PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

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School of Medical Sciences, College of Health Sciences

By

ALEX AGYEKUM

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, KUMASI

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DECLARATION

I declare that this thesis is my own work towards the award of an MPhil in Clinical Microbiology it does not contain any materials previously published by another person. This work has not been submitted for the award of any other degree of any university, except where due acknowledgement has been made in the text.

…………………………………	……………………	……………………..
ALEX AGYEKUM (STUDENT)	SIGNATURE	Date

…………………………………	……………………	……………………..
PROF. YAW ADU-SARKODIE (SUPERVISOR)	SIGNATURE	DATE

…………………………………	……………………	……………………..
PROF. YAW ADU-SARKODIE (HEAD OF DEPARTMENT)	SIGNATURE	DATE

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DEDICATION

I dedicate

this work to God,

my family and friends.

ABSTRACT

Bloodstream infection is a frequent cause of morbidity and associated with mortality in excess of 25%. We aimed to prospectively determine the prevalence of bacteria causing bacteraemia in children under five years of age in a rural African area and their antimicrobial susceptibility patterns. Between 1st January 2008 and 31st December 2008 we studied children under five years of age with medical cases admitted to the Agogo Presbyterian hospital in the Asante Akim North District. One to three (1-3) mls of venous blood were taken from all children admitted in the ward into Becton Dickinson BACTEC™ PEDS PLUS™ culture vials and transported immediately to the laboratory. They were incubated in the BACTEC 9050. Identification of isolates was done by subculturing, cultural morphology, gram staining, biochemical methods, API from biomerieux and several serological testing. Susceptibility testing was by the Kirby-Bauer disk diffusion method and by measuring Minimum Inhibition Concentration (E-test) using the CLSI guidelines. We documented pathogens identified and their susceptibility patterns. During the study period, 1356 patients were admitted and had a blood culture taken. 304 (22.4%) had a positive result and 207 (15.3% overall) were considered a genuine pathogen and 97 (7.1%) were contaminants (Coagulase negative *Staphylococci* and other skin organisms). Four organisms accounted for 60.9% of bacteraemias: Non typhoid salmonellae (35.2%), *Streptococcus pneumoniae* (10.2%), *Staphylococcus aureus (7.9%) and Salmonella* Typhi (7.6%). Majority of non typhoid salmonellae isolates were resistant to chloramphenicol (80.4%), Ampicillin (81.3%) and co-trimoxazole (77.6%), however they were all susceptible to ciprofloxacin and ceftriaxone. Most of the *Klebsiella pneumoniae* (60%) and *Escherichia coli* (50%) produced Extended Spectrum Beta lactamases (ESBLs). Four (16.7%) methicillin resistant *Staphylococcus aureus* (MRSA) isolates were identified. Ceftriaxone and ciprofloxacin turned out to be the best choice as empiric antibiotic therapy. Our study underlines the significance of bacteraemia in this population. Further surveillance of the incidences and the susceptibility patterns of the bacterial pathogens is necessary.

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ABBREVIATIONS

SAM	Ampicillin + Sulbactam
AM	Ampicillin
API	Analytical Profile Index
AST	Antimicrobial Susceptibility Testing
PM	Cefepime
PML	Cefepime + Clavulanic acid
CT	Cefotaxime
CTL	Cefotaxime + Clavulanic acid
TZ	Ceftazidime
TZL	Ceftazidime + Clavulanic acid
TX	Ceftriaxone
CL	Chloramphenicol
CI	Ciprofloxacin
CM	Clindamycin
CLS	Clinical and Laboratory Standard Institute
CoNS	Coagulase Negative Staphylococci
CHRPE	Committee on Human Research Publication and Ethics
TS	Cotrimoxazole
DNAse	Deoxyribonuclease
EM	Erythromycin
ESBL	Extended-Spectrum β-Lactamase
KATH	Komfo Anokye Teaching Hospital
MRSA	Methicillin Resistant *Staphylococcus aureus*

Abbreviation	Meaning
MIC	Minimum Inhibitory Concentration
MOH	Ministry of Health
MIO	Motility Indole and Ornithine medium
MDR	Multi-Drug Resistance
NTS	Non Typhoid Salmonellae
OX	Oxacillin
PG	Penicillin
PBP2a	Penicillin Binding Protein
TC	Tetracycline
TSI	Triple Sugar Iron Agar
UNICEF	United Nations Children's Fund
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Mortality rates in sub-Saharan Africa, particularly of children under 5 years old is estimated to be 100-250 per 1,000 children compared with 10-30 per 1,000 in developed countries (Hill *et al.*, 2007). One of six African children dies before the age of five (UNICEF 2005). The vast majority of these deaths are not fully investigated (Berkley *et al.*, 2005). Bloodstream infection is a frequent cause of morbidity and associated with mortality in excess of 25% (Berkley *et al.*, 2005). The important pathogens causing bloodstream infections are bacteria and parasites (UNICEF/.WHO 2006).

The World Health Organization (WHO) ranks the major causes of mortality in African children younger than five years as neonatal causes (26%), among which the entity "sepsis or pneumonia" contributes a quarter, pneumonia (20%), malaria (18%) diarrhoea (16%) and HIV-infection (6%) (Blomberg *et al.*, 2007).

Pneumonia is responsible for nearly 20% (2 million) of annual worldwide deaths among children under the age of five (UNICEF/WHO 2004). Worldwide, pneumonia contributes to between 750,000 and 1.2 million neonatal deaths and unknown number of stillbirths each year (Duke, 2005) and ranks sixth as the leading cause of death in children (Jao *et al.*, 2003).The causative agents of pneumonia are bacteria or viruses. In developing countries, bacterial pneumonia is more common, especially caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* (UNICEF/.WHO 2006).

Septicaemia is a common cause of morbidity and mortality among children in the developing world (Meremikwu *et al.*, 2005). Children with bloodstream

infection present with fever, difficult breathing, tachycardia, malaise, inability to feed or lethargy, but those with asymptomatic bacteraemia tend to show no obvious sign of illness (Meremikwu *et al.*, 2005). Bloodstream infection and malaria are practically indistinguishable by clinical presentation (Evans *et al.*, 2004).

In malaria- endemic areas, fever is frequently considered synonymous of malaria and treated accordingly, while a particular agent of invasive bacterial or viral infection may remain unrecognised. This leads to a considerable overestimation of the incidence of malaria and subsequently bacteraemia is often incorrectly attributed to malaria (Evans *et al.*, 2004). Meanwhile, there are almost no estimates of incidence, mortality, or hospital burden for the majority of pathogenic bacterial species (Berkley *et al.*, 2005). The local incidence of infections such as meningitis and pneumonia has been estimated, often in relation to vaccine studies (Peltola, 2001).

It is increasingly clear that invasive bacterial infections are a major contributor to mortality in children in developing countries, with incidence rates confirmed to be much higher than those reported in developed countries (Mulholland *et al.*, 2005). However there is not much information concerning the relative contribution of different organisms to bacterial infections in sub-Saharan Africa (Hill *et al.*, 2007).

A recent study in Ghana has demonstrated that invasive bacteraemia was associated with a mortality of 40% and the most common organisms isolated were non–typhoid salmonellae and *Staphylococcus aureus* (Evans *et al.,* 2004). Deaths in children with bacteraemia occur rapidly with bacteria isolates such as *Streptococcus pneumoniae* and *Haemophilus influenzae* (Berkley *et al.*, 2005).

Invasive bacterial infections are detected in up to one third of children with clinical features of severe malaria with a slide negative for malaria (Gwer *et al.*, 2007).

Treatment of bacteraemia is often urgent and may have to be undertaken without definitive identification of the organisms involved and their antimicrobial susceptibilities (Reynolds *et al.*, 2004). Studies have found that inadequate empirical therapy of bacteraemic infections is associated with adverse outcomes such as longer duration of hospital stay in those who survive (Blomberg *et al.*, 2007), and death (Behrendt *et al.*, 1999). Therefore knowledge of the most likely causative organisms and their expected antimicrobial resistance patterns can increase the probability of selecting an effective antimicrobial for empirical treatment (Reynolds *et al.*, 2004).

Blood cultures remain the gold standard test for detecting patients with bacteraemia (Meremikwu *et al.*, 2005). Isolation of the organism from blood confirms the diagnosis and enables identification of the cause of the infection and administration of adequate antimicrobial therapy from antimicrobial susceptibility testing (Pavlovsky *et al.*, 2006). Prompt diagnosis and effective treatment is necessary to prevent death and complications from septicaemia.

1.2 JUSTIFICATION

Among infectious diseases in children in Africa, invasive bacterial infections play an important role. Bacteraemia is considered one of the most important causes linked to morbidity and mortality in children less than five years in Africa (Mulholland *et al.*, 2005). Most of the deaths occurring in these children are not fully investigated because most health facilities lack appropriate diagnostic tools such as culturing and differentiation of bacteria (Berkley *et al.*, 2005).

Due to the urgency associated with the treatment of bacteraemia, there is therefore the need to have knowledge of the causative agents and their susceptibility profiles to come to better decisions of the empirical treatment. Antimicrobial susceptibility testing of isolated bacteria in the study population would help us determine the sensitivity patterns of these isolates, thereby reducing antibiotics misuse and the incidence of microbial drug resistance. The incidence and prevalence of the bacterial pathogens as well as their susceptibility profile will be determined.

Knowledge of bacteria causing bacteraemia and their antimicrobial susceptibility profile would be justifiable as prerequisite for correct treatment and infection control measures.

1.3 RESEARCH QUESTION

What is the aetiology of bacteraemia in children under five years of age attending the Agogo Presbyterian Hospital and what are their Antimicrobial susceptibility profiles?

1.4 AIM OF STUDY

To identify the causative organisms of bacteraemia in children under five years and their antimicrobial susceptibility patterns.

1.4.1 Specific Objectives

- To determine the incidence of specific bacterial pathogens causing bacteraemia in children under five years.
- To determine the antimicrobial susceptibility patterns of the bacteria isolates.
- To make recommendations for the empirical treatment of bacteraemia in children.

CHAPTER TWO

LITERATURE REVIEW

2.1 GENERAL INFORMATION

Bacteraemia is a state in which bacteria circulate through the vascular system, whilst septicaemia (sepsis) is a clinical syndrome characterized by fever, chills, malaise, tachycardia, hyperventilation and toxicity (Parrillo, 1993). Septicaemia results when circulating bacteria multiply at a rate that exceeds their removal by phagocytes (Berger, 1983). Certain infections, such as meningitis, salmonellosis, and endocarditis, have a period of bacteraemia as part of the disease process (Parrillo, 1993).

There are several terms associated with isolation of organisms in blood cultures. First described in 1969, pseudobacteraemia is the term associated with contaminated infusion fluids (e.g., intravenous, hyperalimentation, saline), blood culture bottles, alcohol swabs, syringes and other materials. Most pseudobacteraemias are caused by aerobic Gram-negative bacilli. Occult (unsuspected) bacteraemia predominantly refers to the condition found in children who appear healthy but whose blood culture is positive. This phenomenon is usually observed in children younger than 2 years of age. The most common causes include *Streptococcus pneumoniae* and *Haemophilus influezae type b* (Berger, 1983).

2.2 FORMS OF BACTERAEMIA

The forms of bacteraemia are primary bacteraemia, which is blood stream invasion by bacteria for which no preceding or simultaneous site of infection with the same microorganism can be identified. Secondary bacteraemia is isolation of a microorganism from the blood as well as from other sites in the same patient

before or at the same time, such as pneumonia and urinary tract infections. Nosocomial bacteraemia occurs on or after the third day of hospitalization; with sometimes polymicrobiol bacteraemia, blood cultures yield more than one organism (Berger, 1983).

2.3 BACTERAEMIC EPISODES

Bacteraemic episodes may be transient, intermittent or continuous reflecting several mechanisms by which bacteria enter the blood stream (Mahon *et al.*, 2000). Transient bacteraemia occurs when organisms, often members of the normal flora are introduced into the blood by minimal trauma to membranes (e.g., brushing of teeth, straining during bowel movements or medical procedures (LeFrock *et al.*, 1973). Intermittent bacteraemia occurs when bacteria from an infected site are periodically released into the blood from extravascular abscesses, spreading cellulites or infection of body cavities such as empyema, peritonitis or septic arthritis. Continuous bacteraemia usually occurs when the infection is intravascular, such as infected endothelium (bacterial endocarditis or aneurysms) or infected hardware (arteriovenous fistulas, intraarterial catheters or indwelling cannulas) (Musher *et al.*, 2000). The source of some organisms may not be determined, in up to one third of bacteraemias (Fein, 1999).

The timely detection of bacteraemia, followed by expeditious identification of pathogens and determination of susceptibility to antimicrobial agents has great diagnostic and prognostic importance. Although underlying diseases are important determinants of fatal outcomes, approximately half of the deaths can be attributed directly to the infection (Bryan, 1989). Often there is a focus of infection (e.g. pneumonia, meningitis, soft tissue infection), but it may occur also without a focus, or a focus may develop later (Washington *et al.*, 2006).

2.4 SOURCES OF BACTERAEMIC SPREAD

Pneumonia, pressure sores, skeletal system infection, skin and soft tissue infection have been described to be associated with bacteraemic spread (Mahon *et al.*, 2000). The most common organisms in pneumonia that produce a concurrent bacteraemia include *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* (Washington *et al.,* 2006).

Mahon *et al*., (2000) reported that almost 50% of the cases of bacteraemia could be attributed to pressure sores and the most commonly reported organisms are *Proteus mirabilis, Staphylococcus aureus, Bacteriodes fragilis, Acinetobacter* species, *Bacillus* species and *Corynbacterium* species.

The bones have also been implicated as a source of bacteraemia or consequence of bacteraemic persistence. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and various facultative anaerobes have been implicated in these cases. In patients with osteomyelitis, however, bacteraemia tends to be polymicrobial. *Staphylococcus aureus* is most frequently isolated, with Gram-negative bacilli accounting for more than 33% of the polymicrobial infections (Wieland *et al.*, 1986).

Furthermore skin and soft tissue infections as well as wounds have been described as sources of bacteraemia. These infections tend to be polymicrobial in 2% to 28% of patients. In soft tissue abscess bacteraemias, *Staphylococcus aureus,* beta-haemolytic *Streptococci*, *Pseudomonas aeruginosa* and *Bacteriodes* species, have been reported (Mahon *et al.*, 2000).

2.5 AETIOLOGIC AGENTS OF BACTERAEMIA

Some bacteria responsible for causing bacteraemia in children, their microbiological properties, identification and antimicrobial susceptibility patterns are as follows:

2.5.1 Haemophilus influenzae

Haemophilus influenzae is a common etiologic agent of diseases such as pneumonia and meningitis (Kaplan, 1999). Meningitis caused by *Haemophilus influenzae* occurs almost exclusively in children less than five years of age, and most invasive *Haemophilus influenzae* diseases are caused by organisms with the type b polysaccharide capsule (*Haemophilus influenzae* type b) commonly abbreviated as Hib (Washington *et al.*, 2006).

Various studies have reported *Haemophilus influenzae* as a causative agent of bacteraemia in children. A study in Kenya by Berkley *et al*., (2005) reported 12% *Haemophilus influenzae* isolates as cause of infant bacteraemia. In a similar study in Uganda, Kizito *et al*., (2006) identified 19% of *Haemophilus influenzae* isolates as responsible for causing bacteraemia in children. Maitland *et al*., (2006) isolated *Haemophilus influenzae* in 8% of all children with bacteraemia in Kenya. *Haemophilus influenzae* accounted for 0.5% bacteraemic infection involving children in a study in Nigeria (Komolafe *et al.*, 2008).

Antimicrobial agents recommended for use in antimicrobial susceptibility testing on *Haemophilus influenzae* isolates include ampicillin, amoxicillin + clavulanic acid (augmentin®), ceftriaxone, chloramphenicol and co-trimoxazole (Ajello et al., 2003). Bacteraemia and meningitis due to *Haemophilus influenzae* type b has declined substantially since the licensing of the polysaccharide-protein, seven serotypes conjugated *Haemophilus influenzae* vaccine (Adegbola et al.,

2005; Brook, 2003). In a study conducted at the Korle Bu Teaching Hospital, Ghana, Renner et al., (2007) demonstrated that the introduction of *Haemophilus influenzae* type b conjugate vaccine into routine immunisation programme has resulted in a significant reduction of bacterial meningitis in children younger than 5 years.

2.5.2 Neisseria meningitidis

This organism is the etiologic agent of meningococcal disease, most commonly meningococcal bacteraemia and meningitis. These two clinically overlapping syndromes may occur simultaneously, but meningitis alone occurs most frequently. *Neisseria meningitidis* is an encapsulated bacterium and is classified into serogroups based on the immunological reactivity of the capsule's polysaccharide. The most common serogroups causing disease are A, B, C, Y and W135 (Ajello *et al.*, 2003; Mahon *et al.*, 2000). Diseases in Africa is caused by serogroups A whilst serogroups B and C cause diseases in Western Europe and North America (Urwin *et al.*, 1998). Meningococcal meningitis present a potential epidemic in the sub-Saharan meningitis belt (Peltola, 2001). The development of a vaccine applicable in childhood appears to be the only means of decreasing the mortality and morbidity caused by meningococcal disease. Polysaccharide vaccines are available for serogroups A, C, Y and W-135 (Poolman *et al.*, 1995).

Neisseria meningitidis does not commonly show resistance to many antimicrobial agents. Low-level resistance to penicillin is common in some areas of the world, though the clinical significance of this resistance has not yet been established (Ajello *et al.*, 2003). A study in Ghana on bacterial meningitis in children reported that all *Neisseria meningitidis* isolates were sensitive to ceftriaxone (Commey *et al.*, 1994).

Epidemic response consists of prompt and appropriate case management with oily chloramphenicol or ceftriaxone and reactive mass vaccination of epidemic districts. It is estimated that a mass reactive immunization campaign, when promptly implemented, can prevent up to 70% of cases (WHO, 2010).

2.5.3 Streptococcus pneumoniae

Streptococcus pneumoniae occurs as part of the normal flora in the nasopharynx. However, nasopharyngeal carriage of the organism is considered a risk for several invasive infections, including meningitis, pneumonia and sepsis (Donkor *et al.*, 2010). Meningitis in infants, young children and the elderly is often caused by *Streptococcus pneumoniae* (Michelow *et al.*, 2004). Persons who have sickle cell disease, anatomic asplenia, or are immunocompromised have an increased susceptibility to *Streptococcus pneumoniae* infection (Facklam *et al.*, 2003).

Worldwide, the annual mortality of meningitis and pneumonia due to *Streptococcus pneumoniae* is over 1.6 million (WHO 1999). *Streptococcal pneumoniae* bacteraemia have been reported in 83% of children in America (Segal *et al.*, 2000). A study on bacteraemia in children conducted in Kenya showed that *Streptococcus pneumoniae* isolates accounted for 25% of infection (Berkley *et al.*, 2005). Brent *et al*., (2006) also established that *Streptococcus pneumoniae* was responsible for 50% of all bacteraemias in children. Again in a study conducted in Kenya by Maitland *et al*., (2006), 35% of blood stream infections were due to *Streptocccus pneumoniae* in children. Hill *et al*., (2007) also reported that *Streptococcus pneumoniae* was responsible for 45.2% of all blood stream infections in children in the Gambia. In Malawi, a study by Bronzan *et al*., (2007) identified *Streptococcus pneumoniae* isolates as responsible for 11% of

bacteraemia in children. A study in Nigeria established that *Streptococcus pneumoniae* accounted for 0.5% of bacteraemia (Komolafe *et al.*, 2008). In Ghana at the Komfo Anokye Teaching Hospital, Kumasi, Evans *et al*.,(2004) reported 2% *Streptococcus pneumoniae* infant bacteraemia.

The public health burden related to *Streptococcus pneumoniae* is worsened by the increasing resistance of the organism to essential antimicrobial drugs, particularly penicillin, cephalosporins and macrolides (Van Bambeke *et al.*, 2007). However, a study in Gambia reported that *Streptococcus pneumoniae* isolates were highly susceptible to penicillin (97.5%), ampicillin (100%) and chloramphenicol (97.6%), they were moderately susceptible to tetracycline (65.9%) and 5% susceptible to co-trimozaxole (Hill *et al.*, 2007). Jao *et al*., (2003), established that *Streptococcus pneumoniae* isolates were 100% sensitive to penicillin, ampicillin, tetracycline, erythromycin, chloramphenicol, ceftriaxone and ciprofloxacin. Phetsouvanh *et al*., (2006) identified two of the four *Streptococcus pneumoniae* tested were resistant to oxacillin (1µg) by disc diffusion, however penicillin MICs were determined for 2 isolates to be 0.032µg/ml (sensitive) and 1.0µg/ml (intermediately resistant). A study on epidemiology of invasive pneumococcal disease in Ghana, established that 12% of pneumococci isolates showed intermediate level of resistance to penicillin (Holliman *et al.*, 2007). Again, a recent study in Ghana found 19.4% resistance rate of pneumococcal infections against penicillin (Donkor *et al.*, 2010).

2.5.4 Salmonella enterica **serotype Typhi** *(Salmonella* **Typhi***)*

Salmonella Typhi is the etiologic agent of typhoid fever causes an estimated 16.6 million cases of morbidity and 700,000 deaths worldwide each year (Thong *et al.*, 1994; Washington *et al.*, 2006). Rarely, other serotypes of *salmonella,* such as

Salmonella Enteritidis, can also cause enteric fever (Mahon *et al.*, 2000). Like other enteric pathogens, *Salmonella* Typhi is transmitted through food or water that has been contaminated with faeces from either acutely infected persons, persistent excretors, or from chronic asymptomatic carriers. Humans are the only host for *Salmonella* Typhi; there are no environmental reservoirs. *Salmonella* Typhi is most frequently isolated from blood during the first week of illness, but it can also be present during the second and third weeks of illness, during the first week of antimicrobial therapy, and during clinical relapse (Mahon *et al.*, 2000). In developing countries, typhoid fever is frequently diagnosed solely on clinical grounds; however, isolation of the causative organism is necessary for a definitive diagnosis. Isolation of the agent is also a necessity for the performance of antimicrobial susceptibility testing (Brooks *et al.*, 2007).

Jao *et al*., (2003) established that 9.1% of bacteraemia in children was due to *Salmonella* Typhi in a study at San Lazaro Hospital in the Philippines. Phetsouvanh *et al*., (2006) in another study identified *Salmonella* Typhi as responsible for causing 44% of bacteraemia in children in the Laos. In Africa, Komolafe *et al*., (2008) and Wilkins *et al*., (1997) reported 4.9% and 6.2% *Salmonella* Typhi blood stream infection in children in Nigeria and Ghana respectively.

Chloramphenicol has been the 'first line of defence' for many years, but the emergence of chloramphenicol-resistant strains prompted the use of ampicillin and trimethoprim/sulfamethoxazole, which are considered appropriate alternatives to chloramphenicol (Islam *et al.*, 1993). Unfortunately, resistance to antimicrobial agents ampicillin, trimethoprime-sulfamethoxazole is being increasingly reported among *Salmonella* Typhi isolates because of the emergence of multi-drug resistant

(MDR) strains (Ward *et al.*, 1990). In areas where resistance to these agents is common among circulating *Salmonella* Typhi strains, fluoroquinolones and parenteral third-generation cephalosporin are probably the best choice for empiric treatment of typhoid fever (Bopp *et al.*, 2003). A study in Laos identified 12% ampicillin resistant *Salmonella* Typhi isolates, 11% were resistant to cotrimoxazole and 12% resistant to chloramphenicol (Phetsouvanh *et al.*, 2006). *Salmonella* Typhi isolates in studies conducted in Nigeria and Ghana established that 80-100% of isolates were sensitive to tetracycline, gentamicin, chloramphenicol, cefotaxime, ceftriazone, ciprofloxacin and colistin (Komolafe *et al.*, 2008; Wilkens *et al.*, 1997).

2.5.5 Non typhoid salmonellae (NTS)

Enteric fever is predominantly the result of infection by *Salmonella* Typhi, however gastroenteritis sometimes associated with bacteraemia commonly in immnunocompromised children are caused by non typhoid salmonellae (Wilks *et al.*, 2003). NTS gastroenteritis is usually benign disease, but invasion beyond the gastrointestinal tract occurs in approximately 5% of patients with salmonellosis. The most common manifestation of invasive NTS is bacteraemia, followed by meningitis, osteomyelitis, endocarditis, arthritis, urinary-tract infection and pneumonia (Fierer *et al.*, 2000; Weinberger *et al.*, 2004).The important NTS includes *Salmonella* Enteritidis, *Salmonella* Choleraesuis, and *Salmonella* Typhimurium (Brooks *et al.*, 2007).

NTS have been responsible for bacteraemia in a number of countries; In America, 9% NTS bacteraemia have been reported in children by Segal *et al*., (2000). In the eastern and southeastern part of Africa, Berkley *et al*., (2005); Maitland *et al*., (2006) and Bronzan *et al*., (2007) reported 14.7%, 10% and 58%

incidences of NTS bacteraemia in children in Kenya, and Malawi. Reports from West Africa showed that NTS was responsible for 16%, 8.6%, 2.5% and 59% of infant and child bacteraemia in Ghana, the Gambia and Nigeria (Evans *et al.*, 2004; Hill *et al.*, 2007; Komolafe *et al.*, 2008; Wilkens *et al.*, 1997).

Ten to fifteen years ago, patients with invasive *Salmonellae* infection were successfully treated using either of the 'first line' antibiotics (ampicillin, trimethoprim-sulfamethoxazole or Chloramphenicol (Mills-Robertson *et al.*, 2003), however, as with the trend of many bacterial pathogens in today's world, classical antibiotic treatment regimens are no longer effective (Mourad *et al.*, 1993). A study in Ghana established that 93% of *Salmonella* group B isolated were resistant to 'first line' antibiotics comprising ampicillin, chloramphenicol and trimethoprim/ sulfamethoxazole, however all isolates were sensitive to ceftriaxone and ciprofloxacin (Mills-Robertson *et al.*, 2003). Hill *et al*., (2007) reported that non typhoid salmonellae were susceptible to ciprofloxacin (100%), gentamicin (100%), tetracycline (80%), cotrimoxazole (71%), Chloramphenicol (66.7%) and ampicillin (57%). NTS isolates were 80-100% sensitive to tetracycline, gentamicin, chloramphenicol, ceftazidime, ceftriaxone, ciprofloxacin and colistin according to a study conducted in Nigeria (Komolafe *et al.*, 2008).

2.5.6 Staphylococcus aureus

Staphylococcus aureus is a major pathogen causing pyogenic and toxin mediated infections in humans. They produce a wide variety of extracellular enzymes (Wilks *et al.*, 2003). Infection can result from direct contamination of a wound. When *Staphylococcus aureus* disseminates and bacteraemia ensues, endocarditis, acute hematogenous osteomyelitis, meningitis or pulmonary infection can result. Bacteraemia, endocarditis, pneumonia and other severe infections due

to *Staphylococcus aureus* require prolonged intravenous therapy with a β-lactamase- resistant penicillin (Brooks *et al.*, 2007).

Staphylococcus aureus bacteraemia have been reported in a number of countries; 17.7% *Staphylococcus aureus* have been reported for being the cause of bacteraemia in children in a study conducted in Laos (Phetsouvanh *et al.*, 2006). Meremikwu *et al.*, (2005) in a study conducted in Nigeria reported that *Staphylococcus aureus* accounted for 48.7% of all bacteraemic infection in children. Eight percent (8%) *Staphylococcus aureus* bacteraemia have also been reported in another study in Kenya (Maitland *et al.*, 2006). Kizito *et al.*, (2007) established 60% *Staphylococcus aureus* bacteraemia in children in Uganda. Again Hill *et al.*, (2007) reported 18.3% *Staphylococcus aureus* bacteraemia in children under five years of age. A study in Nigeria established that *Staphylococcus aureus* accounted for 43.6% of bacteraemia in children (Komolafe *et al.*, 2008). Evans *et al.*, (2004) also reported 29% *Staphylococcus aureus* infant bacteraemia in a study at the Komfo Anokye Teaching Hospital, Kumasi, Ghana.

Staphylococcus aureus resistance to penicillin G can be predicted by a positive test for beta-lactamase; approximately 90% of *Staphylococcus aureus* produce beta-lactamase (Brooks *et al.*, 2007). Resistance to oxacillin and methicillin occurs in about 35% of *Staphylococcus aureus* (Mahon *et al.*, 2000).

A study in Nigeria by Meremikwu *et al.*, (2005) on bacteraemia established that the *Staphylococcus aureus* isolated had the highest susceptibility to Ceftriaxone (100%), Cefuroxime (100%), Azithromycin (100%), Erythromycin (90.1%) and Gentamicin (86.6%). A similar study in the Gambia also established that the *Staphylococcus aureus* isolates were all susceptible to cloxacillin, gentamicin and chloramphenicol; moderately susceptible to co-trimoxazole (66.7%) and poorly

susceptible to tetracycline (33%) and 8% for penicillin (Hill *et al.*, 2007). Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates from a bacteraemic study on patients admitted at the San Lazaro hospital in the Philippines showed that 70-90% of them were resistant to commonly used antibiotics such as penicillin, ampicillin, tetracycline and cotrimoxazole. More than 80% of them were sensitive to gentamicin, ceftazidime, ceftriaxone, ciprofloxacin and vancomycin (Jao *et al.*, 2003). Phetsouvanh *et al.*, (2006) reported that all *Staphylococcus aureus* isolates were susceptible to methicillin.

2.5.7 Escherichia coli

They are among the leading causes of meningitis in infants, approximately 75% of *Escherichia coli* from meningitis cases have the K1 antigen. This antigen cross-reacts with the group B capsular polysaccharide of *Neisseria meningitidis* (Brooks *et al.*, 2007).

When normal host defences are inadequate, *Escherichia coli* may reach the blood stream and cause sepsis (Mahon *et al.*, 2000). In Kenya, Berkley *et al.*, (2005) reported 10.7% *Escherichia coli* bacteraemic infections in children. Twelve percent (12%) of *Escherichia coli* infections have also been reported (Maitland *et al.*, 2006). *Escherichia coli* have also been reported as responsible for17.7% bacteraemic infections in children (Phetsouvanh *et al.*, 2006). Hill *et al.*, (2007) reported that *Escherichia coli* accounted for 9.7% of infection in a study conducted in the Gambia. In Nigeria, Komolafe *et al.*, (2008) reported 9.8% *Escherichia coli* bacteraemia in children. In Ghana, Evans *et al.*, (2004) reported 2% *Escherichia coli* infant bacteraemia.

Ampicillin, cephalosporin, fluoroquinolones and aminoglycosides have marked antibacterial effects against *Escherichia coli*. Multiple drug resistance is

common and is under the control of transmissible plasmids (Brooks *et al.*, 2007). The antimicrobial susceptibility pattern of a study in Gambia showed that all *Escherichia coli* isolates were susceptible to ciprofloxacin (100%), they were highly susceptible to gentamicin (88.9%) and moderately susceptible to chloramphenicol (66.7%) and poorly susceptible to co-trimoxazole (22%) and completely non-susceptible to ampicillin (Hill *et al.*, 2007). Again in Nigeria, Komolafe *et al.*,(2008) reported that 85%-95% of *Escherichia coli* isolates were sensitive to gentamicin, colistin, ceftazidime, ceftriaxone and ciprofloxacin.

Many Extended-Spectrum β-Lactamases (ESBLs) are plasmid-mediated derivatives from TEM- and SHV- type enzymes and cause resistance to expanded-spectrum cephalosporins (Gangoue-Pieboji *et al.*, 2005). ESBL strains of *Escherichia coli* have been reported as causing blood stream infection and a prevalence of 17.9% ESBL producing *Escherichia coli* have been identified. Exposure to broad-spectrum cephalosporins was a risk factor for infection with ESBLs producing strains (Kim *et al.*, 2002). Fourteen percent ESBL producing *Escherichia coli* have been reported in Cameroon (Gangoue-Pieboji *et al.*, 2005). A study in Ghana indicated that the commonly used drugs cannot be considered any longer as first-line treatment for *Escherichia coli* infections, however none of these isolates produced ESBL (Djie-Maletz *et al.*, 2008). Ayisi, (2009) reported 44.4% ESBL producing *Escherichia coli* in a study conducted at the Komfo Anokye Traching Hospital in Kumasi, Ghana.

2.5.8 Klebsiella pneumoniae

This organism is present in the feces of about 5% of normal individuals, it causes a small proportion (about 1%) of bacterial pneumonias. Occasionally, it produces urinary tract infection and bacteraemia with focal lesions in debilitated

patients, *Klebsiella pneumoniae* can also cause a variety of extra pulmonary infections, including enteritis and meningitis in infants (Brooks *et al.*, 2007).

Komolafe *et al*., (2008) reported that *Klebsiella pneumoniae* was responsible for 17.1% of bacteraemia in children in a study conducted in Nigeria.

Klebsiella pneumoniae have a tendency to harbour antibiotic-resistant plasmids; thus, infections with multiple antibiotic-resistant strain can be anticipated (Washington *et al.*, 2006). Virtually all clinical strains are resistant to ampicillin, carbenicillin and ticarcillin. Most strains possess plasmids that mediate resistance to extended-spectrum beta lactam drugs (Brooks *et al.*, 2007). This form of resistance is due to the production of beta-lactamase enzymes, referred to as ESBL (Jarlier *et al.*, 1988). These enzymes cause them to be resistant to most beta-lactam drugs, including third generation cephalosporins (Washington *et al.*, 2006). Prevalence of 18.8% and 52.9% ESBLs *Klebsiella pneumoniae* have been reported (Gangoue-Pieboji *et al.*, 2005; Kim *et al.*, 2002). Fifty five percent (50%) ESBL producing *Klebsiella* species have been reported in Ghana (Ayisi, 2009). If an ESBL-producer is detected, it should always be reported as resistant to penicillins and cephalosporins even if *in vitro* tests indicate susceptibility, since these may fail in treatment (CLSI, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

A hospital based longitudinal study was conducted at the Agogo Presbyterian Hospital in the Ashanti Akim North District, Ashanti Region. All children up to 5 years attending the Child Welfare Clinic from January, 2008 to December, 2008 were recruited after fulfilling the inclusion criteria.

3.2 STUDY AREA

The Asante Akyem North District is one of the 21 Districts in the Ashanti Region. The District is located in the eastern part of Ashanti Region. It covers a land area of 1,160 sq.km with an estimated population of 142,434 (projection from 2000 Population Census). Over 40% of the population is under 15 years of age; over 50% is under 20years. Population aged 65years and above consists of 6.4% of the total population (www.asanteakimnorth.ghanadistricts.gov.gh). The vegetation of the study area is mainly rain forest and the climate is tropical. The temperature variation is between 20ºC and 36ºC with monthly rainfall varying from 2.0mm in February to 400mm in July. The major occupation of the people is subsistence farming, animal husbandry and forestry. The sub-districts are Konongo-Odumasi, Agogo, Juansa, Dwease-Praaso and Amanteman. The Agogo Presbyterian Hospital which is the major hospital in the district recorded a total of 111852 outpatient attendance in the 2009 fiscal year. The top 10 cases reported were malaria (16.7%) acute eye disease (13.9%), upper respiratory tract infections (7.0%), acute urinary tract infection (3.1%), skin disease and ulcer (2.5), gynaecological conditions (2.2%), cataract (2.0%), diarrhoea (1.5%), acute ear infections (1.3%), vaginal discharge (1.3%) and other conditions (36.3%)

(Regional Health Directorate, Ashanti Region, 2009).

MAP OF ASANTI AKIM NORTH DIST. OF ASHANTI REGION, GHANA

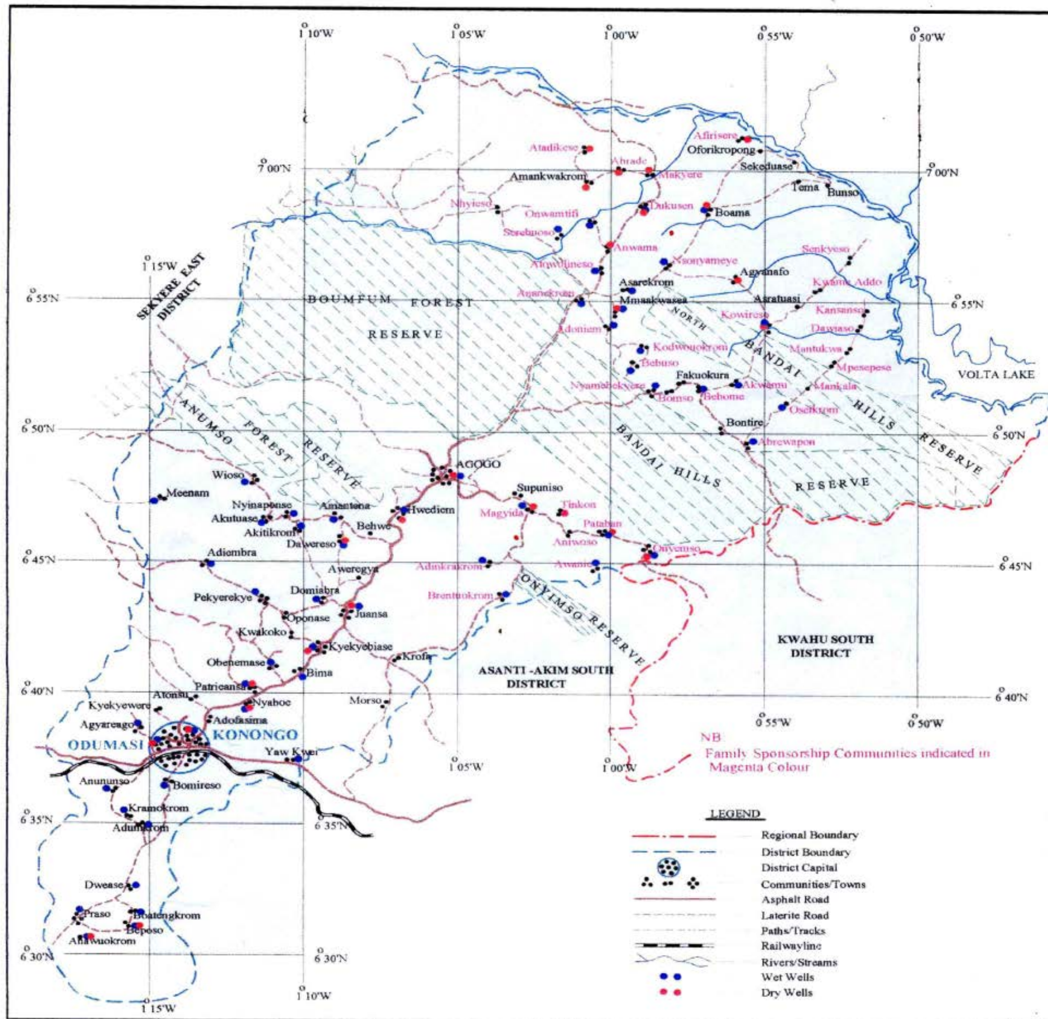

Figure 3.1: Map Showing Asante Akyem North District
(Courtesy: www.Ashanti_districts.png)

3.3 STUDY SITE

The Agogo Presbyterian Hospital is a major hospital serving the Asante-Akyem North District and other parts of the region. The children's ward is managed by a paediatric team of doctors: one (1) Paediatrician, two (2) residents in paediatrics, two (2) medical officers and two (2) house officers, two (2) nurses and three (3) ward and health aids supported by three (3) administrative staff.

Bacterial culture and sensitivity tests were performed at the Agogo Presbyterian Hospital Laboratory. Laboratory department offers diagnostic as well as research services. The department is fully equipped with two automated blood culture incubators (BACTEC 9050, Becton Dickinson, USA), a carbon dioxide incubator and 2 safety cabinets for bacteriological culture and sensitivity testing. There are 6 light microscopes, scientific fridges and freezers as well as centrifuges and water baths.

Laboratory tests are carried out from approved Standard Operating Procedures (SOPs) and every activity undertaken in the laboratory is well documented. The laboratory participates in various External Quality Assessment programme with the National Institute of Communicable Disease and National Health Laboratory (NICD/NHL) in microbiology and parasitology from South Africa.

3.4 ETHICAL APPROVAL

Ethical approval for the study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST, Kumasi. This study was part of a major study on Neglected Infectious Diseases in children in the District.

3.5 STUDY POPULATION

All Children of up to 5 years old and permanently residing in the Asante-Akyem North District who were admitted to the children's ward of the Agogo Presbyterian hospital were included in this study if they fulfilled the inclusion criteria.

3.6 INCLUSION CRITERIA

- All children with body temperature of $\geq 38.0^{o}C$ and signs of very severe disease such as pneumonia, persistent diarrhoea and severe dehydration.
- Signed informed consent was obtained if the potential parent demonstrated understanding of the study and was willing to enroll. In the case of an illiterate parent, a left thumbprint was obtained on the consent forms and a separate witness consent form was signed by a literate witness who had observed the consent processes. The interview was done in Twi which is the local language in the district (appendix 1).

3.7 EXCLUSION CRITERIA

Children referred from other clinics who were already on antibiotic treatment and those whose parents/guardians would not give their consent.

3.8 SAMPLING

One to three (1-3) mls of blood were collected immediately on admission from all children who fulfilled the inclusion criteria and whose parents or guardian gave their consent.

3.8.1 Sampling Method

On admission all children who fulfilled the inclusion criteria were reviewed by the paediatric team for sample collection. The child's skin was cleaned with 70% ethanol and allowed to dry before blood was drawn for culture. Three (3) mls of venous blood was taken and inoculated into commercially produced BD BACTEC^{TM} PEDS PLUS^{TM}/F (Becton Dickinson, USA) culture vials. The culture vial was labelled with a name, barcode, pathology number and time of sample collection.

3.9 LABORATORY INVESTIGATIONS

3.9.1 Sample Processing

Blood culture vials were immediately transferred to the BACTEC 9050 (Becton Dickinson, USA) incubator at Agogo Presbyterian Hospital Laboratory. Blood culture vials were kept in the incubators for five days.

3.9.2 Sample Size

A total of 1356 blood cultures were taken throughout the study period (January 2008-December 2008).

Plate 3.1: Bactec 9050 automated blood culture incubator

3.9.3 Subculturing

Every positive vial flagged by the Bactec 9050 (Becton Dickinson, USA) was removed for subculturing in the safety cabinet. The lid of the positive blood culture vial was disinfected with an alcohol swab and 1ml of blood was aseptically removed from the vial and plated on 5% sheep blood agar, chocolate agar and MacConkey agar. The sheep blood agar and chocolate agar were incubated in 5% CO_2 at 37°C for 24 hours while the MacConkey agar was incubated aerobically at 37°C for 24 hours.

3.9.4 Microscopy

A drop of blood was put on a clean dry labelled slide for smear preparation. The smear was allowed to air dry, and heat fixed by passing the slide three times through a flame, allowed to cool and stained with the Gram stain (appendix 3). The slide was examined with the light microscope x100 objective lens under oil immersion.

3.9.5 Identification of isolates

Isolates were identified based on colonial morphology, biochemical tests, Analytical Profile Index (API) reaction and serology as necessary.

3.9.5.1 Biochemical Test

For Gram positive organisms, the following biochemical tests were done; Catalase test (appendix 4), Coagulase test (appendix 5) and DNAse test (appendix 6).Lancefield grouping kit (Oxoid, Basingstoke, England) was used for Streptococcal identification (appendix 7). Optochin identification disk was put on the Mueller Hinton sheep blood for *Streptococcus pneumoniae* identification (appendix 8).

For Gram negative organisms, triple sugar iron agar (TSI), motility indole and ornithine medium (MIO), oxidase test (appendix 9) were performed. API 20E (Enterobacteriaceae), API 20NE (Non fermenting Gram negative bacilli) and API NH (*Neisseria, Brahamella, Haemophilus*) (Biomerieux, France) were also performed according to the manufacturers instructions. Other serological tests such as enteroclon anti-*Salmonella* A-67 (Sifin, Germany), enteroclon anti-*Salmonella* Vi (Sifin, Germany), enteroclon anti-*Salmonella* Group B (Sifin, Germany) and wellcolex colour *Salmonella* (Remel, United Kingdom) were used in the identification of micro organism including *Salmonella* Typhi and NTS.

3.9.6 Quality Control

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pneumoniae* ATCC 49619, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Typhimurium ATCC 14028 and *Haemophilus influenzae* ATCC 49247 were set up together with the test organism to control media, biochemical tests, potency of antibiotic discs and E-test strips.

3.9.7 Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility pattern was determined by both Kirby-Bauer disk diffusion (CLSI, 2007) and the minimum inhibitory concentrations (MICs) methods. The isolated organisms were tested for susceptibility to antimicrobial agents using Mueller-Hinton agar. However for pneumococci Mueller Hinton sheep blood agar was used.

3.9.7.1 Inoculum preparation for AST

Plates and antibiotic discs were brought to room temperature before use. 4-5 isolated colonies were touched with a straight wire loop and suspended in 5mL

sterile saline to obtain a homogenous suspension. The turbidity was adjusted to 0.5 McFarland turbidity standard.

3.9.7.2 Inoculation and Application of Antibiotic discs

A sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess volume of the inoculum. The entire surface of the agar plate was swabbed evenly in three directions. The plate was left to dry before application of discs. The antibiotic discs were applied to the agar surface using a disc dispenser. Once applied, the discs were not removed.

3.9.7.3 Incubation and Reading

Plates were incubated aerobically at 37°C for 24 hours. Results were scored as susceptible, moderately susceptible or resistant, according to the Clinical and Laboratory Standard Institute (CLSI, 2007) criteria. An Oxacillin (1µg) disk was used to determine penicillin susceptibility of pneumococci.

3.9.8 Methicillin Resistant Staphylococcus aureus (MRSA) Testing

Testing for methicillin resistant *Staphylococcus aureus* (MRSA) was done using cefoxitin disc (30µg). Any growth within the zone of inhibition around the cefoxitin disk was considered as evidence of resistance. All *Staphylococcus aureus* isolates that were resistant to cefoxitin were tested further using penicillin binding protein 2´ (PBP2a´) agglutination kit for MRSA (Oxoid, Basingstoke, England).

Table 3.1 Antibiotic Disc Concentrations Used (Oxoid, Basingstoke, England).

Antibiotic	Concentration (µg/disc)
Ampicillin	10
Ampicillin/Sulbactam	20
Cefoxitin	30
Ceftriaxone	30
Cefuroxime	30
Chloramphenicol	30
Ciprofloxacin	5
Clindamycin	2
Erythromycin	15
Gentamicin	10
Oxacillin	1
Penicillin	10 units
Rifampicin	5
Tetracycline	30
Trimethoprim/Sulfamethoxazole	25
Vancomycin	30

3.9.9 Minimum Inhibition Concentration (MIC)

E-test strips (AB Biodisk, Solna, Sweden) were used for the determination of minimum inhibition concentration (MIC) on Mueller hinton agar. However for pneumococci, Mueller Hinton sheep blood agar was used.

3.9.9.1 Inoculum Preparation for E-test

Isolated colonies from the pure overnight culture were emulsified in 5ml sterile saline. The turbidity was compared to the 0.5 mcfarland standard.

3.9.9.2 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 streaked evenly in three directions. The agar surface was allowed to dry completely before the application of e-test strips.

3.9.9.3 Application of E-test Strips

The E-test strip was applied to the agar surface with the MIC scale facing upward using forceps. It was ensured that the whole length of the antibiotic gradient was in complete contact with the agar surface. Once applied, the strips were not removed.

3.9.9.4 Incubation and Reading

Plates were incubated aerobically at 37°c for 24 hours in an inverted position. MIC values were read where the edge of the inhibition ellipse intersected the strip, results were interpreted according to the CLSI (CLSI, 2007) guidelines.

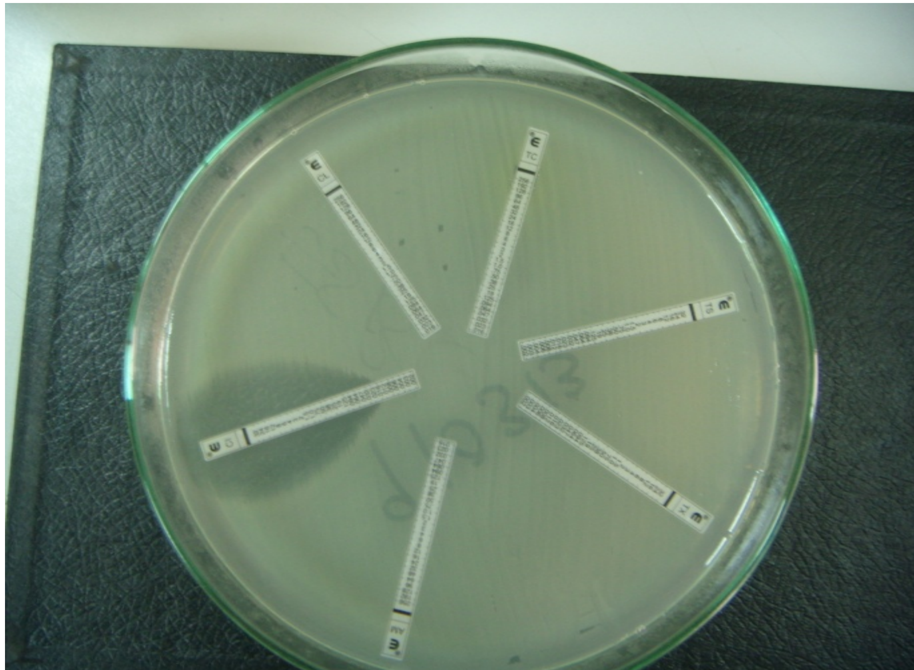

Plate 3.2: E- test results

3.9.10 ESBL testing

Three antibiotics were used for the detection of extended spectrum β-lactamase (ESBL) by E-test;

Cefepime (PM)/cefepime + clavulanic acid (PML), PM ≥ 0.25 and PM/PML≥8 or deformation of ellipse was indicative of extended spectrum β-lactamase (ESBL).

Cefotaxime (CT)/ Cefotaxime + Clavulanic acid- (CTL), CT≥ 0.5 and CT/CTL≥8 or deformation of ellipse confirmed ESBL production.

Ceftazidime (TZ)/ Ceftazidime + Clavulanic acid (TZL), TZ≥1 and TZ/TZL≥8 or deformation of ellipse was ESBL.

Plate 3.3: ESBL detection using E-test

3.10 DATA COLLECTION AND STATISTICAL ANALYSIS

The clinical data were recorded on standardised forms at admission and data collection was entirely embedded into the clinical routine. The admission chart contained a 4-paged admission sheet to be filled in by the admitting doctor. Double data entry was done by data entry clerks using a 4th Dimension Database (© 4D San Jose, California, United States). Data analysis was carried out using STATA 9.2 (©4D College Sation, Texas, United Sates) and excel was used for the graphs. For each pathogen their percentages among all blood cultures and antimicrobial susceptibility profile were determined.

CHAPTER FOUR

RESULTS

4.1 ENROLMENT OUTCOME OF THE STUDY CHILDREN

During the study period, 1356 children were admitted to the hospital and underwent phlebotomy for blood culture. Three hundred and four (304) (22.4%) had a positive blood culture and 207 (15.3%) of these were considered as pathogens whilst 97 (7.1%) were classified as contaminants (coagulase negative *staphylococci* and other skin organisms).Contaminants were excluded from further analysis.

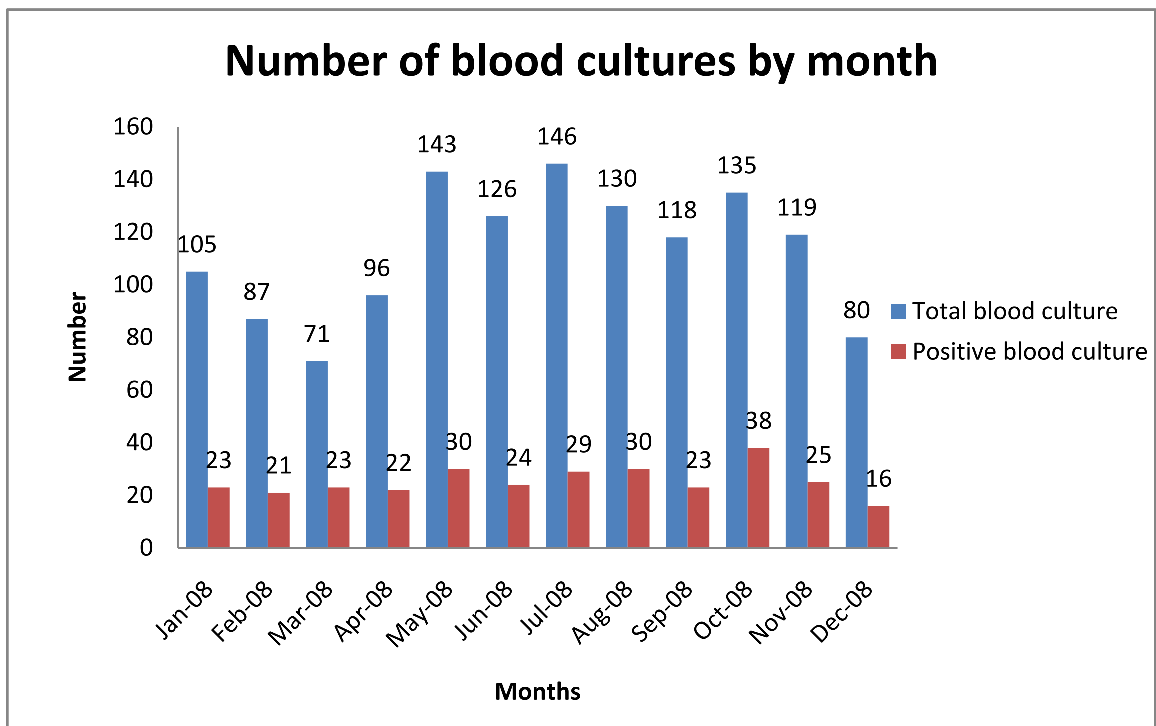

Figure 4.1: Monthly blood cultures.

The highest number of blood culture was taken in the month of July 2008, however the highest number of positive blood cultures was in the month of October 2008 (Figure 4.1).

4.2 GENERAL BACTERIA ISOLATES FOR THE STUDY PERIOD

Four organisms accounted for 60.9% of bacteraemias, a non typhoid salmonellae (35.2%), *Streptococcus pneumoniae* (10.2%), *Staphylococcus aureus* (7.9%) and *Salmonella* Typhi (7.6%), (Table 4.1).

Table 4.1: Blood culture isolates for the study period

Organism	Frequency (n)	Percent (%)
Non typhoid salmonellae	107	35.2
Coagulase negative *Staphylococci*	54	17.8
Streptococcus pneumoniae	31	10.2
Staphylococcus aureus	24	7.9
Salmonella Typhi	23	7.6
Micrococcus species	21	6.9
Bacillus species	17	5.6
Escherichia coli	6	2.0
'Viridans' *Streptococci*	6	2.0
Klebsiella pneumoniae	5	1.6
Streptococcus agalactiae	1	0.3
Group D *Streptococci*	1	0.3
Streptococcus pyogenes	1	0.3
Candida species	1	0.3
Shigella sonnei	1	0.3
Coryneforms	1	0.3
Propioni bacterium species	1	0.3
Pseudomonas fluorescens	1	0.3
Acinetobacter baumannii	1	0.3
Aeromonas hydrophila	1	0.3

Coagulase negative *Staphylococci* (CoNS) and *Micrococcus* species were the predominant contaminants obtained during the study period (Table 4.1).

4.3 INFECTION RATE OF PATHOGENIC BACTERIA IN STUDY CHILDREN

Several pathogens were identified as causative agents of infant bacteraemia. NTS contributed 53% of all pathogens in the study children (Figure 4.2).

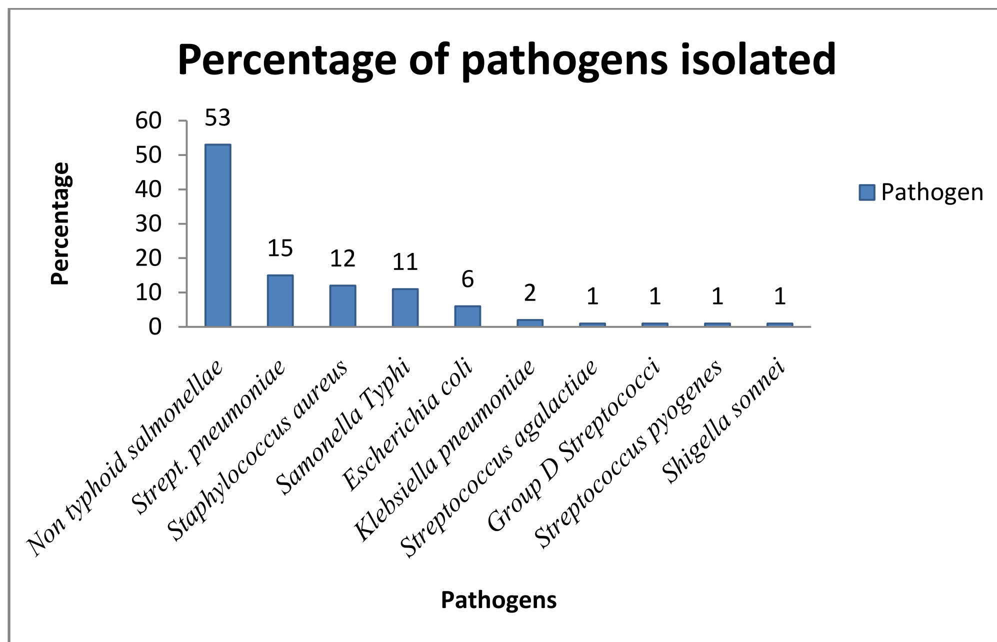

Figure 4.2: Bacteria considered as pathogens isolated from the blood cultures

NTS and *Streptococcus pneumoniae* were considered as the leading cause of bacteraemia contributing 53% and 15% respectively (Figure 4.2).

4.4 BLOOD CULTURE CONTAMINATION RATE IN THE STUDY CHILDREN

CoNS (57%) and *Micrococcus* species (22%) were the frequently isolated contaminant in the study (Figure 4.3).

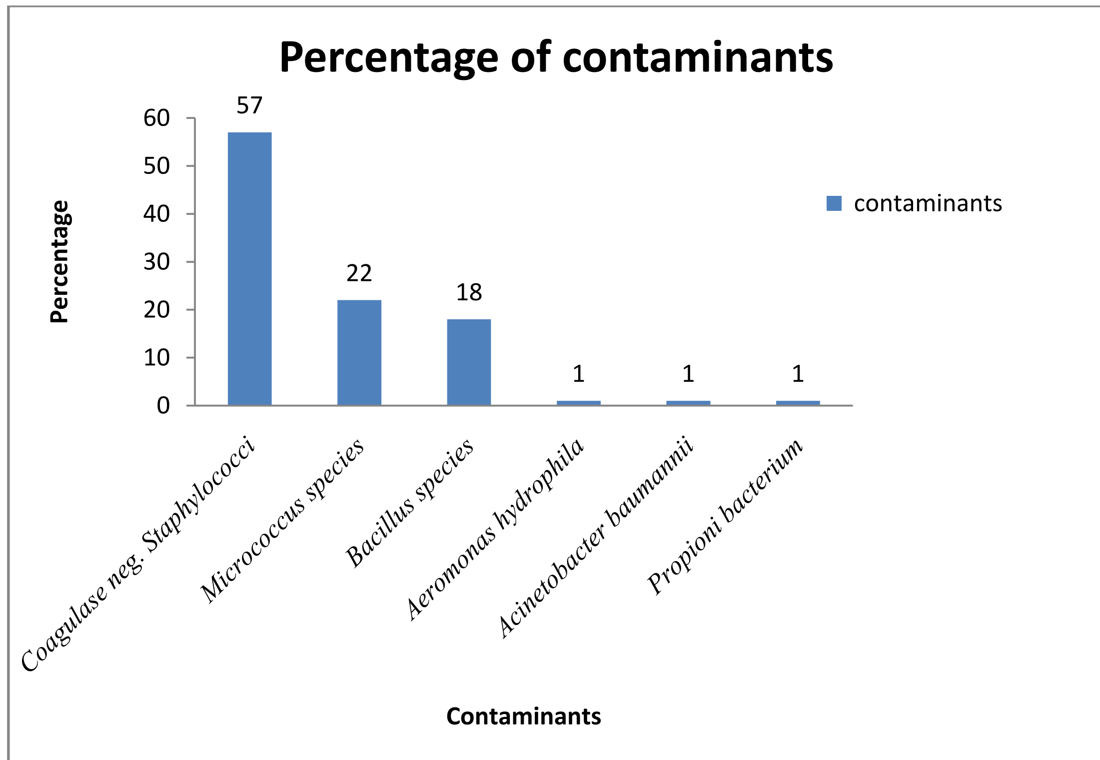

Figure 4.3: Bacteria considered as contaminants isolated from the blood cultures.

4.5 MONTHLY PATHOGENIC INFECTION RATE

The rate of blood cultures with pathogens varied throughout the study period. It ranged from 12.50% to as high as 21.10% (Figure 4.4).

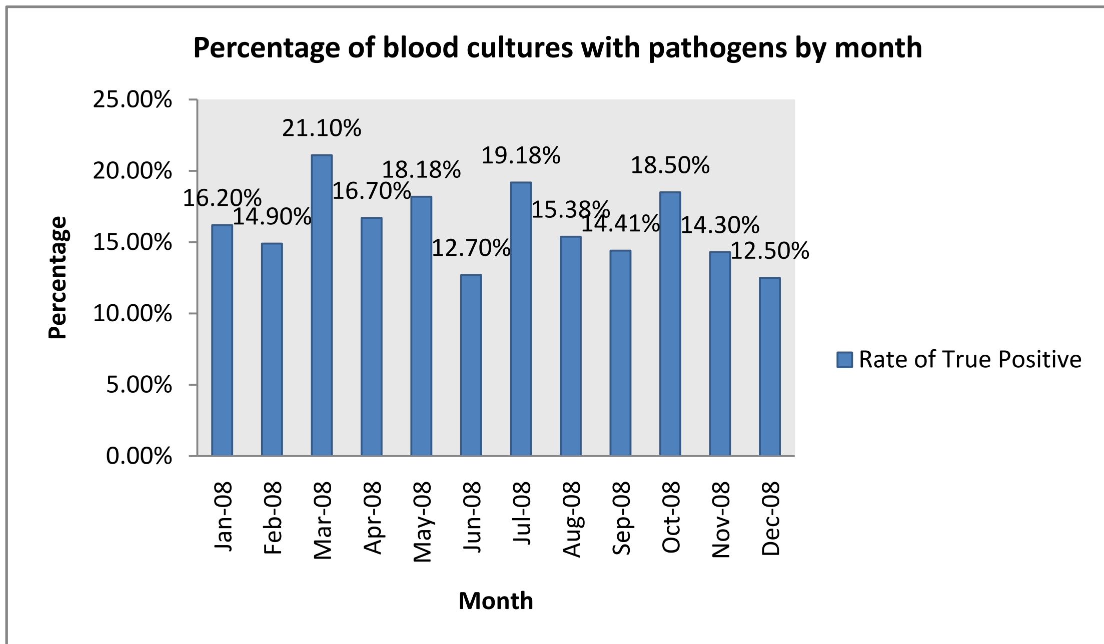

Figure 4.4: Percentages of positive isolates to blood cultures by months.

The highest rate of blood culture with pathogens was in March 2008, whilst the lowest rate was in December 2008 (Figure 4.4).

4.6 MONTHLY CONTAMINATION RATE

The highest contamination rate of 12.60% was obtained in February and October 2008 whilst the lowest contamination rate was in July 2008 (Figure 4.5).

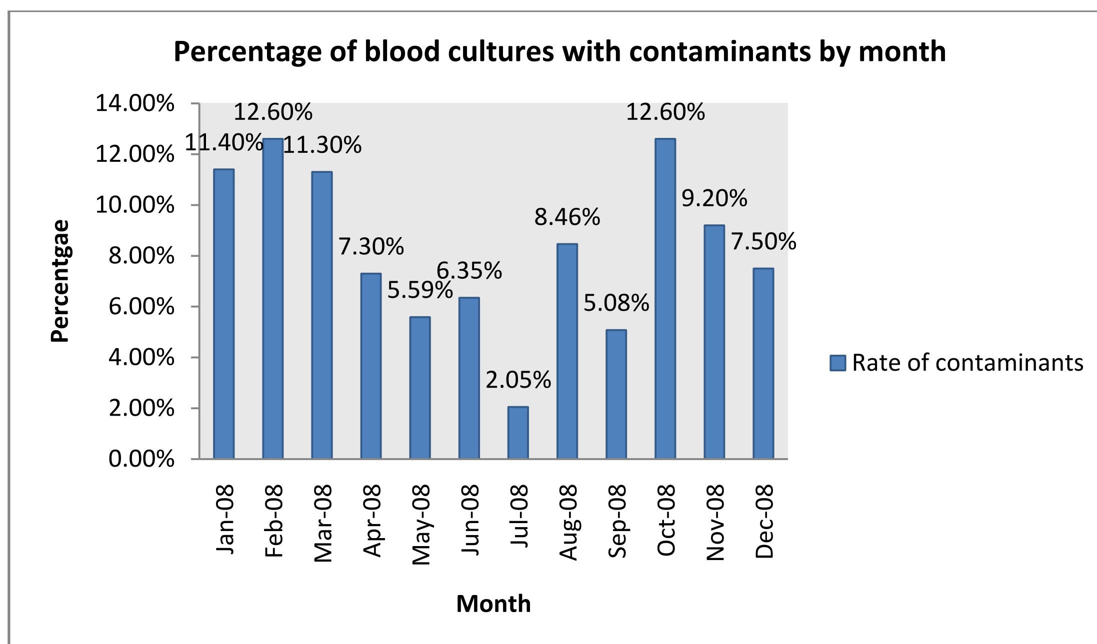

Figure 4.5: Rate of blood cultures with contaminants.

4.7 ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BLOOD CULTURE ISOLATES

The antimicrobial susceptibility pattern of the isolates was determined by both Kirby Bauer disc diffusion method and measurement of minimum inhibition concentration (MIC) using the E-test. The results of the Kirby Bauer disc diffusion method (CLSI guidelines) correlated with the E-test except two isolates of *Streptococcus pneumoniae* that were resistant to penicillin (oxacillin 1µg) by disc diffusion but completely susceptible to penicillin by measuring the MICs using the E-test.

4.7.1 Antimicrobial susceptibility testing by disc diffusion

Antimicrobial susceptibility pattern was determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar and results were interpreted according to the Clinical Laboratory Standard Institute guidelines.

4.7.1.1 Antimicrobial susceptibility of NTS by disc diffusion

All NTS isolates were susceptible to ciprofloxacin and ceftriaxone, however most strains were resistant to Ampicillin (81.3%), chloramphenicol (80.4%) and cotrimoxazole (77.6%) (Table 4.2).

Table 4.2: Antimicrobial susceptibility of 107 isolates of NTS

Antibiotics	Sensitive N (%)	Resistant N (%)
Ampicillin	20 (18.7)	87 (81.3)
Ampicillin+sulbactam	37 (34.6)	70 (65.4)
Ceftriaxone	107 (100)	0 (0)
Cotrimoxazole	24 (22.4)	83 (77.6)
Ciprofloxacin	107 (100)	0 (0)
Tetracycline	97 (90.7)	10 (9.3)
Chloramphenicol	21 (19.6)	86 (80.4)

4.7.1.2 Antimicrobial susceptibility of Salmonella Typhi by disc diffusion

All *Salmonella* Typhi isolates were susceptible to ceftriaxone (100%) and ciprofloxacin (100%). However, they were resistant to Ampicillin (60.9%), chloramphenicol (69.6%) and cotrimoxazole (69.6%) (Table 4.3).

Table 4.3: Antimicrobial susceptibility of *Salmonella* Typhi (n=23)

Antibiotics	Sensitive N (%)	Resistant N (%)
Ampicillin	9 (39.1)	14 (60.9)
Ampicillin+Sulbactam	19 (82.6)	4 (17.4)
Ceftriaxone	23 (100)	0 (0)
Cotrimoxazole	7 (30.4)	16 (69.6)
Ciprofloxacin	23 (100)	0 (0)
Tetracycline	10 (43.5)	13 (56.5)
Chloramphenicol	7 (30.4)	16 (69.6)

4.7.1.3 Antimicrobial susceptibility of Streptococcus pneumoniae by disc diffusion

Table 4.4: Antimicrobial susceptibility of *Streptococcus pneumoniae* (n=31)

Antibiotics	Sensitive N (%)	Resistant N (%)
Penicillin (Oxacillin)	29 (93.6)	2 (6.4)
Ceftriaxone	31 (100)	0 (0)
Erythromycin	31 (100)	0 (0)
Cotrimoxazole	31 (100)	0 (0)
Chloramphenicol	28 (90.3)	3 (9.7)

All pneumococci were susceptible to ceftriaxone, however 2 (6.4%) isolates were resistant to penicillin (Table 4.4).

4.7.1.4 Antimicrobial susceptibility of Staphylococcus aureus by disc diffusion

All *Staphylococcus aureus* isolates were susceptible to ceftriaxone (100%). Four (16.7%) of the *Staphylococcus aureus* isolates were resistant to cefoxitin. 50% of all the *Staphylococcus aureus* isolated were also resistant to penicillin (Table 4.5).

Table 4.5: Antimicrobial susceptibility of *Staphylococcus aureus* (n=24)

Antibiotics	Sensitive N (%)	Resistance N (%)
Penicillin	12 (50)	12 (50)
Ampicillin	12 (50)	12 (50)
Ampicillin+sulbactam (SAM)	20 (83.3)	4 (16.7)
Cefoxitin	20 (83.3)	4 (16.7)
Ceftriaxone	20 (83.3)	4(16.7)
Erythromycin	18 (75)	6 (25)
Cotrimoxazole	13 (54.2)	11 (45.8)
Ciprofloxacin	21 (87.5)	3 (12.5)
Gentamicin	19 (79.2)	5 (20.8)
Tetracycline	9 (37.5)	15 (62.5)

4.7.1.5 Antimicrobial susceptibility of Escherichia coli by disc diffusion

Fifty percent (50%) of the *Escherichia coli* isolates were resistant to ceftriaxone. However, they were all susceptible to ciprofloxacin (Table 4.6).

Table 4.6: Antimicrobial susceptibility of *Escherichia coli* (n=6)

Antibiotics	Sensitive N (%)	Resistant N (%)
Ampicillin	0 (0)	6 (100)
Ampicillin+Sulbactam	2 (33.3)	4 (66.7)
Ceftriaxone	3 (50)	3 (50)
Cotrimoxazole	0 (0)	6 (100)
Ciprofloxacin	6 (100)	0 (0)
Gentamicin	6 (100)	0 (0)
Chloramphenicol	2 (33.3)	4 (66.7)

4.7.1.6 Antimicrobial susceptibility of Klebsiella pneumoniae by disc diffusion

Table 4.7: Antimicrobial susceptibility of *Klebsiella pneumoniae* (n=5)

Antibiotics	Sensitive N (%)	Resistant N (%)
Ampicillin	0 (0)	5 (100)
Ampicillin+ Sulbactam (SAM)	1 (20)	4 (80)
Cefuroxime	2 (40)	3 (60)
Ceftriaxone	2 (40)	3 (60)
Ciprofloxacin	3 (60)	2 (40)
Gentamicin	2 (40)	3 (60)
Chloramphenicol	1 (20)	4 (80)

Sixty percent of the *Klebsiella pneumoniae* isolates were resistant to ceftriaxone, as forty percent were resistance to ciprofloxacin. All *Klebsiella pneumoniae* isolates were resistant to ampicillin and twenty percent sensitive to a combination of Ampicillin and β-lactamase inhibitor (sulbactam) (Table 4.7).

4.7.2. Antimicrobial Susceptibility Testing by MIC

E-test strips (AB Biodisk, Solna, Sweden) were used for determination of the Minimum Inhibition Concentration (MIC) of isolated organisms according to the instructions of the manufacturer. MICs were read at the point where inhibition ellipse intersected the scale on the strip after incubation at 37ºC for 24 hours.

4.7.2.1 Antimicrobial susceptibility of non typhoid salmonellae by MIC

All NTS isolates were susceptible to ciprofloxacin (MICs ≤ 1 µg/ml) and ceftriaxone (MICs ≤ 8 µg/ml) (Table 4.8).

Table 4.8: MIC results for NTS (n=107)

Antibiotic (Code)	MICs (µg/ml) Interpretive criteria			Sensitive N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)		
Ampicillin (AM)	8	16	32	18 (16.8)	89 (83.2)
Ciprofloxacin (CI)	1	2	4	107 (100)	0 (0)
Chloramphenicol (CL)	8	16	32	17 (15.9)	90 (84.1)
Tetracycline (TC)	4	8	16	97 (90.7)	10 (9.3)
Cotrimoxazole (TS)	2	-	4	18 (16.8)	89 (83.2)
Ceftriaxone (TX)	8	16-32	64	107 (100)	0 (0)

4.7.2.2 Antimicrobial susceptibility of Streptococcus pneumoniae by MIC

Table 4.9: MIC results for *Streptococcus pneumoniae* (n=31)

Antibiotic (Code)	MICs (µg/ml) Interpretive criteria			Sensitive N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)		
Penicillin (PG)	0.06	-	0.12	31 (100)	0 (0)
Clindamycin (CM)	0.5	1	2	31 (100)	0 (0)
Erythromycin (EM)	1	2	4	31 (100)	0 (0)
Tetracycline (TC)	2	4	8	10 (32.3)	21 (67.7)

All the *Streptococcus pneumoniae* isolates were susceptible to penicillin (MICs ≤ 0.06 µg/ml) and more than sixty percent were resistant to tetracycline (MICs ≥8 µg/ml) (Table 4.9).

4.7.2.3 Antimicrobial Susceptibility of Salmonella Typhi by MIC

Table 4.10: MIC results for *Salmonella* Typhi (n=23)

Antibiotic (Code)	MICs (µg/ml) Interpretive criteria			Sensitive N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)		
Ampicillin (AM)	8	16	32	10 (43.5)	13 (56.5)
Ciprofloxacin (CI)	1	2	4	23 (100)	0 (0)
Chloramphenicol (CL)	8	16	32	7(30.4)	16 (69.6)
Tetracycline (TC)	4	8	16	11(47.8)	12 (52.2)
Cotrimoxazole (TS)	2	-	4	7 (30.4)	16 (69.6)
Ceftriaxone (TX)	8	16-32	64	23 (100)	0 (0)

All *Salmonella* Typhi isolates were susceptible to ciprofloxacin (MICs ≤ 1 µg/ml) and ceftriaxone (MICs ≤ 8 µg/ml) (Table 4.10)

4.7.2.4 Antimicrobial susceptibility of Staphylococcus aureus by MIC

Table 4.11: MIC results for *Staphylococcus aureus* (n=24)

Antibiotic (Code)	MICs (µg/ml) Interpretive criteria			Sensitive N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)		
Penicillin (PG)	0.12	-	0.25	14 (58.3)	10 (41.7)
Oxacillin (OX)	2	-	4	20 (83.3)	4 (16.7)
Clindamycin (CM)	0.5	1-2	4	24 (100)	0 (0)
Erythromycin (EM)	0.5	1-4	8	20 (83.3)	4 (16.7)
Tetracycline (TC)	4	8	16	10 (41.6)	14 (58.3)

4.7.2.4.1. Methicillin Resistance Staphylococcus aureus (MRSA)

Four (16.7%) of the *Staphylococcus aureus* isolates were resistant to oxacillin (MICs ≥ 4 µg/ml). These isolates were further tested with penicillin binding protein (PBP2a) agglutination kit and tested positive for the kit. They were identified as methicillin resistant *Staphylococcus aureus* (MRSA) (Table 4.11).

4.7.2.5 Antimicrobial susceptibility of Escherichia coli by MIC

Table 4.12: MIC results for *Escherichia coli* (n=6)

Antibiotic (Code)	MICs (µg/ml) Interpretive criteria			Sensitive N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)		
Ampicillin (AM)	8	16	32	0 (0)	6 (100)
Ciprofloxacin (CI)	1	2	4	6 (100)	0 (0)
Chloramphenicol (CL)	8	16	32	2 (33.3)	4 (66.7)
Tetracycline (TC)	4	8	16	1 (16.7)	5 (83.3)
Cotrimoxazole (TS)	2	-	4	1 (16.7)	5 (83.3)
Ceftriaxone (TX)	8	16-32	64	3 (50)	3 (50)

Fifty percent (50%) of *Escherichia coli* isolates were resistant to ceftriaxone (MICs ≥ 64 µg/ml). All isolates were resistant to ampicillin (MICs ≥32 µg/ml). However they were all susceptible to ciprofloxacin (MICs ≤ 1 µg/ml) (Table 4.12).

4.7.2.6 Antimicrobial susceptibility of Klebsiella pneumoniae by MIC

Table 4.13: MIC results for *Klebsiella pneumoniae* (n=5)

Antibiotic (Code)	MICs (µg/ml) Interpretive criteria			Sensitive N (%)	Resistant N (%)
	Sensitive (≤)	Intermediate	Resistant (≥)		
Ampicillin (AM)	8	16	32	0 (0)	5 (100)
Ciprofloxacin (CI)	1	2	4	3 (60)	2 (40)
Chloramphenicol (CL)	8	16	32	1 (20)	4 (80)
Tetracycline (TC)	4	8	16	1 (20)	4 (80)
Cotrimoxazole (TS)	2	-	4	2 (40)	3 (60)
Ceftriaxone (TX)	8	16-32	64	2 (40)	3 (60)

Sixty percent (60%) of the *Klebsiella pneumoniae* isolates were resistant to ceftriaxone (MICs ≥ 64 µg/ml). The isolates showed 100% resistance to ampicillin (MICs ≥32 µg/ml), whilst 40% (MICs ≥ 4 µg/ml) were resistant to ciprofloxacin (Table 4.13).

4.8 ESBL TESTING BY MIC

Susceptibility testing of isolates to cefepime, cefotaxime, ceftazidime alone and in combination with clavulanic acid (4 μg/ml) were carried out to identify ESBL producing strains.

4.8.1 Determination of ESBL of E. coli by MIC

Fifty percent (50%) of the *Escherichia coli* isolates had MIC ratio ≥8 for all the cephalosporins in combination with clavulanic acid. There was also evidence of deformation of ellipse which was indicative of ESBL (Table 4.14).

Table 4.14: ESBL testing of the *Escherichia coli* using E-test (n=6)

Antibiotic (Code)	MICs (μg/ml) Interpretive criteria	Non ESBL Producers N (%)	ESBL Producers N (%)
Cefotaxime(CT)/ Cefotaxime + clavulanic acid (CTL)	CT ≥0.5 and CT/CTL ≥ 8	3(50)	3 (50)
Cefepime(PM)/ Cefepime + clavulanic acid (PML)	PM ≥0.25 and PM/PML ≥8	3 (50)	3 (50)
Ceftazidime(TZ)/ Ceftazidime + clavulanic acid (TZL)	TZ ≥1 and TZ/TZL ≥8	3 (50)	3 (50)

4.8.2 Determination of ESBL of Klebsiella pneumoniae by MIC

Sixty percent (60%) of all *Klebsiella pneumoniae* isolates were found to be ESBL producing strains (Table 4.15).

Table 4.15: ESBL testing of the *Klebsiella pneumoniae* using E-test (n=5)

Antibiotic (Code)	MICs (μg/ml) Interpretive criteria	Non ESBL Producers N (%)	ESBL Producers N (%)
Cefotaxime(CT)/ Cefotaxime+ clavulanic acid (CTL)	CT ≥0.5 and CT/CTL ≥ 8	2 (40)	3 (60)
Cefepime(PM)/ Cefepime + clavulanic acid (PML)	PM ≥0.25 and PM/PML ≥8	2 (40)	3 (60)
Ceftazidime(TZ)/ Ceftazidime+ clavulanic acid (TZL)	TZ ≥1 and TZ/TZL ≥8	2 (40)	3 (60)

CHAPTER FIVE

DISCUSSION

Out of the 1356 blood cultures taken, 15.3% lead to the identification of a pathogen, while 7.1% had contaminants and the remaining 77.6% were sterile. Generally, the low and variable diagnostic sensitivity of blood cultures is a limitation in studies on hospital prevalence. Small volumes of inoculated blood in paediatric patients and use of antibiotics prior to blood sampling may compromise the sensitivity of blood cultures. A study by Berkley *et al*., (2005) in Kenya found that the sensitivity of blood cultures fell by almost one third when cultured samples of 1ml were compared with those of 3ml. It was also observed that recent antibiotic use reduced blood culture yields by 62% to 73% in patients with severe or fatal disease. However, blood culture still remains the benchmark for the diagnosis of bacteraemia in most part of the world. The sensitivity of blood cultures could be improved by increasing the volume of blood cultured and also intensify education on the rational use of antibiotics.

Four organisms accounted for 60.9% of all bacteraemias in the present study; non-typhoid salmonellae (NTS) accounted for 35.2%, *Streptococcus pneumoniae* (10.2%), *Staphylococcus aureus* (7.9%) and *Salmonella* Typhi (7.6%). In consent with other studies in Africa, NTS and *Streptoccoccus pneumoniae* were the predominant causes of bacteraemia in children (Berkley *et al.*, 2005; Hill *et al.*, 2007; Maitland *et al.*, 2006).

The current study corroborates with a study by Evans *et al*., (2004) at the Komfo Anokye Teaching Hospital (KATH) in Ghana that identified NTS as the predominant causative organism of bacteraemia in children. NTS has also been identified as the dominant isolate in bacteraemia elsewhere in Africa (Bronzan *et*

al., 2007; Lepage *et al.*, 1987; Maitland *et al.*, 2006; Nesbitt *et al.*, 1989). In early childhood the high incidence of bacteraemia can mostly be explained by the high incidence of gastroenteritis, since at this age faecal isolation is high and the invasiveness ratio of the common NTS serotypes is low (Weinberger *et al.*, 2004). NTS has also been significantly associated with severe anaemia and malaria (Bronzan *et al.*, 2007; Evans *et al.*, 2004). The effective way to address the high incidence of NTS in Ghana and other parts of Africa is the development of vaccines. This has been demonstrated by dramatic reduction in severe illness caused by *Haemophilus influenzae* type b and *Streptococcal pneumoniae* after the introduction of these vaccines into routine immunisation programmes (Adegbola *et al.*, 2005; Berkley *et al.*, 2005; Cutts *et al.*, 2005).

The current rate of 10.2% *Streptococcus pneumoniae* bacteraemia obtained compares with the 11% reported by Bronzan *et al.*, (2007) in a prospective study involving 1388 children conducted in Malawi. However, studies conducted in Nigeria (Komolafe *et al.*, 2008) and Ghana (Evans *et al.*, 2004) identified only 0.5% to 2.0% *Streptococcus pneumoniae* infant bacteraemia respectively. These results differ from figures obtained in the current study. This could be attributed to the sensitivity of the methods used. The current study employed the use of Bactec 9050 automated blood culture system which is more sensitive compared to the conventional method (glucose broth, thioglycollate broth, brain heart infusion and the cooked meat broth) used in the isolation of the organism in the previous study (Rohner *et al.*, 1999).

Contrary to the present study, Maitland *et al.*, (2006) obtained 35% *Streptococcus pneumoniae* in a study involving 920 children in Kenya. This high rate could be due to the fact that there were more malnourished children in the

Kenya study who are mainly prone to invasive bacteria infections (Eddleston *et al.*, 2008).

Again in another study conducted in Kenya, Brent *et al*., (2006) obtained 50% *Streptococcal pneumoniae* bacteraemia in 1,093 children at the Kilifi District Hospital. This high incidence could also be due to the patient selection criteria. All children presenting to the hospital irrespective of their previous admission status were enrolled in this study, hence they were able to identify children with asymptomatic bacteraemia who did not show obvious sign of illness (Meremikwu *et al.*, 2005) who otherwise would have been missed.

This study has established *Streptococcus pneumoniae* as an important agent of bacteraemia in children under five years in Ghana. The most effective way to tackle these severe bacterial infections in Africa is through vaccination (Peltola, 2001) such as the virtual elimination of *Haemophilus influenzae* type b infection in the Gambia (Adegbola *et al.*, 2005) and the significant reduction of the disease in Ghana (Renner *et al.*, 2007). The success of the 9-valent pneumococcal conjugate vaccine against pneumococcal disease in the Gambia (Cutts *et al.*, 2005) also support the introduction of the vaccine into routine vaccination schedule in Ghana.

The 7.9% *Staphylococcus aureus* observed in the present study agrees with the 8% obtained Maitland *et al*., (2006) in a study conducted in Kenya. However, reports from other African countries including Ghana; Evans *et al*., (2004) 29%, Hill *et al*., (2007) 18.3%, Komolafe *et al*., (2008) 43.6% and Meremikwu *et al*., (2005) 48.7% identified high incidence of *Staphylococcus aureus* infant bacteraemia. The low incidence of this organism in the present study could be due to fact that this organism does not represent a significant clinical disease in the study area.

In the present study, *Salmonella* Typhi was responsible for 7.6% of all blood stream infections. This conforms to studies in Ghana and other parts of the world (Jao *et al.*, 2003; Komolafe *et al.*, 2008; Wilkens *et al.*, 1997). *Salmonella* Typhi disease is a life-threatening disease and treatment options are limited due to the emergence of drug resistant strains in regions with high use of antibiotics (Akinyemi *et al.*, 2007; Brent *et al.*, 2006). Approaches to addressing this concern should therefore focus on surveillance, drug sensitivity surveys and the introduction of vaccines against typhoid fever. Two vaccines are currently available, but they confer limited (50-80%) protection against *Salmonella* Typhi (Guzman *et al.*, 2006). Fluoroquinolones are the most effective drugs for the treatment of typhoid fever (CLSI, 2007).

The current study reported 6 (2%) and 5(1.6%) cases of *Escherichia coli* and *Klebsiella pneumoniae* isolates respectively. This is in agreement with a study in Ghana where 2 (4%) *Escherichia coli* bacteraemia was observed in 251 febrile children (Evans *et al.*, 2004). However, in other parts of Africa high incidences of *Escherichia coli* bacteraemia have been reported (Berkley *et al.*, 2005; Hill *et al.*, 2007; Komolafe *et al.*, 2008; Maitland *et al.*, 2006). This difference in the incidence could be due to the differing criteria for patient selection, Maitland *et al.,* (2006) enrolled children with severe malnutrition, where as Hill *et al*., (2007) and Berkley *et al*., (2005) enrolled all children that were admitted to the hospital. However, the current study enrolled only children with fever (Temperature $\geq$ 38.0ºC).

The in vitro antimicrobial susceptibility profile of the aetiological agents of bacteraemia has revealed that there is a growing emergence of multi-drug resistance microbes. The increasing resistance of bacteria to antibiotics have been

attributed to widespread abuse of these drugs which can be obtained over the counter or even in the open- air markets (Newman, 2001).

NTS isolated were highly resistant to cotrimoxazole (77.9%), chloramphenicol (80.4%) and ampicillin (81.9%), however they were all susceptible to ciprofloxacin and ceftriaxone, which conforms to a similar study in Ghana (Mills-Robertson *et al.*, 2003). This contrasts with another study in Ghana where all *Salmonella* isolates were susceptible to chloramphenicol (Wilkens *et al.*, 1997). This shows that there has been emergence of chloramphenicol resistance NTS isolates in Ghana. Increased susceptibility to chloramphenicol and other antibiotics (cotrimoxazole and ampicillin) have been reported in other African countries (Hill *et al.*, 2007; Komolafe *et al.*, 2008). In Ghana, ciprofloxacin and ceftriaxone are the first line drugs of choice the treatment of NTS bacteraemia (MOH, 2004). The susceptibility of non typhoid salmonellae to ciprofloxacin and ceftriaxone observed in the study support the current treatment regimen in Ghana.

Salmonella Typhi infection is a life-threatening disease and treatment options are limited due to the emergence of drug resistant strains in areas with indiscriminate use of antibiotics, and also due to non-availability of effective drugs for its treatment (Brent *et al.*, 2006). Majority of *Salmonella* Typhi isolates were resistant to cotrimoxazole (69.6%), chloramphenicol (69.6%) and ampicillin (60.9%). However, they were completely susceptible to ciprofloxacin and ceftriaxone. This observation contrasts studies in Nigeria and Ghana where *Salmonella* Typhi showed an increased susceptibility (80-100%) to cotrimoxazole, chloramphenicol and ampicillin (Komolafe *et al.*, 2008; Wilkens *et al.*, 1997). The difference in the susceptibility profile could be due to the emergence of drug resistance strains.

The present study observed that *Streptococcus pneumoniae* isolates were highly susceptible to penicillin (93.6%) by disc diffusion but 100% susceptible by E-test (MICs ≤ 0.06µg/ml). Penicillin has been an important drug in the treatment of *Streptococcus pneumoniae* infections in Ghana. This finding is in agreement with a study conducted in the Gambia (Hill *et al.*, 2007). However, another study in Ghana reported an increase in resistance to penicillin (19.4%) (Donkor *et al.*, 2010). This increase in resistance could be due to the emergence of penicillin resistance strains. Improved commitment to rational use of these antibiotics is needed to sustain this relatively high level of susceptibility to penicillin.

Susceptibility of *Staphylococcus aureus* to ceftriaxone remains high in this study (83.3%). So also is erythromycin (75%) and gentamicin (79.2%).This correlates with a study in Nigeria (Meremikwu *et al.*, 2005). The present study identified 4(16.7%) methicillin resistance *Staphylococcus aureus* (MRSA). MRSA is resistant to β-lactam antibiotics which include penicillin and cephalosporins (Klein *et al.*, 2007). This means patients with MRSA cannot be treated with readily available drugs such as the penicillin and cephalosporins. MRSA bacteraemia is associated with higher mortality rate, longer hospital stay and is a significant independent risk factor for death (Khairulddin *et al.*, 2004). The emergence of MRSA will put lots of pressure on health facilities to stock much more expensive drugs such as Vancomycin and Teicoplanin which are currently used in the treatment of MRSA infections (Schentag *et al.*, 1998). To prevent the spread of MRSA in hospitals, employers should ensure the availability of adequate facilities and supplies that encourage workers to practice good hygiene (Khairulddin *et al.*, 2004). Non-adherence to infection control practices, such as hand hygiene, is the

most potentially modifiable cause of health care-associated infections in hospitals (Asare *et al.*, 2009).

The present study identified 3(50%) and 3(60%) ESBLs producing *Escherichia coli* and *Klebsiella pneumoniae* strains respectively. ESBLs strains have also been reported in other studies (Ayisi, 2009; Gangoue-Pieboji *et al.*, 2005; Kim *et al.*, 2002). However, in a study in the Northern part of Ghana none of the *Escherichia coli* isolates produced ESBL (Djie-Maletz *et al.*, 2008). The high incidence of ESBL producing strains could be due to extensive use of broad-spectrum antibiotics in hospitalized patients which has led to increased carriage of these organisms (*Klebsiella pneumoniae* and *Escherichia coli*) and subsequently, the development of multidrug-resistant strains that produce ESBL. ESBL producers usually carry a multiresistant plasmid, the genes conferring resistance to β-lactam and non-β-lactam antibiotics (Jacoby, 1997). This means that no β-lactam antibiotics except carbapenems must be used for treatment (Bradford, 2001). ESBL infection can be passed from person to person through sub-optimal hygiene. Therefore better infection control and hygiene in hospitals, plus controlled and prudent use of antibiotics, is required to minimise the impact of ESBL and the spread of infections (Paterson *et al.*, 2004).

The current study established that ceftriaxone is active against NTS, *Streptococcus pneumoniae*, *Salmonella* Typhi and *Staphylococcus aureus.* Ciprofloxacin showed improved susceptibility to NTS, *Salmonella* Typhi, *Escherichia coli* and *Klebsiella pneumoniae.* Therefore either ceftriaxone or ciprofloxacin may be prescribed for the initial and empirical treatment of bacteraemia in our environment pending culture and sensitivity reports. However, extensive use of ceftriaxone and ciprofloxacin in hospitalized patients can lead to

the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase (ESBL).

5.1 STUDY LIMITATIONS

For optimum recovery of bacteria from blood cultures in children, 3ml of blood is required for culture. However, due to the difficulty of sample collection from paediatric patients variable volumes (1-3mls) of blood were obtained for culture.

We could not ascertain that patients enrolled in the study had not taken antibiotics prior to admission and sample collection even though it was understood from the parents/guardian that they had not been on antibiotics.

Antibiotics have a greater effect in reducing blood bacteria count compared to bone marrow (Wain *et al.*, 2001), and also despite successful treatment bone marrow samples may remain positive for up to 5 days or longer after starting effective treatment with antibiotics (Gasem *et al.*, 1995). This contributes to the superiority of bone marrow isolation over blood culture in determining prevalence.

5.2 CONCLUSION

Given the high incidence of bacteraemia in this study, blood stream infection should be considered an important childhood illness in patients admitted at the Agogo Presbyterian Hospital in the Asante -Akim North District.

The present study also established that majority of the causative agents of bacteraemia in children in the study area are resistant to majority of locally available antibiotics.

5.3 RECOMMENDATION

1. Due to the high incidence of bacteraemia observed in this study, it is recommended that children under five years who report to the hospital with fever should have blood cultures done as part of the diagnosis.
2. Health workers should ensure that blood for cultures are taken before administration of antimicrobial therapy.
3. The massive emergence of multi-drug resistant organisms (MRSA and ESBLs) in clinical isolates indicates a serious medical and economic burden for our health care systems. It is recommended that educational programmes aimed at ensuring more rational use of antibiotics is increased.
4. ESBL and MRSA testing should be performed routinely in the laboratory. Laboratory scientist should be trained to detect MRSA and ESBL.
5. Hospitals and clinics should perform cultures and antimicrobial susceptibility testing before prescribing antibiotics.

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APPENDICES

APPENDIX 1

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

CONSENT FORM

CHILD WELFARE CLINIC
Version 5.0 (11/11/2007)

Barcode

OPD number | Inpatient-number | Date ____/____/____

Study child data *Registration*

Is the guardian the child's mother? ○ No ○ Yes ○ Alone

Address/Telephone

Family name ____________

Given name ____________

Sex ○ Male ○ Female

Date of birth ___/___/___ or age_____ ○ Months ○ Years

Village ____________

Mother's ethnicity ____________

--> *if parents ethnicities differ from each other:*

Parent's ethnicity differs from each other? ○ No ○ Yes

Father's ethnicity ____________

--> *if the guardian is the father:*

Mother's name ____________ Father's name ____________

Mother's age _____ Father's age _____

Mother's occupation ____________ Father's occupation ____________

Informed consent

We want to collect all the information about the ill children who come to the under fiver clinic and put it on a computer. We will then be able to see what diseases are important in and around Agogo. All the computer information will be anonymous and confidential.

However if we find your child has a certain infection we will inform the doctors in the clinic so you can be prescribed the correct treatment. Sometimes this will be immediately and sometimes this will be some days later.

Do you agree to including your child's information on our computer?
☐ Yes ☐ No

Do you agree to your child providing a stool sample to be tested for different infections?
☐ Yes ☐ No

Do you agree to your child providing a sputum sample to be tested for different infections?
☐ Yes ☐ No

--> If your child is admitted to the paediatric ward:
Do you agree to your child providing a blood sample to look for bacteria in the blood?
☐ Yes ☐ No

Signed/Thumb Print ☐ MOTHER ☐ FATHER ☐ GUARDIAN:

____________ Sign | ____________ Guardian | ___/___/___ Date

Witnessed by

____________ Sign | ____________ Name in block letters | ___/___/___ Date

Consent obtained by

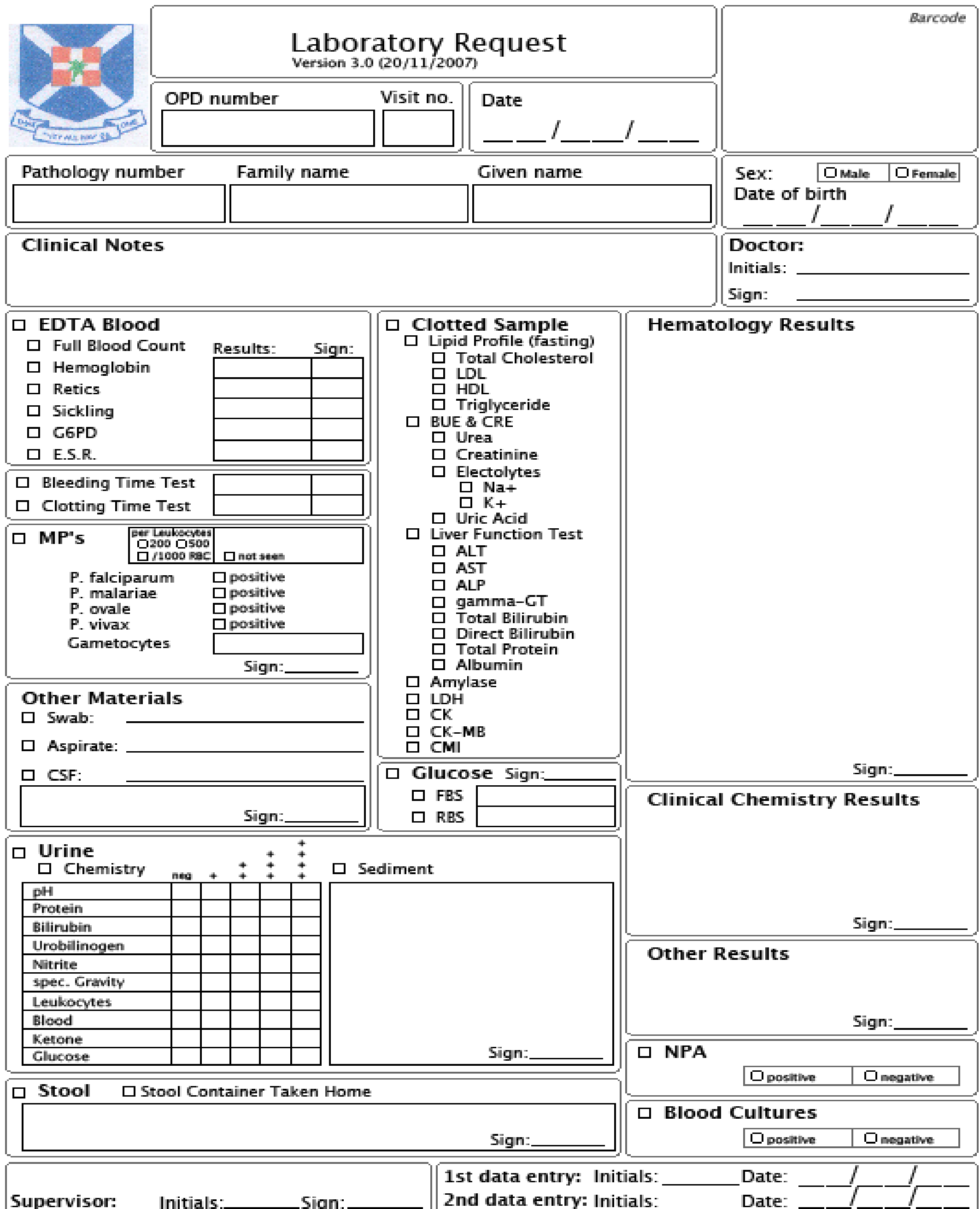
____________ Sign | ____________ Name in block letters | ___/___/___ Date

Registering person: ____________ Sign:________

APPENDIX 2

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

LABORATORY REQUEST FORM



Laboratory Request
Version 3.0 (20/11/2007)

Barcode

OPD number | Visit no. | Date ___/___/___

Pathology number | Family name | Given name

Sex: ○ Male ○ Female
Date of birth ___/___/___

Clinical Notes

Doctor:
Initials: ________
Sign: ________

☐ EDTA Blood

	Results:	Sign:
☐ Full Blood Count		
☐ Hemoglobin		
☐ Retics		
☐ Sickling		
☐ G6PD		
☐ E.S.R.		

☐ Bleeding Time Test
☐ Clotting Time Test

☐ MP's — per Leukocytes ☐200 ☐500 ☐/1000 RBC ☐ not seen

P. falciparum ☐ positive
P. malariae ☐ positive
P. ovale ☐ positive
P. vivax ☐ positive
Gametocytes

Sign: ________

Other Materials
☐ Swab: ________
☐ Aspirate: ________
☐ CSF: ________
Sign: ________

☐ Clotted Sample
- ☐ Lipid Profile (fasting)
  - ☐ Total Cholesterol
  - ☐ LDL
  - ☐ HDL
  - ☐ Triglyceride
- ☐ BUE & CRE
  - ☐ Urea
  - ☐ Creatinine
  - ☐ Electolytes
    - ☐ Na+
    - ☐ K+
  - ☐ Uric Acid
- ☐ Liver Function Test
  - ☐ ALT
  - ☐ AST
  - ☐ ALP
  - ☐ gamma-GT
  - ☐ Total Bilirubin
  - ☐ Direct Bilirubin
  - ☐ Total Protein
  - ☐ Albumin
- ☐ Amylase
- ☐ LDH
- ☐ CK
- ☐ CK-MB
- ☐ CMI

☐ Glucose Sign: ________
☐ FBS
☐ RBS

Hematology Results
Sign: ________

Clinical Chemistry Results
Sign: ________

Other Results
Sign: ________

☐ Urine
☐ Chemistry

	neg	+	++	+++	++++
pH					
Protein					
Bilirubin					
Urobilinogen					
Nitrite					
spec. Gravity					
Leukocytes					
Blood					
Ketone					
Glucose					

☐ Sediment
Sign: ________

☐ NPA ○ positive ○ negative

☐ Stool ☐ Stool Container Taken Home
Sign: ________

☐ Blood Cultures ○ positive ○ negative

Supervisor: Initials: ________ Sign: ________

1st data entry: Initials: ________ Date: ___/___/___
2nd data entry: Initials: ________ Date: ___/___/___

APPENDIX 3

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

GRAM STAIN

PREPARATION OF THE SMEAR

- A thin film of the material was made on a clean glass slide, using a sterile loop. It was air dried and then heat fixed by passing it several times through a flame.

STAINING PROCEDURE

- After fixing, the slide was flooded with crystal violet for 1 minute, and then washed with running tap water for 5 seconds to remove the unbound crystal violet.
- Gram's iodine was added for 1 minute and washed with water.
- It was decolorized with acetone alcohol until thinnest parts of the smear are colourless and washed with water.
- Safranin was added for 1 minute and washed with water.
- It was air dried and examined under the 100x oil immersion objective of the microscope.
- Control slides were stained together with the sample to control the Gram staining solution.

APPENDIX 4

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

CATALASE TEST

PROCEDURE

- A small amount of growth from the culture was placed onto a clean microscope slide.
- One drop of H_2O_2 was added onto the smear.

INTERPRETATION

Positive reaction: immediate and vigorous bubbling of the hydrogen peroxide.

Negative reaction: no bubbling.

APPENDIX 5

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

COAGULASE TEST

PROCEDURE

- Three sterile tubes containing 0.5ml rabbit plasma were prepared and labelled.
- A few colonies of the unknown strain was emulsified in one of the tubes and the positive and negative controls (*Staphylococcus aureus* ATCC 25923 positive, *S. epidermidis* ATCC 12228 negative) in the other tubes.
- The tubes were incubated at 37°C.
- They were examined for coagulation at 1, 3, 6 and 24 hours for the presence of the clot.

INTERPRETATION

Positive reaction ⇒ *S.aureus*

Unknown strain and *S. aureus* reference strain show clot formation, *S. epidermidis* reference strains shows no clot formation.

Negative reaction ⇒ coagulase negative Staphylococcus species.

APPENDIX 6

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

DNAse TEST

PROCEDURE

- A tryptose agar medium containing DNA was labelled with the specimen number and control organisms.
- The plate was inoculated with one colony of the organism under test.
- *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pneumoniae* (ATCC 49619) were inoculated as positive and negative controls.
- The plates were incubated aerobically at 37°C for 18-24 hours.
- After incubation, the plate was flooded with lM HCl. DNA precipitates and turns the medium cloudy. The plates were left for a few minutes.
- The presence of a zone of clearing around the area of growth indicates DNAse production that has hydrolyzed the DNA.

INTERPRETATION

In the presence of hydrochloric acid:
DNAse positive gave a clear zone surrounding the inoculum streak with the rest of the plate remaining opaque.
DNAse negative gave an absence of clear halo around the inoculum streak.

APPENDIX 7

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

STREPTOCOCCI GROUPING

PREPARATION OF CULTURES

- A bottle of Oxoid Streptococcus extraction enzyme (DR593) was reconstituted with sterile distilled water. Test tubes were labelled and 0.4ml of the enzyme was dispensed into each test tube.
- Two colonies of the test organisms were selected with a bacteriological loop and emulsified in the enzyme preparation.
- The tubes were incubated for 10min at 37°C. After 5 min incubation the tubes were removed shaken vigorously for 2-3 seconds, and then incubated again.

TEST METHODS

- The latex reagents were brought to room temperature and mixed vigorously by shaking.
- One drop of each latex reagent was dispensed into the circular rings on the reaction card (DR500).
- A pasteur pipette was used to add 1 drop of the extract to each of the 6 rings.
- The mixing stick provided was used to spread the mixture over the entire area of the ring.
- The card was gently rocked for 1 minute.

INTERPRETATION OF RESULTS

- The test was considered positive when agglutination occurred with one grouping reagent.
- The test was considered negative when no agglutination occurred.

APPENDIX 8

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

OPTOCHIN IDENTIFICATION

PROCEDURE

- With a water insoluble marker, sheep blood agar plate was divided into three sections, and labelled.
- With an inoculating loop, two or three isolated colonies were selected on sheep blood agar and these colonies streaked for confluent growth onto the labelled section of the plate. This was repeated for the control organisms.
- Optochin disk (containing 5 µg of ethylene hydrocupreine hydrochloride) was placed onto the center of each streaked area on the agar surface with a forceps.
- The plate was inverted and incubated for 18-24 hours under CO_2 conditions.
- The plate was observed for zone of inhibition, and the zone sizes were measured.

INTERPRETATION

Positive test: Zone of inhibition ≥ 16 mm.

Negative test: Zone diameter of inhibition < 14 mm.

APPENDIX 9

PREVALENCE OF BACTERIA CAUSING BACTERAEMIA IN CHILDREN UNDER FIVE YEARS IN AGOGO, ASANTE-AKYEM AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

OXIDASE TEST

PROCEDURE

- A sterile wooden stick was used to pick 2-3 colonies from a plate culture onto a piece of filter paper.
- One drop of freshly prepared oxidase reagent was added onto the filter paper.

INTERPRETATION

- A positive reaction appeared bluish-purple that progressively becomes deep purple within 10-15 seconds.
- Negative reaction, no colour change.