AETIOLOGICAL AGENTS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN CHILDREN UNDER FIVE YEARS OF AGE PRESENTING WITH BLOODSTREAM INFECTIONS AT THE KINTAMPO MUNICIPAL HOSPITAL

BY

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DECLARATION

This study was carried out at the Department of Microbiology, University of Ghana, Medical School (UGMS), and at the Clinical Laboratory (Microbiology Unit) of the Kintampo Health Research Center under the joint supervision of Professor Kingsley Twum-Danso and Dr. Japheth A. Opintan.

"I hereby declare that, except for references to other people's work which have been duly acknowledged, this thesis is as a result of my own original investigation. I further certify that the thesis has not been presented to this University or elsewhere for the award of any degree"

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DEDICATION

This work is dedicated to my mentors,

Dr. Seth Owusu Agyei and Dr. Kwaku- Poku Asante

Professor Kingsley Twum Danso and Dr. Japheth A. Opintan

Your exceptional inspirations have been most helpful and very much appreciated.

To God be the glory, for the great things He has done

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ABSTRACT

Introduction: The major organisms that cause septicaemia in children have been established in different geographic locations. However, these causative organisms of paediatric septicaemia and their antibiogram change over time, within and across settings. In addition, increased antimicrobial drug resistance among the organisms has been observed over the last decades. Study of the aetiological agents and their antibiogram is therefore imperative for designing community- based management strategies.

Aim: This study determined the antimicrobial susceptibility patterns in commonly isolated bloodstream organisms among children below five years of age who attend the Kintampo Municipal Hospital in the Brong-Ahafo Region.

Method: Stored and fresh blood culture isolates from a total of 1,965 children below five years of age admitted at the children's ward and clinically diagnosed with septicaemia between 2008 and 2013 at the Kintampo Municipal Hospital were used. Bacteria isolates were identified using biochemical reactions. Antimicrobial susceptibility testing was performed using qualitative method and minimum inhibitory concentration for selected positive blood culture isolates.

Results: Out of the total number of children (1965) enrolled, 47.5% were males and 52.5% were females. Their mean age was 24.6 ± 11.9 months. Three hundred and ninety two children (392) had positive blood cultures of which 261 (66.6%) were considered pathogens and 97 (24.7%), including 34 yeast cells, were considered contaminants. The predominant isolated organisms were non typhoidal *Salmonellae* (NTS) (42.5%), *S. aureus* (38.7%), *S.* Typhi (4.6%) and *S. pneumoniae* (3.8%). Both gram positive and negative bacteria isolates were susceptible to ciprofloxacin (5µg), imipenem (10µg), ceftriaxone (30µg), gentamicin (10µg), amoxicillin clavulanic acid (30mµg), amikacin

(30µg) and cefuroxime (30µg). Sixty five point nine percent (65.9%) *S. aureus* were resistant to ampicillin, 60.8% to cotrimoxazole, 78.9% chloramphenicol, and 60.0% to amoxicillin. Fifty two point eight percent (52.8%) NTS were resistant to amoxicillin, 57.7% to ampicillin, 56.3 to chloramphenicol and 63.9% cotrimoxazole. Eighty seven point five percent (87.5%) of *S.* Typhi were resistant to amoxicillin and 57.7% to ampicillin. Eighty three point three percent (83.3%) of the *S. pneumoniae* isolates were resistant to cotrimoxazole. Ciprofloxacin and ceftriaxone appeared to be the best choice for empiric therapy. Multi- drug resistance (resistance to amoxicillin, chloramphenicol and cotrimoxazole) was observed in NTS (58.6%), *S. aureus* (63.4%) *S.* Typhi (100%) and *S. pneumoniae* (50%).

Conclusion: The investigation undertaken has shown the importance of septicaemia in this population. Continuous monitoring of the incidence and susceptibility patterns of these aetiological agents is therefore important.

TABLE OF CONTENTS

DECLARATION i
DEDICATION ii
ACKNOWLEDGEMENTS iii
ABSTRACT v
TABLE OF CONTENTS vii
LIST OF TABLES vii
LIST OF FIGURES xi
LIST OF ABBREVIATIONS xii
CHAPTER 1 1
INTRODUCTION 1
1.1 BACKGROUND 1
1.2 PROBLEM STATEMENT 5
1.3 JUSTIFICATION 7
1.4 AIM 7
1.5 OBJECTIVES 7
CHAPTER 2 8
LITERATURE REVIEW 8
2.1 DEFINITION OF SEPTICAEMIA 8
2.2 ROUTES OF INFECTION 9
2.3 AETIOLOGICAL AGENTS OF SEPTICAEMIA 9
2.3.1 Non typhoidal *Salmonellae* (NTS) 10
2.3.2 *Salmonella* Enterica serova Typhi 12
2.3.3 *Staphylococcus aureus* 14
2.3.4 *Streptococcus pneumoniae* 16
2.3.5 *Haemophilus influenzae* 20
2.3.6 *Neisseria meningitidis* 22
2.4 EPIDEMIOLOGY OF THE INFECTION 26
2.5 DIAGNOSIS OF SEPTICAEMIA INFECTION 27
2.5.1 Blood Cultures 27
2.6 MANAGEMENT OF SEPTICAEMIA 28
CHAPTER 3 30

MATERIALS AND METHODS 30
3.1 STUDY AREA 30
3.2 SAMPLE SIZE 32
3.3 PARTICIPANT SELECTION 33
3.4 ELIGIBILITY CRITERIA 33
3.5 EXCLUSION CRITERIA 34
3.6 STUDY DESIGN 34
3.7 ETHICAL CONSIDERATION 34
3.8 DATA MANAGEMENT AND ANALYSIS 34
3.9 PROCESSING OF STORED AND FRESH BLOOD SAMPLES 36
3.9.1 Sample Processing from Freshly Collected Blood Cultures 36
3.9.2 Processing Stored Isolates from Previously Suspected Cases of Septicaemia 36
3.10 BIOCHEMICAL TEST 37
3.11 QUALITY CONTROL 38
3.12 ANTIMICROBIAL SUSCEPTIBILITY TESTING 38
3.12.1 Preparation of inoculum 39
3.12.2 Inoculation and application of antibiotic discs 39
3.12.3 Incubation and plate reading 40
3.12.4 Minimum Inhibition Concentration (MIC) 40
3.12.4.1 Inoculum preparation for e-test 41
3.12.4.2 Inoculation 41
3.12.4.3 Application of e-test strips 41
3.12.4.4 Incubation and reading 41
CHAPTER 4 43
RESULTS 43
4.1 ENROLMENT AND DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION 43
4.2 COMMON AETIOLOGICAL AGENTS ISOLATED FROM BLOOD 44
4.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING BY DISC DIFFUSION 46
4.3.1 MINIMUM INHIBITORY CONCENTRATION OF THE MAJOR ISOLATES 48
4.4 BLOOD CULTURE CONTAMINATION RATE IN THE STUDY CHILDREN 52
4.5 RISK FACTORS ASSOCIATED WITH DEVELOPING SEPTICAEMIA 53

CHAPTER 5 55
DISCUSSION 55
CHAPTER 6 63
CONCLUSION AND RECOMMENDATIONS 63
6.1 CONCLUSION 63
6.2 RECOMMENDATIONS 64
REFERENCES 65
APPENDICES 72

LIST OF TABLES

Table 1 Clinical presentation of some serious bacterial infections……6

Table 2 Organisms isolated from blood culture……45

Table 3 Antimicrobial susceptibility profiles of the four most frequent isolated pathogens …….……..….47

Table 4 MIC results for NTS…….……49

Table 5 MIC results for *Staphylococcus aureus*……...50

Table 6 MIC results for *Salmonella* Typhi……..……..51

Table 7 Association of anthropometrical parameters, wealth index and parasitaemia with septicaemia in children below five years of age in Kintampo…….…….54

LIST OF FIGURES

Figure 1 Map of Ghana showing the Brong Ahafo Region……..…31

Figure 2 Levels of sensitivity exhibited by identified organisms against different antimicrobial drugs (using E-test strips)…….42

Figure 3 Flow chart showing the enrolment of children…….….43

Figure 4 Bacteria isolates considered as contaminants in blood…….52

LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
BD	Becton, Dickinson and Company
BSI	Bloodstream Infection (s)
CLED	Cysteine Lactose Electrolyte Deficient
CLSI	Clinical Laboratory Standard Institute
CSF	Cerebrospinal Fluid
CoNS	Coagulase Negative *Staphylococcus*
C. diphtheria	*Corynebacterium diphtheria*
C. freundii	*Citrobacter freundii*
E. coli	*Escherichia coli*
E- test	Epsilometer test
H. influenzae	*Haemophilus influenzae*
Hib	*Haemophilus influenzae* type b
HIV	Human Immunodeficiency Virus
IgG	Immunoglobin G
KHDSS	Kintampo Health Demographic Surveillance System
Kleb. pneumoniae	*Klebsiella pneumoniae*
MIC	Minimum Inhibitory Concentration
MIO	Motility Indole Ornithine Test
MDR	Multidrug Resistance
MRSA	Methicillin Resistant *Staphylococcus aureus*
N. meningitidis	*Neisseria meningitidis*
NTS	Non typhoidal *Salmonellae*

PCV	Pneumococcal Conjugate Vaccine
S. Typhi	*Salmonella* Typhi
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
S. pneumoniae	*Streptococcus pneumoniae*
TSI	Triple Sugar Iron
UNICEF	United Nations Children's Emergency Fund
µg	Microgram
µg/ml	Microgram per milliliter
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Bloodstream infections are important causes of morbidity and mortality in children worldwide (Prabhu *et al.*, 2010). One out of every six African children die before their fifth birthday and the related mortality associated with childhood bloodstream infection is estimated to be twenty five percent (Blomberg *et al.*, 2007). The World Health Organization (WHO) annual report on infectious diseases in 2005, indicated that over 40% of deaths in children occur within 28 days of life while more than 70% of deaths occur within the first year before the age of five years worldwide (Acquah *et al.*, 2013). The causes of these deaths have been attributed to malnutrition and infectious diseases. In Ghana, an estimated number of deaths among children 1-59 months in 2008 were 32,052, while 22,672 deaths were estimated to have occurred among children 0-27 days (Acquah *et al.*, 2013). Children less than one month with septicaemia accounted for 4,923 of these deaths in 2008 (Acquah *et al.*, 2013).

Bloodstream infections are often caused by several microorganisms including fungi, viruses, bacteria and some invasive parasites. Infections caused by fungi and viruses in the blood are often self-limiting or cannot be easily cultured in the laboratory. Fungal bloodstream infections can be very serious and life threatening because diagnosis is difficult and the causative agent is often confirmed only at autopsy (Jain *et al.*, 2010). Patients undergoing organ transplantation or intensive chemotherapy have resulted in more profound levels of immunosuppression that are sustained for long periods and making them susceptible to

systemic fungal infections (Richardson, 2005). Their slow growing nature in culture results in individuals with the infection either losing their lives or recovering by the time culture identification is completed. Bacteria are the most common cause of life-threatening bloodstream infection especially in children and hospitalized patients (Babay *et al.*, 2005). Numerous studies conducted in Africa suggest that bacterial infections contribute to high rates of morbidity and mortality (Babay *et al.*, 2005; Sigaúque *et al.*, 2009). The detection of bacteria is of paramount importance for proper management of bloodstream infections. Usually, bacteraemia progress quickly to septicaemia which can be life-threatening when immediate medical attention is not given (Meremikwu *et al.*, 2005).

Septicaemia is a bloodstream infection usually caused by pathogenic bacteria with the diagnosis often beginning with a clinical suspicion. Septicaemia is a combined clinical and microbiological diagnosis. In children however, the initial symptoms are often non-specific, and usually require urgent medical attention (Ayoola *et al.*, 2003).

The rate of proliferation of most bacteria compels clinicians to commence treatment of severe bacterial infections without often waiting for laboratory results. Hence, empirical treatment with antimicrobials is the best decision for such clinical presentation. Prompt treatment is required especially for septicaemic children at the intensive care unit. Riley and Wheeler indicated that patients at the intensive care unit due to their immunocompromised nature are mostly at risk of septicaemia. The use of intravascular devices in this unit is an important risk factor for acquiring the infection (Riley and Wheeler, 2012).

Septicaemia caused by bacteria, may arise from local infections in children with definite foci such as respiratory tract infection, urinary tract infection, skin tissue infections and diarrhoea if these infections are not properly managed (Blomberg *et al.*, 2007). The importance of septicaemia in terms of mortality is not recognized especially when it arises from localized infections. Instead, mortality is attributed to the underlying disease. Therefore mortality attributable to septicaemia is likely to be greatly underestimated (Riley and Wheeler, 2012).

Septicaemia, usually caused by myriads of bacteria vary from one locality to another (Adeodu and Senbanjo, 2006). Both Gram positive and Gram negative bacteria can be associated with this serious bacterial infection. According to studies conducted in Africa and other parts of the world, *Haemophilus influenzae, Neisseria meningitidis,* non typhoidal *Salmonellae, Salmonella* Typhi and other members of the enterobacteriacae are capable of causing septicaemia in children. Gram positive bacteria such as *Streptococcus pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes* are all implicated in septicaemia. Changes in the etiology of this infection have been observed over the past two decades. In the past few years, Gram negative bacteria were considered as the primary pathogens responsible for septicaemia (Nwadioha *et al.*, 2010). Today, the incidence of Gram positive bacteria has increased (Mayanja *et al.*, 2010) and seems to surpass the Gram negatives as the causative organism of septicaemia (Riley and Wheeler, 2012; Reddy *et al.*, 2010). Several studies across Africa however still maintain that although cases of Gram positive septicaemia have increased, Gram negative bacteria still remain the major pathogens of septicaemia (Ayoola *et al.*, 2003; Reddy *et al.*, 2010; Tsering *et al.*, 2011).

Clinical presentation of septicaemia in young infants may be similar to other serious bacterial infections such as pneumonia and meningitis as shown in Table 1. Therefore thorough investigations are required to arrive at specific diagnosis. Septicaemia infection in many cases is diagnosed using both clinical and microbiological criteria.

Ghana and the rest of Africa continue to suffer from the burden of infectious diseases such as septicaemia and malaria (Sigaúque *et al.*, 2009). Both septicaemia and malaria for instance cause febrile illnesses which are sometimes indistinguishable clinically (Blomberg *et al.*, 2007). In malaria endemic areas, almost all fevers are attributed to malaria and many bacterial infections may remain unnoticed. Until recently, pyrexia of unknown origin in Africa according to Groß *et al*. (2011) has often been treated with antimalarial drugs. Now initial treatment of malaria is followed with broad spectrum antimicrobial drugs such as chloramphenicol probably reinforcing the observation that cases of septicaemia in malaria endemic areas have been under reported (Uwe Groß *et al.*, 2011). Co-infection with malaria parasite and *Salmonella* species especially in areas where malaria is endemic has been reported (Oundo *et al.*, 2002; Eze *et al.*, 2011).

The availability of diagnostic microbiological services for bloodstream infection other than malaria is often limited by cost, personnel constraints and infrastructure (Reddy *et al.*, 2010). Few health facilities in Ghana and the rest of Africa have microbiologic laboratories to identify invasive bacterial infections (Sigaúque *et al.*, 2009).

Despite the major contribution of infectious diseases to hospital admission, the World Health Organization (WHO) guidelines for managing childhood illnesses fail to identify up to half of the cases of bloodstream infections (Reddy *et al.*, 2010; Blomberg *et al.*, 2007). This therefore results in underestimation of the clinical importance of bacterial infections and deaths associated with these infections (Sigaúque *et al.*, 2009).

Consequently, health care providers often rely on empirical treatment and might underestimate or overestimate the likelihood of certain infections like septicaemia (Reddy *et al.*, 2010). Empirical treatment may promote poor clinical outcomes and antimicrobial resistance (Reddy *et al.*, 2010). Information on the aetiological agents and their antibiogram, on the African continent is scarce.

1.2 PROBLEM STATEMENT

Paediatric septicaemia is of serious public health concern, especially in developing countries like Ghana. Worldwide, 70% of the 8.8 million deaths that occur in children below the age of five years are the result of infectious diseases (Nielsen *et al.*, 2012b)**.** Majority of these infections end up with septicaemic complications (Meremikwu *et al.*, 2005). Children have immature immune system making them highly vulnerable to septic complications from these infections. In recent times, the management of septicaemic related illnesses has very much improved compared to two decades ago (Riley and Wheeler, 2012).

Table 1: Clinical presentation of some serious bacterial infections

INFECTIONS	SYMPTOMS
Septicaemia	Temperature > 38°C< 35.5°C
	Respiratory rate > 60 breaths\ minute
	Altered mental state (agitation, lethargy or coma)
	Poor feeding
Meningitis	Lower chest wall in drawing
	A history of convulsions
	A bulging fontanelle
Pneumonia	Slow digital capillary refill
	Lack of spontaneous movement
	Cyanosis

Improvements in antimicrobial therapy regimen might be responsible for the decrease in septicaemic complications and mortality in western countries. However, these successes seemed to be overturned due to the emergence of microbial resistant strains. In sub-Saharan Africa, the case fatality rate of septic complications in children is increasing. Microbial resistance is suspected to be responsible for most septicaemic-related deaths; these could have been prevented with proper knowledge of antimicrobial susceptibility patterns (Omoregie *et al.*, 2009).

There is no recorded data on the aetiological agents in the Kintampo North Municipality and Kintampo South Districts. Most health facilities in these areas rely mostly on empirical

treatment using broad spectrum antibiotics as treatment options. The practice of using broad spectrum antibiotics for the treatment of serious bacterial infections like septicaemia potentially promotes antimicrobial resistance.

1.3 JUSTIFICATION

Most children die of septicaemic infections as a result of inappropriate antimicrobial therapy. Some of these deaths could have been prevented with the appropriate antimicrobial therapy. This study aimed at identifying and determining antibiogram of the major aetiological agents for septicaemia. The study of antimicrobial susceptibility pattern would be pivotal in combating infant mortality caused by septicaemia. This will aid clinical decision on the appropriate selection and use of antimicrobials in septicaemic cases. It would also form the knowledge base for further investigations into antimicrobial treatment of septicaemia.

1.4 AIM

This study determined the antimicrobial susceptibility patterns in commonly isolated organisms from children below five years of age seeking health care at the Kintampo Municipal Hospital in Brong-Ahafo Region of Ghana.

1.5 OBJECTIVES

1. To identify the aetiological agents in children suspected to have septicaemia.
2. To determine their antimicrobial susceptibility patterns
3. To determine the contamination rates of these cultures
4. To determine any risks factors associated with septicaemia.

CHAPTER 2

LITERATURE REVIEW

2.1 DEFINITION OF SEPTICAEMIA

Septicaemia is a systemic infection caused by bacteria constantly multiplying in the bloodstream (Omoregie *et al.*, 2009). The term septicaemia is often used in describing severe bacteraemic infections or a condition in which the blood serves as a site of bacteria multiplication from localized infections. The infection is defined clinically and may be confirmed with positive blood cultures. Cerebrospinal fluid and urine cultures are also used when a localized infection like meningitis are suspected as preceding the septicaemia. Septicaemia is a common condition in children which often leads to serious complications such as disseminated intravascular coagulation and acute renal failure (Komolafe and Adegoke, 2008).

The clinical picture frequently present in septicaemia is that of severe febrile episode with chills, fever, malaise, tachycardia, hypotension, hyperventilation or toxicity, mental confusion and prostration (Komolafe and Adegoke, 2008). In most septicaemic infections, there is an increase on the levels of white blood cells. In addition to the symptoms and signs mentioned, children below age five may also exhibit difficulty in breathing, lethargy and refusal to feed (Prabhu *et al.*, 2010). Septicaemic children may suffer from complications like shock, disseminated intravascular coagulation, multiple organ failure and acute renal failure (Prabhu *et al.*, 2010).

2.2 ROUTES OF INFECTION

Septicaemia arises from infections throughout the body, including infections in the lungs and urinary tract. It may occur before or concurrently with infections such as osteomyelitis, meningitis, septic arthritis and endocarditis. Urinary tract infections are very common lesions that lead to septicaemia in children. Septicaemia occurs when organism causing urinary tract infection travels upstream from the bladder to cause pyelonephritis (Chang and Shortliffe, 2006).

2.3 AETIOLOGICAL AGENTS OF SEPTICAEMIA

A wide range of microorganisms including bacteria and fungi have been named to be responsible for septicaemic infections. These organisms however differ from one locality to the other with varying antimicrobial susceptibility patterns (Meremikwu *et al.*, 2005). Significant differences exist in the causative organisms of septicaemia within Africa (Ayoola *et al.*, 2003). Gram positive organisms according to Phiri *et al*. (2005) are commonly isolated in neonates. This finding corresponds to works done by Omoregie (2009) and Ogunleye (2005) on paediatric septicaemia in Nigeria. Gram positives organisms in these studies had higher prevalence than the Gram negative organisms, although several Gram positives organisms were isolated. This is consistent with the knowledge that the organisms vary from one place to another (Ogunleye *et al.*, 2005; Omoregie *et al.*, 2009). Group B *Streptococcus* according to Phiri *et al*. (2005) is one of the commonest causes of neonatal Septicaemia, followed by Gram negative bacilli. In contrast to the above studies, Ayoola *et al* found Gram negative organisms to be major causative organisms of septicaemia than the Gram positive organisms (Ayoola *et al.*, 2003).

There is a general trend in developing countries of isolating pathogens such as *Staphylococcus epidermidis* which were not previously associated with septicaemia (Phiri *et al.*, 2005; Mugalu *et al.*, 2006). However, in Ghana, Non typhoidal *salmonellae* and *S. aureus* are the predominant aetiological agents of septicaemia (Nielsen *et al.*, 2012b).

2.3.1 Non typhoidal *Salmonellae* (NTS)

Non typhoidal *Salmonellae* is a common cause of bloodstream infection among African children according to several studies conducted in Africa (Graham *et al.*, 2000; Ikumapayi *et al.*, 2007). It is one of the three major causes of invasive disease in children below the age of three (Ikumapayi *et al.*, 2007). Non typhoidal *Salmonellae* cause important infections such as septicaemia and gastroenteritis which result in high morbidity and eventually death (Oundo *et al.*, 2002). Childhood mortality associated with NTS has been reported (Vaagland *et al.*, 2004). Non typhoidal *Salmonellae* infections occur worldwide and their mode of transmission is orofaecal. In Africa Non typhoidal infections occur throughout the year. NTS causes predominantly gastroenteritis in healthy individuals in the developed countries (Kariuki *et al.*, 2006). In sub- Saharan Africa however, Non typhoidal *Salmonellae* consistently cause bloodstream infection in children and adults which may lead to death if prompt and appropriate antimicrobial therapy is not given. A study conducted by Nicholas *et al*. (2012) suggests that NTS causes a 20 to 25% fatality. Kariuki *et al* (2006) found out that NTS (51.2%) was responsible for bloodstream infection in Kenya. Another study conducted in 2006 by Maitland *et al* in Kenya suggested NTS as one of the major causes of invasive

bacterial disease in that region. In Mozambique, NTS (26%) accounts for the highest cause of bloodstream infections amongst paediatric hospital admissions (Sigaúque *et al.*, 2009).

In the developed world, NTS septicaemia is not very common; rather, Non typhoidal *Salmonellae* is responsible for majority of the acute gastroenteritis cases (Ikumapayi *et al.*, 2007). Several risks factors that expose African children to Salmonella septicaemia include malaria, HIV and malnutrition. Reddy et al found out that 58.4% of cases of BSI was caused by NTS (Reddy *et al.*, 2010). Earlier studies in Mozambique indicate that NTS had high rates of resistance to most common antibiotics. Among the NTS isolates, 74% were not susceptible to ampicillin and 66% were also not susceptible to trimethoprim-sulfamethoxazole. Also, 54% were resistant to chloramphenicol. In addition, 38% of NTS isolates were resistant to amoxicillin-clavulanic acid (Sigaúque *et al.*, 2009). Multi resistance amongst NTS has been observed by Kariuki *et al*. (2006). In their study, 59% of the NTS were resistant to ampicillin, chloramphenicol and tetracycline. Ceftriaxone and ciprofloxacin were however active against all NTS isolates. Zaidi *et al*. in 2013 found out from their study in Mexico that, Non typhoidal *Salmonellae* isolates had decreased susceptibility to ciprofloxacin (Zaidi *et al.*, 2013). Also, from their study, they identified a decreased resistance to ceftriaxone (27 to 10%), ampicillin (43- 16%) and trimethoprim–sulfamethoxazole (44 to 26%) which is in contrast to most findings.

Association of NTS and malaria has been observed in Africa (Berkley *et al.*, 1999; Oundo *et al.*, 2002). Oundo *et al* (2002) realized from their study in Kenya that septicaemia infection

caused by some species of *Salmonella* were mostly common and severe at peak season of malaria than any other time.

2.3.2 *Salmonella* Enterica serova Typhi

Typhoid fever, caused by *Salmonella* enterica serovar Typhi is found worldwide and accounts for several cases of morbidities and mortalities. They are mostly found in developing countries where sanitation conditions are very poor. *S.* Typhi infections are not very common in the developed world and may be acquired through emigrants who may be active or passive carriers of the infection (Crump *et al.*, 2003).

Typhoid fever is endemic in both the tropics and subtropics and usually causes systemic infections in these regions. Infections caused by this aetiologic agent may be described as mild or severe but can sometimes be life threatening if proper attention is not given. Typhoid infection is acquired via oro- faecal route through the ingestion of contaminated food or water. Considering the mode of infection, typhoid fever is often associated with poor personal and environmental hygiene (Evans *et al.*, 2004). In Ghana, typhoid fever is predominant in areas with poor sanitary conditions (Nielsen *et al.*, 2012b; Acquah *et al.*, 2013). A study conducted in Ghana by Groß *et al* showed 40.7% of *Salmonella* isolates from septicaemic patients were *S.* Typhi. Increased awareness and proper knowledge on sanitation for healthy living is therefore required to avoid this infection.

Typhoid fever affects people of all age groups but children are more prone to the infection; this is because they possess immature gut lymphoid tissues and have decreased gastric acidity (Evans *et al.*, 2004).

Despite the development and introduction of new antimicrobials, most health practitioners are faced with challenges in treating enteric fevers due to the emergence of multi- resistant strains of *S.* Typhi to most antibiotics. The emergence of antimicrobial resistance, especially among first line treatment options such as ampicillin, chloramphenicol, and cotrimoxazole, has complicated the treatment and management of enteric fever.

Until the emergence of chloramphenicol – resistant strains, chloramphenicol, for many years was used as the first line of treatment for enteric fever. In Ghana, the 2001 national guidelines for treatment stated the use of chloramphenicol as the first line drug for treating typhoid fever (Ghana Ministry of Health, 2004). More than 80% of *S.* Typhi isolates however were resistant to this drug (Uwe Groß *et al.*, 2011). These resistant strains prompted the use of ampicillin and trimethoprim/ sulfamexazole as alternatives. Soon after their introduction as baseline treatment options, a pattern of resistance was observed. The emergence of multi – resistant *Salmonella* strains has rendered most of these common antibiotics ineffective.

Currently, flouroquinolones and 3rd generation cephalosporins are widely used for the treatment of enteric fevers. Florian *et al*. (2010) found out that *S.* Typhi was highly resistant to chloramphenicol (73%), trimethoprim/ sulphamethoxole (70%) and ampicillin (70%) as

compared to tetracycline (64%). Other antibiotics such as gentamicin (46%) and amoxicillin clavulanic acid (24%) showed lower resistance patterns in the study (Marks *et al.*, 2010).

2.3.3 *Staphylococcus aureus*

Staphylococcus aureus is usually found as part of the normal microbiota of the nose, skin, mouth, and other parts of the body. *S. aureus* nasal carriage can be detected in up to 50% of healthy humans (Lowy, 1998; Del Rio *et al.*, 2009), either in a persistent or in an intermittent way. Infections occur when a breach of the skin or mucosal barrier allows *staphylococci* access to adjoining tissues or the bloodstream (Lowy, 1998). Whether an infection is contained or spreads depends on a complex interplay between *S. aureus* virulence determinants and host defense mechanisms (Lowy, 1998). *S. aureus*, a commonly isolated bacterium, is ubiquitous and a common cause of most superficial and invasive infections in healthy and immunocompromised individuals. Diabetic patients are also known to be at high risk of *S. aureus* BSI and its associated complications (Hakeem *et al.*, 2013). Important skin and soft-tissue infections, endovascular infections, septicaemia, pneumonia, septic arthritis, endocarditis, osteomyelitis, metastatic abscesses and wound infections are caused by this organism (Del Rio *et al.*, 2009; Hakeem *et al.*, 2013).

Bloodstream infections caused by *S. aureus* worldwide according to Naber (2009) is very common and often difficult to treat, and therefore the associated morbidity and mortality is relatively high (Shinefield *et al.*, 2002; Naber, 2009). Gram positive organisms including *S. aureus* in the United States were responsible for half of the cases of septicaemia (Kuehnert *et al.*, 2005). Similar observations are made by several studies conducted around Africa

(Awoniyi *et al.*, 2009). *S. aureus* accounted for 48.7% of the isolated cases of septicaemia in Nigeria (Meremikwu *et al.*, 2005). In a similar study, although Gram Negative rods were seen as the major etiological agents, *S. aureus* also contributed significantly to the infection. Another study by Maitland showed that although *S. pneumoniae* (35%) was the major gram positive pathogen in the bloodstream of children in Kenya, *S. aureus* also accounted for a significant number (8%) of the cases (Maitland *et al.*, 2006). Kizito *et al*. found out in a related study that *S. aureus* (60%) was commonly isolated from BSI of children in Uganda (Kizito *et al.*, 2007). Findings from a study conducted in Ghana by Evans *et al*. (2004) have also reported *S. aureus* (29%) as one of the major aetiologic agents. This agrees with studies conducted by Meremikwu (2005) and Awoniyi *et al*. (2009) in Nigeria that also reported *S. aureus* as a major organism isolated representing 48.7% and 28% respectively. In Mozambique (Sigaúque *et al.*, 2009), *S. aureus* (39%) was found as one of the major pathogens isolated from neonates with bloodstream infections followed by group B *Streptococcus* (20%). Another study conducted by Tsering *et al* (2011) in India also revealed that 97% cases of septicaemia in children was caused by *S. aureus*.

Reddy *et al*, in 2010 also reported *Staphylococcus aureus* (9.5%) as one of the aetiological agents of septicaemia. In contrasts to other findings across Africa, Reddy *et al* (2010) indicated that, septicaemia caused by *S. aureus* has been overestimated over the years by some laboratories that have often misidentified coagulase negative *Staphylococcus* as *S. aureus*. This statement however does not support earlier works done.

Over the years, varied sensitivity patterns of antimicrobials to *S. aureus* have been observed. A study conducted in Nigeria by Meremikwu *et al*., (2005) on bacteraemia established that the *S. aureus* isolated had the highest susceptibility to ceftriaxone (100%), cefuroxime (100%), azithromycin (100%), erythromycin (90.1%) and gentamicin (86.6%). A similar study in Nigeria, Komolafe *et al* (2008) also discovered that though 70 to 90% of all *S. aureus* isolates were resistant to penicillin, ampicillin, tetracycline and sulfamexazole trimethoprim, more than 80% of *S. aureus* isolates were however sensitive to gentamycin, ceftazidime, ceftriaxone and vancomycin. Komolafe *et al* (2008).

Studies in the Gambia by Hill *et al* on bacteraemia have shown that all *S. aureus* isolates were susceptible to cloxacillin, gentamicin and chloramphenicol compared to co-trimoxazole (66.7%). The isolates also showed poor susceptibility to tetracycline (33%) and penicillin (8%) (Hill *et al.*, 2007). Also Tsering *et al* (2011) in their study found out that more than 70% of Staphylococci isolated were resistant to penicillin, but were sensitive to clindamycin (70%) and vancomycin (40%).

2.3.4 *Streptococcus pneumoniae*

Streptococcus pneumoniae commonly known as pneumococcus is an important pathogen that causes diseases ranging from upper respiratory tract infections to severe invasive diseases such as pneumonia, septicaemia and meningitis (Bogaert *et al.*, 2004; Donkor *et al.*, 2013). *Streptococcus pneumoniae* is the major cause of invasive bacterial disease in children in places where *Haemophilus influenzae* type b (Hib) disease is almost eliminated by vaccination and also the most frequently reported cause of septic meningitis (Corless *et al.*,

2001). Non encapsulated *S. pneumoniae* is part of the normal bacterial flora found on the mucosal surface of the upper respiratory tract.

In children, the nasopharyngeal flora becomes established during the first months of life and almost all children are colonized at some point in life, with carriage rates highest among infants below age two (Bogaert *et al.*, 2004). Therefore it is very common to find nasopharyngeal carriage in healthy children. Colonization of S. *pneumoniae* at this site is often asymptomatic even though all pneumococcal disease begins with the establishment of colonization (Alter, 2009).

There are over 91 serotypes of pneumococci and 6–11 serotypes are responsible for more than 70% of invasive pneumococcal disease (IPD) in children under age five worldwide (Johnson *et al.*, 2010). Carriage rates however vary widely among the 91 known pneumococcal capsular serotypes, which express structurally and antigenically different capsular polysaccharides (Kadioglu *et al.*, 2008; Donkor *et al.*, 2013). The distribution of serotypes causing disease however varies by age, disease syndrome, disease severity, geographic region, and over time.

Most risk population such as children colonized with *S. pneumoniae* has only one serotype at a time (van der Poll and Opal, 2009). In Ghana and other West African countries, serotypes 1 and 5 are known to be the major causes of invasive pneumococcal disease accounting for more than 30% of all cases in the region (Donkor *et al.*, 2013) . Transmission of pneumococcus is usually by direct contact with contaminated respiratory secretions between

household members, infants, and children. Pneumococcus infections occur across various age groups, though burden of the disease is highest in young children, the elderly, and patients with immune deficiencies.

Streptococcus pneumoniae is the leading cause of death in children less than five years (Isaacman *et al.*, 2010) with about 1.2 million new cases of pneumococci infections emerging annually (Bogaert *et al.*, 2004; Donkor *et al.*, 2013). The disease burden of pneumococci is high in children aged 4–18 months, but in older children and adults, the disease is relatively rare (Trotter *et al.*, 2008). In 2005, approximately one million children below age five died from pneumonia and invasive diseases worldwide (Bogaert *et al.*, 2004; Alter, 2009). Most of these deaths occurred in the developing countries. (Alter, 2009). Following widespread use of the Hib conjugate vaccine in infants, *S. pneumoniae* emerged as the leading cause of bacterial meningitis in children below 2 years of age (Alter, 2009). In the United Kingdom, *S. pneumoniae*, is the commonest bacterial respiratory pathogen responsible for community acquired pneumonia (Kadioglu *et al.*, 2008), with a 20% mortality especially those with concurrent pneumococcal septicaemia.

In the United States, Segal *et al* found *S. pneumoniae* to be the dominant pathogen isolated from children (Segal and Chamberlain, 2000). A study in Kenya also showed that half of the isolates recovered from the bloodstream were *S. pneumoniae* (Brent *et al.*, 2006). Hill *et al*. (2007), in Uganda, established that out of the total number of organisms isolated in children, 45.2 % was *S. pneumoniae*. Studies of septicaemia in the older age in Uganda also reported *S. pneumoniae* as one of the predominant organism (Mayanja *et al.*, 2010). Nielsen *et al*. in

2012 conducted a study in Ghana and concluded that *S. pneumoniae* (9.1%) was among the frequent isolates in blood amongst Ghanaian children. In contrast, Komolafe *et al* (2008), in a related study realized that pneumococcal septicaemia (0.5%) was less in children in Nigeria compared to other studies. This reduction in pneumococcal septicaemia could be as a result of introduction of the pneumococcal vaccine.

Following the introduction of pneumococcal conjugate vaccines in resource-limited countries, there has been a significant reduction in pneumococcal infections. In Ghana, the pneumococcal vaccine, PCV- 13 was introduced in May 2012 (WHO, 2012). The introduction of PCV is expected to reduce the burden of invasive pneumococcal diseases in infants and antimicrobial resistance amongst *S. pneumoniae* serotypes worldwide.

Despite reductions in invasive disease among the vulnerable population, infections with *S. pneumoniae* continue to pose challenges for clinicians (Alter, 2009). Though pneumococcal conjugate vaccines have been introduced in many industrialized and developing countries, the vaccine only target a handful of serotypes (Donkor *et al.*, 2013). Both the upper respiratory tract and invasive infections caused by pneumococcal serotypes not represented in the current conjugate vaccine have been observed with increasing frequency (Alter, 2009). Furthermore, antimicrobial resistance among pneumococcal isolates presents difficulties in treatment (Alter, 2009). A study in Saudi Arabia revealed that forty-three percent of the isolates from patients with invasive pneumococcal infections were resistant to penicillin although 76% were sensitive to ceftriaxone (Twum-Danso *et al.*, 2003). In Nigeria, Adetifa

et al reported that cotrimoxazole (93%) and tetracycline (84%) had high resistance (Adetifa *et al.*, 2012).

2.3.5 *Haemophilus influenzae*

Haemophilus influenzae is one of the major pathogens apart from *S. pneumoniae* and *N. meningitidis* that causes morbidity and mortality amongst children across the globe (Decker and Edwards, 1998; Howie *et al.*, 2007). *H. influenzae* is a member of the normal bacterial flora of the respiratory tract and a major cause of several invasive and non -invasive infections (García-Cobos *et al.*, 2008). Major diseases caused by *H. influenzae* include childhood pneumonia, meningitis, septicaemia, acute otitis media and epiglottitis (Tristram *et al.*, 2007; Resman *et al.*, 2011). *H. influenzae* is mostly a human pathogen and exclusively adapted to the human host with no animal or environmental hosts (Murphy, 2009; Resman *et al*., 2011).

Haemophilus influenzae strains are subdivided into two depending on the absence or presence of one of six polysaccharide capsule (Agrawal and Murphy, 2011); these are the encapsulated and the non-encapsulated strains. Encapsulated strains are reactive with typing antisera (Agrawal and Murphy, 2011) whiles the non- encapsulated strains are non-reactive to typing antisera. Six encapsulated serotypes of *H. influenzae* ranging from a to f have been identified based on their antigenically distinct polysaccharide capsules. (Tristram *et al.*, 2007; Agrawal and Murphy, 2011). Serotype b strains have been well documented as the predominant cause of invasive infections in infants and children (Resman *et al.*, 2011; Agrawal and Murphy, 2011). Hib is commonly found in the nose and throat of healthy

individuals living in areas where vaccination is not carried out. Almost all children who are not vaccinated are exposed to Hib by the age five. Long before the introduction of vaccines, *Haemophilus* type b was responsible for about three million infections worldwide with an annual death of 386,000 occurring mostly in the developing countries (Trotter *et al.*, 2008). The use of Hib conjugate vaccines for the prevention of severe illness in children has significantly decreased the burden of disease globally in spite of the difference of disease epidemiology in these settings (Von Gottberg *et al.*, 2006; Visser and Hoosen, 2012). In Ghana, cases of meningitis amongst children below five years has reduced drastically after the introduction of Hib pentavalent vaccines (Renner *et al.*, 2007). Vaccination has virtually eliminated Hib disease in most industrialized nations (Tristram *et al.*, 2007). Vaccination coverage of Hib has increased tremendously over the years since its introduction; thirty eight countries offered infant immunization against Hib in 1999 and by the end of 2004, eighty nine countries had joined the programme (Tristram *et al.*, 2007). *Haemophilus* type b is spread by exhaled droplets and can invade the bloodstream and cause septicaemic infection or may spread to the meninges through the bloodstream leading to meningitis, and the lungs, causing pneumonia. Most *Haemophilus* type b related mortality is attributed to meningitis and pneumonia, but invasive disease may also present as septicaemia, pericarditis, epiglottitis, osteomyelitis, cellulitis and septic arthritis.

Several strains of non-encapsulated or non-typeable strains of *H. influenzae* have been identified and are common colonizers in the upper respiratory tract. Though often regarded as a commensal, it causes mucosal infections in children and adults. Also, nontypeable strains of *H. influenzae* are responsible to several lower tract respiratory infections (Murphy,

2009). Community acquired pneumonia in adults, acute otitis media, acute sinusitis and acute exacerbations of chronic bronchitis which are typically caused by untypeable or non-capsulated strains are also major diseases caused by *Haemophilus influenzae* (Tristram et al., 2007).

Although the introduction of Hib conjugate vaccines has considerably reduced the incidence of Hib infections amongst children, cases of *Haemophilus* infections are still reported. Work done by Nielsen *et al* (2012) in Ghana suggested that bloodstream infections caused by *H. influenzae* (0.4 %) has significantly reduced. This pattern is also observed in Nigeria, Komolafe *et al*., (2008) identified that 0.5 % *H. influenzae* was the cause of septicaemia in children. Kizito *et al*. conducted a similar study in 2007 and established that 19% of *H. influenzae* isolates were found in Ugandan children. In Kenya, Maitland *et al.*, (2006) suggested that 8% of *H. influenzae* were responsible for invasive bacterial disease although *S. aureus*, *E. coli* and NTS were the major pathogens. In contrast, Berkley *et al.*, (2005) established more than 70% of the isolates from the bloodstream of Kenyan children to be *H. influenzae*.

2.3.6 *Neisseria meningitidis*

Meningococcal diseases have repeatedly caused epidemics (Greenwood, 2007) and continue to be a global health problem affecting all ages, and a major cause of mortality in children (Thompson *et al.*, 2006). It occurs worldwide with incidence rates varying from 1 to 1000 cases per 100,000 (Caugant and Maiden, 2009). *N. meningitidis* is a leading cause of bacterial meningitis and septicaemia worldwide (Antignac *et al.*, 2003). In the United States

of America and some developed countries cases of meningococcal septicaemia and meningitis have been reported (Rosenstein *et al.*, 2001). These two syndromes clinically overlap, usually occurring concurrently, but may frequently occur alone as in the case of meningitis (Caugant and Maiden, 2009). A study on meningococcal in children by Thompson *et al.*, (2006) showed that 296 (66%) out of the total number of children involved in the study, were classified as having predominantly septicaemia. In Sub- Saharan Africa however, *N. meningitidis* is predominantly the cause of meningitis epidemics (Rosenstein *et al.*, 2001; Stephens *et al.*, 2007). Meningococcal disease occurs year-round in the United States and some developed countries with majority of the cases occurring during winter and early spring (Rosenstein *et al.*, 2001) which is in contrast with happenings in Africa. In the African meningitis belt, disease incidence is seasonally dependent, peaking during the dry season and declining rapidly with the onset of the rainy season (Gagneux *et al.*, 2002). "Harsh environmental conditions present in the sub-Saharan meningitis belt during the dry season coupled with high temperature, low absolute humidity, and the Harmattan (a dusty wind that blows from the Sahara) in addition to respiratory coinfections are thought to contribute to an enhanced susceptibility to meningococcal disease by damaging the local mucosal defenses" (Gagneux et al., 2002; Greenwood, 2007).

N*eisseria meningitidis,* commonly called meningococcus is found in the mucosa of the oropharynx of humans and a natural colonizer of the upper respiratory tract (Stephens *et al.*, 2007; Caugant and Maiden, 2009). Humans are the only natural reservoir and therefore infection is spread from man to man, the nasopharynx is the site from which meningococci are transmitted through droplet secretions or via aerosols from an infected person to a

susceptible individual (Rosenstein *et al.*, 2001). Meningococci overcome host defenses and attach to the microvillous surface of nonciliated columnar mucosal cells of the nasopharynx, where they multiply.

About 5 to 10% of persons are asymptomatic nasopharyngeal carriers of strains of *N. meningitidis*, most of which are non-pathogenic (Rosenstein *et al.*, 2001). In a small proportion of carriers, *N. meningitidis* penetrates the mucosa and gains access to the bloodstream, causing invasive meningococcal disease (Rosenstein *et al.*, 2001; Gagneux *et al.*, 2002). In most persons, however, carriage is an immunizing process, resulting in a systemic protective antibody response (Rosenstein *et al.*, 2001).

N. meningitidis is classified into 13 serogroups according to the immunologic reactivity of their capsular polysaccharides, which are the basis for currently licensed meningococcal vaccines. The most common serogroups causing disease are A, B, C, W-135,Y and most recently X (Greenwood, 2007; Stephens *et al.*, 2007). These six known serogroups are the cause of most life-threatening diseases. Serogroups A, B, and C are responsible for more than 90% of invasive meningococcal infections worldwide (Gagneux *et al.*, 2002; Stephens *et al.*, 2007). The A and C serogroups are predominantly found throughout Asia and Africa (Rosenstein *et al.*, 2001). Serogroups X, Y and W- 135 have been reported to cause diseases in Africa as well (Greenwood, 2007; Hedberg *et al.*, 2009). Most large meningitis epidemics are caused by serogroup A meningococci. Such epidemics have been reported to be rare in industrialized countries but occur periodically in the African meningitis belt and in China (Gagneux *et al.*, 2002). Serogroup C meningococci have also been reported as the cause of

many disease outbreaks and occasionally epidemics (Gagneux *et al.*, 2002; Greenwood, 2007). Endemic disease are usually caused by meningococci belonging to serogroups B or C, although Serogroups W135, Y, and X also cause disease occasionally (Gagneux *et al.*, 2002). The serogroups B and C are also responsible for the majority of cases in Europe and the Americas (Rosenstein *et al.*, 2001).

Currently, there are meningococcal vaccines that target serogroup A, C, Y and W135 polysaccharides (Caugant and Maiden, 2009). The introduction of the vaccines have reduced the incidence of meningitis to some extent, however since the vaccines are too expensive for epidemic control (Greenwood, 2007), outbreaks of meningococcal diseases is still reported.

Prompt antimicrobial treatment is crucial for reducing the case fatality rate and risk of developing into a systemic infection of meningococcal disease (Stephens *et al.*, 2007; Hedberg *et al.*, 2009). Most strains of *N. meningitidis* are sensitive to many of the available antibiotics, such as b-lactams, rifampin, chloramphenicol, quinolones, and macrolides (Antignac *et al.*, 2003). Beta -Lactams are the antibiotics of choice for treatment, whereas rifampin, spiramycin, and ciprofloxacin are recommended for the chemoprophylaxis of meningococcal infections (Antignac *et al.*, 2003). In the African meningitis belt however, chemoprophylaxis of close contacts of patients is rarely used (Hedberg *et al.*, 2009).

The general recommendation for treatment during endemic periods in Africa is either administering ceftriaxone in multiple doses to also provide effective treatment of other presumptive etiological agents of bacterial meningitis, such as *Streptococcus pneumoniae*

and *Haemophilus influenzae*; or giving out multiple doses of penicillins (Hedberg *et al.*, 2009). In recent times, there have been reports of reduced susceptibility of penicillins and quinolones among *N. meningitidis* group (Stephens *et al.*, 2007; du Plessis *et al.*, 2008).

During meningococcal epidemics, the recommended treatment is management with a single dose of chloramphenicol in oil or a single dose of ceftriaxone (Hedberg *et al.*, 2009).

2.4 EPIDEMIOLOGY OF THE INFECTION

About twelve million children die as a result of diseases including septicaemia in developing countries (Ezeaka *et al.*, 2003). Septicaemia has a worldwide distribution and predominantly causes morbidity and mortality in children of which majority occur in sub-Saharan Africa (Omoregie *et al.*, 2009; Kamga *et al.*, 2011) One out of every five children in sub Saharan Africa will often lose their lives before their fifth birthday (Ezeaka *et al.*, 2003). The World Health Organization estimates that 85% of neonatal deaths are due to infections like sepsis, pneumonia and tetanus (Omoregie *et al.*, 2009). The rate of mortality among five year olds in developing countries is about 25-100 per 1000 as compared to 10-30 per 1000 deaths in the western world (Kamga *et al.*, 2011). These low mortality rates in the developed countries could be attributed to availability of affordable healthcare. The plight of an African child with septicaemia is the poor quality of healthcare needs. The prevalence of septicaemia according to studies carried out in Nigeria is characterized not only by seasonal changes but also by gender (Omoregie *et al.*, 2009). The conclusion of a study of septicaemia in Nigeria however showed that gender and seasonal variation did not significantly affect the

prevalence of septicaemia, although females were more susceptible to the infection (Omoregie *et al.*, 2009).

2.5 DIAGNOSIS OF SEPTICAEMIA INFECTION

Prompt diagnosis and effective treatment is often required for bloodstream infection to prevent death and complications (Meremikwu *et al.*, 2005; Prabhu *et al.*, 2010). Bacteriological examination is therefore very important for diagnosis of septicaemia. A limitation of bacteriological investigations on suspected cases of septicaemia is that, laboratory diagnostic procedures may take up to a week to complete and may cause most clinicians to depend on empirical treatment (Omoregie *et al.*, 2009).

2.5.1 Blood Cultures

Blood cultures are the mainstay for diagnosis of septicaemia (Prabhu *et al.*, 2010). Physical signs and symptoms may be useful in identifying septicaemic patients but have limited specificity (Kamga *et al.*, 2011). Culture and isolation of specific pathogen in bacteriological cultures is definitive diagnosis for suspected cases of septicaemia (Meremikwu *et al.*, 2005). Positive blood cultures, although are the gold standard for diagnosing septicaemia, however their usefulness in neonates may be limited. Most cases of neonatal septicaemia often produce negative cultures even when there are strong clinical suggestions of septicaemia. Antibiotics administered to pregnant women in the majority of preterm deliveries may suppress the growth of bacteria in culture, yet the neonate may have clinical symptoms and laboratory findings consistent with a diagnosis of septicaemia (Kaufman and Fairchild,

2004). False-negative blood cultures in apparently septicaemic neonates may also result from insufficient sample size (Kaufman and Fairchild, 2004).

2.6 MANAGEMENT OF SEPTICAEMIA

Life threatening infections like septicaemia often requires prompt attention to prevent death or serious complications. Antimicrobials, for a long time have been used to combat septicaemic infections worldwide. A wide range of antimicrobials including cephalosporins, aminoglycosides, flouroquinolones and cabarpenems have been used in the treatment of septicaemia and have generally been successful. In suspected cases of septicaemia, antimicrobial therapy is always initiated empirically. Results of bacteriological cultures and antimicrobial susceptibility tests may take about five days, necessitating initial empirical treatment of the infection. This practice however can add up to the already existing problem of antimicrobial resistance. Antimicrobial resistance occurs when selected antimicrobial agents are over prescribed by medical practitioners, with time; susceptible organisms will be eliminated completely leaving only organisms that have developed resistance to the antimicrobial agents. Usually, antimicrobial resistance is as a result of mutation and transfer of resistance mobile genes (Sheril and Chandrasekharan, 2012). Over the years, several studies conducted both in developing and developed countries all point out that, there have been some significant changes in the antibiogram of common aetiological agents of septicaemia (Ayoola *et al.*, 2003; Tsering *et al.*, 2011).

Since their discovery, antimicrobial therapy has proven remarkably effective for the control of bacterial infections (Newman *et al.*, 2011). Some bacterial pathogens were later found to

be insusceptible to antimicrobial therapy. Some pathogens rapidly became resistant to many of the first effective drugs.

The emergence of resistance to antimicrobial agents is a global public health problem, particularly in pathogens causing nosocomial infections (Sheril and Chandrasekharan, 2012). Drug resistance tends to carry more significance in developing countries, where treatment options are limited and lack of resources constrain implementation of surveillance. In Africa, the incidence of bacterial resistance is known to be very high, probably due to the poor enforcement of drug policies on antibiotic usage (Mayanja *et al.*, 2010). Antimicrobial resistance results in increased and prolonged illness, deaths, and health-care costs (Sheril and Chandrasekharan, 2012). To develop effective guidelines for empirical antimicrobial treatment, knowledge of the type of etiologic agents and the pattern of antibiotic resistance are fundamental (Berkley, 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The laboratory work was carried out at the clinical laboratory of the Kintampo Health Research Center (KHRC), which serves as the clinical research laboratory located in the Kintampo North Municipality. The clinical laboratory (KHRC) is well-equipped with modern facilities required for excellent patient care. Including the Bacteriology Unit, there are currently six active sections within the laboratory.

Samples from patients living in the Kintampo North Municipality and Kintampo South District were selected for this study based on their geographic location, similarity in climatic conditions, population density and structure.

The Kintampo North Municipal and Kintampo South district are located within the forest-savanna transitional ecological zone in the Brong-Ahafo region (figure 1) and have an elevation ranging between 60 and 150 m above sea level. The area under study is located between Latitudes 7°43′N and 8°44′N and Longitudes 1°25′W and 2°1′W respectively. Kintampo North Municipality and Kintampo South District have a total area of 7,162 km^2 and a resident population of approximately 140,000 people. Majority of the population in this area are farmers but some members of the population also engage in commerce.

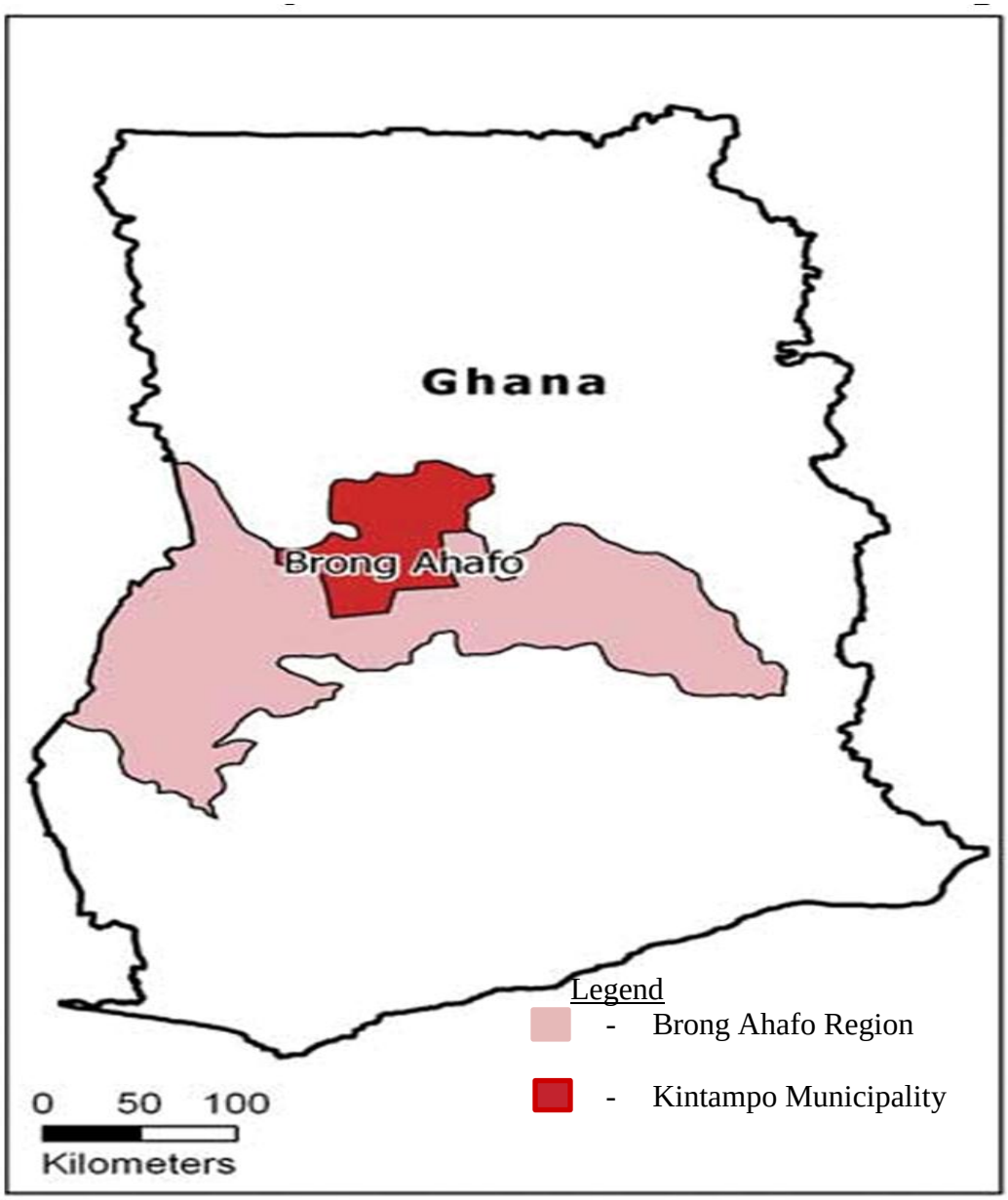

Figure 1: Map of Ghana showing the Brong Ahafo Region (Source: Kintampo Health Demographic and Surveillance System)

The Kintampo Health Research Centre (KHRC) has a Health and Demographic Surveillance System (HDSS) that records and keeps detailed demographics of all residents including pregnancies, births, deaths and migrations (in and out) at a 4 month interval (Owusu-Agyei *et al.*, 2012). Socio- economic status of individuals within the vicinity is also updated every two years. Also, all compounds in these areas have been digitized making the selection and tracing of individuals during research to their homes easy.

3.2 SAMPLE SIZE

It was anticipated that septicaemia prevalence in the Kintampo Municipality will be 19%. At a 95% confidence level and power of 80%, a total of three hundred and ninety eight (398) patients with positive blood isolates will be required to be sampled from this population for this study.

For a one sample test of proportion where the null hypothesis is $P=P_0$ and the alternative hypothesis is $P=P_{A,}$ where the required sample size using (normal approximation) is

$$\mathrm{N} = \left(\frac{z_{1-\frac{\alpha}{2}}\sqrt{P_0(1-P_0)} + z_{1-\beta}\sqrt{P_A(1-P_A)}}{P_{A-}P_0} \right)^2$$

Sampling from this population, I hypothesized that at least 19% positive of septicaemia will be obtained with significant level of 5% (2 sided) and power of 80%, the minimum sample size required is 409 positive blood isolates from children under five in this study, using STATA sample calculator.

Sample size calculation was done using STATA version 12 (Stata Corp, College Park,

TX, USA) as shown below:

Sample size 0.14, 0.19, power (0.80) one sample

Estimated sample size for one-sample comparison of proportion to hypothesized value

Test Ho: p = 0.1400, where p is the proportion in the population

Assumptions:

α (Alpha) = 0.0500 (two-sided)

β (Statistical power)= 0.8000

P_A (Alternative P) = 0.1900

Estimated required sample size:

n = 409 positive isolates

3.3 PARTICIPANT SELECTION

Children less than 5 years of age were recruited into the study. The cases were those with clinical symptoms of septicaemia. The Control group was randomly selected from children who attended the hospital at the same period with other forms of illnesses aside septicaemia. The cases and the controls were age- and sex-matched.

3.4 ELIGIBILITY CRITERIA

Children less than five years, whose caregivers had consented to participate in the study, and presented with the following characteristics were recruited:

1. Clinically diagnosed with septicaemia
2. High (>38°C) or low (<36°C) body temperature

3.5 EXCLUSION CRITERIA

Patients with the following characteristics were excluded from the study:

1. Caregivers unwilling to consent
2. Children with normal body temperature
3. Children above five years of age
4. Children who were not clinically diagnosed with septicaemia

3.6 STUDY DESIGN

A cross-sectional study was carried out at the clinical laboratory of the Kintampo Health Research Center.

3.7 ETHICAL CONSIDERATION

Approval to undertake this study was sought from the ethical review committees of the University of Ghana medical school and Kintampo Health Research Centre.

Written informed consent was duly obtained from caregivers of participants/patients before any activity in the study was started. Each participant was assigned unique identification number to ensure confidentiality of patient information. All relevant source documents used for the study were kept in a secured cabinet.

3.8 DATA MANAGEMENT AND ANALYSIS

Laboratory data collected were entered into a logbook and subsequently transferred onto Microsoft excel spread sheet and finally analyzed using STATA version 12 (Stata Corp,

College Park, TX, USA) statistical software. Demographic information of participants/patients were obtained from the Kintampo Health Demographic Surveillance System (KHDSS) database and as well as additional data collected from the communities of the participants. Information on durable household assets used as proxy for household socio-economic status of caregivers was also collected and linked to existing data of participants on the KHDSS database.

Descriptive statistics such as "means" were used to summarize quantitative variables such as age after examining their distributions, whilst proportions were used to describe categorical and binary variables. Cross-tabulation was used to explore the distributions of pathogens with other categorical variables such as age groupings. A univariate logistic regression model was used to identify relationships between septicaemia and other explanatory variables. All explanatory and confounding variables that were found to be significantly associated with septicaemia ($p<0.05$) from the univariate regression model were adjusted for in a multivariate logistic regression model.

To assess the nutritional status of the children, the Z-scores of the anthropometric indices weight-for-age (underweight), weight-for-height (wasting) and height/length-for-age (stunting) were calculated using 2006 WHO child growth standards. The cut-off points were Z-scores of ±2 as suggested by WHO in 1997. The socio - economics status of the caregivers were obtained by assessing their durable assets.

3.9 PROCESSING OF STORED AND FRESH BLOOD SAMPLES

Both stored and fresh isolates from blood samples from children with septicaemia were used in this analysis. Majority of the isolates used were from stored isolates. In all, three hundred and eighty six (386) stored isolates and only six (6) were fresh blood samples. Single organisms were recovered from patients at a time; no multiple organisms were isolated from these patients. Although, patients with recurrent episodes of the infection were observed.

3.9.1 Sample Processing from Freshly Collected Blood Cultures

1-3ml of venous blood was withdrawn from children less than five years of age who were suspected to have septicaemia and transferred into a culture vial (paeds plus) and incubated in the BACTEC (9050) automated machine. Positive blood cultures were detected 24- 48hrs and vials were declared negative after five days.

3.9.2 Processing Stored Isolates from Previously Suspected Cases of Septicaemia

Bacteria isolates from positive blood cultures suspended in 20% glycerol broth and stored at -80°C freezer were brought to room temperature before use. Using a sterile loop, two to three loopful of blood culture isolates stored in glycerol broth were inoculated into 5mls brain heart infusion broth and incubated in 5- 10% CO_2 at 35°C for 18- 24hrs. Culture plates were then observed for visible growth after incubation. The size and shape, type of elevation, margin, colour and type of haemolysis if any, were observed and recorded to aid in identification.

3.10 CULTURE AND IDENTIFICATION

Isolates were identified using their Gram stain reactions and confirmed by the pattern of biochemical reactions using standard procedures. Smear for Gram stain was prepared by adding a drop of saline onto a clean labeled slide. A desired colony was then picked with the aid of a straight wire and added to the saline to obtain a uniform mixture. The smear was then allowed to air- dry at room temperature and then heat fixed. Gram staining was done using standard procedure (see Appendix 1).

3.10 BIOCHEMICAL TEST

Members of the enterobacteriaceae and other Gram negative rods were identified based on their biochemical reactions using the BBL (BD, Maryland, USA) automated system of identification. Basic tests such as motility, production of indole and the use of cytochrome oxidase also aided in identification.

Gram positive bacteria were identified using the coagulase and catalase test. Catalase activities were detected with BD catalase reagent droppers (BD, Maryland, USA), according to the manufacturer's instruction (see Appendix 3).

The beta- haemolytic and alpha-haemolytic *Streptococci* group were differentiated using bacitracin and optochin disc test respectively. Streptococcal grouping kits (Oxoid, Basingstoke, England) were used to identify streptococci belonging to the Lancefield group A,B,C,D,F or G. Staphylococcal agglutination kits (Oxoid, Basingstoke, England) were used to differenciate *S. aureus* from *Staphylococcus intermedius* and coagulase

negative staphylococci. These tests were performed based on the manufacturer's instructions.

3.11 QUALITY CONTROL

Media prepared in-house and reagents were controlled using known bacteria isolates from the American Type Culture Collection (ATCC) strains (see Appendix 13). Both positive and negative controls were set up. A test organism was cultured onto a plate from a batch of freshly prepared media and incubated overnight. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used to test the quality of Gram stain reagents used.

ATCC strains such as *S. aureus* (ATCC 25923), *E.coli* (ATCC 25922), S. *pneumoniae* (ATCC 49619) , *H. influenzae* (ATCC 49247), *Salmonella* Typhimurium (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853) were set up together with antibiotics (Oxoid, Basingstoke, England) and E- tests strips (bioMerieux SA, Marcy l'Etoile, France) to determine their potency.

3.12 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed on both fresh and stored blood culture bacteria isolates. Antimicrobial susceptibility pattern was determined by the Kirby-Bauer disc diffusion method. The minimum inhibitory concentration of antibiotics was also determined using E- test strips. Sensitivity testing was performed on sensitivity test agar, chocolate agar and blood agar. Organisms such as *Haemophilus influenzae* and the

Streptococcus pneumoniae were cultured on chocolate and blood agar respectively. Multidrug resistance was described as resistance to amoxicillin, cotrimoxazole and chloramphenicol.

3.12.1 Preparation of inoculum

An inoculum was prepared from pure and fresh culture plate, using four to five isolated colonies of bacteria to make a homogeneous suspension in 2ml sterile saline (see Appendix 14).

The turbidity of the inocula was compared to a 0.5 McFarland standard solution (see Appendix 2) using a black card board to aid visualization. Adjustments were then made using sterile saline to obtain a 0.5 McFarland.

3.12.2 Inoculation and application of antibiotic discs

The entire surface of the sensitivity test agar (Oxoid, Basingstoke, England) plate was inoculated using sterile swab sticks. The swab stick was dipped into the inoculum suspension and inoculation was done by swabbing four times, rotating swabbing each time and allowing the plate to stand for at least three minutes before application of the antimicrobial disc.

Excess suspension was removed from the swab stick by gently rotating the swab against the surface of the tube containing the inoculum. The inoculated plates were left at room temperature to dry for 3-5 minutes before disc containing antibiotics were placed onto the

surface of a sensitivity test agar, blood agar or chocolate plate: Six sets of antimicrobial disc were kept onto the plate (10mm x 15mm) at 4mm apart using sterile forceps and placed gently onto the media. Discs were placed firmly onto agar plate using sterile forceps.

3.12.3 Incubation and plate reading

Plates were incubated at 35° C for 18 - 24 hrs. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a ruler and the organisms were classified as susceptible, intermediate or resistant according to the standardized table supplied by the CLSI (2010).

3.12.4 Minimum Inhibition Concentration (MIC)

Epsilometer test strips (bioMerieux SA, Marcy l'Etoile, France) were used for the determination of minimum inhibition concentration (MIC) of the antimicrobial drugs. E-tests strips that were available for use include amoxicillin clavulanic acid, ampicillin, amikacin, ceftriaxone, cefuroxime, ciprofloxacin, imipenem and vancomycin. Sensitivity test agar was used for the organisms that did not require any special nutritional requirements. Blood agar and chocolate agar were used for organisms that had additional nutritional requirements.

3.12.4.1 Inoculum preparation for e-test

Desired single colonies from fresh and pure culture were emulsified in 2ml sterile saline. The turbidity of the inoculum was compared to a 0.5 McFarland standard solution. The inoculum was then adjusted to obtain a turbidity of 0.5 McFarland.

3.12.4.2 Inoculation

A sterile cotton swab was dipped into the inoculum suspension and pressed against the inside wall of the tube to remove excess fluid. The entire agar surface was swabbed evenly in four directions. The agar surface was allowed to dry completely before the application of e-test strips.

3.12.4.3 Application of e-test strips

The E-test strip (bioMerieux SA, Marcy l'Etoile, France) was applied onto the surface of the media with the concentration gradient scale facing upward using sterile forceps.

3.12.4.4 **Incubation and reading**

Plates were incubated aerobically at 35°C for 18- 24 hours in an inverted position. Values of the minimum inhibitory concentration were read where the edge of the inhibition ellipse intersected the strip (figure 2); results obtained were then interpreted according to the CLSI (2010) guidelines.

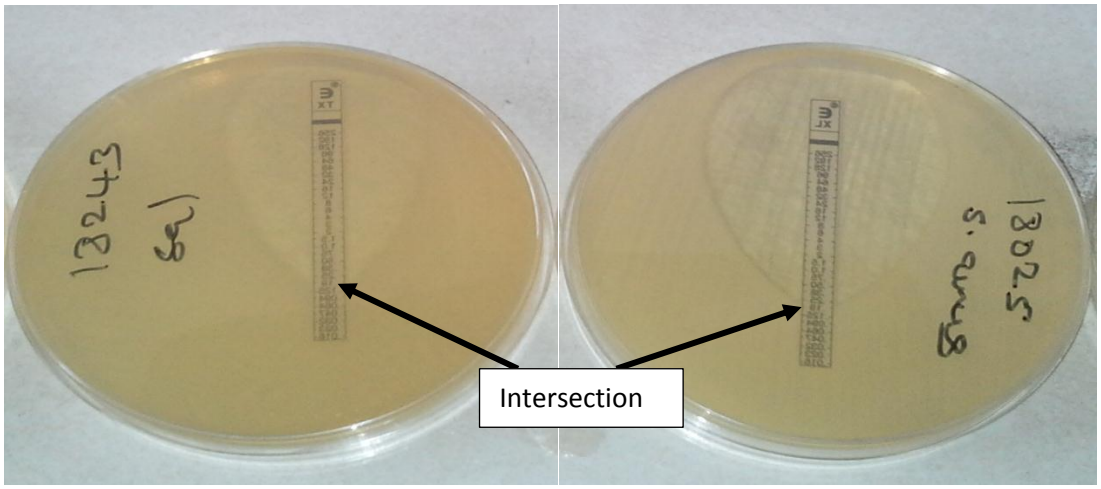

Figure 2: Levels of sensitivity exhibited by identified organisms against different antimicrobials (using E-test strip)

CHAPTER 4

RESULTS

4.1 ENROLMENT AND DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION.

In total, 1,965 children below five years who were admitted to the children's ward of the Kintampo Municipal Hospital between 2008 and 2013 were included in this study. Three hundred and ninety two (392) had positive blood cultures and 261 of these were considered pathogens (figure 3). In 97 children, blood culture isolates were considered contaminants.

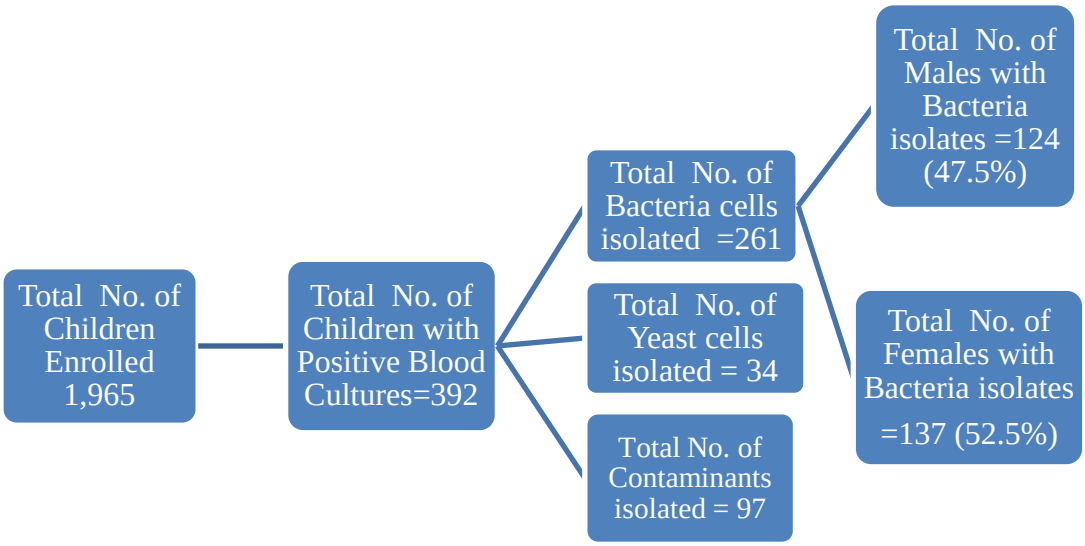

Figure 3: Flow chart showing the enrolment of children

A higher proportion of the children recruited were males 1048 (53.6%) whiles 956 (46.4%) of the number were females. Of the 1,956 patients enrolled, the total number of

males who had septicaemia during this period was 124 (47.5%); females were 137 (52.5%). The mean age of all the children was 24.6 months (SD = 11.9).

4.2 COMMON AETIOLOGICAL AGENTS ISOLATED FROM BLOOD

Overall, non typhoidal *Salmonellae* 111 (42.5%) organisms constituted the predominant bacterial pathogens. *Staphylococcus aureus* isolates were second highest 101 (38.7%) followed by *Salmonella* Typhi 12 (4.6%) and *Streptococcus pneumoniae* 10 (3.8%). These were the four organisms accounting for most of the septicaemia cases as illustrated in Table 2.

Haemophilus influenzae was the only isolate identified in children below one month. The most common organisms isolated from children between 1- 11 months were *Staphylococcus aureus* (50.0%) and non typhoidal *Salmonellae* (26.9%). *Streptococcus pneumoniae* (9.6%) was also found among this age group. Among the 12- 23 months category, non typhoidal *Salmonellae* and *S. aureus* were found to be the major causes of septicaemia in children representing 41.7% and 44.4% respectively. Other organisms such as *S. pneumoniae* (2.8%) and *S. viridans* (4.2%) were also found in children within this age group. Organisms found among children between age 24 and 35 months included NTS (54.6), *S. aureus* (31.8%) and *S.* Typhi (5.7%) isolates whiles *S. agalactiae* (1.1%), *Enterococcus sp.* (1.1%) and *Enterobacter sp*. (1.1%) were the least.

Table 2: Organisms isolated from blood culture

		Age Groups (months)					
Pathogen Isolated (n= 261)	**n (%)**	**<1 n = 1**	**1-11 n = 52**	**12-23 n = 72**	**24-35 n = 88**	**36-47 n = 33**	**48-60 n = 15**
Non typhoidal *Salmonellae*	111 (42.5)	0 (0.0)	14 (26.9)	30 (41.7)	48 (54.6)	15(45.5)	4 (26.7)
Staphylococcus aureus	101 (38.7)	0 (0.0)	26 (50.0)	32 (44.4)	28 (31.8)	11(33.3)	4 (26.7)
Salmonella Typhi	12 (4.6)	0 (0.0)	0 (0.0)	1 (1.4)	5 (5.7)	4 (12.1)	2(13.3)
Streptococcus pneumoniae	10 (3.8)	0 (0.0)	5 (9.6)	2 (2.8)	2 (2.3)	1 (3.0)	0 (0.0)
Escherichia Coli	4 (1.5)	0 (0.0)	1 (1.9)	1 (1.4)	0 (0.0)	0 (0.0)	2 (13.3)
Viridans streptococci	4 (1.5)	0 (0.0)	1 (1.9)	3 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)
Haemophilus influenzae	4 (1.5)	1 (100)	2 (3.9)	0 (0.0)	0 (0.0)	1 (3.0)	0 (0.0)
Pseudomonas aeruginosa	3 (1.2)	0 (0.0)	1 (1.9)	1 (1.4)	0 (0.0)	0 (0.0)	1 (6.7)
Enterobacter sp.	3 (1.2)	0 (0.0)	0 (0.0)	1 (1.4)	1 (1.1)	0 (0.0)	1 (6.7)
Enterococcus sp.	2 (0.77)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	1 (3.0)	0 (0.0)
Listeria monocytogenes	2 (0.77)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.3)	0 (0.0)	0 (0.0)
Klebsiella pneumoniae	1 (0.4)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella oxytoca	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)
Pseudomonas sp.	1 (0.4)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Serratia marcescens	1 (0.4)	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)
Streptococcus agalactiae	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)

Overall, the number and frequency of organisms causing septicaemia decreased among children within the age category 36 – 47 months; 33.3% and 45.5% for *S. aureus* and NTS, respectively (Table 2). In older children (48-60 months age group), NTS (26.7%), *S. aureus* (26.7%) and *E.coli* (13.3%) were also isolated.

4.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING BY DISC DIFFUSION

Susceptibility of the four most frequently isolated organisms to antimicrobial agents tested is listed Table 3. More than 50% of the non typhoidal S*almonellae* isolates showed resistance towards amoxicillin (87.5%), cotrimoxazole (63.9%), ampicillin (57.7%) and chloramphenicol (56.3%). Ceftriaxone (5.9%) and ciprofloxacin (3.0%) were among the antimicrobial agents that showed decreased resistance to the NTS isolates. Imipenem did not show any resistance to the non typhoidal *Salmonellae* isolates (3.0).

Salmonella Typhi showed decreased resistance to ceftriaxone (9.1%), ceftazidime (9.1%) and cefotaxime (9.1%). Most of the *S.* Typhi isolates were however resistant to cotrimozaxole (100%), chloramphenicol (90%) and ampicillin (90%). No resistance was observed in Imipenem to the *S.* Typhi isolates.

Chloramphenicol (78.9%), ampicillin (65.9%), cotrimoxazole (60.8%) and amoxicillin were not very effective against *Staphylococcus aureus*.

Table 3: Antimicrobial susceptibility profiles of the four most frequently isolated pathogens**.**

Antimicrobials	**NTS** (n = 111) Resistant (%)	***S. aureus*** (n = 101) Resistant (%)	***S. pneumoniae*** (n = 10) Resistant (%)	***Salmonella ser.* Typhi** (n =12) Resistant (%)
Amoxicillin	19/36 (52.8)	9/15 (60.0)	0/2 (0.0)	7/8 (87.5)
Ampicillin	30/52(57.7)	27/41 (65.9)	0/1 (0.0)	9/10 (90.0)
Amoxicillin Clavulanic Acid	15/78 (19.2)	10/54 (18.5)	0/4 (0.0)	2/10 (20.0)
Amikacin	2/72 (2.8)	0/2 (0.0)	-	0/11 (0.0)
Gentamicin	3/102 (2.9)	10/76 (13.2)	3/4 (75.0)	0/10 (0.0)
Erythromycin	-	14/63 (22.2)	1/8 (12.5)	-
Vancomycin	-	0/60 (0.0)	0/7 (0.0)	-
Chloramphenicol	45/80 (56.3)	41/52 (78.9)	1/5 (20.0)	9/10 (90.0)
Clindamycin	-	4/54 (7.4)	1/7 (14.3)	-
Ciprofloxacin	3/96 (3.03)	1/55 (1.82)	0/4 (0.0)	0/12 (0.0)
Cotrimoxazole	53/83 (63.9)	49/74 (60.8)	5/6 (83.3)	10/10 (100)
Imipenem	0/74 (0.0)	1/5 (20.0)	-	0/11 (0.0)
Cefuroxime	5/36 (13.9)	4/16 (25.0)	0/2 (0.0)	2/9 (22.2)
Cefoxitin	3/69 (4.3)	9/51 (17.6)	0/3 (0.0)	2/11 (18.2)
Ceftriaxone	5/85 (5.9)	11/58 (18.9)	0/7 (0.0)	1/11 (9.10)
Ceftazidime	11/100 (11.0)	1/3 (33.3)	-	1/11 (9.10)
Cefotaxime	5/84 (5.95)	1/2 (50.0)	-	1/11 (9.10)

Antimicrobial agents such as erythromycin (22.2%), cefoxitin (17.6%), clindamycin (17.4%) and gentamicin (13.2%) showed lower resistance to *S. aureus*. No resistance was observed in vancomycin and amikacin to the *S. aureus* isolates. Most of the *Streptococcus pneumoniae* isolates were seen to be resistant to cotrimoxazole (83.3%) and gentamicin (75.0%). Vancomycin, ceftriaxone and amoxicillin clavulanic acid did not show any resistance to the *S. pneumoniae* isolates. Clindamycin (14.3%) and erythromycin (12.5%) however showed low resistance to *S. pneumoniae.*

Multidrug resistance (resistance to amoxicillin, chloramphenicol and cotrimoxazole) were found in the four main organisms isolated, 58.6% and 63.4% of NTS and S. *aureus* isolates respectively were multidrug resistant. Multidrug resistance (MDR) were found in 50% (5/10) of *S. pneumoniae* isolates. All the *S*. Typhi isolates were multidrug resistant.

4.3.1 MINIMUM INHIBITORY CONCENTRATION OF THE MAJOR ISOLATES

All NTS isolates were susceptible to ceftriaxone (MICs ≤ 1 μg/ml) and imipenem (MICs ≤ 4 μg/ml) (Table 4). Ninety seven point eight percent (97.8%) of non typhoidal *Salmonellae* isolates were sensitive to Ciprofloxacin (MICs ≤ 1 μg/ml). From table 5, 23 (39.7%) of *S. aureus* isolates were sensitive to ampicillin (MICs ≤ 0.25 μg/ml) and all isolates were sensitive to cefuroxime (MICs ≤ 8 μg/ml). Most isolates of S. Typhi were sensitive to the antibiotics tested. All these isolates were sensitive to ciprofloxacin (MICs ≤1), imipenem (MICs ≤4) and ampicillin (MICs ≤8). A total of 91.7% of the S. Typhi isolates were however sensitive to ceftriaxone (Table 6)

Table 4: MIC results for NTS (n=91)

Antimicrobial Agents	MIC (µg/ml) Interpretive criteria			Sensitive N (%)	Intermediate N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)			
Ampicillin (AM)	≤ 8	16	≥ 32	91 (100)	0 (0.0)	0 (0.0)
Amoxacillin Clavulanic Acid (XL)	≤ 8/4	16/8	≥ 32/16	88 (96.7)	1 (1.1)	2 (2.2)
Cefuroxime (XM)	≤4	8-16	≥ 32	75 (82.4)	16 (17.6)	0 (0.0)
Ceftriaxone (TX)	≤1	2	≥ 4	91 (100)	0 (0.0)	0 (0.0)
Ciprofloxacin (CL)	≤1	2	≥ 4	89 (97.8)	2 (2.2)	0 (0.0)
Imipenem (IP)	≤4	8	≥ 16	91 (100.0)	0 (0.0)	0 (0.0)

Table 5: MIC results for *Staphylococcus aureus* (n= 58)

Antimicrobial Agents	MIC (µg/ml) Interpretive criteria			Sensitive N (%)	Intermediate N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)			
Ampicillin(AM)	≤ 0.25	-	≥ 0.5	23 (39.7)	-	35 (60.3)
Amoxacillin Clavulanic Acid (XL)	≤ 4/2	-	≥ 8/4	56 (96.6)	-	2 (3.5)
Cefuroxime (XM)	≤ 8	16	≥ 32	58 (100)	0 (0.0)	0 (0.0)
Ceftiaxone (TX)	≤ 8	16-32	≥ 64	55 (94.8)	1 (1.7)	2 (3.5)
Vancomycin (VA)	≤ 2	4-8	≥ 16	57 (98.3)	1 (1.7)	0 (0.0)

Table 6: MIC results for *Salmonella* Typhi (n= 12)

Antibiotics	MIC (µg/ml) Interpretive criteria			Sensitive N (%)	Intermediate N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)			
Ampicillin(AM)	≤ 8	16	≥ 32	12 (100)	-	0 (0.0)
Amoxacillin Clavulanic Acid (XL)	≤ 8/4	16/8	≥ 32/16	12 (100)	-	0 (0.0)
Cefuroxime (XM)	≤4	8-16	≥ 32	12 (100)	-	0 (0.0)
Ceftriaxone (TX)	≤1	2	≥ 4	11 (91.7)	-	1 (8.3)
Ciprofloxacin (CL)	≤1	2	≥ 4	12 (100)	-	0 (0.0)
Imipenem (IP)	≤4	8	≥ 16	12 (100)	-	0 (0.0)

4.4 BLOOD CULTURE CONTAMINATION RATE IN THE STUDY CHILDREN

Coagulase negative *Staphylococci* 48/ 392 (12.2%) were the major contaminants isolated during the period of study followed by *Bacillus sp.* 40/392 (10.2%) as shown in figure 4. None of these CoNS were isolated from neonates.

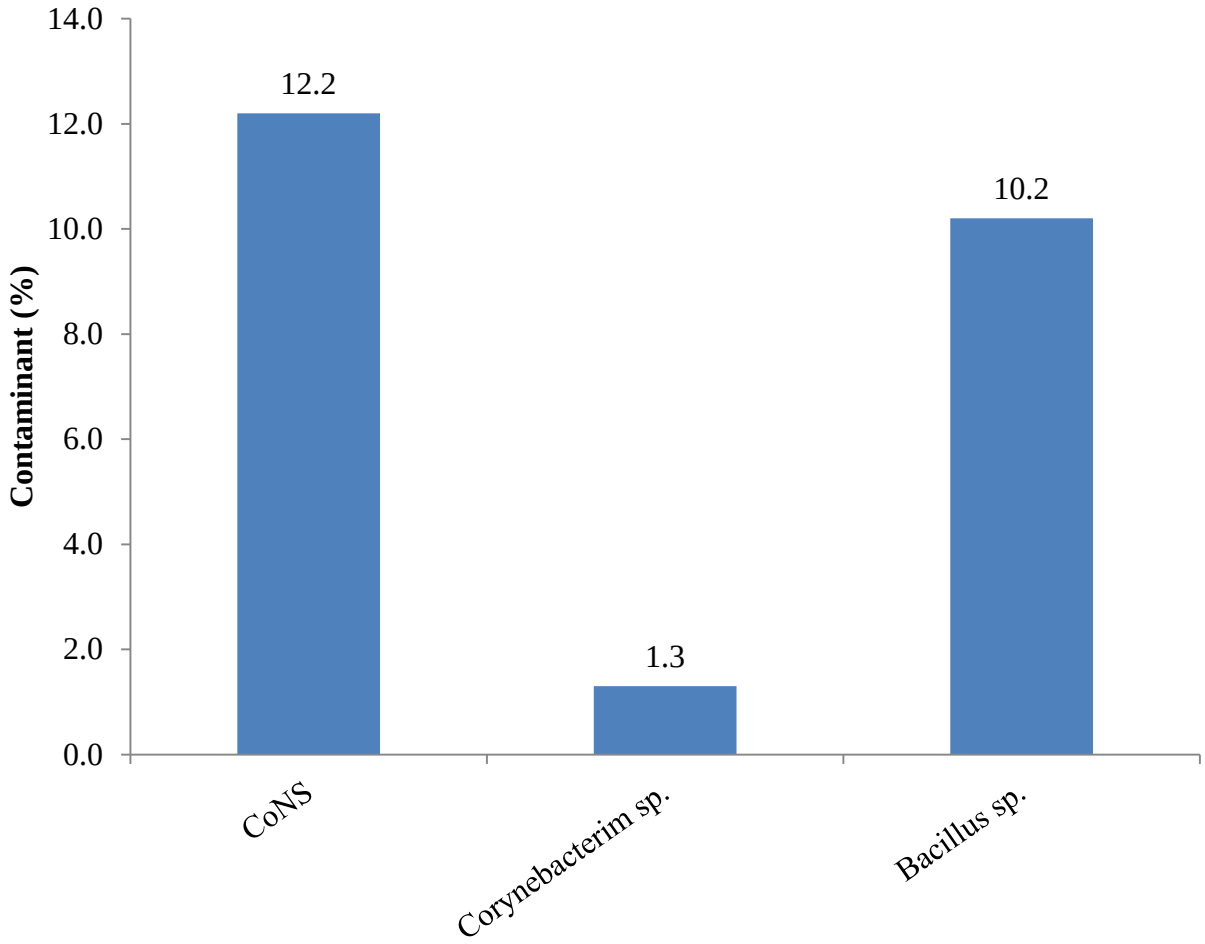

Figure 4: Bacteria isolates considered as contaminants in blood.

4.5 RISK FACTORS ASSOCIATED WITH DEVELOPING SEPTICAEMIA

Risk factors associated with developing septicaemia, were considered using sex, age, parasitaemia, underweight, wasting, stunting and socio-economic status. Univariate and multivariate logistic model were separately analysed (Table 7). Gender was not associated with septicaemia in the patients (OR = 1.36. CI: 0.84- 2.19, P = 0.22). Children above 5 months old were found to have reduced risk of septicaemia. Malaria was also found to be a significant risk factor in the univariate analysis, but this association was attenuated in multivariate analysis after adjustment of other factors. With respect to socioeconomic status, only children from rich background had reduced risk to septicaemia. Also children who were underweight had a higher risk of developing septicaemia (OR = 3.99. CI: 1.77 – 9.01, P = <0.01).

Table 7: Association of anthropometrical parameters, wealth index and malaria parasitaemia with septicaemia in children below five years of age in Kintampo.

	Participants with Septicaemia	Unadjusted logistic regression			Adjusted logistic regression		
Variables	**Yes (%)**	**UOR**	**95% CI**	**P**	**AOR**	**95% CI**	**P**
Sex (n=275)							
Male	49 (41.5)	1			-	-	-
Female	77 (49.0)	1.36	(0.84, 2.19)	0.22	-	-	-
Age in months(n=)							
<3	7 (87.5)	1			1		
3-5	3 (50.0)	0.14	(0.01, 1.99)	0.15	0.19	(0.01, 4.13)	0.28
6-11	9 (29.0)	0.10	(0.01, 0.55)	0.01	0.10	(0.01, 0.70)	0.03
12-23	50 (41.0)	0.10	(0.01, 0.83)	0.03	0.10	(0.01,0.66)	0.02
24-60	53 (51.0)	0.15	(0.02, 1.25)	0.07	0.10	(0.01, 0.66)	0.02
Parasitaemia							
Absent	77 (41.5)	1			1		
Present	49 (41.5)	1.79	(1.02, 3.15)	0.04	1.87	(0.85, 4.09)	0.12
Height for age z-score							
[a] No	106 (51.7)	1			-	-	-
[b]Yes	12 (60.0)	1.40	(0.55, 3.57)	0.48	-	-	-
Weight for age z-score							
[a]No	91 (52.3)	1			1		
[b]Yes	27 (29.7)	2.60	(1.52, 4.46)	<0.01	3.99	(1.77, 9.01)	<0.01
Weight for height z-score							
[a] No	7 (31.8)	1			-	-	-
[b]Yes	105 (54.4)	2.56	(0.99, 6.55)	0.05	-	-	-
Wealth index							
Very rich	17 (44.7)	1			1		
Rich	8 (20.5)	0.32	(0.11, 0.87)	0.03	0.26	(0.10, 0.78)	0.02
Poor	15 (38.5)	0.77	(0.31, 1.91)	0.58	0.60	(0.22,1.62)	0.31
More poor	18 (51.4)	1.31	(0.52, 3.29)	0.56	1.03	(0.38, 2.77)	0.95
Very poor	23 (53.5)	1.42	(0.59, 3.41)	0.43	1.04	(0.40, 2.72)	0.93

[a] Does not meet WHO (2006) growth criteria; [b] Met WHO (2006) growth criteria. UOR:Unadjusted Odds Ratio, AOR: Adjusted Odds Ratio, CI: Confidence Interval, P: p- value

CHAPTER 5

DISCUSSION

In this study, four organisms were found to be predominant (81.2%) in the blood culture isolates and these were, non typhoidal *Salmonellae*, S*taphylococcus aureus, S.* Typhi and *S .pneumoniae*. Nielsen *et al*., (2012) had similar findings in a study conducted between 2007 and 2009 in Ghana. In most developing countries, NTS are commonly isolated from paediatric blood cultures. The results from this study showed that greater than 40% of the total isolates were non typhoidal *Salmonellae*. This is comparable with findings of Kariuki *et al* in 2006 who also found NTS (51.2%) as the predominant cause of septicaemia among Kenyan children below five years. This also confirms results from a study conducted in Kumasi by Evans *et al* (2004), showing 43% of NTS in children with initial diagnosis of severe malaria. Suggestions for the high incidence of NTS among young children may be due to their high vulnerability as results of factors such as decreased gastric acidity or immaturity of gut lymphoid tissue (Evans *et al.*, 2004; Hung *et al.*, 2009). This finding emphasizes the need for increased awareness and knowledge about cleanliness and environmental sanitation for healthy living in the communities. From this study, highest levels of NTS infections were observed in children, after the period of breastfeeding, possibly marking the onset of intake of contaminated water and weaning food (Nielsen *et al.*, 2012a). In contrast to findings from this study, Hill *et al* in 2007 found 8.6% of NTS among Gambian children with bloodstream infections.

Staphylococcus aureus emerged as the second most common isolate greater than 38% in this study. More than half the number of *S. aureus* was found in children less than 2

years of age but none in the neonatal period which contradicts results from Agogo by Nielsen *et al* (2012). From their study, majority of *S. aureus* (26.1%) were found in children below one month (Nielsen *et al.*, 2012a). In Nigeria, Awoniyi *et al* (2009) also found *S. aureus* to be the commonest organism found in neonates (28.0%).

The current study agrees to findings from other developing countries which suggest that *S. aureus* is a major aetiological agent of septicaemia (Evans *et al.*, 2004; Meremikwu *et al.*, 2005; Hill *et al.*, 2007; Kizito *et al.*, 2007; Komolafe and Adegoke, 2008). These studies suggest that infections by this organism constitute a significant threat to child survival in these areas and other developing country settings. In contrast to findings from this study, a systemic review on bloodstream staphylococcal infections in some parts of Africa has been shown to be about 9.9% ((Maitland *et al.*, 2006; Reddy *et al.*, 2010).

The 3.8% *Streptococcus pneumoniae* obtained in this study is much higher than others reported in Nigeria (Komolafe and Adegoke, 2008) and Ghana (Evans *et al.*, 2004) where fewer children were recruited in those studies. Nielsen *et al* (2012) however reported lower prevalence in Ghana, and the success of the PCV- 13 pneumococcal conjugate vaccine against pneumococcal disease can be attributed to this lower rates.

In contrast to the current study, Brent *et al*., in 2006 found 50% *Streptococcus pneumoniae* in 1,093 children at the Kilifi District Hospital. This high incidence could

be attributed to the criteria used for selecting study participant. (Brent *et al.*, 2006). The high prevalence rate by Brent *et al* (2006) of 50% is rather disturbing.

Also, Maitland *et al*., (2006) obtained 35% *S. pneumoniae* in a study involving 920 children in Kenya which disagrees with the 3.8% obtained in this present study. Most of the Kenyan children enrolled in their study were malnourished children and since they are mostly exposed to invasive infections could have accounted for these high numbers.

An effective way to manage severe and invasive bacterial infections in Africa can be achieved through vaccination (Peltola, 2001; Tristram et al., 2007), vaccination successes had resulted in the virtual elimination of Haemophilus influenzae type b infection in the Gambia (Adegbola et al., 2005) and significant reduction of the Hib infections in Ghana (Renner et al., 2007).

Salmonella Typhi accounted for 4.6% of all the invasive blood stream infections in children in this study. This conforms to studies in Ghana and other parts of the world. Komolafe found 4.9% *S.* Typhi among children in Nigeria. *Salmonella* Typhi infections can be life-threatening and treatment options are often limited due to the emergence of drug resistant strains in regions with high use of antibiotics (Brent *et al.*, 2006; Akinyemi *et al.*, 2007).

Resistance of *S.* Typhi and NTS to ciprofloxacin have been reported by (Parry and Threlfall, 2008) and (Zaidi *et al.*, 2013) contrary to the findings in this study. As

observed from the current study, all isolates of *S.* Typhi and NTS were sensitive to Imipenem. Ceftriaxone was found to be effective against all four major organisms. These antimicrobials, Imipenem and ceftriaxone have been on the Ghanaian market for a short period of time and relatively expensive compared to chloramphenicol, amoxicillin and ampicillin as reported by Newman *et al*., (2011). These factors could account for their effectiveness.

Results of MIC from this study has shown that the activities of ciprofloxacin, Imipenem, ceftriaxone, ampicillin and amoxicillin clavulanic acid *in-vitro* are still very effective at lower concentrations and could be used for the treatment Gram negative bacterial infections (Tables 4 and 6). *S.* Typhi showed remarkable susceptibility to ciprofloxacin (100%), imipenem (100%) by disc diffusion method which was confirmed by E Test (MIC ≤1) and (MIC ≤4) respectively (Table 6). More than 90% of NTS and *S*. Typhi showed susceptibility to ampicillin *in-vitro* and could probably be used in the absence of ciprofloxacin. Majority of the *S. aureus* isolates however, were resistant to ampicillin (MIC ≥ 0.5µg/ml) (Table 5). Ampicillin may lead to treatment failure in patients and therefore should not be used for the treatment of Staphylococcal systemic infections. Ceftriaxone (MIC ≤ 8) and cefuroxime (MIC ≤ 8), (vancomycin ≤ 2) were very effective to most of the *S. aureus* isolates (Table 5) and could still be used for the treatment of Staphylococcal infections.

Amoxicillin clavulanic acid was also seen to be active against the four predominant organisms as confirmed by MIC for NTS, *S. aureus* and *S*. Typhi. Therefore ceftriaxone,

ciprofloxacin or amoxicillin clavulanic acid can be used for empirical treatment in children suspected of septicaemia.

Antimicrobial resistance among the four organisms was high in this study. NTS showed low susceptibility to four of the antimicrobial agents namely, cotrimoxazole, chloramphenicol, ampicillin and amoxicillin and these are comparable to results obtained by Sigauque *et al*., (2009). They reported 74% of NTS resistant to ampicillin, cotrimoxazole (66%) and chloramphenicol (54%). *S*. Typhi showed exceptionally high resistance to cotrimoxazole (100%), chloramphenicol (90%) and ampicillin (90%) compared to the work by Florian *et al.*, 2010, which showed more than 70% resistance to chloramphenicol, cotrimoxazole and ampicillin. These are the same agents that (Uwe Groß *et al.*, 2011; Newman *et al.*, 2011; Nielsen *et al.*, 2012a) have also been reported as having low activity against various organisms. This could be the result of misusing these drugs. Though, in this study the organisms exhibited low resistance to ciprofloxacin, several studies report a worrying trend of increased resistance to the flouroquinolones by these organisms. The mechanism of quinolone resistance has been associated with the upregulation of efflux pumps which regulate, pump out quinolones and other antimicrobials out of the bacterial cell (Newman *et al.*, 2011). Also, mutations in the quinolone resistance determining regions of *gyrA* and *parC* are common resistance mechanisms indicating that resistance to one flouroqunolone may extend to other flouroquinolones (Mills-Robertson *et al.*, 2003; Namboodiri *et al.*, 2011). Control of the use of these antibiotics is very important to maintain their effectiveness.

Commonly prescribed antimicrobials such as chloramphenicol (< 70%), ampicillin (< 65%) and cotrimoxazole (< 60%) were not very effective against *Staphylococcus aureus* in this study. Resistance of *S. aureus* to ampicillin in this study was confirmed by the MIC. Komolafe and Adegoke in 2008 also reported that all *S. aureus* isolates in their study were resistant to ampicillin and cotrimoxazole. This study established that both ciprofloxacin and ceftriaxone were active against NTS, *S. aureus*, *S.* Typhi and *S. pneumoniae*.

Antimicrobial resistance is increasingly becoming a problem in Ghana and other countries in Africa (MacPherson *et al.*, 2009; Newman *et al.*, 2011; Uwe Groß *et al.*, 2011) because it leads to treatment failures. From this current study, it has been established that multidrug resistance (MDR) amongst NTS (58.6%), and *S.* Typhi (100%) were very high which corroborates the results obtained by Nielsen *et al*., 2012 who found 77% and 75% MDR in NTS and *S.* Typhi respectively . Kariuki *et al*., in 2006 identified that 59% of NTS isolates were MDR which conforms to findings from this study. All non typhoidal *Salmonellae* and *S.* Typhi isolates were seen to be sensitive to ciprofloxacin which is in agreement with findings elsewhere (Uwe Groß *et al.*, 2011; Nielsen *et al.*, 2012a). The problem of MDR could be reduced by active surveillance, drug sensitivity surveys and the introduction of vaccines against typhoid fever. Though vaccination against typhoid has not been found effective in Ghana and it is not practiced nationwide.

None of the patients recruited in the current study were admitted in the intensive care unit, so all coagulase negative Staphylococci (CoNS) were considered contaminants. At the end of the study, coagulase negative *Staphylococci* were seen as the major contaminants. CoNS being normal skin commensals are common contaminants of blood. This organism usually has little pathogenicity in immunocompetent hosts but is capable of causing infections in premature neonates. Usually, the initial step in the pathogenicity of CoNS would involve adherence of the bacteria to skin, mucosal surfaces, or indwelling artificial devices, such as intravascular catheters and central nervous system shunts, which are commonly used in preterm infants. In recent times, reports of CoNS causing neonatal bloodstream infections are on the increase (Isaacs, 2003; Gwee *et al.*, 2012). A ten year study on neonatal bloodstream infections by Isaacs *et al* (2003) showed that coagulase negative *Staphylococci* were responsible for 1281 cases, comprising more than 57.1% of all late onset infections (Isaacs, 2003). It is to be noted that, none of the CoNS isolated in this study were from the intensive care unit or infants less than 28 days.

Discussions on the routine antimicrobial treatment in children with signs of severe malaria have been extensively discussed, although a clear association between malaria and bacteremia has not been proven (Berkley *et al.*, 1999; Oundo *et al.*, 2002). Oundo *et al*., (2002) proposed that during sequestration in messentric vessels *Plasmodium* parasites make these vessels porous and hence open them up to invasion by salmonella from the gut. The Salmonella finds a medium rich in iron which it needs both for growth and virulence. This explains why an occurrence of both NTS and *Plasmodium* leads to

severe illness and high mortality (Oundo *et al.*, 2002). Although an association between septicaemia and parasitaemia was observed in this present study, it was not statistically significant ($p = 0.12$) which agrees to findings from (Oundo *et al.*, 2002) and (Mackenzie *et al.*, 2010). Nielsen's study also did not observe any positive association between NTS infection and *P. falciparum* parasitaemia.

According to Nielsen *et al*. (2012), children in the circle of poor socio-economic status, malnutrition and a tendency to develop systemic infection due to weak immune response may have both, a higher risk of severe malaria and of complicating bacterial co-infection. In this study, significant association was observed between septicaemia and children from rich background. Children from very rich background had a reduced risk of developing the infection. An association with septicaemia and underweight was also observed in this study. However, this association was not significant ($P < 0.01$) which contradicts findings of Nielsen *et al*. (2012).

The study had some limitations. Due to time factor, the study concentrated more on stored isolates and would have missed new suspected cases of septicaemia at the hospital. Though fresh blood samples were collected, only single blood culture samples were performed from each patient instead of the recommended three (3).

Most of the children with septicaemia may have had coexisting infections such as meningitis and pneumonia. Collection of clinical samples, other than blood, was not done in the present study. The study did not include fungi.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The study identified non typhoidal *Salmonellae*, *Staphylococcus aureus*, *S.* Typhi and *S. pneumoniae* as the predominant aetiological agents of septicaemia in children below five years in the Kintampo North Municipality and Kintampo South District. Given the high incidence of septicaemia in this area, the disease should be considered as an important childhood illness in paediatric patients admitted at the Kintampo Municipal Hospital. Although most of the antimicrobial drugs tested proved active to the organisms, multidrug resistance was observed to be emerging among the antimicrobials tested. High level of resistance to chloramphenicol, ampicillin, amoxicillin and cotrimoxazole which are easily purchased over the counter was seen in the isolates. Misuse and self-prescribed antimicrobial drugs could be monitored through antibiotic surveillance policies. Increasing resistance to amoxicillin clavulanic acid as a result of over prescription of this drug by medical practitioners to patients in this locality has been observed.

CoNS, *Bacillus species* and *Corynebacterium species* were the known contaminants at the end of the study. Most of these contaminants can be found in the environment and could probably mean that disinfection before blood draw was not properly done. CoNS is a known skin contaminant that can easily be introduced into blood cultures during drawing blood and can be mistakenly treated as a pathogen especially when the child

suffers from pyrexia of unknown origin. This problem can be reduced if proper and thorough disinfection of the skin prior to blood sampling.

6.2 RECOMMENDATIONS

Aetiological agents change with time and therefore, there is the need for a continuous review of the common agents of septicaemia in this locality for effective treatment. Continuous monitoring of the activity of antimicrobials and their local patterns of susceptibility is also very important for the management of the disease.

Also, there is the need of constant and continuous training of hospital staff such as the phlebotomist, nurses and doctors on the right procedure of blood sample collection to reduce the levels of contaminations in blood.

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APPENDIXES

APPENDIX 1: Gram Stain Procedure

Reagents:

Crystal violet (BD, Maryland, USA)

Gram's iodine (BD, Maryland, USA)

Acetone/ alcohol; 50/50 (BD, Maryland, USA)

Safranin (BD, Maryland, USA)

Quality Control Used:

Escherichia coli (ATCC 25922) for Gram negative bacteria

Staphylococcus aureus (ATCC 25923) for Gram positive bacteria

Control slides were stained together with test slides to ascertain the quality of Gram stain reagents.

Gloves were worn throughout the procedure.

1. Prepared smear was heat fixed by passing it through a flame three times
2. Fixed smear was flooded with crystal violet stain for 30-60 seconds.
3. The crystal violet stain was then washed off with clean water (tap water).The slide was then tilted to tip off all remaining water.
4. The smear was then flooded with Lugol's iodine for 30-60 seconds and washed off with clean water (tap water).
5. Decolorization was done rapidly (few seconds) with acetone/alcohol (50/50) after flooding slide with Gram's iodine
6. The Gram decolorizer was washed immediately with clean water.

7. Following decolorization, slide was flooded with safranin stain for 30 seconds.
8. The stain was then washed off with clean water and allowed to dry on a rack.
9. The back of the stained slide was blotted with filter paper, and placed on a staining rack to air dry.
10. Slides were examined first with X40 objective lens to observe the staining and the distribution of material and later with X100 oil immersion objective lens to observe the type bacteria and cells present.

APPENDIX 2: Preparation and use of 0.5 McFarland Standard Solution

Reagents:

Deionized water, Concentrated H_2SO_4 and $BaCl_2$ salt.

Materials:

3 volumetric flask (100ml), 0.5ml volumetric pipettes and Spectrophotometer.

Procedure:

1. 1.175% (w/v) of $BaCl_2$ salt was prepared by dissolving 1.175g of the salt in deionized water and made up to the mark in a 100ml volumetric flask.
2. 1.0ml of Concentrated H_2SO_4 was added to 90ml of deionized water in a volumetric flask and made up to the mark with more deionized water.

3. 0.5ml of 1.175% $BaCl_2.2H_20$ was added to 85ml of 1% (v/v) H_2SO_4 in a 100ml volumetric flask. This was made up to the mark with more 1% H_2SO_4 and swirled gently to facilitate reaction.
4. The solution was checked for homogeneity and absence of visible clumps. The optical density was determined at a wavelength of 625nm and found to be approximately 0.09.
5. Volumes were dispensed into small sterilized Bijoux bottles, capped and stored in the cupboard at room temperature.

How to use standard:

Turbidity of inoculums were compared to the McFarland Standard in front of a Wickerham card and adjusted until the turbidity appeared to be the same to the unaided eye using the lines of the card as a guide for standard turbidity.

APPENDIX 3: Catalase Test

Principle:

The enzyme catalase is produced by bacteria that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. Most aerobic and facultative anaerobic neutralizes the bactericidal effects of hydrogen peroxide and protects them. However, anaerobes generally lack this catalase enzyme.

Quality Control Used:

Staphylococcus aureus (ATCC 25923) was used as a positive control

S. epidermidis (ATCC 12228) was used as a negative control.

Procedure:

Using a disposable inoculating needle, a desired colony of a primary culture was transferred onto a clean glass slide.

A drop of 3% hydrogen peroxide (BD Catalase Reagent Droppers) was added to the smear on the slide. The slide was immediately examined for the rapid production of gas bubbles.

Results interpretation;

The rapid and sustained appearance of bubbles was considered as a positive test. Few tiny bubbles forming after 20-30 seconds was considered as a negative test.

No bubble formation is considered a negative test.

APPENDIX 4: Coagulase Test

Principle:

Coagulase is an enzyme that clots blood plasma and the formation of clots around an infection caused by a bacterium likely protects it from phagocytosis. This test is mostly performed on *Staphylococcus aureus* and other Gram-positive bacteria that are catalase positive. The enzyme coagulase is a virulence factor of *S. aureus.* This bacterium *is* known to produce coagulase, which can clot plasma into a gel - like form in a tube or agglutinate cocci on a slide. Most strains of *S. aureus* produce two types of coagulase, free coagulase and bound coagulase. Free coagulase is an enzyme that is secreted extracellularly, and the bound coagulase is a cell wall associated protein. Free coagulase is detected in tube coagulase test and bound coagulase is detected in slide coagulase test.

Slide coagulase test may be used to screen isolates of *S. aureus* and tube coagulase may be used for confirmation. This test is also useful in differentiating *S. aureus* from other coagulase-negative staphylococci.

Quality Control Used:

Positive Control: *Staphylococcus aureus* (ATCC 25923)

Negative Control: *S. epidermidis* (ATCC 12228)

Procedure:

Using the slide method to detect bound coagulase

1. A drop of physiological saline was put on two separate slides labelled 1 and 2.
2. Two to three pure colonies of the suspected organism was emulsified on both slides.
3. A drop of rabbit plasma was added to one of the suspensions on the slide labelled 1 and gently mixed.
4. Clumping was then observed within 10 seconds.
5. The suspension on the slide labelled 2 was observed for any granular appearance. This step was used to differentiate any granular appearance of the organism from the true coagulase clumping.
6. Clumping within 10 seconds was interpreted as bound coagulase produced by *S. aureus*.
7. No clumping within 10 seconds were interpreted as coagulase negative *Staphylococcus* or no bound coagulase produced.

APPENDIX 5: Cytochrome 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:

Indirect paper strip technique was used.

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.

APPENDIX 6: Indole Test

Principle:

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

Quality Control Used:

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

Negative Control: *Klebsiella pneumoniae* (ATCC 700603)

Procedure:

The test organism was inoculated into MIO medium and incubated at 35ºC for 18 to 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.

APPENDIX 7: Simmons Citrate Test

Principle:

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

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

Citrate, a Krebs cycle intermediate, is generated by many bacteria; however, utilization of exogenous citrate requires the presence of citrate transport proteins. Upon uptake by the cell, citrate is cleaved by citrate lyase to oxaloacetate and acetate. The oxaloacetate is then metabolized to pyruvate and CO_2. Further metabolic breakdown is dependent upon the pH of the medium. Under alkaline conditions, pyruvate is metabolized to acetate and formate.

The carbon dioxide that is released will subsequently react with water and the sodium ion in the medium to produce sodium carbonate, an alkaline compound that will raise the pH. In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source.

Procedure:

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

Interpretation:

Citrate positive: growth will be visible on the slant surface and the medium will be an intense Prussian blue. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green color to blue.

Citrate negative: trace or no growth will be visible. No color change will occur; the medium will remain the deep green color of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the

Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant.

APPENDIX 8: 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 9: MIO (Motility Indole Ornithine) Test

Principle:

MIO medium is used for identification of Enterobacteriaceae on the basis of motility, indole production and ornithine decarboxylation in a single tube, where motility is observed as a diffused zone of growth flaring out from the line of inoculation. Indole test is carried out to determine the ability of an organism to split indole from tryptophan by the tryptophanase enzyme. On reaction with Kovacs reagent, indole combines with the colour in the alcohol layer, which is visualized as a red ring. If the test organisms possess the specific decarboxylase enzyme, then ornithine is decarboxylated to putrescine, an amine, resulting in a subsequent rise in the pH of the medium towards alkalinity. This causes the pH indicator bromocresol purple to change from purple to yellow colour.

Procedure:

MIO medium was prepared according to 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 middle of the tube with a stab motion using a wire. Inoculated tubes were incubated for 18 to 24hrs at 35°C in an ambient condition with the cap of the tube were placed loosely. Tubes were examined at 18 - 24 hours for growth, color change, and motility.

Three to four drops of Kovac's reagent was later added after determining motility and ornithine decarboxylase reactions.

Interpretation

Indole Test

Indole positive: Appearance of a pink or red color

Indole negative: formation of yellowish ring or no color change.

Motility Test

Motility is indicated by turbidity of the medium or growth extending from inoculating stab line.

Ornithine Production

A purple colour throughout the medium indicates a positive ornithine reaction.

A yellow colour change in the medium indicates a negative reaction.

Indole is detected by adding Kovac's Reagent to the surface of the medium. A pink or red color indicates an indole-positive culture. Indole is produced from the tryptophan present in the medium.

APPENDIX 10: Staphylococcal Agglutination Test (Using Staphytech Plus, BD)

Principle:

Differentiation between coagulase positive and coagulase negative *Staphylococci* has been performed either with tube coagulase tests that detects extracellular staphylocoagulase or the slide coagulase tests that detects the clumping factor present on the bacterial cell wall. Approximately 97% of human strains of *S. aureus* are said to possess both bound and extracellular staphycoagulase.

Protein A, found on the cell surface of human strains of *S. aureus* has the ability to bind the Fc portion of IgG. Some strains of MRSA express low levels of Protein A and clumping factors that are not easily detected. These strains possess capsular polysaccharides which can mask both protein A and the clumping factor thereby preventing agglutination.

Staphytech Plus (BD, Maryland, and USA) uses blue latex particles coated with porcine fibrinogen and rabbit IgG including specific polyclonal antibodies raised against capsular polysaccharides of *S. aureus.*

When the reagent is mixed on a card with colonies of *S. aureus*, rapid agglutination occurs through the reaction between (i) fibrinogen and clumping factor, (ii) Fc portion of IgG and protein A ,(iii) specific IgG and capsular polysaccharide.

Quality Control Used:

Positive Control: *S.aureus* (ATCC 25923)

Negative Control: *S. epidermidis* (ATCC 12228).

Procedure:

(Latex reagents were brought to room temperature prior to the start of the test procedure)

1. A drop of test latest was dispensed onto one of the circles on a reaction card provided by the manufacturer.
2. A drop of control latest was dispensed onto another circle on the same card.
3. Using a sterile loop, two to three suspected *S. aureus* colonies were picked and smeared in the control latex reagent.

4. With a separate sterile loop, suspected colonies of *S. aureus* were picked and mixed with the test latex reagent.

   (Suspected colonies of *S. aureus* were spread to cover the circles of both the control and test procedure)

5. The reaction card was rocked for 20 second, followed by a thorough examination for agglutination.

Results:

A result is positive if agglutination of the blue latex test particles occurs within 20 seconds and presumptively identifies the test organism as *S. aureus*.

A negative result is obtained if no agglutination remains occurs and a smooth blue suspension remains after 20 second in the test circle. This shows that the test organism is not *S. aureus*.

APPENDIX 11: Streptococcal Grouping Test

Principle:

This is based on the principle releasing of specific antigen from bacteria cell walls by modified nitrous extraction.

Streptococcal grouping (Pro-Lab Diagnostics, Texas, USA) method involves chemical extraction of group specific carbohydrate antigens using specially developed nitrous acid extraction reagents (Extraction reagent 1 and 2). The extraction regents contain a chemical substance that is able to extract the streptococcal grouping antigens at room temperature. An extraction reagent known as neutralizing solution (Extraction reagent 3), contains polystyrene latex particles sensitized with purified group specific rabbit immunoglobulins. This extraction reagent has the ability identify neutralized extracts.

These latex particles agglutinate strongly in the presence of homologous antigens and hence, there will be no agglutination when homologous antigens are absent.

Quality Control Used:

Reagents:

Extraction reagent 1

Extraction reagent 2

Extraction reagent 3

Reagents for Streptococcal groups A, B, C, D, F, G and H

Procedure:

Streptococcal grouping test was done according to manufacturer's instructions.

1. A pure colony of the test organism was put into a labelled test tube.
2. A drop of extraction reagent 1 was added to the test tube.
3. A drop of extraction reagent 2 was added to the suspension in the test tube.
4. The suspension was then taped gently to mix completely and later incubated at room temperature for two minutes.
5. Seven drops of extraction reagent 3 was added to the suspension after 2 minutes.
6. Through mixture of the suspension was done by vortexing for 10 – 15 seconds.
7. Using a sterile Pasteur pipette, a drop of the suspension was transferred onto separate circles on the test card.
8. A drop each of reagents labelled A to G was added to each suspension on the test card separate sterile wooden sticks were used to mix the reagents and suspensions thoroughly on the test card.
9. The test card was gently rocked for two minutes for possible agglutination.

10. Agglutination that was observed after two minutes was considered false positive.

APPENDIX 12: BBL crystal identification system (BD, USA)

Principle:

The BBL crystal identification system is based on microbial utilization and degradation of specific substrates detected by various indicator systems. Fermentation reactions detect the ability of an isolate to metabolize carbohydrates in the absence of atmospheric oxygen, and oxidative reactions are based on the ability of an organism to metabolize the substrate with oxygen as the final electron acceptor. Both reactions are usually detected by the use of a PH indicator in the test substrate. Chromogenic substrates upon hydrolysis produce colour changes that can be detected visually.

The BBL crystal identification system is incorporated with tests that detect the ability of an organism to hydrolyze, degrade, reduce or otherwise utilize a substrate in the BBL identification system. Enteric/ non- fermenter kits was used for the identification of the enterobacteriaceae group.

The BBL crystal identification kits comprises of the BBL crystal panel lids, BBL crystal bases and BBL inoculum fluid.

The lid contains thirty dehydrated substrates on the tips of the plastic wells and the bases have thirty reaction wells.

Procedure:

The test inoculum was prepared by suspending two to three pure colonies of the test organism into the BBL inoculum fluid. The inoculum fluid was then adjusted to a 0.5 McFarland.

All the wells in the base were completely filled with the inoculum fluid. The lids were then aligned to the base and snapped tightly. This step allows the test inoculum to rehydrate the dried substrates and initiate the test reactions.

The Gram negative identification kit was then incubated in a 5% CO_2 at 35°c for 18 – 24hrs.

The kit was then inserted into BBL auto reader for identification.

Gram positive tests kits were incubated for four hours at 35°c in the presence of 5% CO_2.

APPENDIX 13: Quality Control of Media Prepared in- House

	Positive Control	Negative Control
Types Of Media		
Sensitivity Test Agar	*E. coli* (ATCC 25922)	*H. influenzae* (ATCC 49247)
Blood Agar	*S. aureus* (ATCC 25923)	-
MacConkey Agar	*E. coli* (ATCC 25922)	*S. pneumoniae* (ATCC 49619)
Chocolate Agar	*H. influenzae* (ATCC 49247)	-
Brain Heart Infusion	*S. pneumoniae* (ATCC 49619)	-
Triple Sugar Iron	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 25923)
Motility indole Ornithine	*E. coli* (ATCC 25922)	*Kleb. pneumoniae* (ATCC 25923)
Simon's Citrate	*C. freundii* (ATCC 8090)	*E.coli* (ATCC 25922)
Urea Broth	*Kleb pneumoniae* (ATCC 700603)	*E.coli* (ATCC 25922)
Nutrient Agar	*S. aureus* (ATCC 25923)	*H. influenzae* (ATCC 49247)
S.S Agar	*S. boydii* (ATCC 9207)	*S. epidermidis* (ATCC 12228)

APPENDIX 14: Preparation of Inoculum

1. One to two pure colonies of the test organism was suspended into a test tube containing 2ml sterile physiological saline. This was done by touching the surface of the desired colonies with a sterile straight loop.
2. A homogeneous mixture was obtained by touching the inner wall of the test tube containing the saline with a loopful of the test organism to make a paste. The test tube was swirled afterwards to mix the saline and paste together.
3. The turbidity of the prepared inoculum was compared using a 0.5 McFarland standard solution.
4. The turbidity was visualized using a black cardboard at the background.
5. The turbidity of the inoculum was then adjusted until it reached the 0.5 McFarland standard.

APPENDIX 15: Antimicrobial Agents Disc concentrations

Antimicrobial Agents	**Disc Concentration** (µg)
Amikacin	30
Ampicillin	10
Amoxycillin Clavulanic Acid	30
Amoxycillin	25
Cefoxitin	30
Ceftazidime	30
Cetriaxone	30
Cefotaxime	30
Cefuroxime	30
Chloramphenicol	30
Ciprofloxacin	5
Clindamycin	2
Erythromycin	5
Gentamicin	10
Imipenem	10
Trimethoprim/Sulfamethoxazole	25
Vancomycin	30

APPENDIX 16: Questionnaire

KINTAMPO HEALTH RESEARCH CENTRE SEPTICAEMIA STUDY	FORM No.	FORMNO

1. BASIC INFORMATION:

1.1 Subject ID:…… **SCNUM**

1.2 Treatment ID……. **TNUM**

Child's KHDSS Perm_ID
1.3 KHDSS Perm_ID:……. **CHILDID**

1.4 Compound ID:…… **CPDID**

1.5 Community Name:…… **COMM**

1.6 Date Of Birth…… **DOB**

1.7 Gender:……. 1. Male 2. Female **SEX**

Care giver's KHDSS Perm_ID
1.8 KHDSS Perm_ID:…… **CAREID**

1.9 Date Of Birth:…… **CAREDB**

1.10 Gender:…… 1. Male 2. Female **GENDER**

1.11 Date of Interview:……. **DATE**

1.12 Staff Code…… **STAFF**

1. <u>MEDICAL HISTORY OF MOTHER</u>

2.1 Child mothers gestational age. 1. Preterm 2. Term 3. Post term **GEST**

2.2 Mode of delivery:…… 1. Normal 2. CS (Operation) **DELIVER**

2.3 Child's birth weight……. | | | | **WEIGHT**

2.4 Maternal fever within two weeks prior to delivery:……. | 1.Yes | 2. No | **FEVER**

2.5 Did mother experience premature rupture of membrane during pregnancy | 1. Yes | 2. No | **PROM**

2.6 Did you attend any antenatal clinic during pregnancy:…… | 1.Yes | 2. No | **ANTE**

2.7 How often were the visits:…… | 1.Regular | 2. Occasional | 3. NA | **VISIT**

(Check 2.0 from mother's child welfare card)

2. SOCIO-DEMOGRAPHIC CHARACTERISTICS:

3.1 Date of birth: (***dd/mm/yyyy***)…… | | | | | | | | | **DOB**

[Enter 88 if dd=NK, 88 if mm=NK, 2088 if yyyy=NK]

1. What is your age? (in complete years) ***[Enter age=88 if age=NK]***……..... | | | **AGE**

2. What is your highest educational level? **MEDLEV**

1. None	2. Primary school	3. Middle/JHS
4.Technical/Commercial/ SHS	5. Post-middle training – teachers, secretarial, etc	
6. Post-sec training – Nursing, Teacher, Polytechnic, etc.	7. University	

3. Number of years completed at the highest educational level [Enter 00 if No education ie ***(3.3) =1]*.....** | | | **NUMYRS**

4. What is your marital status? **MARRIED**

1. Married	2. Living together with a man, unmarried	3. Widowed
4. Divorced	5. Separated	6. Single, unmarried

5. What is your religion? **RELIGION**

1.Catholic	2. Protestant	3. Pentecostal/ Charismatic	4. Muslim	5. Traditional/ Spiritualist	6. None	7. Other, specify ……..

6. Do you own any land? ……

Yes	No

WOWNLAN

1. Do you have land on which you farm?

1. Yes, my own	2. Yes, part of family land	3. Yes part of husband's	4. Yes, rented land	5. No

FARMLN

2. Which crops do you mainly grow on your land?

1. Food items, mainly for home consumption	2. Food items, mainly for sale on the market	3. Cash crops: tobacco, cashew, cocoa, etc.	9. NA, no farm

CROPS

3. Do you have a regular cash income/are you a salaried worker?

1. Yes, Professional: teacher, nurse, accounts, administrator etc	2.Yes,Clerical/secretarial	3. Yes, trader/food seller/ businesswoman	
4. Yes, seamstress, hairdresser etc.	5. Yes, Farmer/labourer/ domestic worker	6. Other: Specify ……..	7. No

SALARY

THE HOUSEHOLD

Ask participant about their household by explaining what a household is.

4. Who is the household head?

1. Self (Respondent)	2. Husband	3. Father	4. Mother	5. Other, Specify …….

HOUSEHE

1. Date of birth of household head: (dd/mm/yyyy)…
[Enter 88 if dd=NK, 88 if mm=NK, 8888 if yyyy=NK]

HHDOB

2. How old is the household head now (in complete years)? [888= NK]……

HHAGE

3. What is the household head's highest educational level?

1. None	2. Primary school	3. Middle/JHS	
4.Technical/Commercial/SHS	5. Post-middle training – teachers, secretarial, etc		
6. Post-sec training – Nursing, Teacher, Polytechnic, etc.		7. University	8. NK

HHMEDLEV

4. Number of years completed at the highest educational level by household head…….

[Enter 00 if No education ie (2.16)=1]

		HHNUMYRS

5. Does the household head know how to read/write?.

1. Yes	2. No	8. NK	**HHREAD**

6. Does the household head have a regular cash income or salaried job?

1. Professional – teacher, nurse, accounts, administrator etc.	2. Clerical / Secretarial	3. Trader / businessman / driver with own car etc.	4. Employed tradesman, driver without own car, builder, etc.	**HHSALARY**
5. Farmer/labourer/domestic worker	6. Other: Specify……	7. No	8. NK	

7. Do other members of the household do any farming?.....

1. Yes	2. No	**HHFARMIN**

8. Does anyone else in the household own any land?.....

1. Yes	2. No	**HHOWNLAND**

9. Does anyone else in the household own their own farm?.....

1. Yes	2. No	**HHOWNFARM**

10. What is the main source of drinking water for members of your household?

11. Piped into home/compound	12. Public tap	13. Hand pump/ closed bore hole	14. Closed well	15.Open well	16. Stream/ river	**WATER**
17. Lake/dam/pond	18. Water trucks	19. Rain water	20. Sachet/ "Pure water"	21.Bottled water	22. Other, specify ……...	**WATEROTH**

3.24 What kind of toilet facility does your household have?

1. Flush latrine / WC	2. Ventilated improved pit (VIP) /KVIP	3. Other pit latrine	**TOILET**
5. Defecates in house but faeces transferred elsewhere / bucket latrine	5. Open fields	6. Other: Specify ……...	**TOILETOTH**

1. Do you own/rent the house you live in, or have another type of arrangement, such as "perching"?

1. Sole Ownership	2. Joint Ownership	3. Family/relation's house	4. House provided rent free	**OWNHOUSE**
5. Renting	6. Perching	7. Other: Specify……		**OWNHOUSOTH**

Materials used in the construction of the house [observe, if possible]

2.	Floor of sleeping room….....	1. Cement	2. Mud/clay	3. Other:…..….....	**FLOOROTH**
3.	Roofing of sleeping room.....	1. Metal/asbestos	2. Thatch/mud/wood	3. Other:….....	**ROOFOTH**
4.	Wall of sleeping room….....	1. Cement	2. Mud	3. Other…….....	**WALLOTH**

5.	What source of fuel do you use for cooking:	1. Electric	2. Gas	3. Firewood	4. Cow dung	**FUEL**
		5. Charcoal	6. Saw dust	7. Other Specify……		

3.30 Did you sleep under Insecticide Treatment Mosquito Net (ITN) last night	1. Yes	2. No	**ITN**

<u>END OF INTERVIEW.</u>

APPENDIX 17: Consent form

KINTAMPO HEALTH RESEARCH CENTRE AETIOLOGICAL AGENTS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN SEPTICAEMIA IN CHILDREN UNDER FIVES IN KINTAMPO. Information and Informed Consent Form	FORM No.

I agree to take part in the above research project. I have had the project explained to me and I have read the explanatory statement. I understand that agreeing to take part means that I am willing to:

- Be interviewed by the researcher
- Allow the researcher collect my child's blood sample.
- Allow the researchers access to my health records and process information obtained

Explanatory Statement

Septicaemia (germs in the blood) is an infection that causes death in children in both developing and developed countries. Most children are admitted at the hospital as result of germ in the blood. There are several types of germs that cause this condition. A type of germ called bacteria are the most common cause of life-threatening disease in hospitalized patients. Bacteria and the bad chemicals they produce cause changes in the body temperature, heartbeat, and BP (blood pressure), and can result in body's organs working badly. Individuals with germs in their blood are given drugs called antibiotics to kill the germs. However in most people, the drugs cannot kill the germs in the blood because

they have been misused. It is thus important that we understand how germs respond to the drugs (antimicrobial susceptibility patterns). This will enable doctors to give the right and effective drugs for treatment.

Procedure

A small amount of blood (2-3 tablespoons) of venous blood will be taken from your child's arm or ankle and transferred into a special bottle containing nutrients (culture bottle).

•Basic information about your child will be recorded by qualified medical staff

•A small amount of blood (1-2 tablespoons) will be drawn from a vein in your arm (or your child's arm) by a clinician or qualified laboratory staff to be used for blood.

Risk

There is no risk involved in this procedure since blood samples will be collected by qualified personnel.

Benefits

If your child is found to have some positive laboratory results, the results will be given to a clinician who will counsel you and refer to the Kintampo North municipal and South district hospital for appropriate treatment.

Data protection

I understand that all information I provide is confidential and no information that could lead to the identification of any individual will be disclosed in any report or to any Party. To keep the confidentiality of your child, blood samples will be labeled with a study

number, and not your name. All records identifying your child will be kept confidential. If the results are published, you or your child's identity will remain confidential.

Statement of Consent:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and questions I have asked have been answered to my satisfaction. I consent voluntarily to participate (or for my child to participate) as a subject in this study and understand that I have the right to withdraw from the study at any time or decline to answer any questions without it affecting my further medical care. I will be given a copy of this consent form.

Name of participant or Guardian of Child:

……

Signature/Thumbprint of Participant/Guardian of Child:

…….Date…….

Child's name (if applicable)

……..

Witness' name: ……Signature:

……... Date: …….…….

Name of Investigating Team Member: …… Signature:

…..……... Date: …….

Contact: If you have questions concerning this study you may contact Prof. Kingsley Twum – Danso (0243 837817) and Dr. Japhet A. Opintan (0244 789209) all of the Department of Microbiology, UGMS. You can also contact Dr. Seth Owusu- Agyei (0244 560213), Dr. Kwaku Poku Asante (0244 377539) and Miss Louisa F. Iddrisu (0244 469868) all of the Kintampo Health Research Center.