DIVERSITY AND ANTIBIOTIC SUSCEPTIBILITY OF BACTERIA SPECIES ISOLATED FROM BLOOD OF FEBRILE PATIENTS IN ALUPE, WESTERN KENYA

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DECLARATION

I, Njoki Ndegwa Pauline, do hereby declare that this thesis is my original work and has not been presented for a degree in this or any other university.

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ABBREVIATIONS

ANOVA: Analysis of Variance
ATCC: American Type Culture Collection
BD: Becton, Dickinson and Company
BSIs: Blood Stream Infections
CA-MRSA: Community-associated- Methicillin/oxacillin-resistant *Staphylococcus aureus*
CDC: Centre for Disease Control
CIPDCR: Centre for Infectious and Parasitic Diseases Control Research
CMR: Centre for Microbiology Research
DH: District Hospital
ESBLs: Extended-spectrum Beta-lactamases
GNB: Gram negative bacilli
GPC: Gram positive cocci
Hib: *Haemophilus influenza* type b
HIV: Human Immunodeficiency Virus
ICU: Intensive Care Unit
IgG: Immunoglobulin G
KEMRI: Kenya Medical Research Institute
km: Kilometre
MED SUP: Medical Superintendent
MIO: Motility Indole Ornithine
MRSA: Methicillin/oxacillin-Resistant *Staphylococcus aureus*
NCCLS: National Committee for Clinical Laboratory Standards
NDM: New Delhi Metallo-beta-lactamase
NIAID: National Institute of Allergy and Infectious Diseases
NMPDR: National Microbial Pathogen Data Source
NTS: Non-Typhi *Salmonella*
OR: Odds Ratio
OX+: Oxidase positive
OX-: Oxidase negative
P: Probability Value
PCR: Polymerase Chain Reaction
PRSP: Penicillin-Resistant *Streptococcus Pneumoniae*
STD'S: Sexually Transmitted Diseases
SCD: Sickle Cell Disease
TSI: Triple Sugar Iron
UTI: Urinary tract infection
UV: Ultra violet
VRE: Vancomycin-Resistant Enterococci
WHO: World Health Organization

ABSTRACT

Bacterial pathogens cause significant number of deadly diseases and widespread epidemics in man. The mortality associated with these diseases is higher in developing countries where the impact is difficult to estimate as symptoms are similar to other non- bacterial febrile illnesses. Bacteria are transmitted through deep unclean wounds, minor skin breaks or bruises, eyes, oral fecal route, respiratory tract, bites from arthropods and urinary tract. The objective of this study was to determine the diversity of bacteria isolated from the blood of patients presenting with febrile illness seeking health care at Alupe District Hospital and KEMRI CIPDCR clinic in Alupe, Western Kenya. The antibiotic susceptibility profile of drugs commonly used in Alupe was also determined using a standard interpretative table. A total of 200 patients were recruited and their social demographic data collected after obtaining their consent. Thereafter, their blood samples were collected. Laboratory analysis was done at KEMRI-CIPDCR laboratory to identify and characterize bacteria pathogens by conventional methods using microbial culture on various agars and biochemical tests. The youngest and oldest patients were 2 and 82 years, respectively. The proportion of male patients was 38.5% while 61.5% were female. Five bacterial species were detected in 7 of the 200 blood samples. Of the total isolates, 57.1% were Gram- negative whilst 42.9% were Gram-positive. The five bacteria species isolated were *S. aureus* (3 isolates), *E. coli* (2 isolates), *P. mirabilis* and *K. pneumoniae* (1 isolate each). No blood sample yielded more than one species. Data were subjected to analysis using Stata version 10.0 and a standard interpretative table was used for comparison of antimicrobial resistance patterns of the isolates. The odds of bacteremia increased in patients who had a heart murmur (OR = 15.9, p = 0.0047), rash (OR = 6.6, p = 0.0162), severe headache (OR = 4.8, p = 0.0305) and swollen lymph nodes (OR = 28.2, p = 0.0000). The odds of bacteremia were significantly lower in patients who had mild headache (OR = 0.2, p = 0.0338). Overall, all the isolates were 100% susceptible to chloramphenicol and gentamicin with the highest resistance being to erythromycin (71.4%), ampicillin-cloxacillin (57.1%) and cefuroxime (57.1%). The isolates were relatively susceptible to amoxyllin-clavunic acid, ceftazidime and nitrofurantoin and showed the lowest susceptibility to nalidixic acid (14.3%). Bacteremia was not common in febrile patients visiting the two health facilities in Alupe, Western Kenya. This study provided data on specific causative and prevalent bacterial species that presented febrile illness as a symptom and provides guidelines on antibiotic use in the management of bacteremia in Alupe where malaria is endemic. If bacteremia is suspected on admission or at any later stage in management of patients, initial antibiotic therapy with chloramphenicol should be undertaken.

Key words: Age groups, Antibiotic susceptibility, Bacteremia, Bacteria spp., Febrile patients

CHAPTER ONE

1.0 INTRODUCTION

1.1 Definition of bacteremia

Bacteremia is the presence of bacteria in the blood as detected by blood cultures. Blood is normally sterile and hence the presence of bacteria in blood always presupposes an infectious focus elsewhere in the body (the source) from which the blood becomes seeded (Torre *et al*., 2008). Bacteremia occurs when bacteria enter the bloodstream through surgical and clinical procedures, an injection or because of a wound or an infection. It may not cause visible symptoms and may resolve without any treatment on its own due to action of the immune system defenses such as lymphocytes and antibodies. In other cases, it may produce fever and other symptoms of infection. In some cases, bacteremia may lead to septicemia which causes septic shock and multiple organ dysfunctions (Chamberlain, 2004). Sepsis is often life threatening especially in people with weakened immune system or other medical illnesses. Most systemic symptoms of infection are not due to the proliferation of the bacteria but to inflammatory reactions triggered by the infectious process (Root *et al*., 2000).

1.2 Background information

In early1900's, infectious bacterial diseases were the leading cause of morbidity and mortality, and diseases such as tuberculosis and pneumococcal infection were called the "Captain of all these men of death" (Cohen, 2001). Bacterial pathogens have caused a significant number of deadly diseases and widespread epidemics in human civilization. The mortality associated with these diseases is even higher in developing countries due to poor sanitation services, inadequate health facilities, limited health systems, rapid urbanization and population growth. At the beginning of the

twentieth century, pneumonia, tuberculosis and diarrhea were the three leading causes of death (Todar, 2004). Although some bacterial diseases have been conquered, many new bacterial pathogens have been recognized in the past 30 years, while many 'old' bacterial pathogens like *Staphylococcus aureus* and *Mycobacterium tuberculosis* have emerged with new forms of virulence and new patterns of resistance to antimicrobial agents (Todar, 2004). In the last two decades, bacterial diseases have been newly recognized, including: Legionnaires' disease, toxic shock syndrome, Lyme disease, campylobacteriosis, *Escherichia coli* 0157: H7 infections, Helicobacter infections associated with peptic ulcer disease and *Bartonella* infections associated with cat scratch disease. Other diseases, such as meningococcal infections, *Salmonella enteritidis* infections associated with shell-eggs, food borne listeriosis, and tuberculosis have increased in frequency, and in some instances in both industrialized and developing countries (Cohen, 2001).

Sub Saharan Africa contributes significantly to the global mortality of children aged less than 5 years with mortality rates of 100-250 per 1000 (Obaro *et al*., 2011). It is now well established that invasive bacteria disease is the leading cause of childhood mortality in Sub-Saharan Africa. Most of these infections are preventable by the use of vaccines which are already licensed and in routine use in most developed countries. However, few health facilities in Africa have the facilities with capacity to identify invasive bacterial infection (Obaro *et al*., 2011).

The diagnosis of bacterial diseases, especially those that cause fever, on clinical grounds is hard as many of these diseases show similar symptoms to other diseases caused by other microorganisms i.e. protozoa, mycoplasma, rickettsia, viruses, viroids, prions, fungi and Chlamydia (Myers *et al*., 2002; Bhatia *et al*., 2003). From a clinical perspective, enteric fevers and especially non-typhoidal bacteremia are characterized by non-specific symptoms and therefore are not easily distinguished

from other diseases common in endemic areas, such as dengue fever, malaria or leishmaniasis (Porwollik, 2011). This results in misdiagnosis of the exact causative agent and incorrect and/or inadequate prescription in many health centres and hospitals. This in turn results in multi-drug resistance.

Randomized controlled efficacy trials in Gambia and South Africa and effectiveness studies in Malawi uncovered substantial disease burden from *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae*. Population based surveillance studies in Kenya have demonstrated substantial disease caused by *S. pneumoniae* (Obaro *et al*., 2011). While *S. pneumoniae* has been identified as the leading cause of bacteremia or invasive bacteria disease in Kenya, Gambia and Mozambique, this was not the case in Ghana and Malawi where non-typhi *Salmonella* (NTS) predominated (Obaro *et al*., 2011).

Research findings from several parts of Kenya indicate that bacterial diseases that present fever are still misdiagnosed for other diseases. Doctors in Kilifi, Kenya, noticed a high mortality rate in children admitted with high fever. They processed blood cultures in the laboratory for every child admitted between August 1998 and July 2002. They found that bacteremia was the leading cause of deaths in children, responsible for one in three infant deaths and a quarter of deaths in older children (Okeke, 2011). The current study therefore focused on determination of diversity and antibiotic susceptibility of bacteria species isolated from the blood of febrile patients from two health centres in Alupe, Western Kenya.

1.3 Problem statement and justification

Bacteremia is still problematic and common in many parts of rural Kenya. This is due to prevalent unhygienic conditions accompanied by poor sanitation, poor heath and malnutrition, lack of access

to health services, poor infrastructure, other prevailing illnesses such as HIV, and poverty. It has a direct proportionality to the socio-economic level of the affected communities, areas and regions. This is due to costs incurred during treatment and also the time taken for adult patients to recover and get back to their jobs. Children especially, do not recover and most of them succumb to the disease (Root *et al*., 2000). According to a study conducted in Kilifi, Kenya, 103 of 308 deaths in children with bacteremia occurred on the day of admission, 79 occurred the next day and 35 occurred on the third day (Berkley *et al*., 2005).

Resistance to antimicrobial drugs is causing increased mortality and morbidity from bacteremia. There are no documented studies carried out in Kenya on bacteremia of both adult and children population, with majority of the previous studies done on children only. Though only two documented studies have been carried out in Kenya on bacteremia, much still remains unknown about the rate and outcome of bacteremia in Alupe, Western Kenya, the types of bacteria species responsible and resistance of the bacteria species to commonly used antibiotics. Antimicrobial resistance, once thought to be primarily a problem of hospital-acquired infections, is also a problem among community-acquired infections. This study investigated the specific causative and prevalent bacterial species that present febrile illness as a symptom in Alupe, Western Kenya. The data generated will be useful to the ministries of Medical Services and Public Health and Sanitation in Kenya, as well as other relevant authorities and stakeholders in the health sector. This will be useful in developing strategies necessary for managing common bacterial diseases in the region. Additionally, the data will help regulate the use of antibiotics, improve surveillance of the emergence of resistant bacteria, educate and caution the medical personnel on misdiagnosis of bacteremia for other diseases such as malaria and dengue fever.

1.4 Research hypotheses

Null hypothesis: Bacteremia is not common in patients presenting with febrile illness in Alupe, Western Kenya.

Alternative hypothesis: Bacteremia is common in patients presenting with febrile illness in Alupe, Western Kenya.

1.5 Research questions

a) What is the prevalence of bacteremia in Alupe, Western Kenya?
b) What age group is most susceptible to bacteremia in Alupe, Western Kenya?
c) Which are the most prevalent bacterial species isolated from patients with bacteremia?
d) Are the bacteria species that cause febrile illness in Alupe resistant to commonly used antibiotics?

1.6 Objectives of the study

Broad objective

To determine the diversity and antibiotic susceptibility of bacteria species isolated from the blood of febrile patients in two health centers in Alupe, Western Kenya.

Specific objectives

i) To determine the prevalence of bacteria species from blood of patients presenting with febrile illness in Alupe District Hospital and KEMRI-CIPDCR in Alupe, Western Kenya.
ii) To determine the diversity of bacteria species associated with febrile illness from blood of patients presenting fever symptoms in Alupe, Western Kenya.
iii) To determine the antibiotic susceptibility profile of bacteria isolated from patients presenting with febrile illness in Alupe, Western Kenya.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical aspect of bacteremia

Bacterial diseases have been in existence since ancient times with several historically significant pandemics occurring and killing millions of people and clearing populations. Life before the discovery of penicillin was plagued by several bacterial endemics starting from 541 to 751 AD when the Plague of Justinian killed as many as 100 million people, constituting 50% and 60% of Europe's population (Drancourt *et al*., 2004). The plague was caused by *Yersinia pestis* which is the causal agent for a reemerging zoonotic disease transmitted to humans through flea bites and is typically characterized by the appearance of a tender and swollen lymph node, the bubo (Drancourt *et al*., 2004).

The Black Death was also one of the most devastating pandemics of 1347 AD which killed 75 - 200 million people in the world and about 25 million people in Europe over 5 years. The fatalities were estimated to be between 25 and 50% of the populations of Europe, Asia, and Africa (Williman, 1982; Hays, 2005). The plague continued to strike parts of Europe sporadically until the 17th century, each time with reduced intensity and fatality. It was also caused by variant strains; 'Orientalis' and 'Medievalis' of *Yersinia pestis* (Williman, 1982; Hays, 2005).

Pneumonia has been a common disease throughout human history. Pneumonia was first detected in the airways of individuals who died from pneumonia by Edwin Klebs in 1875. The last major pandemic ran from 1855-1896 worldwide, but mostly in China and India, where more than 12 million died. Manchuria in 1910–1911 witnessed about 60,000 deaths due to bacterial pneumonic plague with a repeat in 1920–1921; and a minor outbreak occurred in the summer of 1994 in Surat,

India. *Streptococcus pneumoniae* was found to be the main causal agent. Bacteria that are the most common causes of community acquired pneumonia are: *Streptococcus pneumoniae* which is isolated in nearly 50% of cases, *Haemophilus influenzae* in 20%, *Chlamydia pneumoniae* in 13% and *Mycoplasma pneumoniae* in 3%. Other less common causes of pneumonia include *Staphylococcus aureus*, *Moraxella catarrhalis*, *Legionella pneumophila* and *Klebsiella pneumoniae*. Most of these bacteria infect people with predisposing chronic medical illnesses like diabetes (Sharma *et al*., 2007; Anevlavis *et al*., 2010; Brown *et al*., 2010). Pneumonia is the largest cause of death in children worldwide, where it kills an estimated 1.4 million children under the age of five years each year (WHO, 2012). Other common bacterial diseases worldwide are shown in Table 1.

Most of the diseases have been counteracted by vaccines and drugs e.g. diphtheria which has a vaccine administered during infancy. Despite remarkable progress in treatment and prevention, infectious diseases are still considered as leading causes of death and disability and worsening life quality especially for millions of people in developing countries. Some types of bacteremia have an increasing trend in some regions of the world (Madsen *et al*., 1999), where numerous bacteria have been isolated (Reacher *et al*., 2000; Peltola, 2001).

The most common bacteria that cause bacteremia worldwide include: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, group B Streptococcus, *Streptococcus agalactiae*, *Salmonella enteritidis*, *Proteus mirabilis*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Aeromonas* spp., *Escherichia vulneris, Campylobacter* spp., *Enterobacter cloacae, Enterococcus* spp., *Escherichia coli, Haemophilus influenzae* and *Acinetobacter* spp. (Gorbach *et al*., 2003).

Table 1: Common bacterial diseases experienced worldwide.

Disease	Causative bacteria
Anthrax	*Bacillus anthracis*
Bacillary dysentery	*Shigella dysenteriae*
Bacterial meningitis	*Haemophilus influenzae, Neisseria meningitides, Streptococcus pneumoniae*
Botulism	*Clostridium botulinum*
Brucellosis	*Brucella melitensis*
Cat-scratch disease	*Bartonella hensellae*
Cellulitis, erysipelas, folliculitis, impetigo and scalded skin syndrome	*Staphylococcus aureus, Streptococcus pyogenes*
Chlamydia	*Chlamydia trahomatis*
Cholera	*Vibrio cholera*
Diphtheria	*Corynebacterium diphtheria*
Gangrene	*Clostridium perfringens,* other *Clostridium* spp.
Gonorrhea	*Neisseria gonorrheae*
Heart infections	*Staphylococcus aureus*
Leprosy	*Mycobacterium leprae*
Leptospirosis	*Leptospira interrogans*
Lyme disease	*Borrelia burgdorferi*
Respiratory tract, urinary tract, surgical wounds, skin and soft tissues infections of adult patients	*Serratia marcescens*
Meningicoccal meningitis	*Neisseria meningitides*
Paratyphoid	*Salmonella paratyphi*
Periodontal disease	*Streptococcus mutans*
Pyelonephritis and cystitis	*Proteus, Pseudomonas, Enterobacter*, *Enterococcus*
Rat-bite fever	*Streptobacillus moniliformis*
Strep throat	*Streptococcus pyogenes*
Syphilis	*Treponema pallidum*
Tetanus	*Clostridium tetani*
Trachoma	*Chlamydia trachomatis*
Tularemia	*Francisella tularensis*
Typhoid fever	*Salmonella typhi*
Typhus	*Rickettsia* spp.
Whooping cough	*Bordetella pertussis*

Source: (Myers *et al*., 2002; Bhatia *et al*., 2003; Murray *et al*., 2008).

2.2 Bacteremia

There are two types bacteremia: nosocomial and community acquired bacteremia (Nadel, 2008).

Nosocomial bacteremia is a hospital acquired bacterial infection whose development is favoured

by a hospital environment, such as one acquired by a patient during a hospital stay at the time of admission, which was not present or incubating. These are normally aggravated by the individuals' reduced immune resistance. Most are hard to treat due to their increased antibiotic resistance. Community acquired bacteremia on the other hand refers to bacterial infections contracted outside of a health care setting or an infection present on admission. Most of these infections are usually more antibiotic sensitive. Approximately half of all bloodstream infections (BSIs) occur in children less than one year old, half are community acquired and less than 15% occur in previously healthy children. In developed countries, approximately two thirds of organisms isolated from bacteremic patients are Gram-positive, one quarter are Gram-negative aerobic organisms, and 1% to 5% are fungi. Anaerobes are rarely isolated and constitute 0.4% to 1.4% of the isolates (Nadel, 2008).

The most common Gram-positive infections are methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA) infections. These occur in otherwise healthy people who have not been recently (within the past year) hospitalized nor had a medical procedure (such as dialysis, surgery, catheters) and are categorized as community-associated methicillin/oxacillin-resistant *Staphylococcus aureus* (CA-MRSA) infections. These infections are usually skin infections, such as abscesses, boils, and other pus-filled lesions. About 75% of CA-MRSA infections are localized to skin and soft tissue and usually can be treated effectively. However, CA-MRSA strains display enhanced virulence, spread more rapidly and can cause severe illness and affect vital organs leading to widespread infection (sepsis), toxic shock syndrome and pneumonia. It is not known why some healthy people develop CA-MRSA skin infections that are treatable whereas others infected with the same strain develop severe, fatal infections (Todar, 2009).

Extended-spectrum beta-lactamases (ESBL) producing strains have been isolated from abscesses, blood, catheter tips, lungs, peritoneal fluid, sputum, and throat cultures. They have a world-wide distribution while the rates of isolation vary greatly worldwide and within geographic areas and are rapidly changing over time. The most common ESBL producing species are *Klebsiella* spp. (predominantly *Klebsiella pneumoniae*) and *E. coli*, though other ESBL producing species have been found throughout the *Enterobacteriaeae* family (Todar, 2009).

2.2.1 Transmission of bacteria pathogens

Bacterial pathogens have been isolated from blood, many of which cause diseases that have one common symptom; fever. Bacterial diseases of the circulatory system enter the body through minor skin breaks, oral cavity infections, sexually transmitted diseases, urinary tract infections**,** animal-bite diseases, digestive tract bacterial disease (gastroenteritis), nervous system and respiratory diseases (Tunkel, 2009; Bennett *et al*., 2012).

These bacteria are transmitted through deep unclean wounds, minor skin breaks or bruises, the eyes, the oral route by ingesting spores of the microbe or the microbe itself via contaminated foodstuff or water, the respiratory tract by inhalation of bacterial endospores or the bacteria itself, bites from arthropods e.g. ticks and fleas, the oral fecal route and the urinary tract. Various factors influence the pathogenicity of bacteria e.g. the age of the host, the overall health of the host the type and strain of bacteria. Gastrointestinal diseases are often acquired by ingesting contaminated food and water. Respiratory diseases and meningitis are commonly acquired by contact with aerosolized droplets, spread by sneezing, coughing and talking. Sexually transmitted diseases are acquired through contact with bodily fluids, generally as a result of sexual activity. Some infectious agents may be spread as a result of contact with a contaminated inanimate object, such as a coin passed from one person to another, while other diseases penetrate the skin directly

(Pickering, 2003; NIH, 2007; Right diagnosis, 2011; Bennett *et al*., 2012).

2.2.2 Epidemiology

Blood infections with different bacteria occur worldwide; however, certain bacteremias are more prevalent in different regions than others. The epidemiology of bacteremia differs depending on whether it is community-acquired or nosocomial. Community-acquired bacteremia refers to infections detected within seventy two hours of hospitalization, unless the patient had resided in a long-term care facility. The separation of bacteremia into the two types has implications on the source of bacteria, the organism involved, the complication rate and the mortality (Torre, 2008). A study of bacteremia in African children found distinct differences in the microbiological causes of nosocomial bacteremia compared to community-acquired bacteremia. Nosocomial bacteremia resulted in a higher rate of morbidity and mortality and longer hospital stay (Torre, 2008).

Incidence estimates of community acquired bacteremia are difficult to estimate as most are specific for different bacteria and they also differ regionally. However, it has been found to be more common in infants and in the elderly. The risk of life-threatening bacterial disease is greatest in young infants when their immune system is least mature; they have poor immunoglobulin G (IgG) antibody response to encapsulated bacteria and decreased opsonin activity, macrophage function, and neutrophil activity (Torre, 2008; Bennett *et al*., 2012). In a study done at a University Hospital in Portugal, community acquired bacteremia represented 7% of all intensive care unit (ICU) admissions, with an associated ICU crude mortality of 50%. Respiratory and endovascular focuses of infection were the two most frequent, with Gram-positive microorganisms representing one-half of all isolations (Castro *et al*., 2007). According to the World Health Organization, at least 6 million children die each year of pneumococcal infections (e.g. pneumonia, meningitis, and

bacteremia); most of these fatalities occur in developing countries (Giebink, 2001). In most rural areas of sub-Saharan Africa, 66% of deaths of children between the ages of three months and five years occur outside hospitals (Olipher, 2011). It is quite conceivable, therefore, that the true community-based incidence of bacteremia is at least twice the rate observed (Sharma *et al*., 2008).

Community-acquired bacteremia is a common cause of hospital and intensive care admission, with a case fatality rate of 20–30% worldwide. Its early identification and association with the probable source of infection and agents permits an early and effective antibiotic therapy, and would probably contribute to a decrease in the morbidity and mortality (Castro *et al*., 2007). The burden of community acquired bacteremia in terms of cost and resource use is high, if not treated on time and at times, even when appropriate therapy is given. The presence of individual risk factors substantially affects clinical management. Thus, clinicians need to specifically inquire as to the presence or absence of these risk factors e.g. frequency of lower platelet count, leucopenia, higher serum albumin, etc before making clinical decisions regarding treatment (Naber, 2009; Lin *et al*., 2010; Fowler *et al*., 2012). The incidence and ability of these pathogens to spread in both hospital and community settings has increased. This is due to increased inherent virulence of the pathogens, increased resistance of strains to available antibiotics, increased frequency of invasive surgery, increased use of intravascular devices and increased numbers of patients with immune-compromised status because of HIV infection or immune-suppression. This has led to sharp increases in the incidence of bacteremia over the past 30 years (Biedenbach *et al*., 2004; Shorr *et al*., 2006; Naber, 2009).

A study was conducted on adult patients in Barcelona, Spain to determine the associated factors and outcomes of community-acquired bacteremia and to describe the most frequently isolated microorganisms. It was found that the independent determinants or factors associated with

community acquired bacteremia were male sex and an ultimately or rapidly fatal prognosis for an underlying disease. The most frequently isolated microorganisms in order of frequency were *E. coli*, other enterobacteria and *S. aureus* (Ortega *et al*., 2007). Other specific underlying factors and determinants of community acquired bacteremia found in various studies are diseases and conditions such as diabetes mellitus, liver cirrhosis, HIV and chronic lung diseases. Others include alcoholism, intravenous drug users, use of corticosteroids among others (Naber, 2009; Lin *et al*., 2010).

Community acquired bacteremia increases the risk of developing other disease complications and infections to other body organs. They include infective endocarditis, meningitis, brain abscess, liver abscess, prostatic abscess, lung abscess, thoracic emphysema, deep neck infection, pneumonia, septic arthritis, osteomyelitis, cellulitis, complicated skin and soft tissue infections, sepsis and seeding to other metastatic foci. This increases the risk of mortality and raises the stakes for early, appropriate treatment (Naber, 2009; Lin *et al*., 2010). Also, the concern with occult community acquired bacteremia is that it could progress to a more severe local or systemic infection if left untreated possibly resulting in death. Most episodes of occult bacteremia spontaneously resolve and serious sequels are increasingly uncommon (Bennett *et al*., 2012).

The unknown extent of the disease burden and the changing epidemiology of community acquired bacteremia, especially in Kenya, drive an urgent need for improved strategies and critical planning for bacteremia management and its complications.

2.2.3 Etiology

The presumed mechanism of bacteremia begins with bacterial colonization of the mucosal surfaces; bacteria may then egress into the bloodstream of some patients because of host-specific and organism-specific factors. Once viable bacteria have gained access to the bloodstream, they

may be spontaneously cleared, they may establish a focal infection, or the infection may progress to septicemia; the possible sequelae of septicemia include shock, disseminated intravascular coagulation, multiple organ failure, and death (Bass *et al*., 1993; Harper *et al*., 1993; Bennett *et al*., 2012).

The ability to up-regulate virulence factors under stressful stimuli (e.g. host immune response or circulating antibiotics) is a key factor in enabling of bacteria to persist in the bloodstream, to seed deep tissues, and to form secondary foci of infection. Virulent strains have been effectively able to adhere to and colonize the mucosa of various body organs, to invade the bloodstream, to evade host immunological responses, to form protective biofilms, and to develop resistance to several antibiotics. Consequently, despite the availability of many antibiotics with activity against wild-type strains, many bacteria are still highly successful and increasingly clinically important pathogens (Naber, 2009; Bennett *et al*., 2012). Often, fever is the only presenting sign in patients with bacteremia. Fever is defined as increased temperature caused by resetting the thermoregulatory center in the hypothalamus by action of cytokines (McCarthy, 1998; Bennett *et al*., 2012).

The most frequent source and the most common organisms that cause community acquired bacteremia are *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Streptococcus viridans*, *Neisseria meningitidis, Enterococcus* spp. and *Streptococcus bovis* (Torre, 2008; Naber, 2009). The most likely primary infectious source for *Proteus mirabilis, Enterococcus* spp. and *Pseudomonas aeruginosa* is through urinary tract infections (Torre, 2008). *Escherichia coli* infectious sources are urinary tract infection and the biliary tract (Torre, 2008). *Streptococcus pneumoniae*'s primary infectious source/process is the human upper respiratory tract causing

pneumonia and the next most likely source is meningitis (Torre, 2008; Todar, 2009). *Klebsiella* spp. have the biliary tract as the primary source of infection and the urinary and lower respiratory tract as the secondary source of infection (Torre, 2008; Lin *et al*., 2010). *Staphylococcus aureus* primary infectious source/process is the lower respiratory tract and damaged skin with endocarditis being the secondary source (Torre, 2008; Naber, 2009). *Streptococcus viridans* and other *Streptococcus* spp. have their primary source/process as endocarditis (Torre, 2008). In some cases more than one microorganism may be detected in blood cultures. Once contamination has been excluded, polymicrobial bacteremia suggests the biliary tract, intra-abdominal sepsis, or a urinary tract infection as a source (Torre, 2008).

2.2.4 Clinical features

Bacteremia and sepsis are normally characterized by various symptoms depending on the bacteria responsible. After infection, the following general symptoms are observed, namely; fever lasting less than two-weeks and non-specific symptoms such as malaise, myalgia, chills, hypotension, headache and loss of appetite (Amorn *et al*., 2004). Vomiting, nausea, abdominal pain and diarrhea especially in the case of bacterial diseases of the digestive system are also common (Tunkel, 2009). Sometimes there is a skin rash, break or abscess, furuncle or carbuncle. Bacteremia can evolve to septicemia in which more severe disturbances of temperature, respiration, heart rate or white blood cell count are observed. Septic shock may lead to more serious diseases such as meningitis, and can cause disseminated intravascular coagulation leading to multiple organ failure (Right diagnosis, 2011). Toxic shock syndrome, necrotizing fasciitis ("flesh-eating") and infective endocarditis (which affects the valves of the heart), and bone and joint infections can also arise from virulent bacteremia (Todar, 2009).

2.2.5 Diagnosis

Diagnosis of infectious disease sometimes involves identifying an infectious agent either directly or indirectly. In practice most minor infectious diseases such as warts, cutaneous abscesses, respiratory system infections and diarrheal diseases are diagnosed by their clinical presentations (NIH, 2007).

New rapid techniques for the identification and characterization of bacteria have been developed. This notwithstanding, the bacterium generally must be cultured via blood, sputum, urine, or other body fluid cultures, and cultured in the laboratory in sufficient quantities to perform these confirmatory tests first. Consequently, there is no quick and easy method to diagnose bacteremia infections. Therefore, initial treatment is often based upon 'strong suspicion' by the treating physician, since any delay in treating this type of infection can have fatal consequences (Wawrik, 2002; Filion, 2012).

Blood culture tests for bacteria are done followed by microbiological identification which is considered as the standard for diagnosis of many bacteria e.g. typhoid fever (Baker *et al*., 2010; Ley *et al*., 2011). Since there is a direct relationship between the volume of blood obtained and the yield of a blood culture set, 5ml and 10 ml of blood should be obtained per bottle for children and adults, respectively. A positive blood culture is considered as the gold standard method for diagnosis of a specific bacterial agent that caused fever in that case. However, even under the best conditions, the organisms may not be isolated from blood, especially after antimicrobial treatment commences (Arjunan *et al.*, 2010). Bone marrow and intestinal secretions culture have a higher sensitivity than blood culturing but they require more invasive procedures and therefore they are not commonly done (Rubin *et al*., 1990; House *et al*., 2000).

Diagnostic microbiology laboratories and reference laboratories are key for identifying bacteremia outbreaks. Technologies based upon the polymerase chain reaction (PCR) method will become nearly ubiquitous gold standards of diagnostics of the near future. These technologies include Real-time PCR and Quantitative PCR and are increasingly being employed in clinical laboratories for the rapid detection and identification of bacteremia. The only remaining blockades to the use of PCR as a standard tool of diagnosis are in its cost and application, neither of which is insurmountable (Wawrik, 2002; Filion, 2012). The diagnosis of a few diseases will not benefit from the development of PCR methods, such as some of the clostridial diseases (tetanus and botulism). These diseases are fundamentally biological poisonings by relatively small numbers of infectious bacteria that produce extremely potent neurotoxins. A significant proliferation of the infectious agent does not occur; this limits the ability of PCR to detect the presence of any bacteria (Mackay, 2007). The other common laboratory tests include rapid latex agglutination test that detects a protein (Wawrik, 2002).

2.2.5.1 Differential diagnosis

It is negligent to diagnose a person as having bacteremia when the disease could be malaria, dengue, leishmaniasis etc. It is essential, in a well functioning control programme, to have the diagnosis made by more than one person preferably a Medical Officer, Clinical Officer and Trained Nurse. One must be able to distinguish between bacteremia-like conditions that can be confused with early and/or advanced forms of other diseases like malaria, arthritis, dengue fever, leishmaniasis, cell sarcoma, leukemia, and other viral fevers.

2.2.6 Treatment and control

Previous studies show that despite existence of microbiological facilities in various health centers

and hospitals, causative organisms can be identified only after 24 to 48 hours, by which time most deaths in children with bacteremia will have already occurred (Berkley *et al*., 2005).

Bacterial vaccines are normally used though for prophylaxis and they include vaccines for: diphtheria, influenza, tetanus, pertussis, meningococcal, cholera, anthrax, pneumococcal disease and Lyme disease among others (Ronald *et al*., 2003). Vaccination is effective for preventing certain bacterial and viral diseases in both children and adults. Existing bacterial vaccines can be administered into routine immunization services.

Because bacteria are prokaryotes, it has been relatively easy to find and develop antibacterial drugs that have minimal side effects. These drugs target structural features and metabolic characteristics of prokaryotes that are significantly different from those in eukaryotic cells. Drugs used to treat bacterial diseases can be grouped into categories based on their modes of action. In general, these drugs inhibit cell wall synthesis, protein synthesis, nucleic acid synthesis, or other enzyme-catalyzed reactions (NIH, 2007).

Oral rehydration is used to treat most cases of diarrhea caused by bacteria causing infections in the gastrointestinal tract, e.g. *Vibrio cholera*, *Escherichia coli*, *Clostridium perfringens*, *Clostridium botulinum*, *Clostridium difficule*, *Shigella*, *Salmonella* and *Entamoeba histolytica*. In severe cases, intravenous rehydration is given and sometimes antibiotics are given to shorten the duration of treatment and period of intestinal carriage of the bacteria (Ferraro *et al*., 2000).

Antibiotic treatment depends on the organism system involved (Rivers *et al*., 2001). In general, initial therapy of patients with possible bacteremia is empirical. The choice of a specific antimicrobial agent depends on local susceptibility patterns. Once bacteremia is confirmed, treatment may be modified. The antibiotics may be used as monotherapy or combination therapy.

However, antimicrobial therapy should be provided as soon as bacteremia is suspected and the treatment multi-pronged. Bactericidal and intravenous antibiotics should be used as soon as possible, ensuring good tissue penetration. Therapy should initially begin with coverage for both Gram-positive and Gram-negative bacteria, adjusting as sensitivity tests suggest (Rivers *et al*., 2001).

Although guidelines suggest that antibiotics should be used prophylactically when patients undergo medical procedures associated with bacteremia, these guidelines are aimed at pathogens such as streptococci and enterococci, depending on the procedure (Horstkotte *et al*., 2004).

2.2.7 Complications and their management

Once in the bloodstream, the infection can spread to other parts of body, producing abscesses, peritonitis (inflammation of abdominal cavity), endocarditis (inflammation of the heart), or meningitis. Bacteremia may lead to sepsis or shock, causing a systemic illness with high fever, blood coagulation and eventually organ failure (Bennett *et al*., 2012).

Complications of bacteremia are secondary conditions, symptoms, or other disorders that are caused by bacteremia. In many cases, the distinction between symptoms and complications of bacteremia is unclear or arbitrary (Tunkel, 2009; Bennet *et al*., 2012).

2.3 Identification of bacteria

In diagnosing bacterial infections, the rapid identification of bacteremia at an early stage of the disease is critical for a favorable outcome. During the past decade, there has been unprecedented progress in molecular biology as well as in the application of nucleic acid technology to the study of the epidemiology of human infections. Various methods are used in identification of bacteria in

blood, namely; microbial culturing, biochemical tests, serological tests and molecular diagnostic techniques (Bhatia *et al*., 2007; Millar *et al*., 2007).

Blood specimens are first taken aseptically from patients suspected of bacteremia. Further evidence of bacterial infection is based on the isolation and identification of the organism(s) concerned. These tests are done in parallel with antibiotic sensitivity tests (Gudkova, 2008). Bacterial pathogens may not grow in the culture if there is delay in sending the blood specimens to the laboratory. Delicate bacteria may die from lack of nutrients and differences in environmental conditions or may be outgrown by saprophytes/normal flora (Fischbach *et al*., 2008). Delay in diagnosis of acute infections may have disastrous consequences. Therefore most clinicians opt for the faster diagnostic methods such as serological tests and PCR (Gudkova, 2008).

2.3.1 Microbial culture (Blood culture)

Bacteremia is diagnosed by blood culture, in which a sample of blood is allowed to incubate with a medium that promotes bacterial growth. Since blood is normally sterile, this process does not normally lead to the isolation of bacteria. If, however, bacteria are present in the bloodstream at the time the sample is obtained, the bacteria will multiply and can thereby be detected. Any bacteria that incidentally find their way to the culture medium will also multiply, hence the need to draw blood aseptically (Tunkel 2009; Bennett *et al*., 2012). Detection of bacteria with blood culture starts with testing if there are actually bacteria in blood or not. Only growth-positive cultures are used in subsequent analyses. Thereafter, pathogenic organisms are isolated using agar plates (Gudkova, 2008).

After carrying out a blood culture, the growth-positive cultures are then sub-cultured on other various differential and selective media so as to isolate the bacteria. The pathogens in culture can

then be described and identified based on morphological characteristics of the colonies i.e. either mucoid, slimy, rough edged, etc (Shimeld *et al*., 1999).

2.3.2 Biochemical tests

Gram staining is based on the chemical and physical properties of bacteria cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in the periplasmic space in the cell wall of Gram-positive bacteria. The peptidoglycan layer in the cell wall of Gram-negative bacteria is thinner than in Gram-positive bacteria. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell envelope), which is stained purple by crystal violet, whereas Gram-negative bacteria have a thinner layer (10% of cell envelope) which is stained pink/red by the counter-stain as it fails to retain the crystal violet purple stain (Alberts *et al*., 2002).

Simmon's citrate test is based on the principle of pH change and substrate utilization. Simmon's citrate agar slants contain sodium citrate (the only carbon source) and ammonium ions (the sole nitrogen source). A pH indicator, Bromothymol blue is also included. Bromothymol blue is green at pH < 7.0 and blue at pH > 7.6 (Cheesbrough, 2006a).

Motility-indole-ornithine (MIO) medium is a semisolid medium useful in the identification of members of the *Enterobacteriaceae*. It tests the ability of a microbe to degrade the amino acid tryptophan to produce indole by a hydrolase called tryptophanase which splits the amino acid tryptophan into indole, pyruvic acid and ammonia. It also tests for ornithine production. The casein and gelatin peptones, yeast extract and dextrose provide nitrogenous and carbonaceous substances, vitamins and minerals essential for bacterial metabolism (Cheesbrough, 2006a).

Many organisms use carbohydrates differently to obtain energy depending on their enzyme complement. Some organisms are capable of fermenting sugars such as glucose anaerobically while others use the aerobic pathway. Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is often used in the selective identification of enteric bacteria including but not limited to *Salmonella* and *Shigella* spp. based on the ability to reduce sulfur and ferment carbohydrates. Examples of sulfide-producing bacteria include *Salmonella*, *Proteus*, *Citrobacter* and *Edwardsiella* spp. (Cheesbrough, 2006a).

Urea medium contains urea, a very small amount of nutrients for the bacteria, and the pH indicator phenol red. Urea Agar was devised by Christensen in 1946 for use as a solid medium for the differentiation of enteric bacilli (Cheesbrough, 2006a).

The oxidase test identifies organisms that produce the enzyme cytochrome oxidase. Cytochrome oxidase participates in the electron transport chain by transferring electrons from a donor molecule to oxygen. Most Enterobacteriaceae are typically oxidase negative (Cheesbrough, 2006a).

The catalase test is used by microbiologists to detect presence of catalase enzyme in the test isolate which is detected using hydrogen peroxide. If the bacteria possess catalase, they are catalase-positive and if they do not possess the catalase enzyme they are catalase-negative. This test is primarily used to distinguish among Gram-positive cocci (Cheesbrough, 2006a).

The coagulase test identifies an organism that produces the exoenzyme coagulase. Coagulase causes the fibrin of blood plasma to clot. Organisms that produce catalase can form protective barriers of fibrin around themselves, making themselves highly resistant to phagocytosis, other immune responses, and other antimicrobial agents (Cheesbrough, 2006a).

2.3.3 Serological tests

These tests rely on antibodies which identify pure bacterial isolates. When these antibodies are mixed with a suspension of the bacteria, a clumping or an agglutination reaction will occur, and the particular 'serotype' of the organism identified (Struthers, 2003). The antibodies bind to proteins and other cell components. They are highly selective, meaning that a particular antibody will only bind to a particular species of bacteria; where some even differentiate between strains of the same species (Struthers, 2003).

2.3.3.1 Enzyme-linked immunosorbent assay (ELISA)

A specific antigen or antibody attached to wells of plastic plates is used to capture the antibody or serum corresponding to the antigen in a specimen, usually serum. An indicator antibody/enzyme is used to label the plates so as to measure the formation of antigen/antibody complexes adsorbed to the solid surface (Evans *et al*., 1998). The enzyme then links to the complex and after washing, the amount of enzyme activity is proportional to amount of antigen/antibody present (Evans *et al*., 1998).

2.3.3.2 Immunofluorescence

It uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell. The antibody is labeled with a fluorescent dye (fluorochrome) and therefore allows visualization of the distribution of the target molecule through the sample. The resulting reaction can then be read visually using a spectrophotometer (Evans *et al*., 1998).

2.3.3.3 Radio immunoassay

This technique is very similar to immunofluorescence, the only difference being that it uses a radio isotope to label and detect the antibody other than a fluorescent dye (Evans *et al*., 1998).

2.3.3.4 Rapid latex agglutination test

It involves antigen detection using a simple slide agglutination test for the simultaneous detection of protein and clumping factor using latex particles coated with human plasma. Some formulations of latex tests include protein and/or clumping factor but also detect various surface antigens, which improve sensitivity of the tests but at some expense to specificity (Liu, 2011).

2.3.4 Molecular diagnostic techniques

Unlike diagnostic virology which detects target DNA as well as target mRNA, most molecular diagnostic assays in medical bacteriology have been based on the amplification of DNA in a target gene rather than mRNA or other nucleic acid signals (Kessler, 2010).

2.3.4.1 Polymerase chain reaction (PCR)

Polymerase chain reaction is a technique used for enzymatic *in vitro* amplification (PCR amplification) of specific DNA sequences without utilizing conventional procedures of molecular cloning. It allows the amplification of a DNA region situated between two convergent primers and utilizes oligonucleotide primers that hybridize to opposite strands. Primer extension proceeds inward across the region between the two primers. The product of DNA synthesis of one primer serves as a template for the other primer; repeated cycles of DNA denaturation, annealing of primers, and extension result in an exponential increase in the number of copies of the region bounded by the primers. The sequence of the double-stranded DNA fragment can be identified indirectly by hybridization to allele-specific oligonucleotide probes representing the various alleles studied or whose sequence can be determined (O'Connor, 2006; Kessler, 2010). It utilizes the Taq enzyme as it has a high thermostability enabling the reaction mixture to be re-heated to 95 ^{o}C so as to separate newly synthesized DNA strands from template. The reaction can be continued through 30-40 cycles thus amplifying DNA over several million-fold in a few hours.

Specific primers can be used to amplify a specific gene. The PCR product and a known standard pathogen are then run on an electrophoretic gel. Observation is done visually or using a spectrophotometer (O'Connor, 2006; Liu, 2011).

2.3.4.2 Real time polymerase chain reaction (Real time PCR)

This technique is similar to PCR but avoids the use of gels altogether. Real time PCR assays utilize fluorescent resonance energy transfer probes. It amplifies and sequences genes hence enables the identification of organisms in a shorter time to (Liu, 2011).

2.3.4.3 Gel Electrophoresis

Separation of proteins is done by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel. This is a very useful way to determine a protein. It utilizes a slab of gel made of agarose. The DNA is digested with restriction enzymes and the digested DNA samples are placed in slots in the gel (Kessler, 2010; Liu, 2011). Standard DNA from the test organism is then included. The gel is placed in an electric field and since DNA is negatively charged, DNA fragments move according to their molecular weight. The resulting reaction can then be read visually using a UV light/spectrophotometer (Kessler, 2010; Liu, 2011).

2.3.4.4 Restriction fragment length polymorphism (RFLP)

This method relies on the digestion of DNA with site-specific restriction endonucleases. Differences in DNA nucleotide sequences between organisms will result in differences in the number and size of the restriction fragments produced (restriction fragment length polymorphism or RFLP) (Kessler, 2010; Liu, 2011). Since the distribution of sites recognized by any particular restriction endonuclease is determined by the DNA nucleotide sequence of the organism, the size

distribution of the restriction fragments generated will be characteristic for the genotype (Kessler, 2010).

2.3.4.5 Southern blot

It is a technique used in molecular biology research to study gene expression by detection of DNA in a sample. Southern blotting involves the use of electrophoresis to separate DNA samples by size and detection with a hybridization probe complementary to part of or the entire target sequence (Kessler, 2010; Liu, 2011). The DNA is first digested using restriction enzyme and probes labeled with DNA and superimposed over nitrocellulose filters. Hybridization is then done with test DNA from the organism/pathogen of interest (Liu, 2011).

2.3.4.6 Colony blots

It is used to detect a given nucleotide sequence in a crude preparation of genomic DNA obtained from colonies grown on agar plate. This method can easily determine which colonies on the plate contain the sequence of interest by formation of plaques (Kessler, 2010).

2.4 Antimicrobial resistance in bacteremia

Antimicrobial chemotherapy has increased the life expectancy of many people who would have otherwise succumbed to diseases. However, bacteria that have become resistant to antibiotic drug therapy are an increasing public health challenge (Todar, 2009). About 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs. An alarming increase in resistance of bacteria that cause community acquired infections has also been documented, especially in the staphylococci and pneumococci (*Streptococcus pneumoniae)*, which are prevalent causes of disease and mortality. In

a recent study, 25% of bacterial pneumonia cases were shown to be resistant to penicillin, and an additional 25% of cases were resistant to more than one antibiotic (Todar, 2009).

Wound infections, gonorrhea, tuberculosis, pneumonia, septicemia and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. This is due to bacteria and other microbes that cause these infections becoming remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs. Other common causes are the increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture (Todar, 2004).

Multiple drug resistant organisms are resistant to treatment with several, often unrelated, antimicrobial agents. Some of the most important types of multiple drug resistant organisms that are commonly encountered are: Methicillin/oxacillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant enterococci (VRE) which are multiple drug resistant organisms in patients residing in non-hospital healthcare facilities, such as nursing homes and other long-term care facilities (Todar, 2004). Penicillin-resistant *Streptococcus pneumoniae* (PRSP) are more common in patients seeking care in outpatient settings (Todar, 2004). Extended-spectrum beta-lactamases (which are resistant to cephalosporins and monobactams) are most often encountered in the intensive care hospital setting, but MRSA and VRE also have significant nosocomial ecology (Todar, 2004).

Recently, new strains of antibiotic resistant bacteria have been detected in USA, namely: New Delhi metallo-beta-lactamase (NDM)-producing *Klebsiella pneumoniae* which is highly resistant to antibiotics and easily spread. It is rare in the United States, but more common in India, Pakistan, Cambodia and other Asian countries (CDC, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The study area

The study was conducted between November 2011 and April 2012 in Alupe which is located in Busia County, Western Kenya (Figure 1). Alupe is located at latitude 0.5° and longitude 34.1166667° coordinates and has an altitude of 1189 m above sea level. It has a main seasonal stream called Alupe (Maps of Alupe – Kenya, 2012). The communities in Alupe practice subsistence farming as the main economic activity. Main sources of water include boreholes, wells, seasonal shallow rivers and springs. Most households lack proper human waste disposal facilities and at times inhabitants use bushes. There are several predominant communities found in Alupe including the Bakhayo, Marachi, Samia, Bunyala and Teso. Other communities found in Alupe in lower population are the Luo, Kikuyu, Somalia and Kamba (Busia County Kenya, 2012).

Busia County borders Uganda to the west and Lake Victoria to the south west, north and northeast. The Bungoma County and Kakamega town border Busia County towards the east. The population in Busia County based on the 2009 Kenya census is about 743,946 people (male - 48%, female - 52%) constituting 1.8% of the national population, with a distribution of 439 people per km^2. Busia town is approximately 431 km by road west of Nairobi, Kenya's capital city. The town has an area of 1,695 km^2. Busia County consists of Amagoro, Budalangi, Butula, Funyula, Nambale, Matayos, Teso North and Teso South constituencies. The main economic activities of the county are fishing, trade, tourism and agriculture. The county experiences moderate rainfall throughout the year which helps the county's population in farming their cash and subsistence crops. Crops that are grown here are mainly sugarcane, tobacco and cotton. Lake Victoria provides for a fishing

hub in the flood prone Budalangi area (Busia County Kenya, 2012).

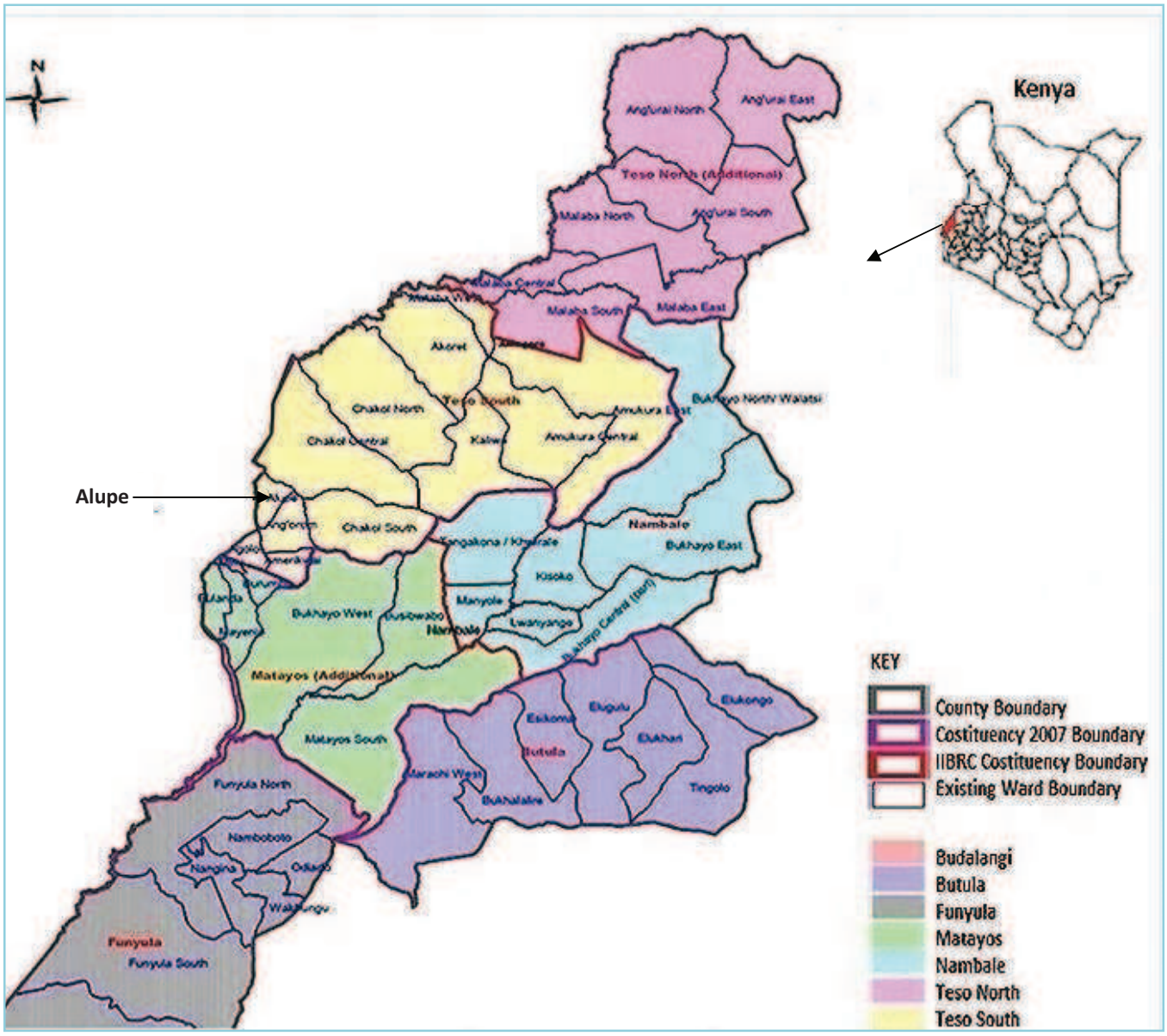

Figure 1: Detailed map of Busia County showing Alupe, existing wards and constituencies.

Source: (Kenya Mpya, 2012; Flickr, 2012).

Busia County is accessible mainly through road and railway network. Being a major entry point to the great lakes region of landlocked Uganda, Rwanda, Burundi and Democratic Republic of

Congo, it supports numerous exports to and from these areas (Busia County, Kenya, 2012).

The prevalent diseases found in the county are caused by arboviruses especially Chikungunya which is common in Western Kenya (Mwau *et al*., 2012). Children in Alupe are also exposed to West Nile, Chikungunya, Yellow Fever and Dengue viruses (Onyango *et al*., 2012). Malaria is also endemic in Alupe. Fungal infections and various skin infections are also common (KEMRI, 2012).

3.1.1 Alupe District Hospital and Alupe KEMRI Clinic

Alupe District Hospital is owned by Kenya's Ministry of Health. It is located in Western Province, Teso District, Busia County, Angorom Location, Alupe Sub-location in Amagoro Constituency. It has 130 beds and the services offered include**:** antenatal, antiretroviral therapy, basic emergency obstetric care, caesarean section, comprehensive emergency obstetric care, growth monitoring and promotion, immunization, integrated management of childhood illnesses, radiology services (e.g. x-ray, ultra scan, etc) and tuberculosis diagnosis. The Hospital is located at the latitude and longitude coordinates of 0.5° and 34.130556° (eHealth-Kenya, 2012; NGA, 2012). It has an average flow of 40 patients per day and has one doctor, 14 clinical officers, 18 nurses and 2 laboratory technicians.

Kenya Medical Research Institute (KEMRI) Centre for Infectious and Parasitic Diseases Control Research (CIPDCR) clinic in Alupe is an outpatient clinic run by one of the 12 centers of KEMRI. The KEMRI CIPDCR clinic is also located in Western Province, Teso District, Busia County, Angorom Location, Alupe Sub-location in Amagoro Constituency. The centre was established in 1947 as the Alupe leprosarium hospital when the government of Kenya set up a hospital for this purpose in Alupe. In 1979, the East Africa Leprosy Research Centre was taken over by KEMRI following the breakup of the East African Community. Its name was changed to the Alupe Leprosy and Skin Disease Research Centre and its mandate was appropriately expanded to foster research

in that field. In 2000, the centre's name changed to Centre for Infectious and Parasitic Diseases Control Research (CIPDCR). Its mandate was expanded to include research on: HIV/AIDS, sexually transmitted diseases (std's), tuberculosis, leprosy research, emerging and remerging diseases research, malaria research, geo-helminthes research and fungal infections. The clinic is also located at the latitude and longitude coordinates of 0.5° and 34.130556° (KEMRI, 2012; NGA, 2012). It is the only centre where bacterial cultures are done in the whole of Busia, South Teso and North Teso Districts. The clinic has an average flow of 20 patients per day and has 2 clinical officers, 2 nurses and 7 laboratory technicians.

The two health facilities are situated 6 km north of Busia, along the Busia-Malaba road in Western Kenya. This study was sited at Alupe KEMRI-CIPDCR which is strategically located hence there was easy access to diagnostic and laboratory services.

3.2 Inclusion and exclusion criteria

The inclusion criteria for patients was diagnosis of fever which is a symptom of bacteremia; patients of ≥18 months of age; and patients or guardian willing to participate in the study by consenting to provide socio-demographic details and a blood sample. Patients were excluded from the study if they had no fever (which is a symptom of bacteremia); were <18 months of age; were unwilling to participate in the study; and if a blood sample tested positive for malaria.

3.3 Sample size determination

Assuming bacteremia isolation rate of 13.5% (Reddy *et. al*., 2010) and a level of significance of 5%, sample size was calculated using the Fisher's formula:

$$n = \frac{z^2PQ}{d^2}$$

Where:

n = minimum sample size required

z = 1.96 (standard normal deviate at the required confidence level)

P = 0.135 (isolation rate in bacteremia)

Q= 1-P

d = 0.05 (level of significance)

$$n = \frac{1.96^2 \times 0.135(1-0.135)}{0.05^2} = 179 \text{ blood samples}$$

Two hundred blood samples were taken for the study.

3.4 Collection of background data

A structured questionnaire (Appendix V) was used to obtain social demographic data from the patients through face to face interviews. The social demographic data collected from the patients included gender of patient, age, place of residence, catchment areas of patients and occupation of patient or of the guardian and the health care facilities where patients were registered. The patients were aged 18 months and above and the health facilities were Alupe DH and KEMRI-CIPDCR clinic.

Additionally, a clinical examination questionnaire with 33 listed items including the general condition of the patient and the vital signs - temperature, blood pressure, pulse and respiration - was used and it had symptom subscales for the respiratory system, abdominal system, central nervous system, cardiovascular system and other ungrouped symptoms (Appendix VI). It was used

to record disease symptoms that the patients presented with at the time of their recruitment in the study.

3.5 Sampling procedure

Two hundred patients were enrolled for the study. The samples were collected sequentially, one patient after another. The patients taking part in the study were examined clinically for signs and symptoms of fever by qualified medical personnel. Those whose clinical outcomes suggested febrile illness were requested to participate in the study. Two hundred of them accepted to take part in the study. A written informed consent was obtained from the patients for participation in the study (Appendix I - IV). The consent form was read and explained in the language best understood by the participant. A standardized structured questionnaire was used to obtain socio-demographic data. A general clinical examination was undertaken to assess the symptoms present. Other observed symptoms were also recorded. A clinical examination form was completed for every patient. After consent, the patients' skin was cleaned with 70% ethanol and allowed to dry before venous blood was collected aseptically for culture. A venipuncture was then done aseptically on each patient and 5ml and 10 ml of blood obtained from children and adult patients, respectively using standard phlebotomy procedures (Isenberg, 2004). After collection, the blood was immediately inoculated into blood culture broth media and the blood specimens were taken for laboratory investigations at the KEMRI laboratory in Busia.

3.6 Culture media preparation

All the media used in the study (MacConkey agar, blood agar, chocolate blood agar, mannitol salt agar, Mueller Hinton agar, Simmons citrate agar, TSI agar, urea agar and MIO medium) were from Himedia and were all prepared according to the manufacturer's instructions (Himedia, 2012).

3.6.1 MacConkey agar

MacConkey medium (51.63 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was cooled to 45 – 50°C and poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation.

Composition

Ingredients	**Grams/ Litre**
Peptic digest of animal tissue	20.000
Lactose	10.000
Bile salts mixture	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

3.6.2 Blood agar

Blood agar medium (40.0 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was cooled to 50°C and 5% (v/v) sterile defibrinated blood aseptically added. It was mixed well and poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation.

Composition

Ingredients	**Grams/ Litre**
Proteose peptone	15.000
Liver extract	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

3.6.3 Chocolate blood agar

Blood agar medium (40.0 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was cooled to 80°C and 5% (v/v) sterile defibrinated blood aseptically added. The blood was added at this high temperature so the red blood cells could be lysed. It was mixed well and poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation.

Composition

Ingredients	**Grams/ Litre**
Proteose peptone	15.000
Liver extract	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

3.6.4 Mannitol salt agar

Mannitol salt agar medium (111.02 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was then poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation.

Composition

Ingredients	**Grams/ Litre**
Proteose peptone	10.000
Beef extract	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

3.6.5 Mueller Hinton agar

Mueller Hinton agar medium (38.2 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath and gently swirled to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was mixed well and then poured into sterile Petri plates. The agar was allowed to settle and the surface of the agar had to be dry before inoculation.

Composition

Ingredients	**Grams/ Litre**
Casein acid hydrolysate	17.500

Beef heart infusion	2.000
Starch, soluble	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.2

3.6.6 Simmons citrate agar

Simmons citrate agar medium (24.28 grams) was suspended in 1000 ml distilled water in a conical flask. It was then heated to boiling in a water bath to dissolve the medium completely. It was dispensed as desired into sterile tubes and sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was then allowed to cool with the tubes in a slanting position.

Composition

Ingredients	**Grams/ Litre**
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

3.6.7 Triple sugar iron (TSI) agar

Triple sugar iron agar medium (55.3 grams) was suspended in 1000ml distilled water in a conical flask. It was then heated to boiling in a water bath to dissolve the medium completely. It was

mixed well and distributed into sterile tubes and sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was then allowed to cool with the tubes in a slanting position.

Composition

Ingredients	**Grams/ Litre**
Peptic digest of animal tissue	20.000
Meat extract	3.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Glucose	1.000
Ferric citrate	0.300
Sodium chloride	5.000
Sodium thiosulphate, pentahydrate	0.300
Phenol red	0.024
Agar	12.000
Final pH (at 25°C)	7.4±0.2

3.6.8 Urea agar

Urea medium (24.01 grams) was suspended in 1000ml distilled water in a conical flask. It was then heated to boiling in a water bath to dissolve the agar completely. The medium was then sterilized by autoclaving at 10 lbs pressure at 115°C for 15 minutes. It was then cooled to 50°C and 50ml of sterile 40% Urea Solution (FD048) was added aseptically and mixed well. The agar was then dispensed into sterile tubes and allowed to cool with the tubes in a slanting position. Care was taken not overheat the medium as urea decomposes very easily.

Composition

Ingredients	Grams/ Litre
Peptic digest of animal tissue	1.000
Dextrose	1.000
Sodium chloride	5.000
Monopotassium phosphate	2.000
Phenol red	0.012
Agar	15.000
Final pH (at 25°C)	6.8±0.2

3.6.9 Motility-indole-ornithine (MIO) medium

Motility-indole-ornithine medium (31.02 grams) was suspended in 1000ml distilled water in a conical flask. It was then heated to boiling in a water bath to dissolve the medium completely. The medium was then sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. It was dispensed into sterile tubes and allowed to cool with the tubes in an upright position.

Composition

Ingredients	Grams/ Litre
Casein enzymic hydrolysate	10.000
Peptic digest of animal tissue	10.000
Dextrose	1.000
Yeast extract	3.000
L-Ornithine hydrochloride	5.000
Bromocresol purple	0.020
Agar	2.000
Final pH (at 25°C)	6.5±0.2

3.7 Blood culture

Blood (5ml and 10 ml for children and adults, respectively) was inoculated into blood culture bottles containing commercially prepared broth media (HiMedia HiCombi Dual Performance Medium, Mumbai, India) after the needle was changed; and the top was cleaned with 70% ethanol and allowed to dry. The bottles were incubated at 37°C and observed daily for bacterial colony growth, turbidity and haemolysis for up to 21 days.

Reference strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028 and *Salmonella typhi* ATCC 19430) obtained from KEMRI Centre for Microbiology Research (CMR), Kenyatta Hospital, Nairobi were used as controls and included in each step.

Blood cultures were then subcultured onto blood agar and MacConkey agar. Selective and enriched media were also used for sub-culturing: chocolate blood agar and mannitol salt agar. The cultured blood (1 ml) was aseptically drawn from the culture bottle using a sterile hypodermic needle and syringe and a drop placed on the agar. A sterile inoculating wire was then used to streak the inoculum on the surface of the agar. Bacitracin and optochin discs were placed in the blood agar after streaking for the detection of *Streptococcus pyogenes* and *Streptococcus pneumoniae*, respectively. All the plates were incubated overnight at 37°C then examined for growth and if no growth was observed, the plates were further incubated for an additional 24 hours.

For the MacConkey agar, the medium was used to distinguish between the lactose-fermenting coliforms from the lactose-non-fermenting *Salmonella* and dysentery groups, where the lactose-fermenting coliforms were expected to produce a visible pinkish color on growth. For the blood agar, it was used for growth of fastidious organisms producing haemolysin which would give

visible haemolytic zones on the medium. Different haemolytic patterns i.e. alpha haemolysis, beta haemolysis and gamma haemolysis were used to distinguish among the various fastidious microorganisms.

Chocolate blood agar was used to primarily isolate *Haemophilus influenzae* which normally utilizes nutrients from lysed red blood cells in the medium hence would be detectable by growth. For mannitol salt agar, the medium was used to isolate *Staphylococci* spp. where *Staphylococcus aureus* was expected to ferment mannitol hence producing yellow-colored colonies surrounded by yellow zones, whereas, other *Staphylococci* spp. which are mannitol non-fermenters were expected to produce pink to red colonies surrounded by red-purple zones (Cheesbrough, 2006a).

3.8 Identification of bacteria pathogens

Standard procedures for identification and characterization of bacterial pathogens included microscopic morphological identification of colonies (i.e. coccus, bacillus), differential staining by Gram stain, growth on selective and enriched media and biochemical tests.

3.8.1 Morphological identification

This involved distinguishing between cocci and bacilli by using a microscope after performing Gram staining. Cocci bacteria were distinguished by either being in clusters and tetrads, in long chains, diplococci or as coccobacilli while bacilli were distinguished by either being in diphtheroids, slender, curved, short or long bacilli.

3.8.2 Gram staining

Gram staining (or Gram's method) is a method for differentiating bacterial species into two large groups; Gram-positive and Gram-negative. Gram-positive bacteria appear stained purple by crystal violet; whereas Gram-negative bacteria are stained pink/red by the counter-stain (Fig 2).

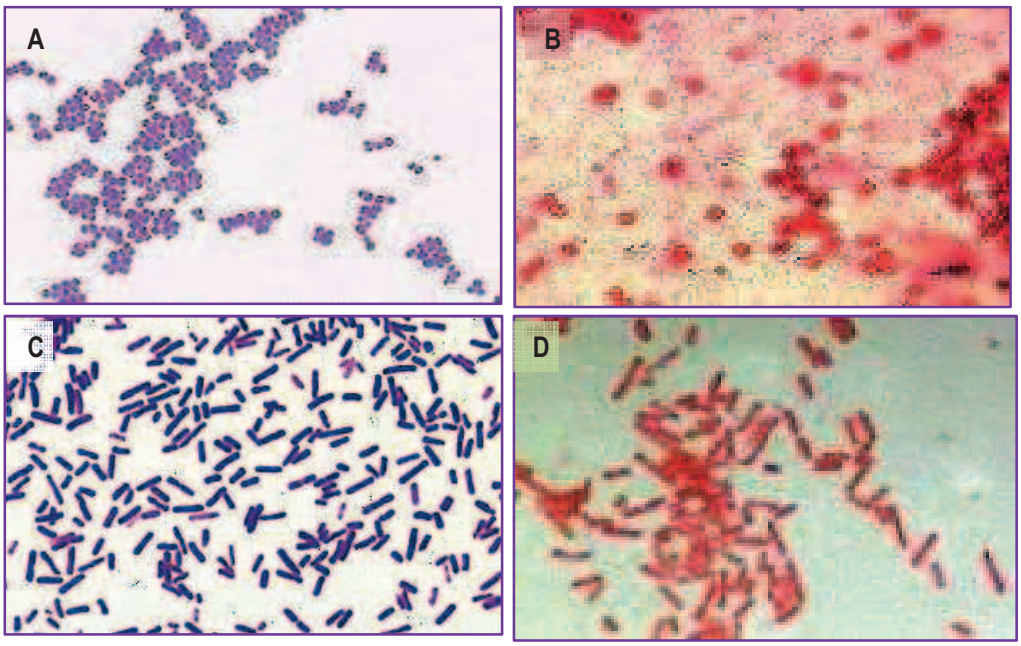

Figure 2: Images showing interpretation of Gram staining; where A: Gram-positive cocci, B: Gram-negative cocci, C : Gram-positive bacilli and D: Gram-negative bacilli.

Source: (NMPDR, 2012; NIAID, 2012).

A drop of normal saline was placed on a microscope slide. A colony was then picked using a sterile wire loop and emulsified using the normal saline on the slide. The bacterial culture smear was then heat-fixed over a flame. The slide was then gently flooded with crystal violet which is the primary stain and left for 1 minute. The slide was then rinsed gently with deionized water and flooded with iodine and left for 2 minutes. The slide was gently rinsed using deionized water and rapid decolorization was done with acetone for 10 seconds. The slide was rinsed again using deionized water and a counterstain, safranin poured over the slide and left for 45 seconds. The safranin was rinsed off the slide using deionized water and the slide left to air dry and then examined under oil immersion lens.

3.9 Biochemical tests

Five tests were used for identification of Gram-negative bacilli; namely, Simmon's citrate test, triple sugar iron test, urea test, motility-indole-ornithine test and oxidase test. Catalase and coagulase tests were used for identification of Gram-positive cocci. All tests were performed according to standard microbiological procedures (Cheesbrough, 2006).

3.9.1 Simmon's citrate test

Simmon's citrate test tested the use of sodium citrate as the only carbon source. Organisms which can utilize citrate as their sole carbon source used the enzymes citrase and citrate permease which facilitated transport of citrate into the cell. These organisms also converted the ammonium dihydrogen phosphate to ammonia and ammonium hydroxide, which created an alkaline environment in the medium. Thus, a positive result for citrate utilization was the formation of a blue color while a negative result was indicated by a green color. This test was used to differentiate among the Gram-negative bacilli in the family Enterobacteriaceae. *Enterobacter* and *Klebsiella* spp. were citrate positive while *E.coli* is negative.

A tube containing the Simmon's citrate media was inoculated by touching a colony using a sterilized inoculating wire then streaking the slanted surface of the agar after which the tube was loosely capped. The tube was then put in a test-tube rack and incubated overnight at 37˚C for 24 to 48 hours. The slants were obtained from the incubator and the color changes observed. The results had to be well defined (either blue or green). If results were equivocal, the slants were incubated for an additional 24 hours.

3.9.2 Motility-indole-ornithine medium (MIO) test

Motility-indole-ornithine medium was used to demonstrate motility, indole production and

ornithine decarboxylase activity for the differentiation of enterobacteria. When ornithine decarboxylase was present, ornithine was decarboxylated to putrescine which caused a rise in the pH and corresponding color change of the Bromocresol purple from yellow to purple. Decarboxylation of ornithine was indicated by the development of a turbid purple to a faded yellow-purple color. A negative reaction was indicated by a yellow color. Motility was indicated by growth extending from the line of inoculation. Non-motile organisms grew only along the line of inoculation. Indole production was indicated by the formation of a pink to red color after the addition of three or four drops of Kovacs' reagent to the surface of the medium and gentle shaking. A negative reaction was indicated by the development of a yellow color or brown layer. Table 2 shows how results obtained from MIO medium tests were interpreted.

Inoculation was done by touching a colony using a sterilized straight inoculating wire then stabbing the agar in a single down and up motion in the centre of the agar going three-fourths of the way down the tube and keeping the wire as vertical as possible. The tubes were loosely capped and then put in a test-tube rack and incubated overnight at 37˚C for 24 hours. The tubes were obtained from the incubator and examined for growth, color changes, presence of motility and for ornithine decarboxylase. Subsequently, 3–4 drops of Kovacs' Reagent were added to the surface of each tube to test for indole production. If the indole reaction was negative, the tubes were incubated for an additional 24 hours after which the tubes were examined color changes.

Table 2: Parameters used for interpretation of results obtained from motility-indole-ornithine medium test.

Organism	Motility	Indole	Ornithine
Escherichia coli	+	+	+
Enterobacter aerogenes	+	-	+
Klebsiella pneumoniae subsp. *pneumoniae*	-	-	-
Salmonella enterica subsp. *enterica* serotype Typhi	+	-	-

Source: (Becton, Dickinson and Company, 2012).

3.9.3 Triple sugar iron agar (TSI) test

The purpose of this test was to determine whether an organism could ferment a specific carbohydrate with production of acid and/or gas. Triple sugar iron (TSI) agar was used for the identification of Enterobacteriaceae by the rapid detection of the fermentation of lactose, glucose (with or without gas production) and of sucrose as well as the production of hydrogen sulfide.

Bacteria that could ferment any of the three sugars in the medium produced acid byproducts which caused a colour change of the red pH-sensitive phenol red to a yellow color due to lowering of the pH of the medium. Position of the color change distinguished the acid production associated with glucose fermentation from the acidic byproducts of lactose or sucrose fermentation. Many bacteria that ferment sugars in the anaerobic butt of the tube are enterobacteria. If the bacteria did not ferment the carbohydrate then the media remained red. If gas was produced as a byproduct of fermentation, then the tube had a bubble in it.

Some bacteria utilized thiosulfate anion as a terminal electron acceptor, reducing it to sulfide. If this occurred, the newly-formed hydrogen sulfide gas (H_2S) reacted with ferrous sulfate in the

medium to form ferrous sulfide, which was visible as a black precipitate. Hydrogen production may have lifted the agar from the butt of the tube or fractured the agar. Carbon dioxide, if produced, may not have showed as bubbles because it is far more soluble in the medium. Table 3 shows the criterion that was used for interpretation of common enteric bacteria using TSI agar tests while Table 4 shows how results obtained from TSI agar tests were interpreted.

Table 3: Typical reactions of selected enteric bacteria in triple sugar iron agar test.

Organism	Slant	Butt	H_2S
Escherichia coli	Yellow	Yellow /Gas	-
Klebsiella pneumoniae	Yellow	Red or Yellow /Gas	-
Salmonella typhimurium	Red	Yellow /Gas	+
Salmonella typhi	Red	Yellow	+
Shigella dysenteriae	Red	Yellow	-
Aerobacter aerogenes	Yellow	Yellow /Gas	-
Citrobacter freundii	Yellow	Yellow /Gas	+
Proteus vulgaris	Yellow	Yellow /Gas	+
Pseudomonas aeruginosa	Red	Red	-
Alcaligenes faecalis	Red	Red	-

Source: (Fankhauser, 2001).

Inoculation of the colonies was done by touching a colony using a sterilized straight inoculating wire then stabbing to the bottom of the tube with a single down and up motion. After stabbing, the surface of the agar was streaked immediately and the tube loosely capped. The tube was then put in a test-tube rack and incubated overnight at 37˚C for 24 to 48 hours. The slants were obtained from the incubator and any color changes, production of a black precipitate and presence of air bubbles were observed.

Table 4: Interpretation of results in triple sugar iron agar test.

Color status	Interpretation
Slant color:	
Red	Does not ferment either lactose or sucrose
Yellow	Ferments lactose and/or sucrose
Butt color/condition	
Red	No fermentation of glucose
Yellow	Some fermentation of glucose has occurred, acid has been produced
Gas formed	Seen as cracks in the agar, bubbles, or entire slant may be pushed out of the tube. (C*aution:* these gassy fermenters may have bacteria close to the opening.)
Black	H_2S has been produced

Results (slant/butt)	Symbol	Interpretation
Red/yellow	K/A	Glucose fermentation only; Peptone catabolized
Yellow/yellow	A/A	Glucose and lactose and/or sucrose fermentation
Red/red	K/K	No fermentation; Peptone catabolized
Red/no color change	K/NC	No fermentation; Peptone used aerobically
Yellow/yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation; Gas produced
Red/yellow with bubbles	K/A,G	Glucose fermentation only; Gas produced
Red/yellow with bubbles and black precipitate	K/A,G, H_2S	Glucose fermentation only; Gas produced; H_2S produced
Red/yellow with black precipitate	K/A, H_2S	Glucose fermentation only; H_2S produced
Yellow/yellow with black precipitate	A/A, H_2S	Glucose and lactose and/or sucrose fermentation; H_2S produced
No change/no change	NC/NC	No fermentation

Note: A=acid production; K=alkaline reaction; G=gas production; H_2S =sulfur reduction

Source: (Fankhauser, 2001; Austin Community College, 2012).

3.9.4 Urea agar test

Urea agar test is a differential medium that was used to test the ability of an organism to produce an exoenzyme called urease that hydrolyzes urea to ammonia and carbon dioxide. Phenol red is yellow in an acidic environment and turns bright pink in an alkaline environment. If the urea in the medium was degraded and ammonia was produced, an alkaline environment was created and the media turned bright pink. A color change from yellow to bright pinkish-red was positive; lack of color change was a negative result. It differentiated between rapid urease-positive *Proteus mirabilis* and other urease-positive organisms: *Citrobacter*, *Enterobacter* and *Klebsiella pneumoniae.*

Inoculation was done by touching a colony using a sterilized straight inoculating wire then stabbing 3 times into the agar and the tube loosely capped. The tube was then put in a test-tube rack and incubated overnight at 37˚C for 24 to 48 hours. The slants were obtained from the incubator and observed for color changes.

3.9.5 Oxidase test

The oxidase reagent contains a chromogenic reducing agent, which is a compound that changed color when it became oxidized. If the test organism produced cytochrome oxidase, the oxidase reagent turned blue or purple, to dark-blue within 15 seconds. If negative there was no colour change. *Vibrio*, *Pseudomonas*, *Campylobacter*, *Helicobacter*, *Neisseria*, *Moraxella* and *Legionella*, are typically OX+ while Enterobacteriaceae are typically OX-.

Each oxidase disk was made wet with about 4 inoculating loops of de-ionized water. A loop was used to aseptically transfer a mass of pure bacteria to each oxidase disk. The disk was observed for up to 3 minutes. If the area of inoculation turned blue or purple, to almost dark-blue, then the result

was Oxidase positive. If a color change did not occur within three minutes, the result was Oxidase negative.

3.9.6 Catalase test

When a small amount of bacterial isolate was added to hydrogen peroxide, oxygen was released which formed bubbles or froth. Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. This test was primarily used to distinguish among Gram-positive cocci; members of the genus *Staphylococcus* were catalase-positive, and members of the genera *Streptococcus* and *Enterococcus* which normally are catalase-negative.

A drop of hydrogen peroxide was placed on a microscopic slide. Using a sterile wire loop, a colony was picked and then smeared into the hydrogen peroxide drop. Production of bubbles or froth was observed and if present, the organism was considered catalase positive and catalase negative if there were no bubbles.

3.9.7 Coagulase test

The coagulase slide test was used to identify the presence of bound coagulase or clumping factor, which is attached to the cell walls of the bacteria. Bound coagulase reacts with the fibrinogen in plasma, causing the fibrinogen to precipitate. This caused the cells to agglutinate, or clump together, which created the “lumpy” look of a positive coagulase slide test. Free coagulase which is an enzyme excreted outside the cell wall by the bacteria was detected by doing a tube coagulase test. If the slide test was negative, the tube test was carried out. The coagulase test was used to differentiate *Staphylococcus aureus* from coagulase-negative *Staphylococci*. *Staphylococcus aureus* produces two forms of coagulase (i.e., bound coagulase and free coagulase).

Two drops of saline were put on a microscopic slide. The saline drops were emulsified with the

test colonies by using a sterile wire loop. A drop of coagulase reagent (rabbit plasma) was placed on the inoculated saline drop and mixed well with wooden applicator stick. The slide was gently rocked for about 10 seconds. If the slide coagulase test was negative, a tube test followed as a confirmation test where a tube with rabbit plasma was inoculated with the staphylococcal colony. The tube was then incubated at 37°C for one and a half hours. If negative the incubation was continued for up to 18 hours.

3.10 Antibiotic susceptibility testing

Testing of all the isolates obtained from the study for antibiotic susceptibility was performed as described by Dalsgaard *et al*. (1996) and Scrascia *et al*. (2003). Mueller Hinton agar was used as the medium. *Escherichia coli* ATCC 25922 was used as a control strain. A sterile inoculating wire was used to pick a colony of the test organism from the agar plates. The colony was emulsified in a sterile bijou bottle using normal saline. A sterile non-toxic cotton swab was then dipped into the inoculum in the bijou bottle and rotated to drain excess liquid. It was then used to streak the inoculum on the entire surface of the agar. This was repeated for all the bacteria isolates obtained. The inoculum was allowed to dry for 5-10 minutes with the lid in place.

Twelve commercially prepared single antibiotic discs of different potencies/concentration were used to test for drug resistance and drug susceptibility and included: Amoxicillin-Clavunic acid, Ampicillin-Cloxacillin, Chloramphenicol, Ceftazidime, Ciprofloxacin, Co-trimoxazole, Cefuroxime, Erythromycin, Gentamicin, Nalidixic acid, Nitrofurantoin and Tetracycline. The commercial names and potencies of the antibiotics are as shown in Table 5. A sterile pair of forceps was then used to place the commercially prepared single antimicrobial discs on the surface of the streaked Mueller Hinton agar. Four to eight discs were used per plate. It was ensured that the discs were placed at a minimum of 2mm apart from each other as described by Scrascia *et al*.,

(2003). The plates were incubated overnight at 37°C then examined for bacterial growth. Those with no growth were further incubated for a further 12 hours.

The plates were examined for zones of inhibition which were measured using a ruler in millimeters after removing the plates from the incubator and the results recorded. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the Muller Hinton agar medium. The zones of inhibition were read and results interpreted using standardized thresholds for defining susceptibility as described by NCCLS (2002). The results were reported as sensitive, resistant or intermediate. There were three replications for the antibiotic susceptibility test for each isolate with four to eight antimicrobial discs in each plate.

Table 5: Common trade names of antibiotics used.

Antibiotic/Agent Name	Synonym Generic Name or Trade Name	Manufacturer	Class/Subclass or Microbial Derivation	BBL™ Discs Catalog No Package/Carton	Sensi-Disc™ Code
Amoxicillin - Clavunic acid	Augmentin™	GlaxoSmithKline	β-lactam/ β-lactamase inhibitor combination	231628 / 231629	AmC-30
Ampicillin - Cloxacillin	*Amplicox™ *Amplicox™-D	GlaxoSmithKline GlaxoSmithKline	See components		
Chloramphenicol	Chloromycetin™	Pfizer	Phenicol	230729 / – 230733 / 231274	C-5 C-30
Ceftazidime	Ceptaz™ Fortaz™ Tazicef™ Tazidime™	GlaxoSmithKline GlaxoSmithKline Hospira Lilly	Cephem/ Cephalosporin III	231632 / 231633	CAZ-30
Ciprofloxacin	Cipro™	Bayer	Fluoroquinolone	231657 / 231658	CIP-5
Co-trimoxazole (*Septrin™)	Composition Sulfamethoxazole/ Trimethoprim	GlaxoSmithKline	Folate pathway Inhibitor	231536 / 231539	SXT
Cefuroxime (sodium)	Kefurox™ Zinacef™	Lilly GlaxoSmithKline	Cephem/ Cephalosporin II	231620 / 231621	CXM-30
Erythromycin	Emgel™ Erythrocin™ Ilotycin™	Elan Abbott Dista	Macrolide	– / 231289 230793 / 231290	E-2 E-15
Gentamicin	Garamycin™ Gentocin™	Schering	Aminoglycoside	231227 / 231299 231693 / –	GM-10 GM-120
Nalidixic acid	NegGram™ *Wintomylon™	Sanofi-Aventis	Quinolone	230874 / 231311	NA-30
Nitrofurantoin	Furadantin™ Macrodantin™ Macrobid™	Procter & Gamble	Nitrofuran	– / 231292 231149 / 231293	F/M-100 F/M-300
Tetracycline	Panmycin™ Polycycline™ Tetracyn™ Tetrex™	Pfizer Bristol-Myers Pfizer Bristol-Myers	Tetracycline	– / 231343 230998 / 231344	Te-5 Te-30

Source: (Becton, Dickinson and Company, 2012).

An interpretive table (Table 6) was used to determine whether an isolate was resistant, intermediate or sensitive to the different antibiotics commonly used in the two health centres at Alupe, Western Kenya.

Table 6: Interpretative table of zone inhibition sizes showing antimicrobial agent disc diffusion breakpoint.

Antimicrobial agent	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Ampicillin-cloxacillin (30µg)	≤28[1]	-	≥29[1]
	≤11[2]	12-13[2]	≥14[2]
Amoxycillin-clavulinic acid	<13[1]	13-18[1]	>18[1]
(30µg)	<19[2]	19-20[2]	>20[2]
Chloramphenicol (30µg)[a]	≤12	13-17	≥18
Ceftazidime (30µg)[a]	≤14	15-17	≥18
Ciprofloxacin (5µg)[a]	≤15	16-20	≥21
Co-trimoxazole (25µg)[a]	≤10	11-15	≥16
Cefuroxime (30µg)[a]	≤14	15-17	≥18
Erythromycin (15µg)[a]	≤13	14-22	≥23
Gentamicin (10µg)[a]	≤12	13-14	≥15
Nalidixic acid (30µg)[a]	≤13	14-18	≥19
Nitrofurantoin (300µg)[a]	≤14	15-16	≥17
Tetracycline (30µg)	≤14[1]	15-18[1]	≥19[1]
	≤10[2]	11-18[2]	≥19[2]

[µg] potency of drug in micrograms
[1] zone size for gram negative bacteria
[2] zone size for gram positive bacteria
[a] value for both gram negative and positive bacteria

Source: (CLSI, 2006).

3.11 Data analysis and presentation

All data (social demographic and clinical data) was analyzed using Stata V.10.0 (Texas, USA: StataCorp LP). Categorical variables were presented as percentages and continuous variables as

means. Descriptive statistics were generated and used to analyze the socio-demographic variables. Differences in the age of the participants across gender were analyzed using ANOVA and means separated using Chi-square test. Comparison of antimicrobial resistance patterns of the isolates was analyzed using a standard interpretative table. Odds ratios were generated. An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure (Szumilas, 2010). The level of significance for all the analysis was set at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic data of patients included in study

4.1.1 Catchment areas of patients

The patients who were recruited in the study were drawn from ten administrative divisions in Kenya and one from Uganda (Table 7). Majority (43%) of the patients came from Chakol division of South Teso district, a division with a high population density of approximately 427 persons per km^2 (National Census, 2009). The other divisions where a high percentage of patients were recruited were Amukura (17%) and Busia town (10.5%).

Table 7: Catchment areas of the patients recruited in the study.

Division	No. of patients	Percentage
Chakol	86	43.0
Amukura	34	17.0
Busia town	21	10.5
Amagoro	15	7.5
Matayos	14	7.0
Nambale	13	6.5
Angurai	7	3.5
Funyula	7	3.5
Budalangi	1	0.5
Butula	1	0.5
Butambala*	1	0.5
Total	200	100.0

* District located in Uganda

Of the patients, 72.5 % were registered at KEMRI CIPDCR Alupe Clinic while 27.5 % were registered at Alupe District Hospital (Figure 3).

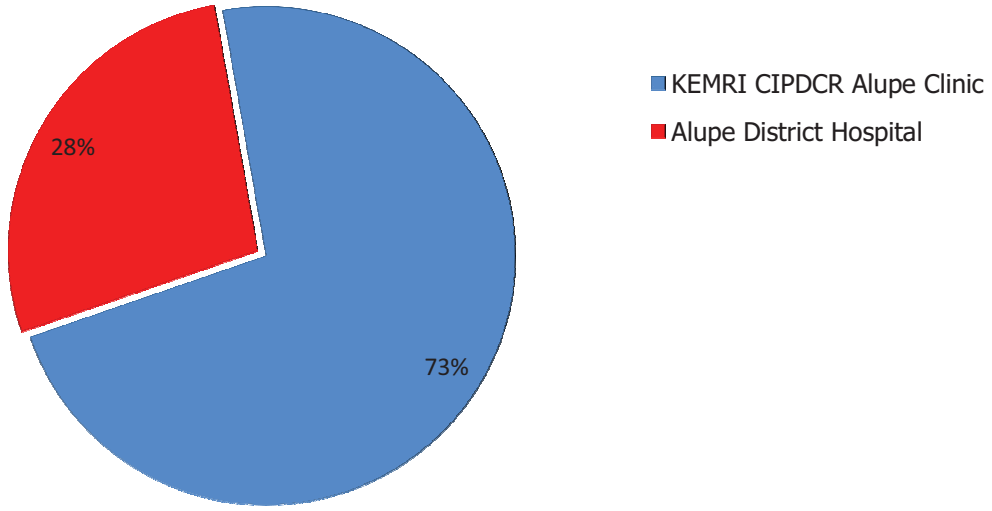

Figure 3: Proportion (%) of patients registered at KEMRI CIPDCR Clinic and Alupe District Hospital.

4.1.2 Age and gender of patients

The youngest and oldest patients in the study were 2 and 82 years old, respectively (Figure 4). The mean age of the patients interviewed was 37.0 years while the median age was 38 years which was normally distributed. The standard deviation of the age was 19.6. There was no significant difference in the age of the participants across gender ($X^2 = 0.18$).

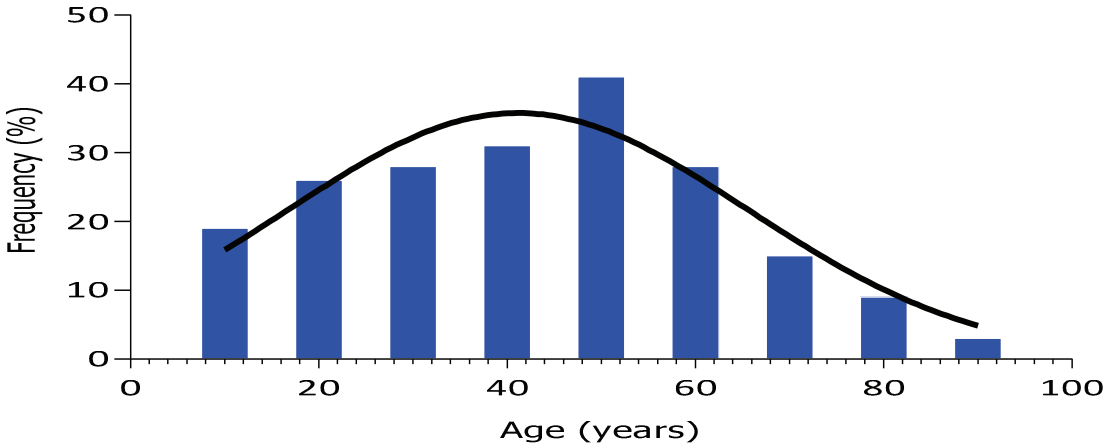

Figure 4: Age distribution of patients interviewed at Alupe District Hospital and KEMRI CIPDCR Clinic.

The age group with the highest number of patients was 40-49 years constituting 20.5% of all patients involved in the study (Table 8). Of the patients, 61.5% were female while 38.5% were males.

Table 8: Proportion (%) of age distribution and gender of patients interviewed at Alupe District Hospital and KEMRI CIPDCR Clinic.

Age group (years)	Male (%)	Female (%)	Total (%)	n
1.5– 9	5.0	4.5	9.5	19
10 – 19	4.0	9.0	13.0	26
20 – 29	6.5	7.5	14.0	28
30 – 39	5.0	10.5	15.5	31
40 – 49	6.5	14.0	20.5	41
50 – 59	6.0	8.0	14.0	28
60 – 69	2.5	5.0	7.5	15
70 – 79	2.0	2.5	4.5	9
80 – 89	1.0	0.5	1.5	3
Total population (%)	38.5	61.5	100.0	-
Total (n)	77	123	-	200

4.1.3 Occupation of the patients

A third (33%) of the recruited patients were unemployed. Only 10% were in formal employment while 32.5% were self employed (Figure 5). Patients in other categories included students (16%), retirees (2.5%) and infants (6%).

Figure 5: Proportion (%) of recruited patients in different occupations.

4.2 Bacteria species isolated from blood cultures of patients

A total of 200 blood culture samples were collected, out of which 7 were culture positive. Three out of 7 bacteria isolates were Gram-positive while 4 were Gram-negative. *Staphylococcus aureus* was predominant and the only Gram-positive bacteria isolate in blood culture of patients who remained febrile after clearance of malarial parasitemia. *Escherichia coli* was the predominant (50%) Gram-negative bacteria with 2 bacteria isolates. *Klebsiella pneumoniae* and *P. mirabilis* each had one isolate hence accounted for 25% of Gram-negative isolates each. All bacteria isolated had overall individual isolation rates from positive blood cultures in the following

decreasing order: *S. aureus* (42.9%), *E. coli* (28.6%), *P. mirabilis* (14.3%) and *K. pneumoniae* (14.3%). No blood sample yielded more than one bacteria species (Figure 6).

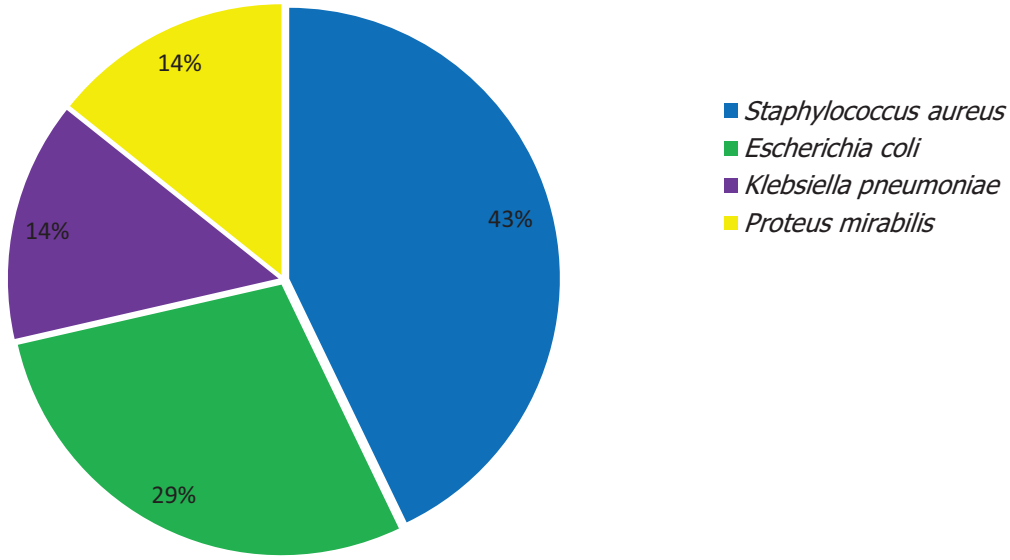

Figure 6: Proportion (%) of bacteria species isolated from the blood culture samples of patients presenting with febrile illness.

The bacteria isolates showed different morphological and Gram staining characteristics (Table 9). *Escherichia coli* cells were coccobacillary, short rods, arranged singly and in pairs and Gram stained negatively. *Staphylococcus aureus* cells were Gram-positive, cocci and arranged singly/pairs or in irregular clusters. *Proteus mirabilis* cells were coccobacillary, short rods, arranged singly, in pairs and in short chains and Gram stained negatively. *Klebsiella pneumoniae* cells were short rods arranged singly and in pairs and Gram stained negatively.

Table 9: Morphological and staining characteristics of bacteria species isolated from positive blood cultures.

Patient ID No	Bacteria species isolated	Shape	Cell arrangement	Gram stain
CIP/09	*P. mirabilis*	Coccobacillary, short rods	Single, pairs, and in short chains	Gram-negative
CIP/198	*K. pneumoniae*	Small rods	Single and in pairs	Gram-negative
CIP/52	*E. coli*	Coccobacillary, short rods	Single and in pairs	Gram-negative
CIP/122	"			
CIP/34	*S. aureus*	Cocci	Single, pairs and in irregular clusters	Gram-positive
CIP/80	"			
CIP/87	"			

The bacteria isolates showed different cultural characteristics (Table 10). The cultural characteristics for *E. coli* were production of lactose fermentative colonies on MacConkey agar which gave circular, smooth and convex colonies pink colour. On blood agar, it was non-haemolytic.

Staphylococcus aureus gave yellowish smooth raised and glistening colonies with circular and entire margin. It produced beta haemolysis of red blood cells on blood agar whereas it did not grow on MacConkey agar medium. It fermented mannitol salt agar turning the medium yellow.

When cultured, *K. pneumoniae* colonies were lactose fermentative on MacConkey agar and gave circular and convex colonies with pink colour. It produced a distinctive yeasty odor and the bacterial colonies had a viscous/mucoid appearance. It showed no haemolysis on blood agar.

Proteus mirabilis formed round, flat, large swarming colonies on blood agar and gave off a faint ammonia / fishy odor. It showed no haemolysis on blood agar and did not metabolize lactose on

MacConkey agar.

Table 10: Cultural characteristics of bacteria species isolated from positive blood cultures.

Patient ID No	Bacteria species isolated	Morphological characteristics
CIP/09	*P. mirabilis*	Formed clear films on blood agar. Swarming ability throughout agar plate and was motile. It produced a very distinct fishy odour.
CIP/198	*K. pneumoniae*	Produced a distinctive yeasty odor and colonies had a viscous or mucoid appearance on all agars
CIP/52	*E. coli*	On blood agar, non-haemolytic while on MacConkey agar, it gave circular,
CIP/122	"	smooth and convex colonies with pink colour.
CIP/34	*S. aureus*	Produced fairly large golden-yellow colonies on blood agar with regions of
CIP/80	"	clearing around colonies caused by lysis of red cells in the agar (beta
CIP/87	"	hemolysis).

Cultures of *S. aureus*, *K. pneumoniae*, *P. mirabilis* and *E. coli* isolates growing on different media are shown in Figure 7 and 8.

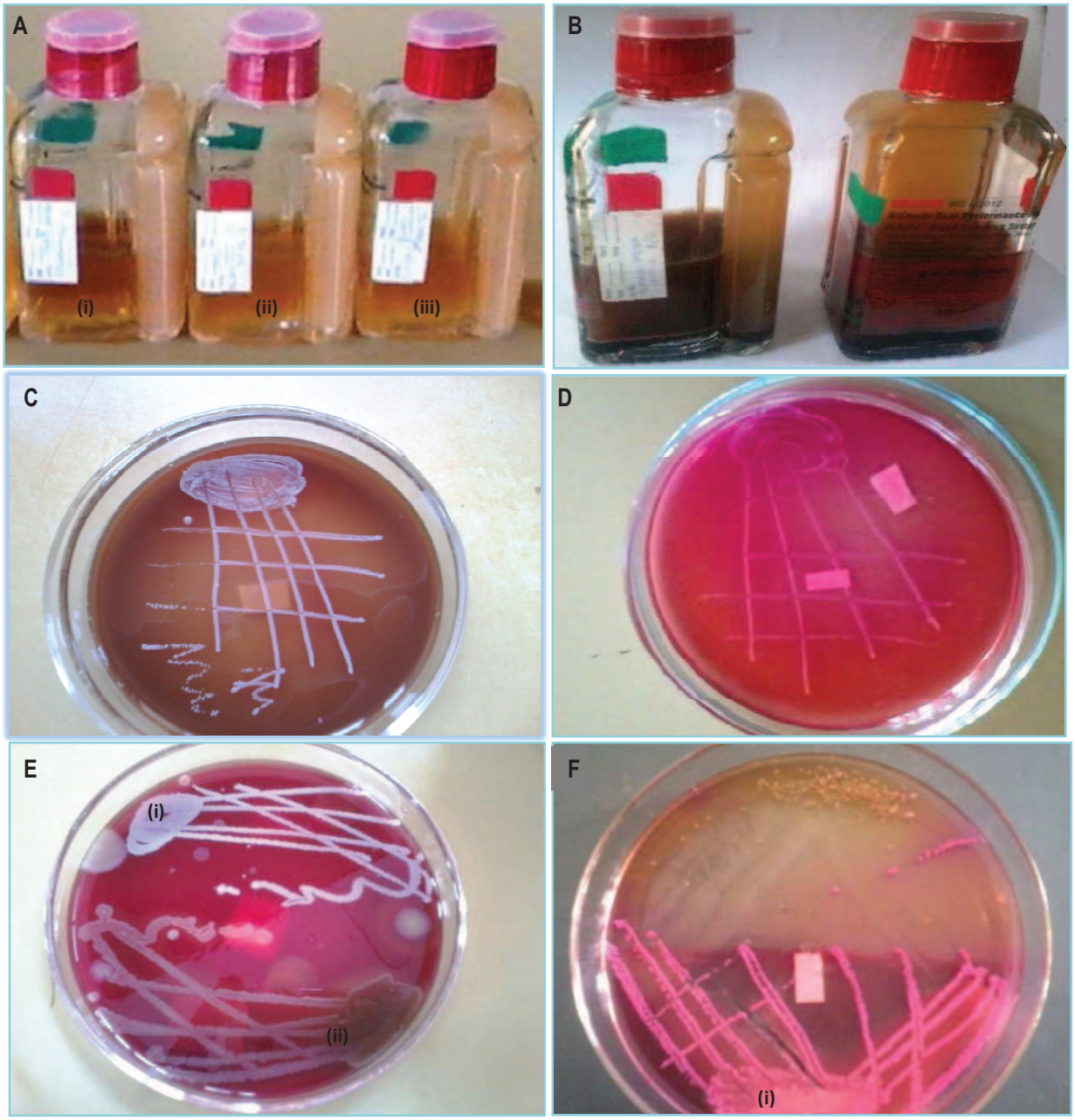

Figure 7: Blood cultures and cultures of various bacteria species isolated on different media. A - Control strains in broth media used for culturing blood where (i) *S. aureus* ATCC 25923, (ii) *P. aeruginosa* ATCC 27853 and (iii) *S. typhimurium* ATCC 14028; B - Culture of blood samples in broth media; C – *S. aureu s* on Chocolate Blood Agar; D – *S. aureus* on Mannitol Salt Agar; E - (i) *K. pneumoniae* and (ii) *P. mirabilis* on Blood Agar and F - (i) *E. coli* on MacConkey Agar.

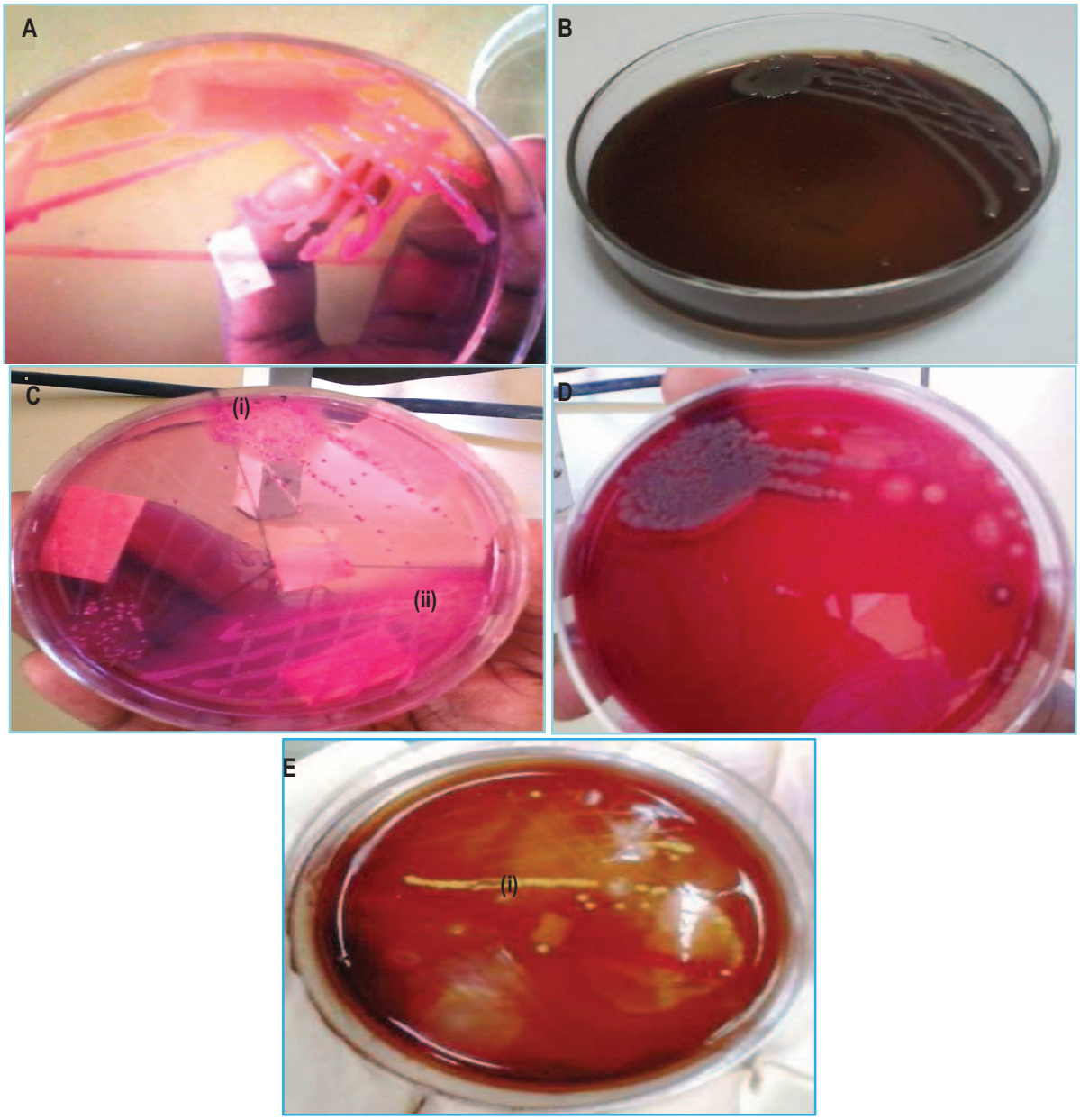

Figure 8: Cultures of various bacteria species isolated on different media. A - *K. pneumoniae* on MacConkey Agar; B - *P. mirabilis* on Blood Agar; C - (i) *E. coli* and (ii) *K. pneumoniae* on MacConkey Agar; D - Beta hemolysis as seen on Blood Agar by *S. aureus*; and E (i) - *S. aureus* ATCC 25923 on Blood Agar.

Biochemical tests (Simmon citrate test, urea test, oxidase test, catalase test and coagulase test) were carried out to identify the bacteria isolates as shown in Figure 9. A rapidly positive urease test by *P. mirabilis* was indicated by a color change to bright pink throughout the urea agar slant as

compared to the non-inoculated control. A delayed positive reaction by *K. pneumoniae* was indicated by a color change only along the slant.

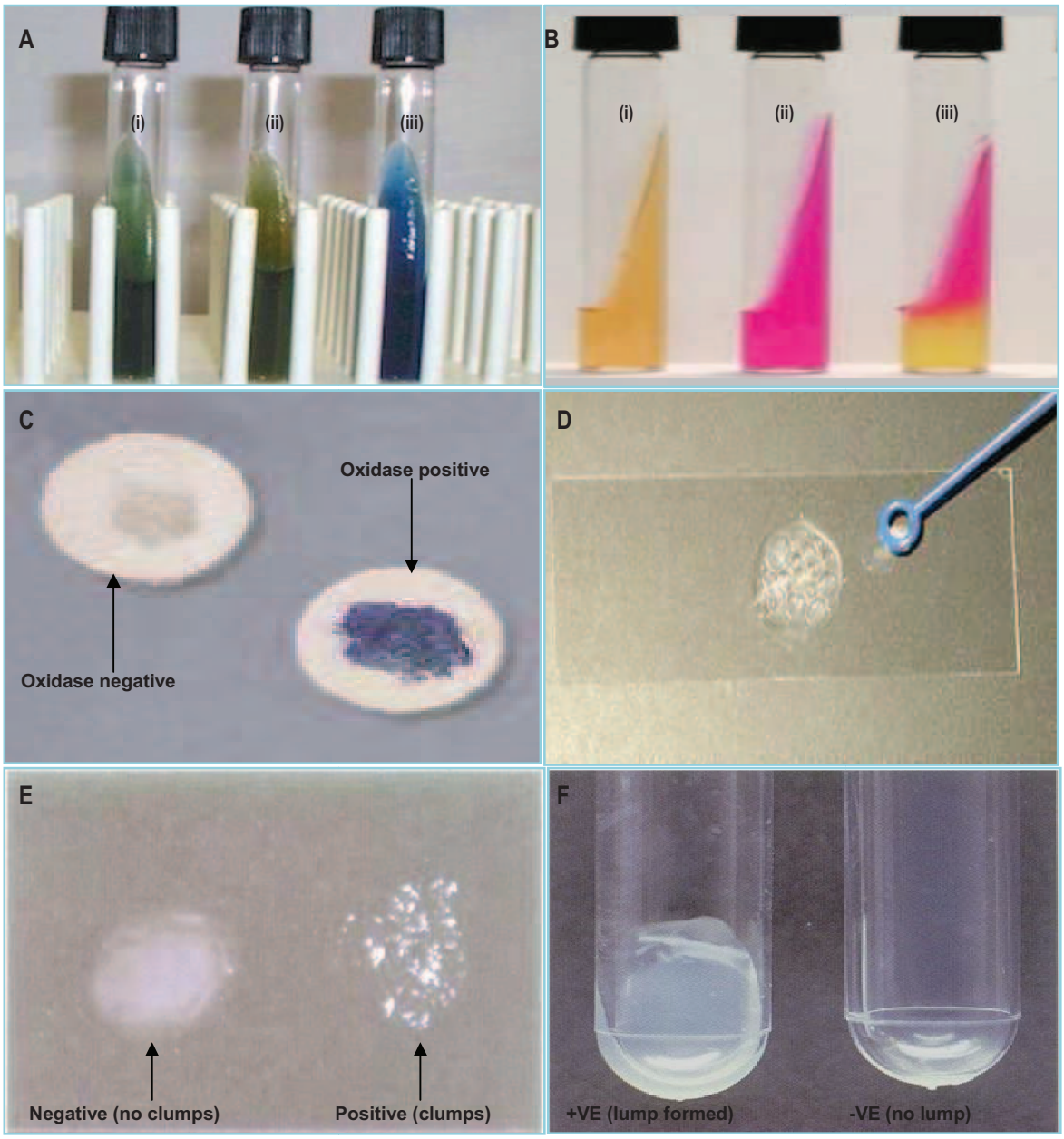

Figure 9: Results of different biochemical tests. A - Color changes in Simmon Citrate medium where (i) non-inoculated medium, (ii) negative result and (iii) positive result; B- color changes in a Urease test where (i) non-inoculated medium, (ii) positive and (iii) rapidly positive; C - Color changes in an oxidase test; D - A positive catalase reaction; E - A coagulase slide test and; F - A coagulase tube test.

4.2.1 Symptoms associated with the bacteria isolates

A single species was isolated from each positive blood culture and various diagnostic symptoms were observed from such patients (Table 11). Out of the patients with a bacteriologic diagnosis of *S. aureus* bacteremia, all presented with chills, 66% presented with clinical diagnosis of myalgia, severe headache or enlarged lymph nodes while only 33.3% presented a clinical diagnosis of nausea, rash, heart murmurs and bronchial breathing. For patients with clinical diagnosis of *E. coli* bacteremia, all presented with chills and myalgia whilst 50% presented with rash, abdominal pain and mild headache. For *P. mirabilis* and *K. pneumoniae,* bacteremia presented various symptoms.

Table 11: Symptoms observed in patients from whose blood different bacteria species were isolated.

Organism	Symptoms observed
Proteus mirabilis	Myalgia, abdominal pain, severe headache
Staphylococcus aureus	Chills, myalgia, nausea, severe headache
Escherichia coli	Chills, myalgia, abdominal pain, mild headache
Staphylococcus aureus	Chills, rash, enlarged lymph nodes, heart murmurs
Staphylococcus aureus	Chills, myalgia, enlarged lymph nodes, bronchial breathing, severe headache
Escherichia coli	Chills, rash, myalgia, abdominal pain
Klebsiella pneumoniae	Chills, body weakness, myalgia, enlarged lymph nodes, productive cough, rapi breathing, chest pain, abdominal pain, mild headache, dizziness

4.2.2 Distribution of bacteria species among age groups

Bacteria species were isolated from patients in age groups 18 months - 9yrs, 20 - 29, 30 - 39, 40 - 49 and 60 – 69 (Table 12). The proportion of bacteria isolated from the respective age groups was 28.6%, 14.3%, 14.3%, 28.6% and 14.3%. *Staphylococcus aureus* was the only species isolated from patients in age groups 18 months - 9yrs and 20 – 29. Two *S. aureus* isolates were obtained from patients in age group 18 months - 9yrs and one isolate from a patient in age group 20 – 29.

Escherichia coli accounted for 50% of the isolates from patients in age group 40 – 49 and was the only species isolated from patients in age group 60 – 69. *Klebsiella pneumoniae* also accounted for 50% of the isolates from patients in age group 40 - 49. *Proteus mirabilis* was the only species isolated from patients in age group 30 - 39.

Table 12: Bacteria species isolated from patients of different ages and gender in the study.

Age bracket (years)	Age of patient (years)	Gender of patient	Bacteria species isolated	Number of bacteria isolates
1.5– 9	7	Male	*S. aureus*	2
	2	Female	*S. aureus*	
10 – 19	-	-	-	-
20 – 29	29	Female	*S. aureus*	1
30 – 39	36	Female	*P. mirabilis*	1
40 – 49	41	Female	*E. coli*	2
	40	Female	*K. pneumoniae*	
50 – 59	-	-	-	-
60 – 69	62	Female	*E. coli*	1
70 – 79	-	-	-	-
80 – 89	-	-	-	-

4.3 Susceptibility of isolated bacteria species to antibiotics

4.3.1 Antibiotic susceptibility profile of the isolates

Antibiotic susceptibility tests were carried out to determine the sensitivity of bacteria isolates to the different antibiotics. The bacteria isolates differed in their sensitivity patterns to the different antibiotics (Figure 10).

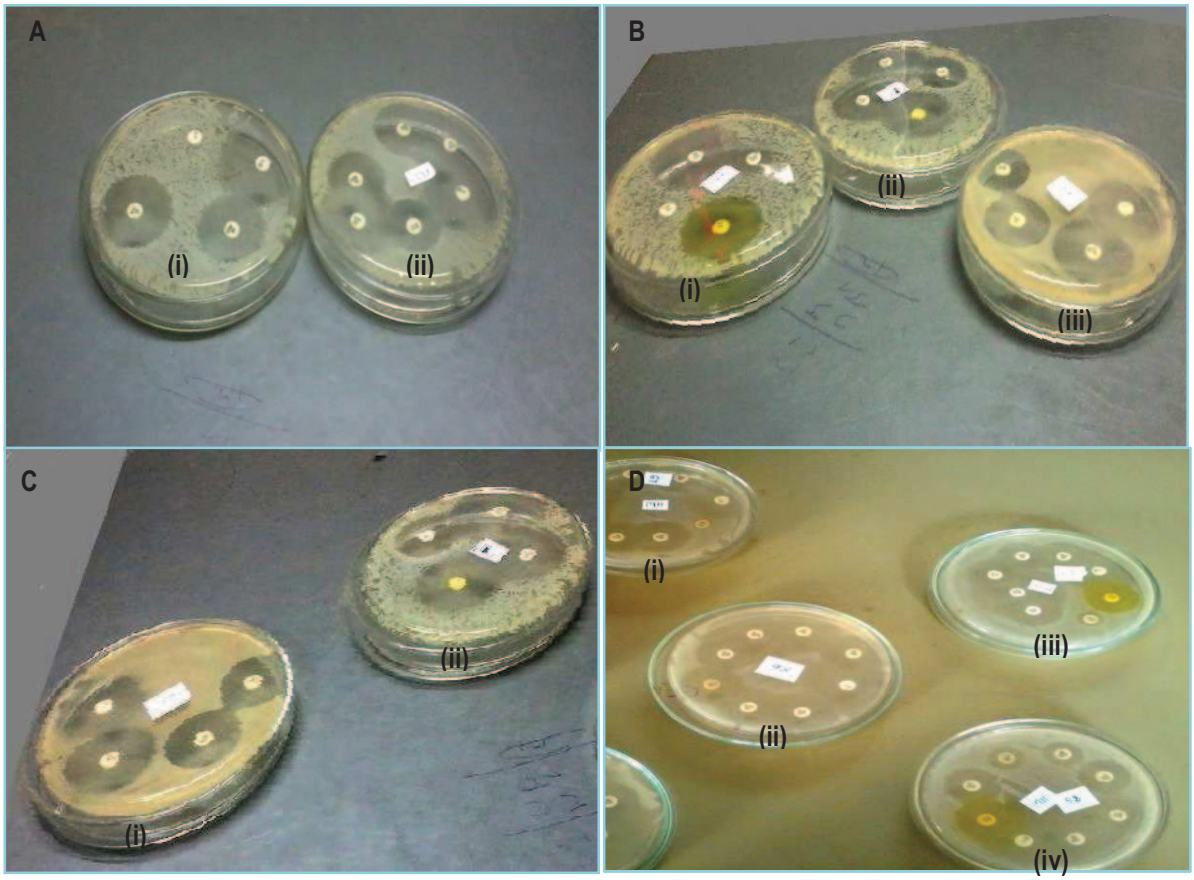

Figure 10: Sensitivity of bacteria isolates to different antibiotics using Mueller Hinton agar. A – (i) *E. coli* ATCC 25922 and (ii) *E. coli* from CIP/52, B – (i) *P. mirabilis*, (ii) *S. aureus* from CIP/34 and (iii) *K. pneumoniae*, C – (i) *S. aureus* from CIP/80 and (ii) *E. coli* from CIP/122, D – (i) *S. aureus* from CIP/87, (ii) *E. coli* from CIP/52, (iii) *S. aureus* from CIP/80 and (iv) *P. mirabilis*.

The bacteria isolates showed varied susceptibility to the twelve antibiotics (Table 13). Chloramphenicol, ciprofloxacin and gentamicin had the highest mean inhibition zones of 27.4, 25.7 and 23.3 mm, respectively while nalidixic acid, cefuroxime, erythromycin and ampicillin-cloxacillin had the lowest mean inhibition zones of 13.4, 13.4, 13.1 and 12 mm, respectively.

Table 13: Antibiotic susceptibility profile (zone of inhibition in millimeters) of different bacteria isolates to commonly used drugs.

Antibiotic	Bacteria isolate							Mean inhibition (mm)
	P. mirabilis	*S. aureus*	*E. coli*	*S. aureus*	*S. aureus*	*E. coli*	*K. pneumoniae*	
Amc	6	14	6	13	15	21	9	12.0
Ax	6	25	6	21	27	20	6	15.9
C	32	28	32	27	22	25	26	27.4
Caz	21	28	16	16	18	26	7	18.9
Cip	18	29	36	28	23	29	17	25.7
Cot	12	6	18	18	36	14	10	16.3
Cxm	12	25	6	15	6	23	7	13.4
E	12	27	10	10	15	9	9	13.1
Gen	18	28	28	31	25	21	16	23.3
Na	18	14	22	10	14	8	8	13.4
Nit	14	22	15	10	17	19	18	16.4
Te	6	10	26	18	14	19	12	15.0

Key: Amc: Ampicillin-Cloxacillin, Ax: Amoxyllin-Clavunic Acid, C: Chloramphenicol, Caz: Ceftazidime, Cip: Ciprofloxacin, Cot: Co-trimoxazole, Cxm: Cefuroxime, E: Erythromycin, Gen: Gentamicin, Na: Nalidixic Acid, Nit: Nitrofurantoin, Te: Tetracycline.

The susceptibility patterns of the isolates to the twelve antibiotics are as shown in Figure 11. Overall, all the isolates were 100% susceptible to chloramphenicol and gentamicin with the highest resistance being to erythromycin (71.4%), ampicillin-cloxacillin (57.1%) and cefuroxime (57.1%). The isolates were relatively susceptible to amoxyllin-clavunic acid, ceftazidime and nitrofurantoin and showed the lowest susceptibility to nalidixic acid (14.3%). Chloramphenicol and gentamicin were 100% effective against both Gram-positive cocci (GPC) and Gram-negative bacilli (GNB). Ciprofloxacin was 100% effective against GPC and 50% effective against GNB, respectively.

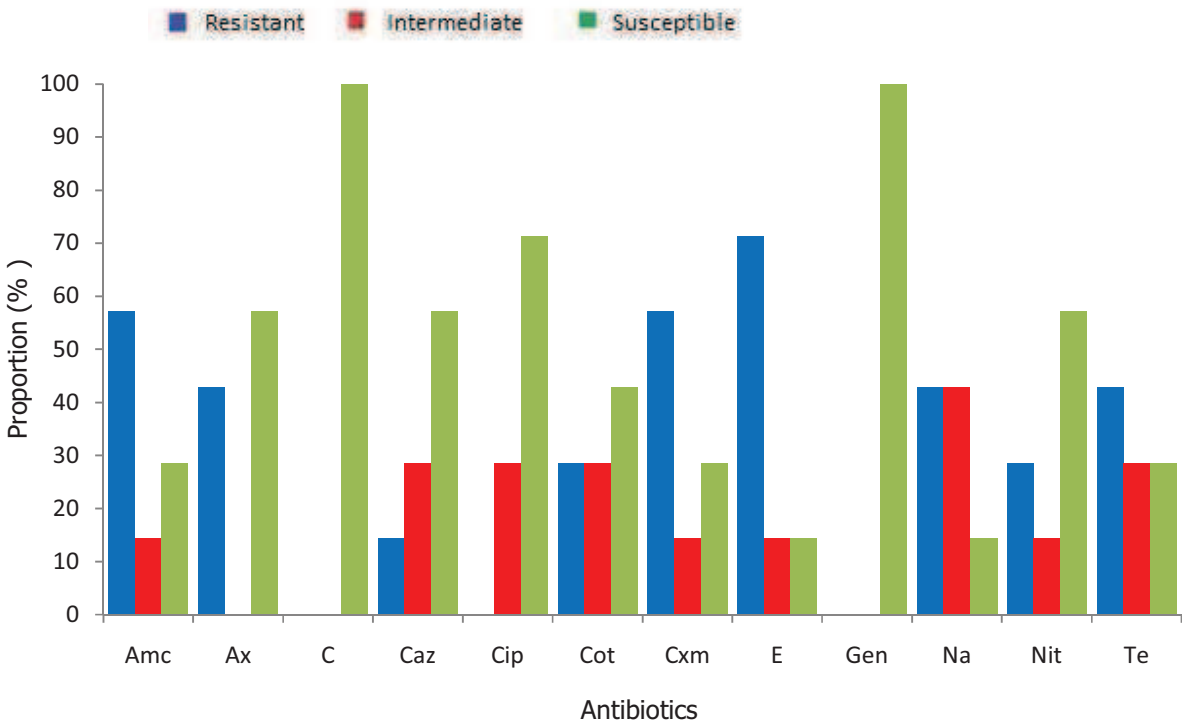

Figure 11: Susceptibility (%) patterns of the seven bacteria isolates to antibiotics commonly used in Alupe, Kenya.

Key: Amc: Ampicillin-Cloxacillin, Ax: Amoxyllin-Clavunic Acid, C: Chloramphenicol, Caz: Ceftazidime, Cip: Ciprofloxacin, Cot: Co-trimoxazole, Cxm: Cefuroxime, E: Erythromycin, Gen: Gentamicin, Na: Nalidixic Acid, Nit: Nitrofurantoin, Te: Tetracycline.

4.3.2 Antibiotic susceptibility profiles of the individual bacteria isolates

4.3.2.1 *Proteus mirabilis*

The isolate showed high resistance to ampicillin-cloxacillin, amoxyllin-clavunic acid, co-trimoxazole, cefuroxime, erythromycin, nitrofurantoin, and tetracycline (Figure 12). However, it showed high susceptibility to chloramphenicol, ceftazidime and gentamicin. Intermediate susceptibility to ciprofloxacin and nalidixic acid was observed.

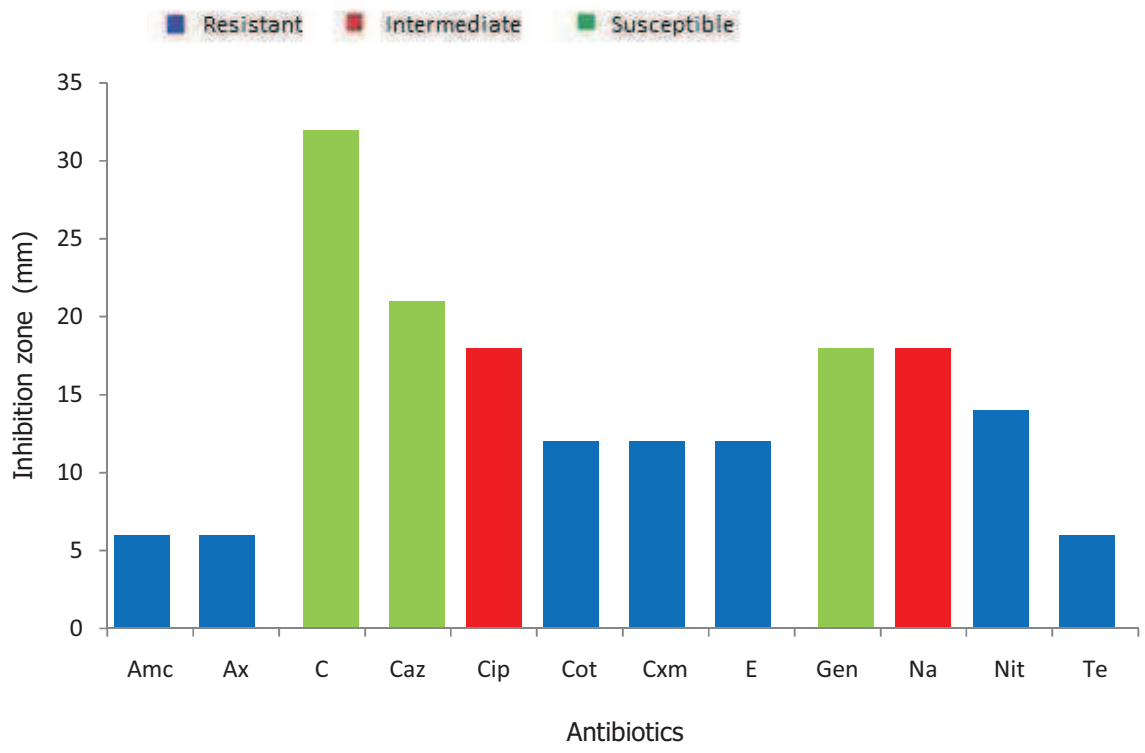

Figure 12: Susceptibility pattern of *Proteus mirabilis* to antibiotics commonly used in Alupe, Kenya.

Key: Amc: Ampicillin-Cloxacillin, Ax: Amoxyllin-Clavunic Acid, C: Chloramphenicol, Caz: Ceftazidime, Cip: Ciprofloxacin, Cot: Co-trimoxazole, Cxm: Cefuroxime, E: Erythromycin, Gen: Gentamicin, Na: Nalidixic Acid, Nit: Nitrofurantoin, Te: Tetracycline.

4.3.2.2 *Staphylococcus aureus*

There were three isolates of this species each isolated from a different patient. The bacteria isolates showed high susceptibility to gentamicin, ciprofloxacin, chloramphenicol, amoxicillin-clavunic acid, ceftazidime and co-trimoxazole (Figure 13). The isolates were relatively susceptible to ampicillin-cloxacillin and showed intermediate susceptibility to erythromycin, nitrofurantoin, cefuroxime and tetracycline. The isolates showed high resistance to nalidixic acid.

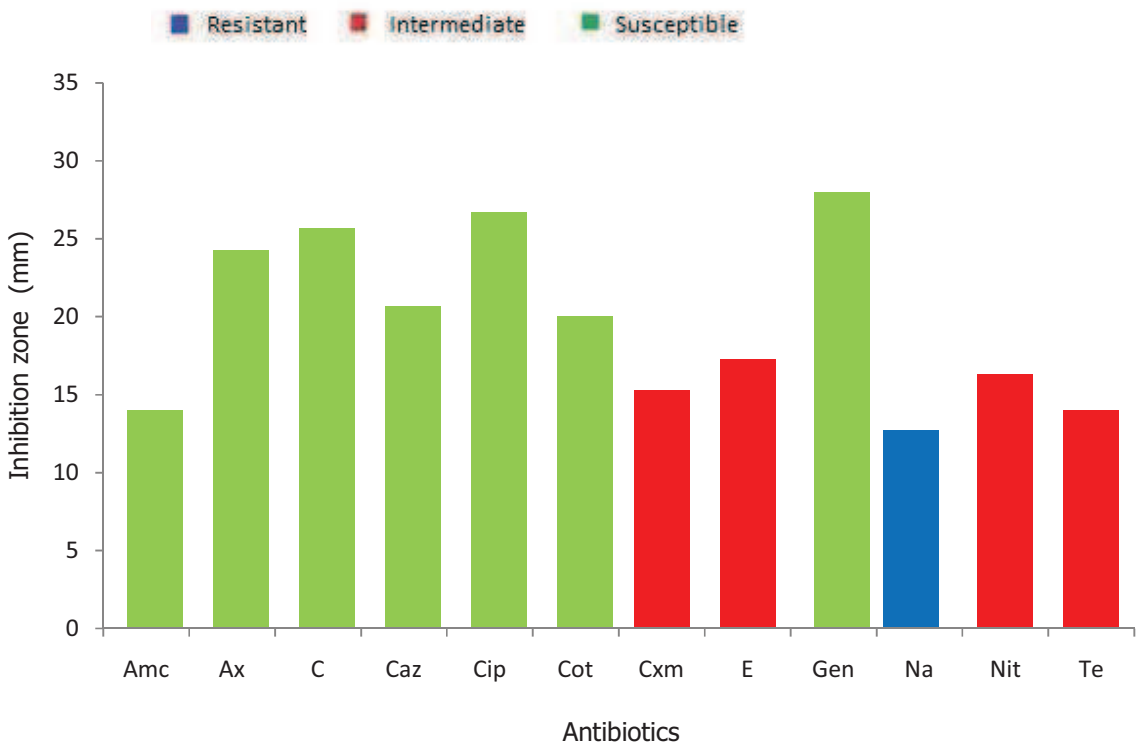

Figure 13: Susceptibility pattern of *Staphylococcus aureus* to antibiotics commonly used in Alupe, Kenya.

Key: Amc: Ampicillin-Cloxacillin, Ax: Amoxyllin-Clavunic Acid, C: Chloramphenicol, Caz: Ceftazidime, Cip: Ciprofloxacin, Cot: Co-trimoxazole, Cxm: Cefuroxime, E: Erythromycin, Gen: Gentamicin, Na: Nalidixic Acid, Nit: Nitrofurantoin, Te: Tetracycline.

4.3.2.3 *Escherichia coli*

Two isolates of *E. coli* were isolated from different patients. The isolates were highly resistant to ampicillin-cloxacillin, cefuroxime and erythromycin (Figure 14). The species were highly susceptible to ciprofloxacin, chloramphenicol, gentamicin, tetracycline and ceftazidime and showed intermediate susceptibility to amoxicillin-clavunic acid and nalidixic acid. They were relatively susceptible to nitrofurantoin and co-trimoxazole.

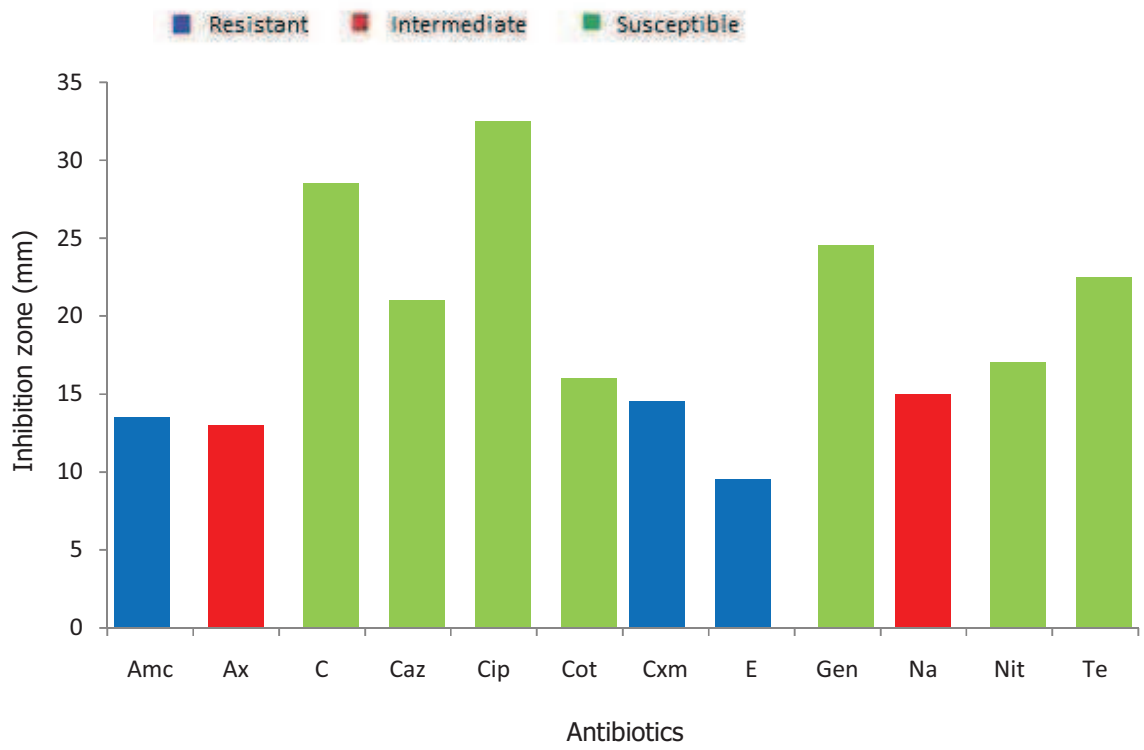

Figure 14: Susceptibility pattern of *Escherichia coli* to antibiotics commonly used in Alupe, Kenya.

Key: Amc: Ampicillin-Cloxacillin, Ax: Amoxyllin-Clavunic Acid, C: Chloramphenicol, Caz: Ceftazidime, Cip: Ciprofloxacin, Cot: Co-trimoxazole, Cxm: Cefuroxime, E: Erythromycin, Gen: Gentamicin, Na: Nalidixic Acid, Nit: Nitrofurantoin, Te: Tetracycline.

4.3.2.4 *Klebsiella pneumoniae*

Klebsiella pneumoniae isolate showed high resistance to ampicillin-cloxacillin, amoxyllin-clavunic acid, ceftazidime, co-trimoxazole, cefuroxime, erythromycin and nalidixic acid (Figure 15). The isolate showed high susceptibility to chloramphenicol, nitrofurantoin and gentamicin while intermediate susceptibility to ciprofloxacin and tetracycline was observed.

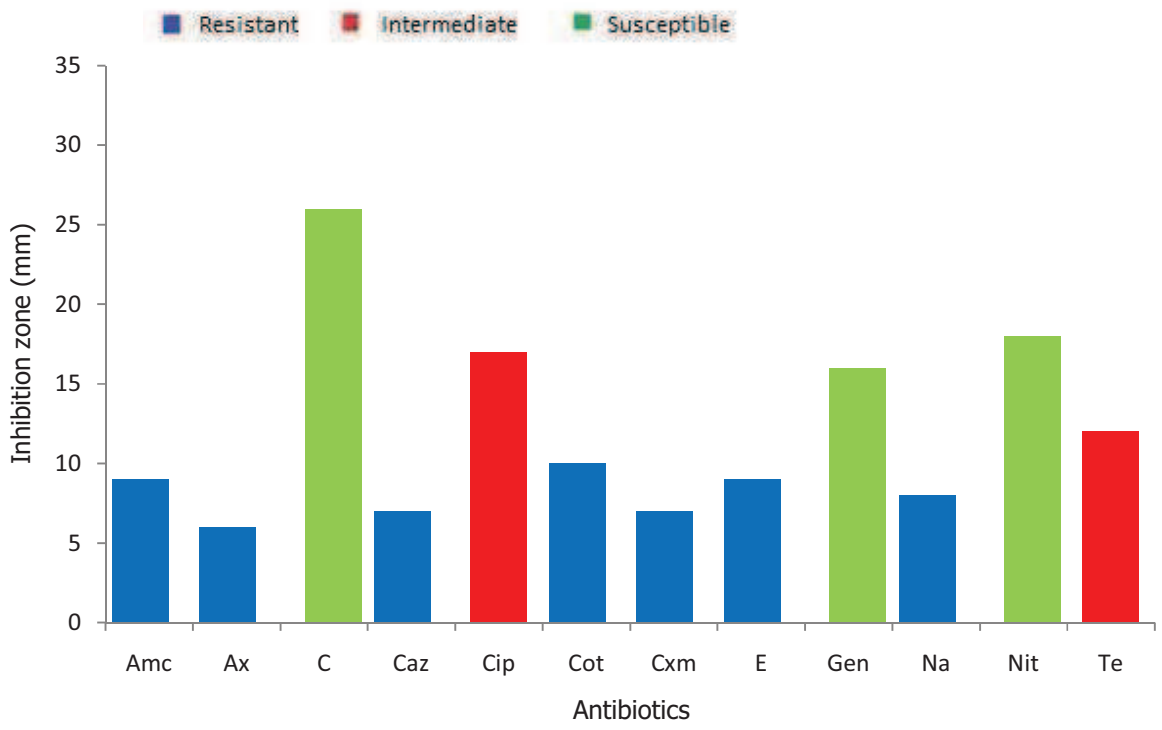

Figure 15: Susceptibility pattern of *Klebsiella pneumoniae* to antibiotics commonly used in Alupe, Kenya.

Key: Amc: Ampicillin-Cloxacillin, Ax: Amoxyllin-Clavunic Acid, C: Chloramphenicol, Caz: Ceftazidime, Cip: Ciprofloxacin, Cot: Co-trimoxazole, Cxm: Cefuroxime, E: Erythromycin, Gen: Gentamicin, Na: Nalidixic Acid, Nit: Nitrofurantoin, Te: Tetracycline.

4.4 Risk factors for bacteremia

The odds of bacteremia were increased in patients who had a heart murmur (Odds ratio, OR = 15.9, p = 0.0047), rash (OR = 6.6, p = 0.0162), severe headache (OR = 4.8, p = 0.0305) and swollen lymph nodes (OR = 28.2, p = 0.0000). The odds of bacteremia were significantly lower ($p \leq 0.05$) in patients who had mild headache (OR = 0.2, p = 0.0338). Bacteremia was not significantly ($p < 0.05$) related to gender, occupation, general condition, pallor, jaundice, dehydration, oedema, vomiting, dryness of skin, body weakness, chills, abscesses, myalgia, nausea, joint pains, dry cough, productive cough, bronchial breathing, crepitations or rhonchi,

rapid breathing, chest pains, retention, palpable masses, abdominal pain, diarrhea, constipation, dizziness, antibiotics taken prior to the hospital visit, analgesics taken prior to the hospital visit or other drugs taken prior to the hospital visit (Table 14).

Table 14: Various variables observed during the study versus bacteremia.

Variable	Odds ratio	Confidence interval (95%)	p value
Gender	3.9	0.45 - 33.51	0.18
General condition (good)	1.52	0.29 - 8.08	0.62
General condition (fair)	0	.	0.31
General condition (sickly)	1.21	0.23 - 6.46	0.83
Pallor	0	.	0.52
Jaundice	0	.	0.79
Dehydration	0	.	0.61
Oedema	0	.	0.52
Chills	4.53	0.53 - 39.01	0.13
Vomiting	0	.	0.34
*Rash	6.62	1.12 - 39.23	0.02
Dryness of the skin	0	.	0.52
Body weakness	1.37	0.16 - 11.97	0.78
Abscesses	0	.	0.58
Myalgia	2.91	0.34 - 24.94	0.31
Nausea	0.94	0.11 - 8.16	0.96
*Enlarged lymph nodes	28.2	4.28 - 185.64	0.00
Joint pains	0	.	0.50
Dry cough	0	.	0.31
Productive cough	3.85	0.41 -36.45	0.21
Crept/rhonchi	0	.	0.71
Bronchial breathing	5.19	0.53 - 51.18	0.11
Rapid breathing	3.85	0.41 - 36.45	0.20
Chest pains	6.27	0.62 - 63.77	0.08
Retention	0	.	0.58
Palpable masses	0	.	0.56
Abdominal pain	0.41	0.09 - 1.89	0.24
Diarrhea	0	.	0.21
Constipation	0	.	0.40
*Mild headache	0.19	0.04 - 1.05	0.03
*Severe headache	4.82	1.00 - 23.28	0.03
Dizziness	2.13	0.24 - 19.11	0.49
*Heart murmurs	15.92	1.19 - 212.85	0.00
Antibiotics prior to hospital visit	0	.	0.85
Analgesics prior to hospital visit	0	.	0.23
Others prior to hospital visit	0	.	0.54

* Significant values

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Bacteremia continues to be a health burden in most developing countries. This is mainly attributed to poor diagnosis since bacteremia presents with signs and symptoms similar to those of other common febrile illnesses (Bhatia *et al*., 2003). Blood culture which is the standard method used for diagnosis of bacteremia was done aseptically to detect presence of bacteria in blood of patients with febrile illness in two health facilities in Alupe. Screening of malaria parasites was also done to all patients to eliminate malaria as the primary cause of fever as the study area is endemic to malaria (Olipher, 2011).

Bacteremia was not common in febrile patients visiting KEMRI CIPDCR Clinic and Alupe District Hospital in Alupe, Western Kenya. No other known bacteremia studies have been done in Kenya on both adult and children population. According to a study conducted in Kilifi, Kenya, 103 of 308 deaths in children with bacteremia occurred on the day of admission, 79 occurred the next day and 35 occurred on the third day (Berkley *et al*., 2005).

Bacteria were most likely to be isolated from patients with a heart murmur, a rash, severe headache or swollen lymph nodes. It is possible that these symptoms represent general clinical dispensation due to other unknown underlying health factors such as HIV, malnutrition, sickle cell disease (SCD), respiratory distress and anaemia (Were *et al*., 2011) hence development of bacteremia. These factors have been found to be associated with bacteremia in studies by Berkley *et al*. (2005), Lin *et al*. (2010) and Fowler *et al*. (2012). These conditions would make the patients more vulnerable to bacterial invasion. Other specific underlying factors and determinants of community

acquired bacteremia found in various documented studies are diseases such as diabetes mellitus and liver cirrhosis. Other conditions include alcoholism, intravenous drug users, use of corticosteroids among others (Naber, 2009; Lin *et al*., 2010).

The rate of bacteria isolation from the blood culture of patients in the current study was relatively low (3.5%) compared to a previous study carried out in Kilifi, Kenya where the isolation rate of bacteria was 6.6% (Berkley *et al*., 2005). The difference in isolation rate of bacteria between the two studies could be attributed to the fact that the study in Kilifi only involved children, a total of 19,339 inpatients which included infants under a year old where, out of a total of 1783 infants who were under 60 days old, 228 had bacteremia (12.8%) as did 866 of 14,787 children who were between 60 days of age and 5 years (5.9%). The high isolation rate (12.8%) of bacteria in infants in the study by Berkley *et al*. (2005) may have been due to an immature and/or underdeveloped immune system as children, especially infants, generally show lower resistance to disease than adults (Givon-Lavi *et al*., 1999).

Seasonal variability could also have played a role as the current study was carried out during a dry season in Alupe hence reduced respiratory viral infections such as common cold which compromise the patients' immune system to secondary bacterial infections (Bennett, 2012). Delayed diagnosis of fever could also have influenced the low yield of the blood cultures. Essentially, blood cultures produce best results when performed in the first week of infection by bacteria (Chamberlain, 2004). This requires early presentation of the patient to the hospital and keen diagnosis by the clinician.

In Alupe, there were delayed hospital visits by patients until the disease had progressed. Additionally, most of the patients were peasant farmers, who may not afford the cost of treatment

in hospital and only visited the hospital when there was severe disease progression. It was also observed that most patients had several pre-treatment options usually with available herbal medicine, local concoctions and analgesics with the hope that the infection would clear away. The community used these options to cut cost incurred during hospital visits and this may explain the delayed hospital visits by the study participants. Some of the local herbs like acacia stem bark have bactericidal effects which could in turn affect the blood culture yields since the bactericidal compounds suppress the growth of bacteria (Zaitoun *et al*., 2012).

The higher incidence of bacteremia in the age group 18 months – 9 years may have been related to chronic malnutrition which could have impaired the immune response hence predisposed the children to invasive bacterial infection as children generally show lower resistance to diseases than adults (Givon-Lavi *et al*., 1999). Age and nutritional status of the child have been found to be associated and closely linked with bacteremia in a study by Nielsen *et al*. (2012). The age group consisting of neonates and younger infants (below one year) where most bacteremia, septicemia and fever of unknown origin (FUO) occur was also not included in the study hence the rates of bacteremia could be higher than what this study recorded.

There was no evidence of variation in the overall prevalence of bacteremia according to age of patients. This was similar to a study on bacteremia on 13,043 patients in the USA where the patients' age was not an independent risk factor for bacteremia (Metersky *et al*., 2004). The findings of the current study however differed with previous studies on some aspects. A study carried out in Nigeria recorded high infant and under-five years' mortality rates of 97 and 189 per 1000, respectively due to community-acquired bacteremia (Obaro *et al*., 2011). Findings of a study carried out in Kilifi, Kenya showed that community-acquired bacteremia was responsible for at least one third of deaths in infants and one quarter of deaths in children over one year of age

(Berkley *et al*., 2005). The study also found that 4.9% of hospital deaths in children between the ages of 2 months and 5 years were attributable to *Haemophilus influenza* bacteremia, and 8.7% to *Streptococcus pneumoniae* bacteremia (Berkley *et al*., 2005). The difference in age being an independent risk factor of bacteremia among the studies could be attributed to the fact that the studies in Kilifi and Nigeria both involved children, including neonates, as the study population (under 10 years for Kilifi study and under 15 years for Nigeria study) where infants below one year old recorded high bacteremia rates. Children, especially infants below one year have an immature and/or underdeveloped immune system and generally show lower resistance to disease than adults and this has been associated with clinical malnutrition as one of the predisposing factors (Givon-Lavi *et al*., 1999; Berkley *et al*., 2005; Nielsen *et al*., 2012).

The origin of infection of the bacteria suggests that bacteremia associated with *S. aureus* from patient's ID CIP/87 and *K. pneumoniae* was respiratory due to the chest complications observed; *S. aureus* from patient's ID CIP/80 was cardiovascular due to heart murmurs experienced; *P. mirabilis*, *E. coli* from patient's ID CIP/52 and *E. coli* from patient's ID CIP/122 could either have been intra-abdominal or from the urinary tract. *Proteus mirabilis* is almost always a urinary tract infection, UTI (Torre, 2008). *Escherichia coli* having been isolated from female patients could have been a UTI since it is a common cause of these infections especially in women (Torre, 2008). It could also have been intra-abdominal due to the abdominal discomforts experienced by the patient.

The likely focus of infection of *S. aureus* from patient's ID CIP/34 could have been the respiratory tract. The primary infectious source of most *S. aureus* is the lower respiratory tract with endocarditis being the secondary source (Torre, 2008). The role of underlying conditions such as SCD and HIV was evaluated only in patients who had an established diagnosis hence it was not

possible to determine the role of these conditions in promoting the risk for specific bacteremia. Adults or children with functional or anatomic asplenia, particularly those with sickle cell disease, and children with HIV infection are at high risk for invasive bacterial diseases, with rates in some studies more than 50 times higher than those among children of the same age without these conditions (Winn *et al*., 2006; CDC, 2012). Additional investigations have established that HIV and SCD were important predictors of bacteremia in children residing in areas where *Plasmodium falciparum* is endemic (Berkley *et al*., 2005; Williams *et al*., 2009; Were *et al*., 2011).

A large proportion of febrile patients in Alupe which is endemic to malaria are assigned a clinical diagnosis of malaria (Olipher, 2011). Therefore, most patients who present with an acute febrile illness are treated empirically for malaria. However, it is of concern that poor diagnosis continues to hinder effective malaria and bacteremia treatment in the tropics. It is important therefore to state that erroneous diagnosis leads to mismanagement of the patient resulting in major morbidity and mortality (Uneke, 2008). This leads to unnecessary expenditure and exposure of patients to the side-effects of anti-malarials. In addition, misdiagnosis often results in delayed diagnosis and treatment of other acute febrile illnesses such as bacteremia leading to disease progression (Nsutebu *et al*., 2002; Uneke, 2008).

Staphylococcus aureus was the dominant bacteria species isolated from blood cultures sampled from febrile patients. Dominance of *S. aureus* species could have been due to environmental factors like seasonal variation, antibiotic usage or patient selection. This may also in part be due to the high occurrence of skin conditions and diseases, both fungal and bacterial in the settlements and areas around Alupe which in general had high incidence of wounds reported in the health centres during the study. The results of the current study concurred with those of a recent bacteremia study done at Siaya District Hospital in Western Kenya where the most common

isolate in children was *S. aureus* (Were *et al*., 2011). The results of the current study were also consistent with those of another study which showed that *S. aureus* was the most frequent cause of community-acquired bacteremia caused by Gram-positive organisms in infants and young children in Nigeria and Mozambique, respectively (Johnson *et al*., 2008; Sigauque *et al*., 2009).

Bacteremia caused by Gram-negative bacteria had overall prevalence from the blood cultures sampled. This observation was similar to others reported elsewhere in Africa especially in children which describe a preponderance of Gram-negative bacteria (Gwee *et al*., 2012). *Escherichia coli* was the dominant Gram-negative species isolated in the current study. This finding differed with previous findings from other areas in sub-Saharan Africa where malaria is endemic, which showed that nontyphoidal *Salmonella* sp. (NTS) was the most common invasive Gram-negative bacteria (Nadjm *et al*., 2010; Were *et al*., 2011; Nielsen *et al*., 2012). Contrary to the dominance of *S. aureus* in the current study, NTS was the most common invasive Gram-negative bacteria in a previous study conducted in Kilifi District, Kenya, an area of malaria transmission hyperendemicity (Were *et al*., 2011). However the current findings were similar to a study done in a tertiary hospital in Nigeria where *E. coli* dominated Gram-negative bacteria isolation (Nwadioha, 2010). Predominance of either Gram-positive or Gram-negative bacteria could be influenced by geographical location (Nwadioha, 2010).

Isolation of *P. mirabilis*, *E. coli* and *K. pneumoniae* from febrile patients in the current study was in agreement with studies done in Tanzania (Moyo *et al.,* 2010; Crump *et al.,* 2011) and Kenya (Berkley *et al*., 2005; Were *et al*., 2011), where the three bacteria pathogens have been shown to cause bacteremia. The findings of this study show the importance of Gram-negative bacteria in causing bacteremia, similar to the findings of Kang *et al.,* (2005) who reported increasing incidences of blood stream infections as a result of antibiotic resistance Gram-negative bacilli.

Blood cultures were done using commercially prepared media, incubated at the recommended temperature and routinely observed for bacteria growth. The media used was also stored under the right conditions as recommended by the manufacturer. Before the study began, a run of quality control check was performed on all the media used. The media used during the study supported growth of the control strains, namely *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* serovar Typhi ATCC 19430, *Salmonella enterica* serovar Typhimurium ATCC 14028 and *Escherichia coli* ATCC 25922. The quality control checks indicated that there were no contaminants in the media which could have inhibited growth of bacteria present in blood samples. Therefore the low yield of blood cultures could not be attributed to technical laboratory errors during media preparation. However, it is possible that robust organisms such as *E. coli* and *S. aureus* were more likely to be isolated than the fastidious ones. To address this possibility, culture techniques were consistent across groups to avoid biased comparisons and growth between groups.

Bacteria isolated from the blood of febrile patients in Alupe were resistant to erythromycin, cefuroxime and ampicillin-cloxacillin. The high resistance to the above antibiotics could be attributed to selective pressure generated by overuse of the drugs in the study location. This observation is similar to the findings of a study done in Nigeria on community acquired bacteremia in young children from where bacteria isolated were resistant to cefuroxime (Obaro *et al*., 2011). The isolates showed moderate resistance to tetracycline which is commonly used in adults, findings which concur with those of a study done in Tanzania to establish the causative agents of blood stream infections and their antimicrobial susceptibility. In the study, resistance to tetracycline was reported to be 63.8% (Moyo *et al*., 2010). Antibiotics with relative susceptibilities and were useful in clearing bacterial infections in the study area included amoxicillin-clavunic

acid, ceftazidime and nitrofurantoin. It is highly unlikely that the resistance patterns observed could have been as a result of self medication with antibiotics. From the results, it was evident that the communities self medicate with anti-malarial drugs which is in part useful as it serves to reduce the widespread multi-drug resistance to antibiotics (Olipher, 2011).

All bacteria isolated from the blood of febrile patients in Alupe were highly susceptible to chloramphenicol, gentamicin and ciprofloxacin. The findings concur with results from a study carried out in a tertiary hospital in Nigeria where ciprofloxacin and gentamicin were found to be effective to the isolates (*E. coli*, *S.aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. pneumoniae* and *H. influenzae*) obtained (Nwadioha, 2010). Chloramphenicol, a prototypical broad-spectrum antibiotic also known as chlornitromycin, is effective against a wide variety of Gram-positive (including most strains of MRSA) and Gram-negative bacteria, including most anaerobic organisms (Falagas *et al*., 2008). Due to resistance and safety concerns, it is no longer a first-line agent for any infection in developed nations, although it is sometimes used topically for eye infections. Nevertheless, the global problem of advancing bacterial resistance to newer drugs has led to renewed interest in its use (Falagas *et al*., 2008).

Gentamicin, a relatively cheap and easily available antibiotic is routinely used synergistically with a beta-lactam antibiotic or vancomycin for empirical therapy and is used to particularly treat bacterial infections caused by Gram-negative organisms (Falagas *et al*., 2008). Ciprofloxacin is administered intravenously, orally, or intravenously and followed by the oral route is effective therapy in the treatment of severe bacteremic infections (Bouza *et al*., 1989). Ciprofloxacin is not routinely recommended for pediatric use except in special cases where the benefits out–weigh the short term risks of joint toxicity, such as in cystic fibrosis (Feigin, 2003).

Based on the findings of this study, chloramphenicol should be used as a drug of choice for empirical treatment of bacteremia in patients in Alupe, Western Kenya. It was the most effective against all the bacterial isolates tested in the study. Gentamicin came second in effectiveness while ciprofloxacin was the third most effective. However, ciprofloxacin is more toxic than chloramphenicol and gentamicin and has adverse side effects such as irreversible liver failure, peripheral neuropathy (irreversible nerve damage), tendon rupture, pseudomembranous colitis, heart problems, rhabdomyolysis (muscle breakdown) among others and hence is not suitable as a first line agent for bacteremia (Aronson, 2012). In the absence of chloramphenicol, gentamicin should be the second option.

5.2 Conclusion

In conclusion, bacteremia was found to be uncommon among febrile patients visiting Alupe District Hospital and KEMRI CIPDCR Clinic in Alupe, Western Kenya. The rate of bacteremia among the recruited patients was 3.5%. *Staphylococcus aureus* was the predominant bacterial isolate responsible for bacteremia in febrile patients in Alupe, Western Kenya with *Escherichia coli* as the second highest.

The study also showed that bacteremia was positively correlated with heart murmur, rash, severe headache and swollen lymph nodes. Bacteremia was however not positively correlated to age, gender, occupation or the other observed symptoms.

This study demonstrated the public health significance of etiologic diagnosis of acute febrile illness in a setting where clinical malaria is over-diagnosed, non-prescription antibiotics are available and empirical prescription of antibiotics by physicians is the standard clinical practice. The findings of this study from a region of holoendemic *P. falciparum* transmission demonstrate

that bacteremia which shows similar symptoms to malaria parasitemia, could be the cause of febrile illnesses.

The data generated on susceptibility of bacteria isolates to commonly used antibiotics in Alupe, Western Kenya should guide antibiotic use in the management of bacteremia in a locality where malaria is endemic. If bacteremia is suspected on admission or at any later stage of management of patients, initial antibiotic therapy with chloramphenicol should be undertaken. In the absence of chloramphenicol, gentamicin should be the second option. Empirical treatment with antibiotics is recommended only in life threatening cases.

5.3 Recommendations

1. The etiologic agents and their susceptibility patterns to commonly used antibiotics observed in this study raise concerns about the appropriateness of the current treatment offered at the two health centres studied where ampicillin, erythromycin and cefuroxime which have shown resistance are still used as the first line treatment for most bacterial conditions. This requires revision given the high prevalence of resistance of the isolates to these antibiotics, hence, chloramphenicol and gentamicin should be considered as a first choice of reliable antibiotics for empirical treatment of bacteremia in Alupe and its environs.

2. Improved clinical microbiology services and reassessment of empirical treatment guidelines that account for the epidemiology of bloodstream infections might contribute to better outcomes.
3. KEMRI-CIPDCR clinic is the only health centre that offers laboratory blood culture services in Busia and Teso districts. Additional laboratory services in various medical hospitals and heath

centres should be provided for identification of bacteria in blood. Providing the diagnostic service will improve the quality of care and improve clinical outcome.

5.4 Further studies

There is a need to intensify surveillance of bacteremia both at rural and urban settlements and to expand population surveillance to include neonates and infants below two years. Such surveillance activities will be critical in informing the need for introduction of specific bacterial vaccines in the medium term and improvement of health care infrastructure in the long term. This expanded surveillance should also provide additional data on the disease burden caused by each bacteria isolate. It is also necessary to determine if the observed pattern of infection in the current study is similar during other seasons in Alupe in other geographic locations in the country.

Co-infection of bacteremia with viral diseases and malaria in this region of holoendemic *P. falciparum* transmission should also be investigated further.

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APPENDICES

APPENDIX I

KENYA MEDICAL RESEARCH INSTITUTE (KEMRI)

INFORMED CONSENT FORM (BACTEREMIA STUDY)

AGE≥16 YEARS

Scientists from the Kenya Medical Research Institute (KEMRI) are conducting a study to find out the bacterial pathogens found in blood in Alupe. In order to do this, we are asking you to give a small sample of your blood (10 mls) which will be used for the analysis. The results will be availed to your doctor and will be used in the management of your illness. The process of sample collection is harmless though mild pain might be experienced during blood collection. You are free to choose to participate and withdraw at anytime. Your refusal in the participation of the study or withdrawal from this study at any stage will not affect the quality of health care given to you. In case of complaints from this study, you are free to seek advice from Director CIPDCR, KEMRI-Busia (Tel 055-22232).

DECLARATION

I understand that the study is investigating the above mentioned phenomenon and will lead to the identification and characterization of the bacterial pathogens found in blood for diagnosis and management of bacterial diseases. I am being voluntarily asked to participate. My participation will involve taking samples of 10 mls of my blood. I understand sample collection is harmless and being conducted by qualified personnel. I further understand that my participation in this study is voluntary and I may withdraw if and when I choose to anytime unconditionally.

I have read and understood the information stated above.

I sign this consent form willingly:

Name of the patient…….Sign……Date…….

Witness……Date…….

Name of officer/s……Sign……Date……

NB: Translation to local languages will be done if need be by field workers.

APPENDIX II

TAASISI YA UTAFITI WA UTABIBU YA KENYA

FOMU YA KUPATA KIBALI (UTAFITI WA MAGONJWA YA BAKTERIA)

UMRI ≥ MIAKA 16

Wanasayansi kutoka Kenya Medical Research Institute (KEMRI) wanafanya utafiti ili kujua bakteria zinazopatikana kwa damu. Ili kufanya hivyo, sisi tunachukuwa sampuli ndogo ya damu yako (10 mls), ambayo itatumika kwa ajili ya uchambuzi. Matokeo itapewa daktari wako na itatumika katika usimamizi wa ugonjwa wako. Mchakato wa kukusanya sampuli ni wa upole ingawa unaweza hisi maumivu kidogo wakati wa ukusanyaji wa damu. Wewe uko huru kuchagua kushiriki na kuondoka wakati wowote. Kukataa kushiriki au kujiondoa kutoka utafiti huu katika hatua yoyote, hakutaaathiri huduma za afya utakayopewa. Iwapo utakuwa na malalamiko kutoka kwa utafiti huu, uko huru kutafuta ushauri kutoka kwa Mkurugenzi CIPDCR, KEMRI-Busia (Tel 055-22,232)

TANGAZO

Ninaelewa ya kwamba, utafiti huu unahusu kuchunguza mambo yaliyotajwa hapo juu, na utasababisha kitambulisho na tabia ya vijidudu vya magonjwa vya bakteria vinavyopatikana katika damu kwa ajili ya utambuzi na usimamizi wa magonjwa ya bakteria. Ushiriki wangu utahusisha kuchukua sampuli ya 10 mls ya damu yangu. Naelewa ukusanyaji ni wa upole na unafanywa na wafanyakazi wenye sifa. Pia naelewa kuwa ushiriki wa utafiti huu ni kwa hiari yangu na naweza kuondoka wakati wowote bila masharti. Nimesoma na kuelewa habari ilivyoelezwa hapo juu.

Mimi nimeweka sahihi kwa fomu hii kwa hiari yangu:

Jina la mgonjwa ... ... Sign ... ... Tarehe ... ...

Shahidi ... ... Tarehe ... ...

Jina la afisa / s ... ... Sign ... ... Tarehe ... ...

NB: Tafsiri kwa lugha za kienyeji itafanyika kama kuna haja ya kuwa na wafanyakazi wa shamba.

APPENDIX III

KENYA MEDICAL RESEARCH INSTITUTE (KEMRI)

INFORMED CONSENT FORM (BACTEREMIA STUDY)

AGE<16 YEARS

Scientists from the Kenya Medical Research Institute (KEMRI) are conducting a study to find out the bacterial pathogens found in blood. In order to do this, we are asking for your consent to allow your child to give a small sample (5 mls) of his/her blood, which will be used for analysis. The results will be availed to his/her doctor and will be used in the management of his/her illness. The process of sample collection is harmless though mild pain might be experienced during blood collection. You are free to allow for your child to participate and withdraw at any time. Your refusal for your child to participate in this study at any stage will not affect the quality of health care given to him/her. In case of complaints from this study, you are free to seek advice from Director CIPDCR, KEMRI-Busia (Tel 055-22232).

DECLARATION

I understand that the study is investigating the above mentioned phenomenon and will lead to the identification and characterization of the bacterial pathogens found in blood for diagnosis and management of bacterial diseases. I am being voluntarily asked to allow my child to participate. My child's participation will involve taking samples of his/her 5 mls of blood. I understand sample collection is harmless and being conducted by qualified personnel. I further understand that my child's participation in this study is voluntary and I may allow his /her withdrawal if and when I choose to anytime unconditionally.

I have read and understood the information stated above.

I sign this consent form willingly:

Name of the parent/ Guardian…….Sign……Date…….

Witness……Date…….

Name of officer/s……Sign……Date……

NB: Translation to local languages will be done if need be by field workers.

APPENDIX IV

TAASISI YA UTAFITI WA UTABIBU YA KENYA

FOMU YA KUPATA KIBALI (UTAFITI WA MAGONJWA YA BAKTERIA)

UMRI <MIAKA 16

Wanasayansi kutoka Kenya Medical Research Institute (KEMRI) wanafanya utafiti ili kujua bakteria zinazopatikana kwa damu. Ili kufanya hivyo, sisi tunachukuwa sampuli ndogo ya damu yako (10 mls), ambayo itatumika kwa ajili ya uchambuzi. Matokeo itapewa daktari wako na itatumika katika usimamizi wa ugonjwa wako. Mchakato wa kukusanya sampuli ni wa upole ingawa unaweza hisi maumivu kidogo wakati wa ukusanyaji wa damu. Wewe uko huru kuchagua kushiriki kwa mtoto wako na kumwondosha wakati wowote. Kukataa kushiriki kwa mtoto wako au kumwondoa kutoka utafiti huu katika hatua yoyote, hakutaaathiri huduma za afya atakayopewa. Iwapo utakuwa na malalamiko kutoka kwa utafiti huu, uko huru kutafuta ushauri kutoka kwa Mkurugenzi CIPDCR, KEMRI-Busia (Tel 055-22,232)

TANGAZO

Ninaelewa ya kwamba, utafiti huu unahusu kuchunguza mambo yaliyotajwa hapo juu, na utasababisha kitambulisho na tabia ya vijiduduvyamagonjwavya bakteria vinayopatikana katika damu kwa ajili ya utambuzi na usimamizi wa magonjwa ya bakteria. Ushiriki wangu utahusisha kuchukua sampuli ya 10 mls ya damu yangu. Naelewa ukusanyaji ni wa upole na unafanywa na wafanyakazi wenye sifa. Pia naelewa kuwa ushiriki wa utafiti huu ni kwa hiari yangu na naweza kuondoka wakati wowote bila masharti. Nimesoma na kuelewa habari ilivyoelezwa hapo juu.

Mimi nimeweka sahihi kwa fomu hii kwa hiari yangu:

Jina la mzazi / Guardian ... ... Sign ... ... Tarehe ... ...

Shahidi ... ... Tarehe ... ...

Jina la afisa / s ... ... Sign ... ... Tarehe ... ...

NB: Tafsiri kwa lugha za kienyeji itafanyika kama kuna haja ya kuwa na wafanyakazi wa shamba.

APPENDIX V

KENYA MEDICAL RESEARCH INSTITUTE (KEMRI)

PERSONAL DATA (BACTEREMIA STUDY)

1. Date…….
2. Study site ……

KEMRI ☐ Alupe District Hospital ☐

3. Sex: Male ☐ Female ☐
4. Age: Years Months Days

5. Place of residence
   a) Village……
   b) Sub-location……
   c) Location……..
   d) Division……..
   e) District……

6. Socio-medical data
   a) Occupation (specify)…….
   b) If child, Father`s/Mother`s/Guardian`s occupation…….

APPENDIX VI

KENYA MEDICAL RESEARCH INSTITUTE

CLINICAL EXAMINATION (BACTERIAL DISEASES STUDY)

Date ……

Health facility…….Study No…….

Major Presenting Complaints

1.....

2…….....

3……

4……

5……

General condition Good ☐

Fair ☐

Sickly ☐

Wasted ☐

Observed clinical signs on examination

Vital signs: Temp…….BP……Pulse…….Res……..

Other signs:

		Yes=1 No=2
1	Pallor	
2	Dehydration	
3	Jaundice	
4	Oedema	
5	Chills	
6	Vomiting	
7	Rash	
8	Dryness of the skin	
9	Body weakness	
10	Abscesses	
11	Myalgia	
12	Nausea	

13	Enlarged lymph nodes	
14	Joint pains	

Specific systems respiratory

		Yes=1 No=2
1	Dry cough	
2	Productive cough	
3	Crept/Rhonci	
4	Bronchial breathing	
5	Rapid breathing	
6	Chest pains	
7	Any added sound	

Per abdominal examination:

		Yes=1 No=2
1	Retention	
2	Palpable masses: Spleenomegally Hepatomegally	
3	Abdominal pain	
4	Diarrhea	
5	Constipation	
6	Other abdominal disorders	

Central nervous system

		Yes=1 No=2
1	Mild headache	
2	Severe headache	
3	Dizziness	
4	Others	

Cardiovascular system

		Yes=1 No=2
1	Heart murmurs	
2	Others	

Drug Management

Drug management prior to this visit (if any)

		Yes=1 No=2	Specify
1	Antibiotics		
2	Analgesics		
3	Other		

Drug management offered during this visit (if any)

		Yes=1 No=2	Specify
1	Antibiotics		
2	Analgesics		
3	Other		

Examined by…….……Sign…….Date

Appendix VII

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541 , 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: kemri-hq@nairobi.mimcom.net; director @ kemri. org; website: www.kemri.org

ESACIPAC/SSC/4917 **10th September, 2009**

Matilu Mwau

Thro'
Director, CIPDCR
P.O. Box 3
BUSIA (K)

REF: SSC No.1698 (New) – Novel approaches to the diagnosis, characterization and surveillance of priority infectious diseases.PI:Matilu Mwau (CIPDCR).

I am pleased to inform you that the above mentioned proposal, in which you are the PI, was discussed by the KEMRI Scientific Steering Committee (SSC), during its **160th meeting** held on **Tuesday 8th September, 2009** and has since been approved for implementation by the SSC.

The SSC however, advises that work on this project can only start when ERC approval is received.

DR. C. S. MWANDAWIRO

C. Mwandawiro, PhD
SSC SECRETARY

In Search of Better Health

Appendix VIII

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI, Kenya
Tel: (254) (020) 2722541, 2715349, 0722-205901, 0733-400005; Fax: (254) (020) 2720030
E-mail: kemri-hq@nairobi.mimcom.net; director @ kemri. org; website: www.kemri.org

KEMRI/RES/7/3/1 **October 15, 2009**

TO: **DR. MATILU MWAU,**
THE DIRECTOR, CIPDCR
BUSIA
EMAIL: Matilu.mwau@gmail.com, mmwau@kemri.org

RE: **SSC PROTOCOL NO. 1698 (*INITIAL SUBMISSION*): NOVEL APPROACHES TO THE DIAGNOSIS, CHARACTERIZATION AND SURVEILLANCE OF PRIORITY INFECTIOUS DISEASES.**

This is to inform you that during the 171st meeting of KEMRI/National Ethics Review Committee held on Tuesday 13th October 2009, the abovementioned study was reviewed.

The Committee commends you for this timely and useful study/program that will enable the capacity building of the Centre for Infectious and Parasitic Diseases Control and Research and wishes you the best in your endeavor.

Due consideration has been given to ethical issues and the study is hereby granted approval for implementation effective this **15th day of October 2009,** for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on **Thursday, 14th October 2010.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **Thursday, 2nd September 2010**.

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the ERC prior to initiation. You may embark on the study.

Yours sincerely,

R.C. Kithinji

R. C. KITHINJI,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

In Search of Better Health