT FORM

CONSENT FORM

SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA

Participant ID.....

Date.....

Malaria and Typhoid Fever Co-infection: A study among Patients presenting with febrile illnesses in Ga West Municipal Hospital

General Information about the research

Febrile illness is an acute illness characterized by a rise in body temperature. Fever, vomiting, diarrhea, headache, lethargy, malaise, skin rash, loss of appetite, constipation and anemia may be caused by malaria, Typhoid fever or both. Malaria and typhoid fever often present with mimicking symptoms especially in the early stages. Thus it is very common to see patients undergoing both typhoid and malaria treatments even if their diagnosis has not been confirmed.

The present study will provide an epidemiological data on co-infection of malaria with Typhoid fever which may aid development of preventive strategies to control and manage co-infections as well as contribute to implementation of the guided empirical treatment of common bacteria isolates causing febrile illnesses.

You will be asked to take part in this study which will contribute to this aim. In connection with this exercise and if you agree to participate, we will collect about 2-5ml blood sample (children/adults). We will test the blood sample for the presence of malaria parasites, salmonella and to carry out susceptibility testing on salmonella isolates if present with various antibiotics that work best to treat your bacterial infection.

Possible Risks and Discomforts

Collection of blood is considered a low-risk procedure and will be conducted by experienced Laboratory Technicians and Biomedical Scientist

Possible Benefits

The individual patient will receive a malaria test and an investigation for bacteria in the blood, as well as antibiotic testing for free. The community will benefit from development of preventive strategies to control and manage co-infections as well as contribute to implementation of the guided empirical treatment of common bacteria isolates causing febrile illnesses.

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 receive in this facility.

Confidentiality

All information collected will be kept secured, confidential and would be accessible only to the Principal investigator and his supervisors.

Voluntary Participation and Right to Leave the Research

Your participation is voluntary, and you can leave the study at any time. You are assured that refusing to participate in this study will not in any way affect your treatment in this facility.

Contacts for Additional Information

For questions about the research and in case of research-related health concerns, please call
Name... ..Tel.....

If you agree to allow your ward or to participate in the study please write your name and sign in the spaces below.

Name.....

.....

Signature /Thumbprint

APPENDIX B: QUESTIONNAIRE

Patient ID:

Date.....

A: Personal data:

Location of residence.....

1. Age years: ☐ (1- 10) ☐ (11- 20) ☐ (21- 30) ☐ (31- 40) ☐ (41-50) ☐ (51-60) ☐ (61-70) ☐ (>71)

2. Sex: Male ☐ Female ☐

3. Marital Status: ☐ Married ☐ Single ☐ Divorced ☐ Cohabiting ☐ N/A

4. Educational level: ☐ Primary ☐ JHS ☐ SHS ☐ Tertiary ☐ Others ☐ N/A

5. Occupation: ☐ Teacher ☐ Trader ☐ Student ☐ Farmer ☐ Others

If Others, Specified.....

B: Clinical data:

I. Fever: Yes ☐ No ☐

II. Vomiting: Yes ☐ No ☐

III. Diarrhoea: Yes ☐ No ☐

IV. Anaemia: Yes ☐ No ☐

V. Convulsion: Yes ☐ No ☐

VI. Vomiting: Yes ☐ No ☐

VII. Headache: Yes ☐ No ☐

VIII. Abdominal Pain: Yes ☐ No ☐

IX. History of blood transfusion: Yes ☐ No ☐ X. Antibiotic use within the week: Yes ☐ No ☐

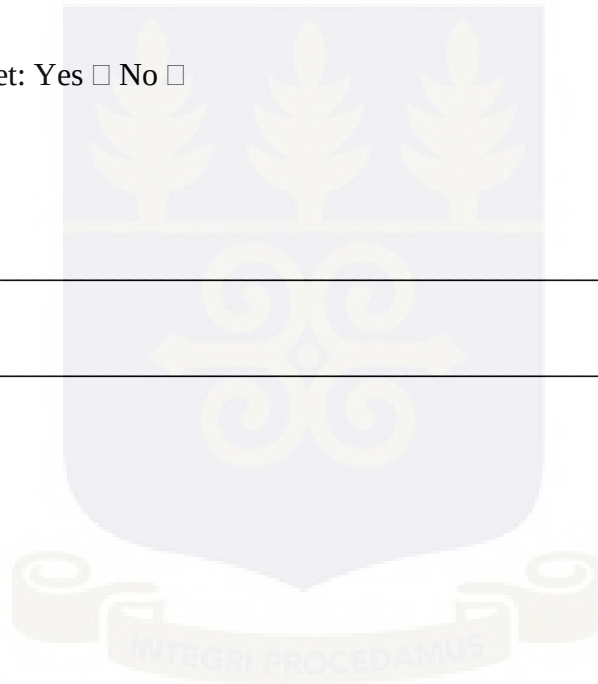
XI. Others: Yes ☐ No ☐ If Others, Specified.....

Environmental

1. Number of times you have eaten outside within the week:
2. Number of persons in households
3. Do you have toilet facility in house: Yes ☐ No ☐
4. What is the sources of household water: ☐ tap water ☐ borehole ☐ river ☐ packaged water
5. Bed net Usage: Yes ☐ No ☐
6. Impregnation of bed net: Yes ☐ No ☐

Date _____

Signature/thumbprint _____



APPENDIX C: MEDIA AND STANDARD SOLUTIONS

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

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

With the agar plates, dehydrated powders were dissolved in appropriate volumes of distilled water according to manufacturer's instructions. Substances were mixed thoroughly and gently heated to completely dissolved and autoclaved. When cooled to about 50-55°C, approximately 25ml volumes were dispensed into 90 cm sterile Petri dishes, left to set and agar surfaces dried.

For the agar slopes, dehydrated powders were dissolved in appropriate volumes of distilled water according to manufacturer's instructions. Substances were mixed thoroughly and gently heated to completely dissolve. Appropriate volumes were then dispensed into appropriate tubes before autoclaving. Autoclaved tubes were slanted at appropriate gradients during setting for the agar slopes.

Quality and sterility of prepared media were ascertained by incubating randomly selected media for growth due to contamination and also inoculating with *Escherichia coli* positive control strains.

a) Mueller Hinton Agar

Composition gms/Ltr

Beef, infusion 300.0

Casein acid hydrolisate 17.5

Starch 1.5

Agar 17.0

pH 7.3 ± 0.1

Preparation

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

b) MacConkey Agar

Composition gm/Ltr

Peptic digest animal tissue 20.0

Lactose 10.0

Sodium taurocholate 5.0

NaCl 5.0

Neutral red 0.04

Agar 20.0

pH 7.4 ± 0.2

Preparation

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



c) Blood Agar

Composition gm/Ltr

Beef heart infusion (Beef Extract) 10.0

Tryptose 10.0

Sodium Chloride 5.0

Agar 15.0

ii) 5-10 % Sheep Blood 50 ml/Ltr

pH 7.3± 0.2

Preparation

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

d) Triple Sugar Iron (TSI)

Composition gm/Ltr

Peptic digest of animal tissue 10.0

Casein enzymic hydrolysate 10.0

Yeast extract 3.0

Beef extract 3.0

Lactose 10.0

Sucrose 10.0

Dextrose 1.0

Sodium Chloride 5.0

Ferrous Sulphate 0.20

Sodium Thiosulphate 0.30

Phenol Red 0.024

Agar 12.0

pH 7.4± 0.2

Preparation

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

e) Urea agar

Composition gm/Ltr

Peptic digest of animal tissue 1.0

Dextrose 1.0

Sodium chloride 5.0

Disodium phosphate 1.2

Monopotassium phosphate 0.8

Phenol red 0.012

Agar 15.0

pH 6.8 ± 0.2

Preparation

Prepared according to the manufacturer`s (Biomark Laboratories, India) instructions. The completely dissolved mixture was autoclaved and allowed to cool. 5ml of filtered sterilized 40%

urea solution was then added aseptically to the autoclaved mixture before dispensed into sterile tubes and sloped.

f) Citrate Agar

Composition gm/Ltr

Magnesium sulphate 0.2

Ammonium di-hydrogen phosphate 1.0

Di-potassium phosphate 1.0

Sodium citrate 2.0

Sodium chloride 5.0

Bromothymol blue 0.08

Agar 15.0

pH 6.9

Preparation

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



h) Peptone water

Composition gm/Ltr

Peptone 10.0

NaCl 5.0

pH 7.1 ± 0.2

Preparation

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

i) Kovac's indole reagent

Composition

p-dimethylaminobenzaldehyde 5.0g

Amyl alcohol 75ml

Conc. HCl 25ml

Preparation

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

j) McFarland 0.5 Turbidity Standard (per 100ml)

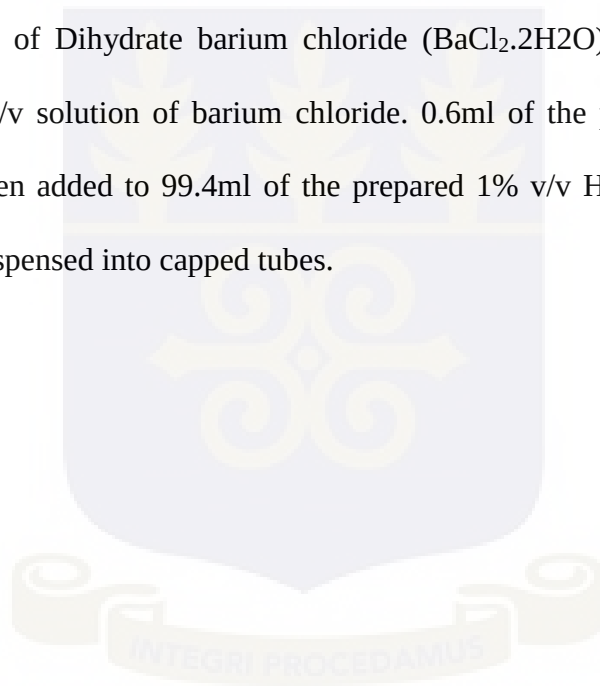
Composition

1ml Conc. H₂SO₄

0.5g Dihydrate barium chloride (BaCl₂·2H₂O)

Preparation

1ml of Conc. H₂SO₄ was added to 99 ml of distilled water and thoroughly mixed for 1% v/v solution of H₂SO₄. 0.5g of Dihydrate barium chloride (BaCl₂·2H₂O) was dissolved in 50ml distilled water for 1% v/v solution of barium chloride. 0.6ml of the prepared 1% v/v barium chloride solution was then added to 99.4ml of the prepared 1% v/v H₂SO₄ solution. Solutions thoroughly mixed and dispensed into capped tubes.



APPENDIX D: STAINING PROCEDURES

a) Gram stain

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

Results

Gram positive bacteria.....	Dark purple
Yeast cells.....	Dark purple
Gram negative bacteria.....	Pale to dark red
Nuclei of pus cells.....	Red
Epithelial cells.....	Pale red

b) Giemsa stain

Giemsa stain is used to differentiate nuclear and/or cytoplasmic morphology of platelets, RBCs, WBCs and parasites. It is the most dependable stain for blood parasites particularly in thick films

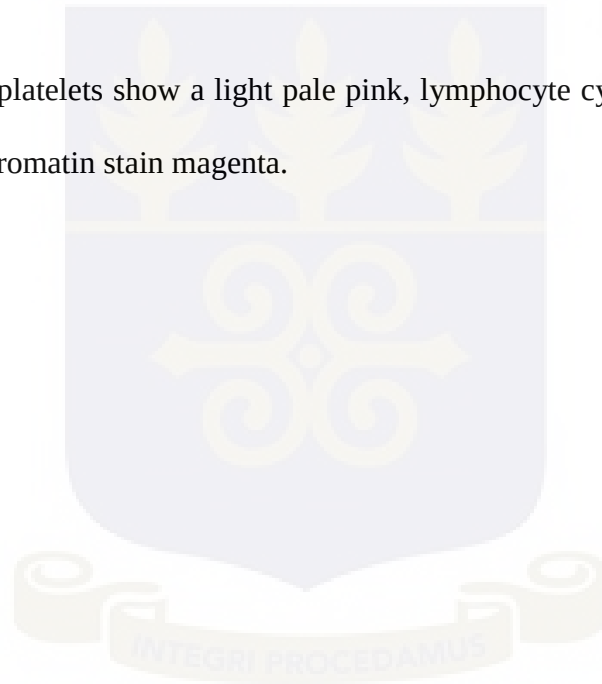
is the Giemsa stain containing azure B. The stain must be diluted for use with water buffered to PH 7.2.

Procedure

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

Results:

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



APPENDIX E: BIOCHEMICAL TESTS

a)OXIDASE TEST

Principle:

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

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

Quality Control Used:

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

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

Procedure:

1. Two to three drops of the reagent was dropped onto a filter paper strip.
2. Using a sterile plastic loop, a pure colony of the test organism was smeared onto the area on the filter paper containing the reagent.
3. Bacterial colonies observed to have developed a deep blue color at the inoculation site within 10 seconds were considered to have a cytochrome oxidase activity. The test organism was therefore positive for oxidase test.

b) INDOLE TEST

Principle:

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

Quality Control Used:

Positive Control: *Escherichia coli* (ATCC 25922)

Negative Control: *Klebsiella pneumoniae* (ATCC 700603)

Procedure:

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

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

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

c) CITRATE TEST

Principle:

This test is commonly used as part of a group of tests that distinguish between members of the Enterobacteriaceae based on their metabolic by-products. The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on

the generation of alkaline by-products of citrate metabolism. A subsequent increase in the pH of the medium is demonstrated by the color change of a bromothymol blue pH indicator.

In most common formulation, citrate is the sole source of carbon in the Simmons citrate medium while inorganic ammonium salt ($\text{NH}_4\text{H}_2\text{PO}_4$) is the sole fixed nitrogen source. When an organic acid such as citrate is used as a carbon and energy source, alkaline carbonates and bicarbonates are produced. The visible presence of growth on the medium and the change in pH indicator color due to the increased pH are the signs that an organism can import citrate and use it as a sole carbon and energy source; such organisms are considered to be citrate positive.

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

Procedure:

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

d) TRIPLE SUGAR IRON (TSI) AGAR FERMENTATION

Principle:

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

Procedure:

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

APPENDIX F: PICTURES OF STANDARD MANUAL BIOCHEMICAL TEST AND ANTIMICROBIAL TESTING



Figure 6: Biochemical reactions of gram negative organisms



Figure 7: Antimicrobial testing showing zone of inhibition