

**ETIOLOGY OF DIARRHEA IN CHILDREN UNDER
5 YRS IN MBAGATHI DISTRICT HOSPITAL,
NAIROBI PROVINCE //**

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I57/11040/04**

DEPARTMENT OF PUBLIC HEALTH

**A RESEARCH THESIS SUBMITTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE
AWARD OF THE DEGREE OF MASTER OF PUBLIC
HEALTH IN THE SCHOOL OF HEALTH SCIENCES
OF KENYATTA UNIVERSITY**

MARCH 2009

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*Etiology of diarrhea
in children under 5*



2009/339428

DECLARATION

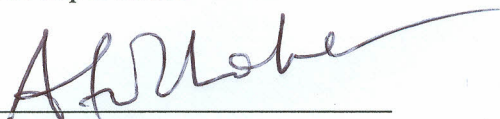
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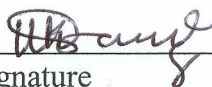
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DEDICATION

This work is dedicated to promotion of health through surveillance of infectious diseases in developing countries.

ACKNOWLEDGMENT

I acknowledge my supervisors, Dr. Augustine Afullo, Dr. Margaret Keraka and Dr. Willie Sang for their guidance, advise and great support in the whole duration of this course. Many thanks to the GEIS Program, Walter Reed Project-KEMRI for all the support in the successful completion of this graduate program, this achievement would not have been possible without GEIS. I also appreciate the support from the Enterics Laboratory –WRP GEIS especially for all the laboratory work without which my study would not have been possible. Special thanks to Bonventure Juma for his assistance with microbiology aspects of the study.

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Abstract

Diarrhea is a major public health problem with adverse effects on children's health. After acute respiratory illnesses, pediatric diarrhea is the most important cause of disability-adjusted life years lost; it has the single greatest adverse effect on children's growth and development. Epidemiology of diarrheal illness in many areas remains poorly understood. Two hundred and forty six children who presented to Mbagathi district hospital in the months May-Aug 2007 were randomly selected and screened for bacterial agents causing diarrhea. Descriptive cross sectional survey design was used for this study. Main objective of study was to identify and characterize bacterial causes of diarrhea among children under 5years. Identification was done through stool culture, biochemical tests, serotyping and multiplex PCR. Demographic data were collected using a standardized questionnaire. Physical examination and clinical symptoms in patients were assessed to determine association with diarrheal illness. Main risk factors associated with diarrhea in children less than 5 years are: area of residence and water sources . Data management was by MS-Access(2003) and data analysis was done using STATA (StataCorp. 2005. *Stata Statistical Software: Release 9*. College Station, TX: StataCorp LP version 8.0) software. Dependent variable was bacterial pathogen and independent variables were: area of residence and water sources of study households. Seventy six patients (93.83%) harbored *E.coli*, 3 (3.7%) had *Salmonella* and 2 (2.46%) were positive for *Shigella*. *E.coli* was found to be the most common bacterial pathogen associated with acute diarrhea among children below 5 years of age. *E.coli* being the most isolated bacteria Chi-square tests of associations with the different areas of residence gave the following p-values: Kibera p=0.415, South B p=0.478, Kawangware p=0.209. Diarrheal illnesses in the hospital during the study period ranked third among the most common diseases. Chi-square tests of association for well as water source gave p value of 0.74 and a cross tabulation of municipal water source and isolated bacteria pathogen did not prove any interaction. Prevalence of acute diarrhea among children less than 5 years of age was 20%. There is need to create awareness on maintenance of hygiene among mothers/caretakers of children less than 5 years to control occurrence of *E.coli* infections. It is important for MoH to conduct continuous surveillance and reporting of diarrheal pathogens to promote better prevention and therapeutic measures for diarrheal illnesses among children under 5 years.

LIST OF ACRONYMS AND ABBREVIATIONS

APW	Alkaline Peptone Water
CDC	Centre for Disease Control
CFA	Colonization Factor Antigens
CT	Cholera Toxin
DNA	Deoxyribonucleic Acid
EAGG/EAEC	Enteroggregative <i>Escherichia coli</i>
EC	<i>Escherichia coli</i>
EHEC	Enterohemorrhagic <i>Escherichia coli</i>
EINV/EIEC	Enteroinvasive <i>Escherichia coli</i>
EPEC	Enteropathogenic <i>Escherichia coli</i>
ETEC	Enterotoxigenic <i>Escherichia coli</i>
GEIS	Global Emerging Infections and Response System
GI	Gastro-intestinal
GMP	Guanosine monophosphate
HIV	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
HUS	Hemolytic Uraemic Syndrome
JICA	Japan International Cooperation Agency
KEMRI	Kenya Medical Research Institute
KOH	Pottasium Hydroxide
LT	Heat-labile toxin
MOH	Ministry of Health
ST	Heat stable toxin
ORT	Oral rehydration therapy
PCR	Polymerase chain reaction
pH	Measure of acidity/alkalinity
SOP	Standard Operating Procedure
<i>Spp</i>	Species
ST	Heat stable toxin
STEC	Shigatoxigenic <i>Escherichia coli</i>
TCBS	Thiosulfate Citrate Bile Salts Sucrose
VP	Voges-Prosakuer (test)
WHO	World Health Organization
WRP	Walter Reed Project
XLD	Xylose Lysine Deoxycholate

CHAPTER 1

1.0 INTRODUCTION

1.1. Background

Worldwide, diarrhea claims several million of lives, mostly those of infants (Field, 2003). Diarrhea accounts for 1.6–2.5 million deaths annually, each child in the developing world experiences an average of three episodes of diarrhea per year. Diarrhea remains one of the principal causes of morbidity and mortality in children (Kosek *et al*, 2003).

More than one billion diarrhea episodes occur every year among children younger than 5 years of age in socioeconomically developing countries causing 2 to 2.5 million deaths (UNICEF, 2004). A wide array of viral, bacterial, and parasitic enteropathogens is currently associated with acute diarrhea. Rotavirus and diarrheagenic *Escherichia coli* are the most common pathogens responsible for acute diarrhea episodes in children; *Shigella*, *Salmonella*, *Campylobacter jejuni/coli*, *Vibrio cholerae*, *Aeromonas*, and *Plesiomonas* occur more commonly in poorer areas and infections caused by protozoa and helminthes occur mainly in areas where environmental sanitation is significantly deteriorated (O'ryan *et al*, 2005).

Acute infectious diarrhea is the second most common cause of death in children living in developing countries, surpassed only by acute respiratory diseases accounting for approximately 20% of all childhood deaths. The major etiologic agents that account for the estimated 1.5 million deaths per year are enterotoxigenic *Escherichia coli* (ETEC), rotavirus, *Vibrio cholerae*, and *Shigella* sp; all are known to be endemic in essentially all

developing countries (Firdausi *et al*, 2005). According to the WHO world report of year 2004 a total of 707,000 deaths due to diarrheal diseases were reported in Africa in 2002 (WHO, 2004).

In Kenya, diarrhea is among the 5 illnesses with high mortality rates among children below 5 years of age. The disease mainly affects children who live in squalid environments and those in rural areas. Environmental conditions favoring the spread of communicable diseases among children in the urban setting include inadequate safe water supply, poor sanitation, inadequate disposal of solid wastes, inadequate drainage of waste water, poor personal and domestic hygiene, unsafe and inadequate housing resulting in overcrowding (MoH Kenya, 2004).

1.2. Problem Statement

Bacterial agents are known to be the leading causes of diarrhea among children. Viral and parasitic causes are present but are not as frequent as bacterial causes (WHO, 2004). In a prevalence study conducted in Egypt 45.6 % of diarrhea was as a result of bacterial infections, 12.2% of cases were of mixed infections, and 34.6% by Rotavirus (Sheikh, 2001). Numerous studies have shown an increasing trend of more virulent diarrhea causing pathogens and antibiotic resistance by these agents including multi-drug resistance; this presents a problem during treatment. In Kenya, there is no recent study done to estimate the prevalence of bacterial diarrhea among children below 5 years of age. The last study done was in rural Western Kenya in 1997-2003 (Brooks *et al*, 2006).

In Mbagathi hospital no study has been done to estimate prevalence of bacterial diarrhea among children.

1.3. Study Purpose

To determine the etiology of diarrhea in children less than 5 years in a hospital serving both low and average income households in an urban setting.

1.4. Objectives

1.4.1 Main Objective

To identify and characterize bacterial causes of diarrhea among children below 5 years of age.

1.4.2 Specific Objectives

1. To determine the prevalence rate of acute diarrhea among children below 5 years in an urban setting.
2. To determine the prevalence of bacterial agents causing diarrhea among children below 5 years of age.
3. Identify risk factors for diarrhea among children.

1.5. Research Questions

1. Which bacterial agents cause diarrhea among children in Mbagathi district hospital?
2. What is the prevalence of diarrheal illnesses among children presenting to Mbagathi district hospital?

1.6. Study Hypothesis

Escherichia coli, *Shigella* and *Campylobacter* are not the most common pathogens responsible for acute diarrhea infections among children below 5 years of age who present to Mbagathi district hospital.

1.7. Study Justification

New and more virulent diarrhea causing agents keep emerging and therefore a clear understanding of the agents that cause diarrheal illnesses in children is needed so as to implement effective preventive and therapeutic measures. Current data on pathogen prevalence are important for development of effective drugs and aid in vaccine development efforts. This study will identify bacterial agents that cause diarrhea among children and the prevalence rate of diarrheal illness in children in an urban setting.

1.8. Study Limitations

This study was limited to examining bacterial causes of diarrhea other non-bacterial causes of diarrhea among children were not studied. Rotaviruses are also known to be common among children. This study was limited in terms of costs of reagents for laboratory diagnosis for rotaviruses. Research studies have shown that bacterial diarrhea presents more challenges to human health in terms of prevention and treatment hence the focus of this study on bacterial causes of diarrhea.

CHAPTER 2

2.0. LITERATURE REVIEW

2.1. EPIDEMIOLOGY OF DIARRHEA IN CHILDREN

Diarrhea is the passage of three or more watery stools in 24 hrs. It is an increase in the number, volume and water content of stools. It is a global cause of much illness and death and a major factor in childhood malnutrition (O'ryan *et al*, 2005). Bacterial enteric infections have a huge impact on human health, particularly among the pediatric population. Despite the expansion of knowledge on the pathogenesis of bacterial enteric infections experienced in the past decade, the number of diarrheal episodes and childhood deaths reported continue to increase in many areas of the world. Specific antimicrobial therapy is indicated for certain confirmed infections, notably shigellosis, enterotoxigenic and enteroinvasive *Escherichia coli* infections, typhoid fever and cholera (Kosek *et al*, 2003). Antimicrobial therapy may have a role in severe and prolonged gastrointestinal illness caused by nontyphoid *Salmonella* and *Campylobacter*. However, the use of antimicrobial agents may increase the risk of hemolytic uremic syndrome in children with *E. coli* O157:H7 infection. Bacterial genome sequencing and better understanding of the pathogenic mechanisms involved in the onset of diarrhea are leading to new preventive interventions, such as enteric vaccines, which may have a significant impact on the magnitude of this public health concern (Chao *et al*, 2006). Diarrhea pathogens are generally spread by faecal-oral transmission through:

- i. Contamination of food,
- ii. Faecal contamination of drinking water,
- iii. Direct person to person spread (WHO, 2004).

2.2. ANTIMICROBIAL RESISTANCE IN BACTERIAL ENTERIC PATHOGENS

Antimicrobial resistance is a global pandemic that has been associated with increased morbidity, mortality and healthcare costs. Important enteric pathogens are becoming increasingly resistant to the major antibiotics that are needed for optimal treatment of patients (WHO 2004). *Shigella* spp show a pattern of steadily increasing resistance to antibiotics. In order to ensure appropriate treatment, continuous surveillance is required to determine which antibiotics are still active. This strategy of “trying to keep one step ahead” implicates the continual development and testing of new antibiotics, which inevitably are more expensive (Sack *et al*, 2003). Determining the susceptibility of individual isolates is not cost-effective, nor would the results be available rapidly enough to be clinically useful. Therefore, a surveillance system is needed to determine the predominant patterns of resistance in a given locality.

Previously efficacious drugs such as sulphonamides, tetracycline, ampicillin, and trimethoprim-sulfamethoxazole have become largely ineffective against prevalent *Shigella* strains. The recently reported emergence of ciprofloxacin resistance further narrows the choice of effective antimicrobials (Lorenz *et al*, 2006). Multidrug resistance is increasing in ETEC due to the widespread use of chemotherapeutic agents in countries where diarrhea is endemic (WHO 2004).

2.3. ETIOLOGY OF DIARRHEA AMONG CHILDREN

Diarrhea continues to be the most common cause of morbidity and mortality among infants and children in developing countries (O’ryan *et al*, 2005). The causes of diarrhea

include a wide range of viruses, bacteria, and parasites. Among the bacterial pathogens, *Escherichia coli* plays an important role. *E.coli* is the predominant nonpathogenic facultatively anaerobic member of the human intestinal microflora. Some *E.coli* strains, however, have developed the ability to cause diseases of the gastrointestinal, urinary, and central nervous systems in the human host (Trung *et al*, 2005).

The major etiologic agents that account for the estimated 1.5 million deaths per year are enterotoxigenic *Escherichia coli* (ETEC), rotavirus, *Vibrio cholerae*, and *Shigella* spp.; all are known to be endemic in essentially all developing countries (Firdausi *et al*, 2005).

As suggested in the above study and other studies conducted, ETEC is recognized as an extremely important cause of diarrhea in the developing world where there is inadequate clean water and poor sanitation; however this may not be the case in all areas (Podewils *et al*, 2004).

In a collaborative survey of bacterial diarrheal diseases in Kenya under the JICA/KEMRI Research Project, Sang *et al.*, (1996) found the first confirmed case of hemorrhagic colitis due to *E.coli* 0157:H7 in Kenya. In this case, a 2 year old boy, presenting with severe diarrhea was admitted to the pediatric ward at Malindi district hospital. The patient presented with bloody diarrhea, but neither fever nor vomiting, The patient was treated with trimethoprin-sulpamethoxazole and discharged on the fourth day of admission. Routine laboratory investigation yielded negative results for both viral (rotavirus) and parasitic pathogens. Bacterial investigation showed no enteropathogenic bacteria other than *E.coli*, which sero-typed as 0157:H7. The vero cell test showed cytotoxic effect of the *E.coli* isolate as a result of verotoxin –production. Both DNA probes and PCR

confirmed the presence of the *vt2* gene but no *vt1* gene in the isolate. To date this has been the only documented report of STEC infection in Kenya (Sang, 2007).

Several studies on etiologic agents of diarrhea have been done in Kenya and most of the findings have been published. In a study by Brooks *et al* (2003) in Western Kenya on bloody diarrhea, *Salmonella spp*, *Campylobacter* and *Shigella* were identified as the major causes of diarrhea. In another study by Nyaundi *et al* (2007) on the prevalence of enteric pathogens among international travellers with diarrhea acquired in Kenya, it was found that ETEC was the most common pathogen followed by *Shigella* and *Salmonella* within the study group.

In a study conducted in rural Kenyan community from May 1997 through April 2003 to identify bacterial enteric pathogens, *Shigella* species was most commonly isolated. *Campylobacter* species and *E.coli* predominated among children less than 5 years and were progressively replaced by *Shigella* species with increasing age (Brooks *et al*, 2006). This study was conducted in a rural area and therefore may not be generalized to an urban setting.

2.3.1. Bacterial causes of Diarrhea

2.3.1.1. Description of the family Enterobacteriaceae

Most organisms in the family Enterobacteriaceae share the following properties: they are gram negative and rod shaped; do not form spores; are motile by peritrichous flagella or nonmotile; grow on peptone or meat extract media without the addition of sodium chloride or other supplements; grow well on MacConkey agar; grow both aerobically and anaerobically; are active biochemically; ferment (rather than oxidize) D-glucose and

other sugars, often with gas production; are catalase positive and oxidase negative; reduce nitrate to nitrite; contain the enterobacterial common antigen; and have a 39 to 59% guanine-plus-cytosine (G+C) content of DNA(4). Species in the family should also be more closely related (by techniques that measure evolutionary distance) to *Escherichia coli*, they type species of the type genus of the family, than they are to organisms in other families (Murray *et al*, 1999).

2.3.1.2. Natural Habitats

Enterobacteriaceae are widely distributed on plants and in soil, water and the intestines of humans and animals. Some species occupy very limited ecological niches. *Salmonella typhi* causes typhoid fever and is found only in humans. In contrast, strains of *Klebsiella pneumoniae* are distributed widely in the environment and contribute to biochemical and geochemical processes. However, strains of *K. pneumoniae* also cause human infections, ranging from asymptomatic colonization of the intestinal, urinary, and respiratory tracts (Bui *et al*, 2007).

2.3.1.3. Clinical Significance

Strains of Enterobacteriaceae are associated with abscesses, pneumonia, meningitis, septicemia, and infections of wounds, the urinary tract, and the intestines. They are a major component of the normal intestinal flora of humans but are relatively uncommon at other body sites. Several species of Enterobacteriaceae are important causes of nosocomial infections. Enterobacteriaceae may account for 80% of clinically significant isolates of gram-negative bacilli and 50% of clinically significant bacteria in clinical microbiology laboratories. They account for nearly 50% of septicemia, more than 70% of urinary tract infections and a significant percentage of intestinal infections (Murray *et al*, 1999).

2.3.2. *Escherichia coli*

Shiga toxin-producing *Escherichia coli* (STEC) are bacterial pathogens that result in both outbreak and sporadic occurrences of human mortality and disease. Symptoms can include bloody and non-bloody diarrhea, and children are susceptible to renal failure due to haemolytic uraemic syndrome. STEC are transmitted to humans by consumption of contaminated food or water, person-to-person contact or animal-to-person contact, where natural reservoirs include cattle, pigs and sheep (Karch *et al*, 2005).

Pathogenicity of *E.coli* is a complex multi-factorial mechanism involving a large number of virulence factors, which vary according to the pathotype. The virulence factors include attachment functions, host cell surfaces, modifying factors, invasion characteristics, toxins, adhesions, and capsule production as well as secretion systems, which export other virulence factors and pilot them to the target cells. Virulence factors are often organized into large genetic blocks on the chromosomes (pathogenicity islands), on large plasmids or on phages and can be transmitted horizontally between strains (Firdausi *et al*, 2007). Enteric pathogenic *E.coli* have been broadly divided and classified into several groups based on their mechanism of pathogenicity. These are enterotoxigenic *E.coli* (ETEC), enteropathogenic *E.coli* (EPEC), shiga toxin producing *E.coli* (STEC), enteroinvasive *E.coli* (EIEC) and enteroaggregative *E.coli* (EAEC). Each class falls within a serological subgroup and manifests distinct features in pathogenesis.

2.3.2.1. Clinical Significance

Among five *Escherichia* species, *E.coli* is the species most commonly isolated from human specimens. It is part of the bowel flora of healthy individuals: however certain

strains may cause extra-intestinal and intestinal infections in immuno-compromised as well as healthy individuals.

2.3.2.2. Enterotoxigenic *E.coli* (ETEC)

Enterotoxigenic *E.coli* is an important cause of diarrhea in infants and travelers in underdeveloped countries or regions of poor sanitation. The diseases vary from minor discomfort to a severe cholera-like syndrome. ETEC are acquired by ingestion of contaminated food and water, and adults in endemic areas evidently develop immunity. The disease requires colonization and elaboration of one or more enterotoxins. Both traits are plasmid-encoded (Qadri, 2000).

Enterotoxins produced by ETEC include the LT (heat-labile) toxin and/or the ST (heat-stable) toxin, the genes for which may occur on the same or separate plasmids. The LT enterotoxin is very similar to cholera toxin in both structure and mode of action. It is an 86kDa protein composed of an enzymatically active (A) subunit surrounded by 5 identical binding (B) subunits. It binds to the same identical ganglioside receptors that are recognized by the cholera toxin (i.e., GM1), and its enzymatic activity is identical to that of the cholera toxin (Pacheco, 2001).

The ST enterotoxin is actually a family of toxins which are peptides of molecular weight about 2,000 daltons. Their small size explains why they are not inactivated by heat. ST causes an increase in cyclic GMP in host cell cytoplasm leading to the same effects as an increase in cAMP. STa is known to act by binding to a guanylate cyclase that is located on the apical membranes of host cells, thereby activating the enzyme. This leads to secretion of fluid and electrolytes resulting in diarrhea (Firdausi, 2005).

Symptoms ETEC infections include diarrhea without fever, abdominal cramps sometimes accompanied by nausea and headache but with little vomiting. Although ETEC is usually associated with relatively mild watery diarrhea, illness in recent ETEC outbreaks has been notable for its prolonged duration. The bacteria colonize the GI tract by means of a fimbrial adhesin, e.g. CFA I and CFA II, and are noninvasive, but produce either the LT or ST toxin (Pacheco, 2001).

2.3.2.3. Enteroinvasive *E.coli* (EIEC)

EIEC closely resemble *Shigella* in their pathogenic mechanisms and the kind of clinical illness they produce. EIEC penetrate and multiply within epithelial cells of the colon causing widespread cell destruction. The clinical syndrome is identical to *Shigella* dysentery and includes a dysentery-like diarrhea with fever. EIEC apparently lack fimbrial adhesins but do possess a specific adhesin that, as in *Shigella*, is thought to be an outer membrane protein. Also, like *Shigella*, EIEC are invasive organisms. They do not produce LT or ST toxin and, unlike *Shigella*, they do not produce the shiga toxin (Sethabutr *et al*, 2002). EIEC strains invade cells of the colon and produce a general watery but occasionally bloody diarrhea by a pathogenic mechanism similar to that of *Shigella*.

2.3.2.4. Enteropathogenic *E.coli* (EPEC)

EPEC induce a watery diarrhea similar to ETEC, but they do not possess the same colonization factors and do not produce ST or LT toxins. They produce a non fimbrial adhesin designated intimin, an outer membrane protein, that mediates the final stages of

adherence. Although they do not produce LT or ST toxins, there are reports that they produce an enterotoxin similar to that of *Shigella*. Other virulence factors may be related to those in *Shigella* (Giron *et al*, 2005).

Adherence of EPEC strains to the intestinal mucosa is a very complicated process and produces dramatic effects in the ultrastructure of the cells resulting in rearrangements of actin in the vicinity of adherent bacteria. The phenomenon is sometimes called "attaching and effacing" of cells. EPEC strains are said to be "moderately-invasive" meaning they are not as invasive as *Shigella*, and unlike ETEC or EA_gEC, they cause an inflammatory response. The diarrhea and other symptoms of EPEC infections probably are caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins (Firdausi *et al*, 2005).

Some types of EPEC are referred to as Enteroadherent *E. coli* (EAEC), based on specific patterns of adherence. They are an important cause of traveler's diarrhea in Mexico and in North Africa. The symptoms of severe, prolonged and nonbloody diarrhea, vomiting, and fever in infants or young toddlers are characteristic of EPEC illness. Infection with EPEC has been associated with chronic diarrhea; sequelae may include malabsorption, malnutrition, weight loss and growth retardation.

2.3.2.5. Enteroaggregative *E.coli* (EA_gEC)

The distinguishing feature of EA_gEC strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhea in young children. They resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhea without invading or causing inflammation. This

suggests that the organisms produce a toxin of some sort. Recently, a distinctive heat-labile plasmid-encoded toxin has been isolated from these strains, called the EAST (EnteroAggregative ST) toxin. They also produce a hemolysin related to the hemolysin produced by *E. coli* strains involved in urinary tract infections. The role of the toxin and the hemolysin in virulence has not been proven. The significance of EAaggEC strains in human disease is controversial (El Sheikh *et al*, 2001).

2.3.2.6. Enterohemorrhagic *E. coli* (EHEC)

Enterohemorrhagic *E. coli* are represented by a single strain (serotype O157:H7), which causes a diarrheal syndrome distinct from EIEC (and *Shigella*) in that there is copious bloody discharge and no fever. A frequent life-threatening situation is its toxic effects on the kidneys (hemolytic uremia). Infection caused by *Escherichia coli* O157:H7 has become a significant public health problem world-wide. The forms of transmission are animal-to-person, waterborne and person to person. Cattle faeces has been recognized as the principal reservoir of the microorganism in waterborne and food-borne *E. coli* O157:H7 outbreaks and sporadic infections. Enterohaemorrhagic *E. coli* (EHEC) O157:H7 is the dominant shiga toxin producing strain that is known to be associated with both outbreak and sporadic cases of human diseases ranging from uncomplicated diarrhoea to hemorrhagic colitis and hemolytic uraemic syndrome (HUS). Their ability to cause severe diseases is related to their capacity to secrete shiga toxins also called verotoxins (Beutin *et al*, 2003).

Enterohemorrhagic *E. coli* has recently been recognized as a cause of serious disease often associated with ingestion of inadequately cooked hamburger meat. Pediatric diarrhea caused by this strain can be fatal due to acute kidney failure hemolytic uremic

syndrome [HUS]. EHEC are also considered to be "moderately invasive". Nothing is known about the colonization antigens of EHEC but fimbriae are presumed to be involved. The bacteria do not invade mucosal cells as readily as *Shigella*, but EHEC strains produce a toxin that is virtually identical to the Shiga toxin. The toxin plays a role in the intense inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause HUS. The toxin is phage encoded and its production is enhanced by iron deficiency (Firdausi, 2007).

2.3.3. *Shigella*

Shigella is a genus of the bacterial family *Enterobacteriaceae*. *Shigellae* are Gram-negative, nonmotile, non-spore forming, rod-shaped bacteria, very closely related to *Escherichia coli*. Shigellosis is an infectious disease caused by various species of *Shigella*. People infected with *Shigella* develop diarrhea, fever, and stomach cramps starting a day or two after they are exposed to the bacterium. The diarrhea is often bloody. Shigellosis usually resolves in 5 to 7 days, but in some persons, especially young children and the elderly, the diarrhea can be so severe that the patient needs to be hospitalized. A severe infection with high fever may also be associated with seizures in children less than 2 years old. Some persons who are infected may have no symptoms at all, but may still transmit the *Shigella* bacteria to others (*Bull World Health Organization*, 2004).

Shigella were discovered over 100 years ago by a Japanese microbiologist named Shiga, for whom the genus are named. There are four species of *Shigella*: *boydii*, *dysenteriae*, *flexneri*, and *sonnei*. *Shigella sonnei*, also known as "Group D" *Shigella*, accounts for

over two-thirds of the shigellosis in the United States. *Shigella flexneri*, or "group B" *Shigella*, accounts for almost all of the rest. Other types of *Shigella* are rare in this country, although they are important causes of disease in the developing world. One type, *Shigella dysenteriae type 1*, causes deadly epidemics in many developing regions and nations (Escheverria *et al*, 2002).

2.3.3.1. Clinical Significance

Shigella causes bloody diarrhea (dysentery) and non bloody diarrhea. Shigellosis often begins with watery diarrhea accompanied by fever and abdominal cramps but may progress to classic dysentery with scant stools containing blood, mucus, and pus. All four species of *Shigella* species are capable of causing dysentery, but *S.dysenteriae 1* has been associated with a particularly severe form of illness thought to be related to its production of Shiga toxin. Infection can also be asymptomatic, particularly infection with *S.sonnei* strains. Although these organisms are very important as causes of gastro-intestinal infections, they rarely cause other types of infections.

2.3.4. *Salmonella*

Salmonella is a Gram-negative facultative rod-shaped bacterium in the same proteobacterial family as *Escherichia coli*, the family *Enterobacteriaceae*, trivially known as "enteric" bacteria. *Salmonella* is nearly as well-studied as *E. coli* from a structural, biochemical and molecular point of view, and as poorly understood as *E. coli* from an ecological point of view. *Salmonellae* live in the intestinal tracts of warm and cold blooded animals. Some species are ubiquitous. Other species are specifically

adapted to a particular host. In humans, *Salmonella* are the cause of two diseases called *salmonellosis: enteric fever (typhoid)*, resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a food borne infection/intoxication.

2.3.4.1. Clinical Significance

Strains of non-typhoidal *Salmonella* usually cause an intestinal infection (accompanied by diarrhea, fever, and abdominal cramps) that often lasts 1 week or longer. Persons of all ages are affected: the incidence is highest in infants. *Salmonella* is ubiquitous in animal populations, and human illness is linked to foods of animal origin.

2.3.5. *Campylobacter*

Campylobacters are bacteria that are a major cause of diarrheal illness in humans and are generally regarded as the most common bacterial cause of gastroenteritis worldwide. In developed and developing countries, they cause more cases of diarrhoea than, for example, food borne *Salmonella* bacteria. In developing countries, *Campylobacter* infections in children under the age of two years are especially frequent, sometimes resulting in death. In almost all developed countries, the incidence of human *Campylobacter* infections has been steadily increasing for several years. The reasons for this are unknown (WHO, 2004).

2.3.5.1. Clinical Significance

C.jejuni and *C.coli* have been recognized as agents of gastrointestinal infection. A spectrum of illness is seen during *C.jejuni* or *C.coli* infection; patients may be asymptomatic to severely ill. Symptoms and signs usually include fever. Abdominal cramping and diarrhea (with or without blood or fecal leucocytes) that lasts for several

days to more than 1 week. Symptomatic infections are usually self-limited but relapses may occur in 5 to 10% of untreated patients. *Campylobacter* infection may mimic acute appendicitis and results in unnecessary surgery.

Campylobacters are mainly spiral-shaped, S-shaped or curved, rod-shaped bacteria. There are 16 species and six subspecies assigned to the genus *Campylobacter*, of which the most frequently reported in human disease are *C. jejuni* (subspecies *jejuni*) and *C. coli*. *C. laridis* and *C. upsaliensis* are also regarded as primary pathogens, but are generally reported far less frequently in cases of human disease. Most species prefer a micro-aerobic (containing 3-10% oxygen) atmosphere for growth. A few species tend to favour an anaerobic environment, although they will grow under micro-aerobic conditions also (Bolton *et al*, 2004).

2.3.6. *Vibrio Cholerae*

Vibrio cholerae is a species of bacteria. Some strains of *Vibrio cholerae* cause cholera, a severe diarrheal illness. *Vibrio cholerae* has many different types or serogroups, only two of which can cause epidemic cholera. Those two serogroups are called serogroup O1 and serogroup O139 (O139 is found only in Asia) and can cause epidemic cholera if they produce the cholera toxin. The other serogroups are known collectively as non-O1 and non-O139 *Vibrio cholerae*. These serogroups can cause a diarrheal disease which is less severe than cholera and does not have epidemic potential (Inacio *et al*, 2007). Non-O1 and non-O139 *Vibrio cholerae* are the third most commonly reported group of *Vibrio* bacteria. On the average, 44 cases of non-O1 and non-O139 *Vibrio cholerae* were reported to the CDC each year since 2000. Infections are seasonal with a peak in the late

summer and early fall, coinciding with the warmest water temperatures (Gascon *et al*,2000).

2.3.6.1. Clinical Significance

V.cholerae serogroup 01 is the etiologic agent of epidemic cholera. The severity of disease resulting from *V.cholerae* infections ranges from asymptomatic or inapparent to the most severe form, referred to as “cholera gravis”. Incubation period of cholera ranges from several hours to 5 days, depending on the inoculum size. The buffering effect of food on gastric acidity reduces the infective dose of *V. cholerae* 01 to about 10^6 vibrios, whereas it is 10^{11} CFU at normal gastric activity. Cholera symptoms result from the action of cholera toxin (CT), which is chromosomally mediated, heat-labile enterotoxin. Initial symptoms of cholera are an increase in peristalsis followed by loose stools, which rapidly progress to the watery, mucus-flecked, “rice water” stools characteristic of cholera. Vomiting often occurs in the early stages of cholera. There’s little abdominal pain, although abdominal cramping may result from dehydration and electrolyte imbalance. Dehydration, hypovolemic shock, hypoglycemia, and metabolic acidosis must all be managed to prevent death in patients with cholera (Bui *et al*,2005).

2.4. PREVENTION AND CONTROL OF CHILDHOOD DIARRHEA

There are numerous ways recommended in the prevention and control of pediatric diarrhea: environmental sanitation, hand washing, health education, food safety, and safe water supply (WHO, 2003) However an integrated approach that includes the above methods and active surveillance on pathogen prevalence and antibiotic resistance is important for effective prevention and control of diarrhea among children.

Very few treatments for specific diarrheal pathogens exist. In many parts of the world, diarrhea is routinely treated with antibiotics, regardless of the underlying cause (WHO 2004). However, antibiotics are ineffective against many pathogens, and indiscriminate use of such drugs contributes to resistance in many different bacterial pathogens (Stoycheva *et al*, 2006). Based on the great impact of ETEC infections on morbidity and mortality, and probably also on nutritional status, particularly of children in areas where they are endemic, an effective ETEC vaccine is highly desirable. Such a vaccine is feasible since epidemiologic evidence and results from experimental challenge studies with human volunteers have demonstrated that specific immunity against homologous strains follows ETEC infection (Firdausi *et al*, 2005).

2.5. CURRENT EFFORTS AND APPROACHES IN CONTROL AND PREVENTION OF CHILDHOOD DIARRHEA

Nearly all the countries in Sub-Saharan Africa now have diarrheal disease control programs, at least on paper. These have largely employed a WHO-endorsed case-management strategy which emphasizes ORT, probably the easiest intervention to implement. Additional measures including improved nutrition with a focus on breastfeeding and safe weaning foods, better personal and domestic hygiene, and the provision of safe water supplies have been implemented. These are all more difficult to effect (Child Health Report 2003).

2.6. DIARRHEA SURVEILLANCE

Disease surveillance is the collection, analysis, and interpretation of data to determine disease trends and patterns. Disease surveillance provides information such as:

- Disease incidence, morbidity, and mortality, and progress in achieving disease control goals ;
- Changes in patterns of morbidity and mortality among different age groups in different geographical areas and among different economic, social, or cultural groups ;
- Impact of immunization strategies on disease incidence;
- Disease trends .

A functional disease surveillance system is useful for priority setting, planning, resource mobilization and allocation, prediction and early detection of epidemics and monitoring and evaluation of intervention programs.

In most developing countries disease surveillance is non existent or ineffective in meeting the above objectives. The WHO regional office for Africa proposes an integrated approach to disease surveillance. The approach aims at coordinating and streamlining all surveillance activities and ensuring timely provision of surveillance data to all disease prevention and control programmes (WHO,2003). There is need for diarrhea surveillance efforts in developing countries. It is important to monitor trends of enteric pathogens and antimicrobial drug resistance patterns .

2.7. CURRENT EFFORTS IN DIARRHEA SURVEILLANCE

A literature search on diarrhea surveillance in Kenya yielded results of 1 study carried out in Western Kenya between the years 1997-2003. This means that there are no ongoing surveillance efforts on childhood diarrhea.

CHAPTER 3

3.0 METHODOLOGY

3.1 Research Design

This is a descriptive cross sectional survey on the bacterial causes of diarrhea among children who presented to Mbagathi District Hospital. The study design was chosen because this study seeks to find the prevalence of enteric bacterial pathogens in children who are 5 years and below at a single point in time. This design is appropriate for assessing prevalence of acute conditions in a population.

3.2 Variables

The dependent variable or outcome of interest was bacterial pathogen. Independent variables were factors of acute diarrhea: area of residence and water source of study households.

3.3 Study Area

The study area was Mbagathi district hospital, Nairobi. A public health facility serving both low and average income households in Nairobi. The hospital serves total population of 500,000. The most common diseases observed in this hospital are HIV/AIDS related illnesses, malaria, diarrhea and respiratory infections. On average the hospital attends to 250,000 outpatients per year and 100,000 inpatients. (Mbagathi February 2007 monthly report).

3.4 Target Population

The target population was all children under 5 years of age.

3.5 Study Population

The study population was drawn from both middle-level and low-level income earning groups residing in diverse areas within Nairobi city. Study participants were children below 5 years of age presenting with diarrhea to Mbagathi District Hospital. The reason this age bracket was chosen is because children are an immunologically naïve population and therefore easy to identify and diagnose disease causing pathogens within the age group.

3.5.1 Inclusion Criteria

1. All outpatient children under the age of 5 years observed at Mbagathi district Hospital;
2. Patients experiencing acute diarrhea (3 or more loose or watery stools) in a 24 hour period;
3. Patients experiencing diarrhea episodes lasting up to 14 days (duration);
4. Repeat visit accepted if end of first episode and the onset of the second are 3 or fewer days apart;
5. Patients in above categories experiencing an episode of acute diarrhea with visible blood in the stool.

3.5.2 Exclusion Criteria

1. Patients unwilling to give stool or consent for participation in the study;
2. Diarrhea lasting more than 14 days;
3. Inpatients.

3.6 Sample Size Determination and Sampling Procedures:

3.6.1 Study site selection and sampling procedure

Convenience sampling procedure was used to select Mbagathi district hospital. The hospital was chosen because of its proximity to KEMRI laboratories and also because the hospital forms part of a surveillance network for research on infectious diseases. Mbagathi district Hospital is a government health facility that provides health services to mostly low and middle income residents in Nairobi. Most patients are drawn from Kibera slum who are more exposed to risk factors for diarrheal illnesses.

Simple random sampling technique was used to enroll children under 5 years who met inclusion criteria. Every second patient meeting inclusion criteria was enrolled into study at limit of 5 patients per day. Mothers or guardians accompanying children who met the inclusion criteria and who were willing to have their children enrolled into study were respondents to questions in the questionnaire. Consent for participation of children in the study was sought from mothers who accompanied the children.

3.6.2 Sample size determination

The fisher *et al.* 1998 equation below was used to calculate the number of children to be sampled:

$$n = \frac{Z^2 pqD}{d^2}$$

where n = the desired sample size for population attending Mbagathi District hospital

Z = the standard deviate of a normally distributed population,

which corresponds to 95% confidence levels and is usually given as 1.96

- d = the standard deviation of the desired sample size usually taken to be 0.05
- p = the proportion of the target population estimated to have the desired characteristic
- q = 1-p
- D= design effect. Since random sampling will be used, design effect is one

Since estimated population of children visiting Mbagathi hospital is 20% of Nairobi's children population, p becomes 0.20 and q becomes 0.80, Z is 1.96 and d is 0.05. Since random sampling will be used, D is 1. Substituting these values in the formula above will give

$$n = \frac{1.96^2 \times 0.20 \times 0.80 \times 1}{0.05^2}$$

$$= 245.8624$$

$$= 246$$

A total of 246 children were sampled from Mbagathi district hospital.

3.6.3 Ethical Considerations

This study was passed by KEMRI's Ethical Review Committee. Consent for participation of children in this study was sought from mothers/guardians accompanying the children.

Informed consent was provided by the clinical officer attending to the patients.

Demographic and social information was provided by the mother/guardian accompanying the child. All consent forms and questionnaires were kept under lock and key at all times

to ensure privacy and confidentiality of patients was maintained. Electronic databases were password protected and only unique identifier-study number was used in the data base and not patient names.

3.7 Laboratory Analysis of stool specimens

Laboratory procedures were conducted according to GEIS Standard Operating Procedures authorized for year 2007 (GEIS 2007).

3.7.1 Validity

Laboratory analyses was validated by use of set and approved Standard Operating Procedures GEIS-KEMRI. Validity of questionnaires was ensured by training Clinical Officer who did case identification as per inclusion criteria and enrolment. To ensure consistent and uniform administration of questionnaires , all questionnaires were assessed and checked to ensure they met required quality assurance and quality control procedures.

3.7.2 Bacterial Identification

Representative colonies of different morphologies from each plate were inoculated into biochemical media using the standard methods. The specimen was plated on Sorbotol-MacConkey, to screen for *E.coli*. The specimen was plated directly on XLD and incubated at 35° C-37°C for 18-24 hours to distinguish between the *Salmonella* spp, *Shigella* spp. To enhance the growth of *Salmonella* and *Shigella* spp, specimens were inoculated into Selenite-F broth, incubated for 18-24 at 35-37°C followed by plating onto xylose lysine deoxycholate (XLD) agar.

For the detection of *Vibrio* spp, the specimen was plated directly onto thiosulphate citrate bile sucrose agar(TCBS), and enriched in alkaline peptone water(APW) prior to plating on TCBS.

Thereafter, a single colony suspected as *Salmonella* spp, *Shigella* spp. *Vibrio* spp and approximately *E.coli* colonies were picked and plated onto nutrient agar plates. The suspect colonies were identified by conventional biochemical testing before selecting for serotyping with respective antisera.

The specimens were screened for the presence of thermotolerant *Campylobacter* spp by plating on campylobacter blood agar and incubated in a microaerophilic environment using the BBL Campy Puch Microaerophilic System, followed by biochemical reactions to distinguish between the species. This was complemented with multiplex PCR to allow for speciation of the suspected isolates.

3.7.3 Biochemical Identification

All isolates were characterized on the basis of their biochemical reactions using BBL enterotubes. An enterotube is a prepared test system contained in a half round, molded plastic tube divided into 8 compartments, each containing a slant of a standard biochemical test medium.

Enterotubes permit the inoculation of media and the subsequent performance of 15 standard biochemical tests from a single bacterial colony: glucose, gas production from glucose, lysine decarboxylase, ornithine decarboxylase. H₂S, indole, adonitol, lactose,

arabinose, sorbitol, voges-Proskauer, dulcitol, phenylalanine deaminase, urea and citrate. Resulting combinations of reactions together with computer coding system, allow identification of Enterobacteriaceae. Before using the Enterotubes, the specimen was cultured in a manner as to obtain well-isolated colonies on nutrient agar plates. One colony was picked from the plate. The tube was inoculated by twisting through all twelve compartments using a turning motion and without pulling the needle all the way out of the tube. The needle was then re-inserted into the tube until the notch was aligned with the opening of the tube, and needle broken off at the notch by bending. With the broken off part of the needle, a hole was punched through the plastic covering the air inlets in each of the last eight compartments (adonitol through the citrate). This was done so that air can enter these compartments. The broken off part of the needle discarded. Both the caps replaced . The tubes were incubated at 35-37° C for 18-24 hrs with the tube on its flat surface. The Voges-Proskauer (VP) test was utilized as confirmatory test . To perform the VP test , 3 drops of naphthol solution and 2 drops potassium hydroxide were injected through the air inlet into the VP compartment of the tube. Colour change was observed after 10 minutes.

Table 3.1: Interpretation of the Enterotube

Compartment	Reaction	Negative	Positive
1	Glucose gas	Red/orange	Yellow
2	lysine	yellow	purple
3	ornithine	yellow	purple
4	Hydrogen sulfide production	Beige	Black
5	Indole production	Colorless	Red
6	Adonitole fermentation	Red/orange	Yellow
7	Lactose fermentation	Red/orange	Yellow
8	Arabinose fermentation	Red/orange	Yellow
9	Sorbitol fermentation	Red/orange	Yellow
10	Voges-Proskauer	Colorless	Red
11	Dulcitol fermentation	Not yellow	Yellow

Compartment 1

- Glucose- Any degree of yellow is positive. Acid end products from glucose fermentation turn the pH indicator from red (alkaline) to yellow (acid);
- Gas- Positive is a definite and complete separation of the white wax overlay from the surface of the glucose medium. Detects gas from glucose fermentation.

Compartment 2

Any degree of purple is positive. An alkaline end product from the decarboxylation of lysine changes the pH indicator from pale yellow (acid) to purple (alkaline).

Compartment 3

Any degree of purple is positive. An alkaline end product from the decarboxylation of ornithine changes the pH indicator from pale yellow (acid) to purple(alkaline).

Compartment 4

- Hydrogen sulfide-Only a true black is positive. Reduction of thiosulfate produces hydrogen sulfide, which reacts with iron salts to produce black ferric sulfide
- Indole- This test was not interpreted until all other compartments had been read. To perform the indole test, the tube was laid, its flat surface pointing upward, on the table; dropped in 2-3 drops of Kovac's reagent directly under the plastic film into the H₂ S/Indole compartment. Indole, produced from the breakdown of tryptophan, reacts with Kovac's reagent turning it red.

Compartment 5

Any degree of yellow is positive. Acid end products from adonitol fermentation turn the pH indicator from red(alkaline) to yellow (acid).

Compartment 6

Any degree of yellow is positive. Acid end products from lactose fermentation turn the pH indicator, phenol red, from pink to yellow.

Compartment 7

Any degree of yellow is positive. Acid end products from arabinose fermentation turn the pH indicator from red (alkaline) to yellow (acid).

Compartment 8

Any degree of yellow is positive. Acid end products from sorbitol fermentation turn the pH indicator from red (alkaline) to yellow (acid).

Compartment 9

This test is not used unless required later as a confirmatory test. Acetone produced during the production of butylene glycol from glucose fermentation reacts with added reagents KOH and alpha-naphthol and turns red.

Compartment 10

Dulcitol-Yellow or pale yellow is positive. Any other color is negative. Acid from dulcitol fermentation turns the pH indicator from green(alkaline) to yellow (acid).

Compartment 11

Hydrolysis of urea forms ammonia, which causes the pH indicator to turn from yellow(acid) to red/purple (alkaline).

Compartment 12

Any degree of blue is positive. Utilization of citrate produces alkaline products turning the pH indicator from green (acid) and to blue (alkaline). Multiplex PCR was performed to detect virulence factors that characterize *E.coli* strains based on the methods. Primers for amplifying segments of the Vero toxins (VT1, VT2, VT2e), cytotoxin necrotizing factors (CNF1 and CNF2) attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanism (Eagg), enteroinvasive mechanism (Einv).

The virulence mechanisms that characterize *Escherichia coli* are genetically coded for by chromosomal, plasmid, and bacteriophage DNAs and include heat-labile (LTI, LTIIa, and LTIIb) and heat-stable (STI and STII) toxins, verotoxin types 1, 2, and 2e (VT1, VT2, and VT2e, respectively), cytotoxic necrotizing factors (CNF1 and CNF2), attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanisms (Eagg), and enteroinvasive mechanisms (Einv).

3.7.4 PCR (Polymerase chain reaction) PROCEDURE

This was conducted according to GEIS-KEMRI Laboratory PCR procedure. (PCR SOP)

3.7.4.1 DNA Template preparation.

3.7.4.1.1 DNA extraction

Method 1

Day 1

Grow cells at 37°C overnight in 2 ml broth medium.

Day 2

1. Pellet cells from 500µl of bacterial culture by centrifugation at 12000rpm for 30 secs and remove spent media. Resuspend pelleted cells in 500 µl of sterile distilled water.
2. Heat in a water bath at 95°C for 5 minutes.
3. Freeze at -80°C for 30minutes then taken for PCR.
4. Use 10µl of the sample (template) for PCR.
5. The template can then be stored at -80°C or -20°C until further analysis.

Method 2

1. Starting with a liquid suspension sample of 10^2 - 10^4 cells, pellet them at 1200-1500xg for 10mins.
2. Resuspend the cells in 1.5ml PBS and pellet as before. If necessary, re-pellet the sample and resuspend the cells again to remove traces of the original suspension buffer.

3. Resuspend the cells gently in 25-50 μ l distilled water. Incubate at 95-100°C for 3-5 mins.
4. Centrifuge briefly to collect any condensate.
5. Optional: pellet the cellular debris at 12000 xg for 3 mins and transfer the cleared lysate to a new tube.
6. Use lysate as template for PCR.
7. The template can then be stored at -80°C or -20°C until further analysis.

3.7.4.2 Colony PCR template preparation

1. Label microcentrifuge tube with sample identifying number.
2. Dispense 25 μ l of distilled water to the tubes.
3. Pick a tiny amount of a single colony from a fresh culture (18-24hours)
4. Transfer to the respective microcentrifuge tube and emulsify.
5. Use 2 μ l for template.
6. Do not reuse this template. Always prepare fresh template.

3.7.5 PCR Amplification: Detection of virulence factors in *Escherichia coli* and *Shigella spp.*

- Virulence factors of interest:
 - Shiga toxin 1 and 2 (stx1 and stx2).
 - Heat labile toxin I and II (LTI and LTII).

Table 3.2: Components of PCR Analysis

<i>Components</i>	Volume (20µl)	Final Concentration
Distilled water*	15.2	Variable
10x PCR Buffer**	2.5	1x
MgCl ₂ solution	1.5	1.5-3.0mM
DNTPs mix (25mM each nucleotide)	0.5	200µM
Primer	1.0 each	0.1-1.0µM
Taq DNA polymerase	0.3	1Unit/µl
Genomic DNA template*	4	Variable.

- Heat stable toxin I (sta) and II (stb).
- Cytotoxic necrotizing factor I (CNFI) etc.

Primer sequences for the above genes – refer to Attachment 3: Multiplex PCR Primer sequences.

The protocol next page is carried out in a 25µl reaction volume.

- 2µl for Colony PCR. Adjust water volume accordingly.
- Because of the small volumes involved, it is convenient to make a cocktail of the first six ingredients for each primer pair to be used. For instance, if 10 PCR reactions are to be performed from 10 different genomic DNA templates using one primer pair, then a cocktail may be made (including a slight excess) for 12 reactions by mixing together each of the volumes above multiplied by 12.

Add a 21µl aliquot of the cocktail to each tube. 4.0 µl of DNA is then added to each tube.

Steps:

- a) Plan experiment before adding any reagents (number of primer pairs to be used, number of DNA templates, etc.). Appropriate cocktail/s prepared and mixing done by tapping the tube and quick spinning.
- b) Pipet 21.0 μl of the appropriate cocktail directly into the bottom of a sterile microeppendorf tube for each reaction. Label tubes using a permanent marker.
- c) Add 4.0 μl of the DNA directly into the drop of cocktail in each tube and ensure adequate mixing. Quick spin to collect the reaction mixture in the bottom of the tube.
- d) Place the tightly capped tubes in the temperature block and make sure each is firmly seated by pressing on the tubes individually.
- e) Create or select a program from memory and proceed to "RUN" the program.
Refer to the SOP #: GEI 107 V1 and GEI 142 V1 for operation of the thermal cyclers. Run the program "MUL 2" (on the Peltier Thermal cyclcer or MUL 1 on the 9700 Thermal cycler) .

Programme

95°C for 30 s
72°C for 1 min for 5 cycles;

95°C for 30 s
63°C for 30 s
72°C for 30 s for 20 cycles;

72°C for 5 min when isolated DNA was amplified.

The PCR program is to be preceded by 5 min at 95°C to lyse the bacteria when bacterial suspensions are examined (colony PCR).

After completion remove the tubes and ensure labeling markings are still clearly visible.

- f) The reaction products are conveniently separated according to size by gel electrophoresis through a 1.2% agarose “EAST CAST™” gel at 170V for 45minutes-1hour, and visualized after staining the gel with ethidium bromide. Refer to SOP #: GEI 164 V1 for procedures on gel electrophoresis.

3.7.6 Serotype Identification

The serologic classification of *E.coli* is generally based on two types of antigens: the O antigen (somatic) and the H antigen (flagellar). Strains identified as *Salmonella*, *Shigella*, or *E.coli* by their colonial morphology and biochemical properties were serotyped using O-antigen and H-antigen antisera. These antigens are most commonly identified by the tube agglutination test with antisera prepared against the different antigenic components. The O and H antigens of *E.coli* are stable and reliable strain characteristics. Determination of the O and H serotypes of *E.coli* strains implicated in diarrheal illness is particularly useful in epidemiologic investigations as well as in the identification of certain classes of diarrheagenic *E.coli* (Murray *et al*, 1999).

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Demographic characteristics of study population

The general demographic characteristics of study participants who presented to Mbagathi district hospital are as shown in Table 4.1:

Table 4.1: Demographic characteristics of study population

Characteristics	n =246	Percent	
Sex			
Female	115	46.7	
Male	131	53.2	
Age			
Under 12 months	3.54(mean)	<1-11 (range)	SD 3.8
Over 1 year	1.01 yrs(mean)	<1-4.92(range)	SD 1.27
Specific area of residence			
Kibera	112	45.5	
South B	26	10.6	
Kawangware	12	4.9	
Kariobangi	9	3.7	
Embakasi	9	3.7	
Other areas	78	31.7	
Water source			
Municipal	240	97.6	
Well	4	1.6	
Rainwater	3	1.2	
Borehole	3	1.2	

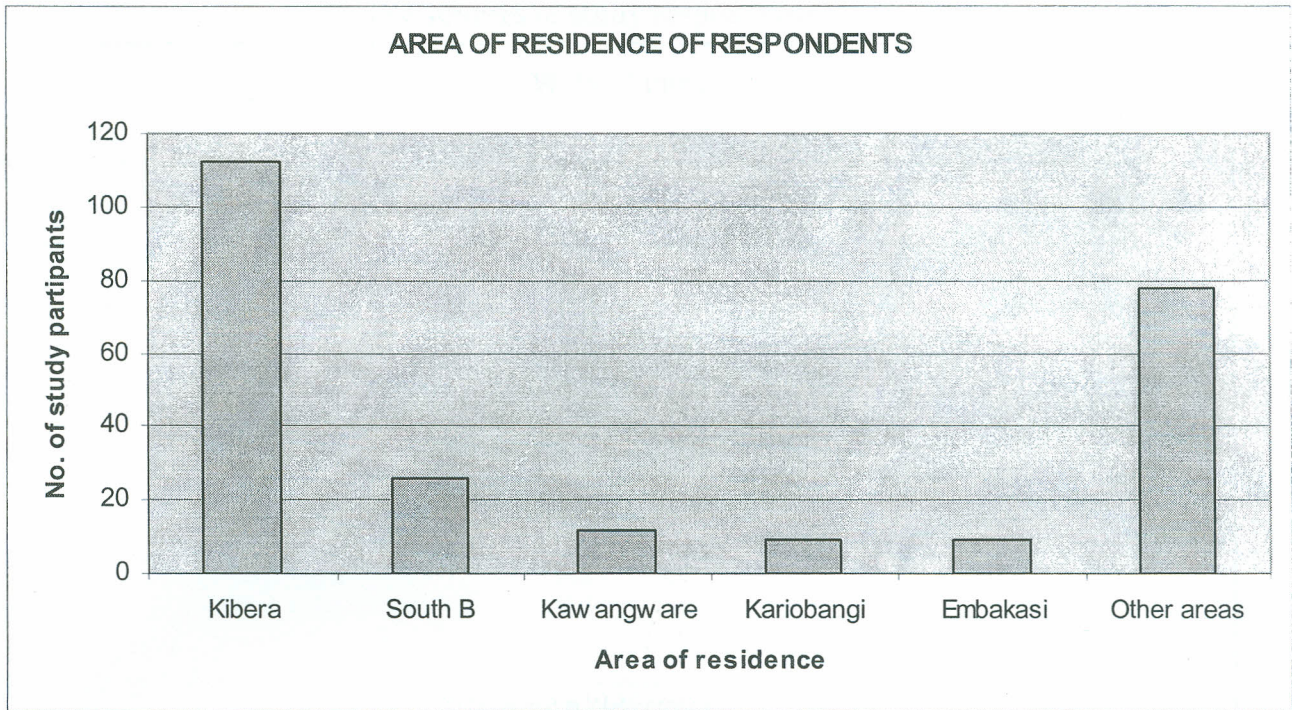


Figure 4.1: Area of residence of respondents' n=246

The figure 4.1 above shows that most of the study respondents were drawn from Kibera 112(45.53%) and the rest from South B 26 (10.57%), Kawangware 12 (4.88%), Kariobangi 9 (3.66%), Embakasi 9 (3.66%) other areas 78 (3.1%). There were 115 females and 131 males.

Water sources of study respondents

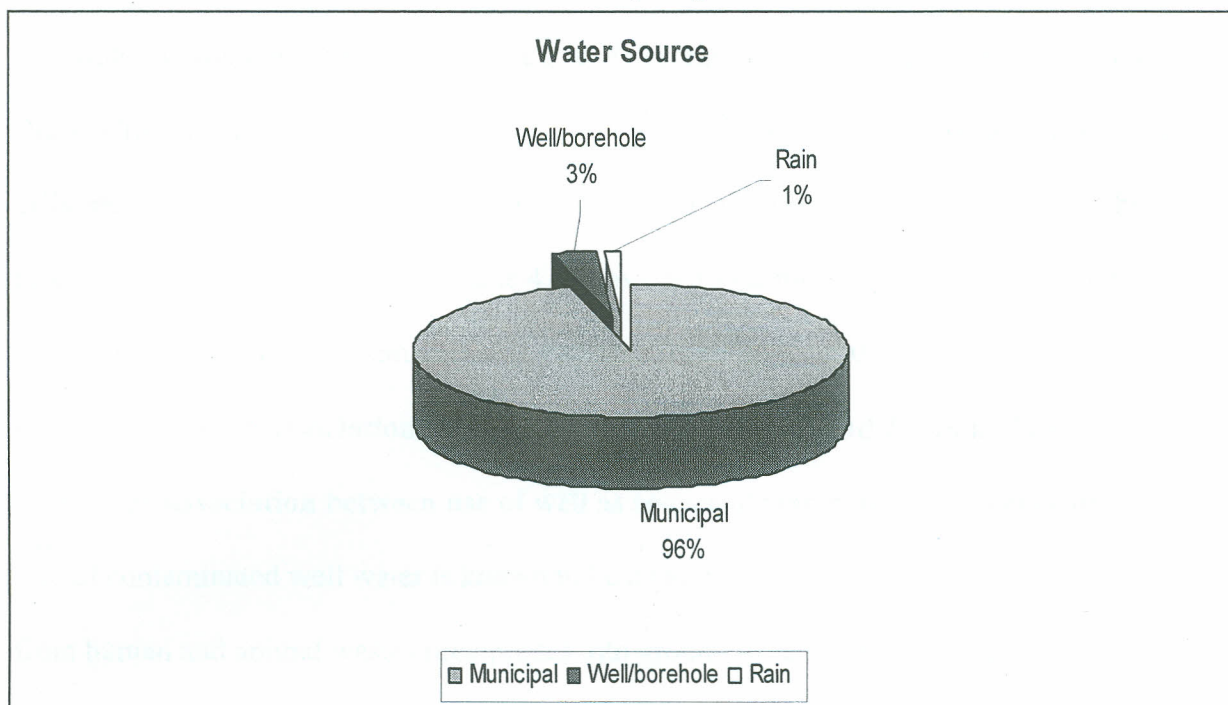


Fig4.2: Water source n=246

Figure 4.2 above shows that majority of the households (96%) obtained their water from the municipal council. One percent(1%) used rain water, three percent (3%) used water from the well and borehole.

Comparison of occurrence of *E.coli* and use of municipal water source n=246

Contaminated water is a factor for *E.coli* infections. 96% of study participants indicated that they use municipal water.

Table 4.2: Cross tabulation *E.coli* and municipal water source

Municipal	<i>E. coli</i>		Total
	Neg	Pos	
No	4 (2.4%)	2 (2.4%)	6 (2.4%)
Yes	160 (97.6%)	80 (97.6%)	240 (97.6%)
Total	164 (100%)	82 (100%)	246 (100%)

Table 4.2 shows similar percentages in each category , this is an indication of no interaction between occurrence of *E. coli* and use of municipal water. This means that

water provided by the municipal council is not a risk factor for occurrence of *E.coli* infections. Cross-tabulation can be used to determine causal agent for factors under study. Cross-tabulation gives a basic picture of how 2 variables inter-relate. If certain cells show disproportionate values (large or small) of cases, then this suggests there might be a pattern of interaction. Table 4.2 above shows same values in all cells hence an indication of no interaction among the 2 variables (Gordis, L. 2000).

Chi-square test of association between: well (water source) and *E.coli* n=246

Table 4.3: Association between use of well as source of water and occurrence of *E.coli*

Use of contaminated well water is known to be a factor for infection with *E.coli*. *E.coli* from human and animal wastes precipitates into ground water causing contamination.

<i>E.coli</i>	Well (water source)		Total
	No	Yes	
Neg	162 (66.9%)	2 (50%)	164 (66.7%)
Pos	78 (32.2%)	2 (50%)	80 (32.5%)
Total	242 (100%)	4 (100%)	246 (100%)
Chi square(x^2) =0.5845 Df=1 p=0.747			

The above test of association in table 4.3 gives $p= 0.747$ proving that there is no significant association between use of well water and occurrence of *E.coli*. This study shows that residents who use well water are not exposed to *E.coli* through contaminated water. *E.coli* may be found in water sources such as wells that have been contaminated with feces from infected humans or animals. In any situation where drinking water and sanitation are inadequate *E.coli* is known to be common, this study however shows no significant association between source of water and occurrence of *E.coli* therefore water is not a risk factor for *E.coli* infections isolated in patients seen at Mbagathi hospital.

4.2 LABORATORY RESULTS

4.2.1 Bacterial causes of diarrhea

Table 4.4: Pathogenic microorganisms isolated from children in Mbagathi district hospital

This study identified *E. coli*, *Salmonella* and *Shigella* pathogens in study population.

Pathogen	n=246	Percentage of infected patients
<i>E.coli</i>	76	93.8
<i>Salmonella</i>	3	3.7
<i>Shigella</i>	2	2.5
Total	81	100

Laboratory analysis shows *E.coli* pathotypes were the microorganisms frequently associated with diarrhea among children seen at Mbagathi District Hospital. Numerous studies have proved that *E.coli* pathogen is common in low income communities (Chao *et al* 2006, Firdausi *et al* 2005) . Table 4.4 shows that 76 patients had *E.coli*, 3 had *Salmonella* and 2 had *Shigella* infections respectively.

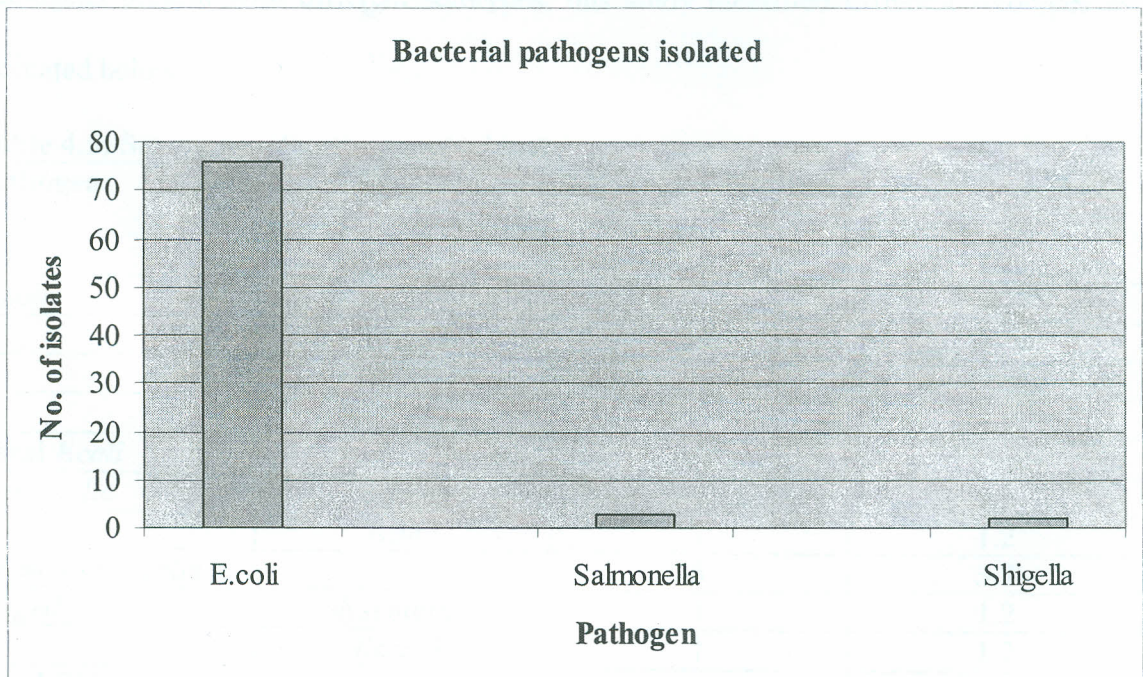


Fig4.3: Bacterial pathogens isolated from study population

E.coli is a common cause of acute watery diarrhea in children in developing countries. Numerous studies show that in densely populated urban housing areas, the majority of children suffer from diarrhea ranging from a minimum of 1 to 14 episodes and *E.coli* was the major cause (Qardi *et al*, 2005). Figure 4.3 shows 93.83% of diarrheal cases in this study were attributed to *E.coli* evidence that the pathogen is more prevalent among children in urban areas. Contaminated weaning foods are likely causes of *E.coli* in children in developing countries.

In this study diarrhea cases of *Salmonella* and *Shigella* were low 3.7% and 2.47% respectively. The low rates of isolation of these pathogens are not attributed to methodological differences but possibly to age factor as observed in other studies (Kosek *et al*, 2003).

Bacterial pathogens and strains isolated from study population

Pathogens present with different serotypes, this study identified different serotypes as indicated below.

Table 4.5: Serotypes of pathogens isolated

Pathogen	Strains	No. of positive isolates n=81	% of infected patients
<i>E.coli</i>	ETEC	31	38.3
	EAGG	21	25.9
	EINV	20	24.7
	STEC	4	4.9
Total Ecoli		76	93.8
<i>Salmonella</i>	<i>arizona</i>	2	2.5
	<i>typhii</i>	1	1.2
Total Salmonella		3	3.7
<i>Shigella</i>	<i>dysenteriae</i>	1	1.2
	<i>flexneri</i>	1	1.2
Total Shigella		2	2.5
Total isolates		81	

The enteric *E. coli* are divided on the basis of virulence properties:

- i. enteroaggregative *E. coli* - EAGG
- ii. enteroinvasive *E.coli*- EINV
- iii. enterotoxigenic *E.coli*- ETEC
- iv. shigatoxigenic *E.coli*- STEC

ETEC is a multivalent pathogen producing the heat stable (ST) and/or heat-labile toxin (LT) as well as over 25 colonization factors (CFs). The ST phenotype of ETEC has been shown to be predominant. Two virulence attributes that characterize ETEC are the colonization factors(CFs) of the small intestine surface and the production of enterotoxins that induce a net secretion of electrolytes and water into the gut lumen. 24.69% of ETECs were isolated in this study as shown in table 4.5.

Salmonella is a genus of rod-shaped Gram-negative enterobacteria that causes typhoid fever, paratyphoid fever and food borne illnesses. There are numerous strains of *Salmonella* but in this study *Salmonella typhii* and *arizona* were isolated. *Salmonella typhii* causes typhoid fever. 3.7% of diarrhea in this study was attributed to *Salmonella*.

Shigella is a genus of Gram-negative, non-motile, non-spore forming rod-shaped bacteria closely related to *Escherichia coli* and *Salmonella*. *Shigella* infection is mainly via fecal-oral contamination. *Shigella* has numerous strains *dysenteriae* and *flexneri* are strains isolated in this study.2.46% of *Shigella* were isolated in this study.

4.2.2 Pathogen prevalence in study population

Prevalence rates of bacteria isolated are as indicated below.

Table 4. 6: Prevalence of bacterial pathogens

Pathogen	n=246	Prevalence
<i>E.coli</i>	76	30.9
<i>Salmonella</i>	3	1.2
<i>Shigella</i>	2	0.8
Negative	165	67

As indicated in Table 4.6, *E.coli* is more prevalent in children who presented with diarrhea to Mbagathi hospital (30.9%). Infections with *E.coli* are mainly prevalent among young children caused mainly by contaminated food or water. This bacteria is known to be common especially among children being weaned when hygienic food handling procedures are not practiced. Results of this study give a true picture of high prevalence of *E.coli* among children which is a common trend in most cases.

Prevalence of *Salmonella* in this study was 1.22%. This bacterium is mainly associated with food poisoning. Incidents of food poisoning are rare in most cases and known to occur especially in food establishments. Hence the low prevalence of this bacteria among children who were enrolled into the study. Many studies carried out indicate that prevalence of *Salmonella* among children especially in developing countries is not responsible for large fraction of episodes.

In this study *Shigella* bacteria have the lowest prevalence -0.81%. Occurrence of *Shigella* pathogen is rare in most cases unless during outbreaks.

4.2.3 Prevalence of acute diarrhea in Mbagathi hospital

In the period of data collection a total of 3068 children less than 5 years were examined and within this period the following diseases were observed:

Most common diseases among children under 5 years

Table 4.7 : Common diseases diagnosed in Mbagathi hospital during study period

Disease	No. of patients total 3068	% of total patients	Prevalence rate
HIV/AIDS related illnesses	1453	47.36	0.5
Malaria	600	19.56	0.2
Diarrhea	563	18.35	0.2
Pneumonia	452	14.73	0.2

Table 4.7 shows that HIV/AIDS related illnesses ranked top among diseases commonly diagnosed among children under 5 years, followed by malaria, diarrhea then pneumonia. Diarrheal illnesses were the third most common disease among this age group with prevalence rate of 0.18. A study done at the Mukuru slum in Nairobi showed that diarrheal infections ranked second among the most common diseases with 197 cases in a period of one month (Svabova *et al*, 2007). Comparing the cases in this study and cases in Mbagathi Hospital diarrheal illnesses is common among children under 5 years of age. Disease burden of diarrheal illnesses among children presenting to Mbagathi hospital is lower compared to HIV/AIDS related illnesses and malaria, nevertheless, diarrheal illnesses are a common occurrence among children under 5 years of age.

4.2.4 Distribution of symptoms within diarrheal cases

Infections with bacterial pathogens cause symptoms. Below are common symptoms observed in study participants.

Table 4.8 : Symptoms associated with diarrhea

Symptom	Positive	Negative
Loss of appetite	235 (95.5%)	11 (4.5%)
Fever	209 (84.9%)	37 (15%)
Vomiting	181 (73.6%)	65 (26.4%)
Mucus in stool	168 (68.3%)	78 (31.7%)
Abdominal cramp	114 (46.3%)	132 (53.7%)
Bloody stool	29 (11.8%)	217 (88%)
Nausea	2 (0.8%)	244 (99.2%)
Headache	2 (0.8%)	244 (99.2%)

Table 4.8 demonstrates loss of appetite as the most common symptom reported among the study population followed by fever, vomiting and mucus in stool. These symptoms present when infected with any of the enteric pathogens however, there are some distinct symptoms which are characteristic of infection by specific bacterial pathogen. Mucus and blood in stool are distinct symptoms for infection with *Shigella* bacterium.

4.2.5 Association Between Symptoms and Bacteria

Chi-square test of association between : mucus in stool and *Shigella* n=246

Mucus in stool is a main symptom that presents with *Shigella* infection.

Table 4.9: Association between symptom mucus in stool and isolation of *Shigella*

	Neg	Pos	Total
Mucus in stool			
No	78 (31.9%)	0 (0%)	78 (31.7%)
Yes	166 (68.1%)	2 (100%)	168 (68.3%)
Total	244 (100%)	2 (100%)	246 (100%)
Chi square (χ^2)=0.9362 DF=1 p= 0.33			

Mucus in stool and bloody stool are symptoms associated with *Shigella* pathogen.

However, in this study presentation of these symptoms and occurrence of *Shigella* are not directly associated. There is no significant association between symptom mucus in stool and isolation of *Shigella* pathogen $p=0.33$. Possible reason for this observation is the few number of *Shigella* cases observed in the study- only 2 cases were identified.

Chi-square test of association between: bloody stool and *Shigella* n=246

Bloody stool is also a main symptom associated with *Shigella*.

Table 4.10: Association between symptom bloody stool and isolation of *Shigella*

	Neg	Pos	Total
Bloody stool			
No	216 (88.5%)	1 (50%)	217 (88.2%)
Yes	28 (11.5%)	1 (50%)	29 (11.8%)
Total	244 (100%)	2 (100%)	246 (100%)
Chi square(χ^2)=2.8312 DF=1 p=0.0902			

The Table 4.10 shows no significant association between symptom bloody stool and isolation of *Shigella* pathogen $p=0.09$. In this study both symptoms are not significantly associated. Possible reason for this observation is because of sample size since this pathogen is rarely isolated in most cases.

4.2.6 Association Between Area of Residence (Kibera) and Isolated Enteric Bacteria

Chi-square test of association between : Kibera and *E.coli* $n=246$

Table 4.11: Association between area of residence (Kibera) and *E.coli*
Kibera

<i>Ecoli</i>	No	Yes	Total
Neg	93 (68.9%)	71 (63.9%)	164 (66.7%)
Pos	42 (31.1%)	40 (36%)	82 (33.3%)
Total	135 (100%)	111 (100%)	246 (100%)
Chi-square(x^2)=0.6649 DF= 1 $p=0.415$			

In Table 4.11, there is no significant association between residing in Kibera and isolation of *E.coli* pathogen. Chi-square test of association gives a p-value of 0.415 which is greater than 0.05 and therefore no significant association. This study shows that residence in Kibera slum is not a risk factor for *E.coli* infections. The squalor conditions in the slum are not directly related to *E.coli* infections. *E.coli* infections in children from Kibera slum are more likely attributed to poor hygiene in handling of food and water given to the children.

Chi-square test of association between: Kibera and *Salmonella* n=246

Table 4.12: Association between area of residence (Kibera) and *Salmonella*

Kibera			
<i>Salmonella</i>	No	Yes	Total
Neg	133 (98.5%)	110 (99.1%)	243 (98.8%)
Pos	2 (1.5%)	1 (0.9%)	3 (1.2%)
Total	135 (100%)	111 (100%)	246 (100%)
Chi square(x^2)=0.1704 DF=1 P=0.680			

Table 4.12 shows a Chi square test of association between residence and occurrence of *Salmonella* which gives a p value of 0.680. This shows there is no significant association between residence in Kibera and isolation of *Salmonella*. *Salmonella* is usually transmitted to humans by foods contaminated with animal feces, poorly cooked meat/poultry, fruits and vegetables not washed properly. The mentioned risk factors for *Salmonella* are not associated with Kibera.

Chi-square test of association between : Kibera and *Shigella* n=246

Table 4.13: Association between area of residence (Kibera) and *Shigella*

Kibera			
<i>Shigella</i>	No	Yes	Total
Neg	134 (99.3%)	110 (99.1%)	144 (99.2%)
Pos	1 (0.7%)	1 (0.9%)	2 (0.8%)
Total	135 (100%)	111 (100%)	246 (100%)
Chi square(x^2)=0.0194 DF=1 p=0.889			

P value of 0.889 shows that there is no significant association between residence in Kibera and isolation of *Shigella*. Tables 4.11-4.13 indicate that there is no significant association between residence in Kibera and enteric bacteria isolated from patients. Squalor conditions are known in most cases to be predisposing factors for diarrheal illnesses. However, in this study the isolated pathogens are not directly linked to

residence in Kibera slum. Therefore residence in Kibera is not a risk factor for diarrheal illnesses. Possible factors for causes of diarrheal illnesses in Kibera include: food handling and storage procedures, mother's or caretaker's level of education which determines level of hygiene which children are exposed to (WHO, 2004).

4.2.7 Association Between Area of Residence (South B) and Isolated Enteric Bacteria

Chi-square test of association between: South B and *E.coli* n=246

Table 4.14: Association between area of residence (South B) and *E.coli*
South B

<i>E.coli</i>	No	Yes	Total
Neg	147 (67.4%)	17 (60.7%)	164 (66.7%)
Pos	71 (32.6%)	11 (39.3%)	82 (33.3%)
Total	218 (100%)	28 (100%)	246 (100%)
Chi square(χ^2)=0.5038 DF=1 p=0.478			

Table 4.14 above shows no significant association between residence in South B and occurrence of *E.coli* bacteria. South B is an area of residence for both low and middle income earners in Nairobi. *E.coli* is commonly isolated in areas with squalor conditions due to poor hygiene. Compared to Kibera slums levels of hygiene are better in South B hence residence in the area is not a predisposing factor for *E.coli* infections.

Chi-square test of association between: South B and *Salmonella* n=246

Table 4.15: Association between area of residence (South B) and *Salmonella*
South B

<i>Salmonella</i>	No	Yes	Total
Neg	217 (99.5%)	26 (92.9%)	243 (98.8%)
Pos	1 (0.5%)	2 (7.1%)	3 (1.2%)
Total	218 (100%)	28 (100%)	246 (100%)

Chi-square(x^2)=9.2027 DF=1 p=0.002

Table 4.15 above shows a significant association between residence in South B and occurrence of *Salmonella* bacteria. *Salmonella* occurs through contamination of foods with animal faeces. Food may also be contaminated by unwashed hands of an infected food handler. This means that population in South B are exposed to risk factors for *Salmonella* and as a results the positive association between residence in this area and isolation of *Salmonella*.

Chi-square test of association between : South B and *Shigella* n=246

Table 4.16: Association between area of residence (South B) and *Shigella*
South B

<i>Shigella</i>	No	Yes	Total
Neg	217 (99.5%)	27 (96.4%)	244 (99.2%)
Pos	1 (0.5%)	1 (3.6%)	2 (0.8%)
Total	218 (100%)	28 (100%)	246 (100%)

Chi-square(x^2)=2.9813 DF=1 p=0.084

Table 4.16 above shows no significant association between residence in South B and occurrence of *Shigella*. *Shigella* infection is typically via ingestion (fecal-oral contamination) This happens when basic hygiene and hand washing habits are inadequate. South B is an area of residence where basic hygiene is observed hence no association between residence in this area and isolation of *Shigella*.

4.2.8 Association Between Area Of Residence (Kawangware) and Isolated Enteric Bacteria

Chi-square test of association between : Kawangware and *E.coli* n=246

Table 4.17: Association between area of residence (Kawangware) and *E.coli*
Kawangware

<i>E.coli</i>	No	Yes	Total
Neg	158 (67.5%)	6 (50%)	164 (66.7%)
Pos	76 (32.5%)	6 (50%)	82 (33.3%)
Total	234 (100%)	12 (100%)	246 (100%)
Chi-square(x^2)=1.5769 DF= 1 p=0.209			

Table 4.17 above shows *E.coli* infections are not directly associated with residence in Kawangware. This is an area of residence for both low and middle income earners in Nairobi. *E.coli* is associated with poor hygiene however in this study, residence in Kawangware is not a predisposing factor for *E.coli* infections meaning that the population in this area observe basic hygiene practices.

Chi-square test of association between : Kawangware and *Salmonella* n=246

Table 4.18: Association between area of residence (Kawangware) and *Salmonella*
Kawangware

<i>Salmonella</i>	No	Yes	Total
Neg	231 (98.7%)	12 (100%)	243 (98.8%)
Pos	3 (1.3%)	0 (0%)	3 (1.2%)
Total	234 (100%)	12 (100%)	246 (100%)
Chi-square(x^2)=0.1557 DF=1 p=0.693			

Table 4.18 indicates that there is no significant association between residence in Kawangware and occurrence of *Salmonella*. Risk factors for *Salmonella* include contamination of foods with animal feces and poor food handling practices. Residents in this area possibly observe safe handling practices and as a result there is no direct association between residence in this area and isolation of *Salmonella*.

Chi-square test of association between : Kawangware and *Shigella* n=246

Table 4.19: Association between area of residence (Kawangware) and *Shigella*

<i>Shigella</i>	Kawangware		Total
	No	Yes	
Neg	232 (99.2%)	12 (100%)	244 (99.2%)
Pos	2 (0.9%)	0 (0%)	2 (0.8%)
Total	234 (100%)	12 (100%)	246 (100%)
Chi-square(x^2)=0.1034 DF=1 p=0.748			

Table 4.19 above shows there is no significant association between residence in Kawangware and occurrence of *Shigella*. Fecal-oral contamination that is commonly associated with *Shigella* is not a predisposing factor for residents of Kawangware.

4.2.9 DISCUSSION

There are no reliable data on incidence or prevalence of diarrhea among the under-fives in Nairobi and in the whole country. Diarrhea is one of the major health problems facing children under fives in both urban and rural areas. This study aimed at studying the prevalence of enteropathogens to determine the extent of bacterial diarrhea in children under 5 years. According to this study prevalence of acute diarrhea in children under 5 was 20%. This rate is consistent with findings on occurrence of acute diarrhea among children in Sub-Saharan Africa. Studies have shown a prevalence rate of between 10.5 to 19% (Child Health Report, 2003).

As with most studies *E.coli* was the predominant pathogen (98.3%) isolated from children who presented to Mbagathi district hospital. In this study the presence of ETEC in the study group was highest(38.3%) compared to other *E.coli* groups. ETEC is a

common cause of acute watery diarrhea in children in developing countries. World Health Organization (2000) reported ETEC to be a childhood disease, due to its substantially higher incidence in early childhood than in older age groups. The prevalence of ETEC is particularly high in tropical and developing countries where standards of hygiene are compromised. This finding is consistent with most studies as *E.coli* has been found to be common among children below 5 years of age. This occurs as a result of poor hygiene standards when children are weaned.

Isolation of *Salmonella* and *Shigella* pathogens in this study were low 3.7% and 2.5% respectively. These pathogens are known to be more common among children older than 5 years hence the low isolation rates of both pathogens. Other bacterial pathogens tested in this study but not isolated are *Campylobacter* and *Vibrio Cholerae* species. *Campylobacter* is known to be prevalent in children under 12 months and few children in this study were in this age bracket and therefore the zero isolation rate of the bacteria. *Vibrio Cholerae* is known to occur frequently during disease outbreaks. During this study no diarrhea outbreak was observed and therefore a justification for no isolation of the pathogen.

The main risk factors which predispose children to acute diarrhea are: area of residence, water source. It would be assumed that residence in squalor conditions would be a factor for acute diarrhea among children. Most study participants were drawn from Kibera slums. Assumption would be that residents of Kibera would be more predisposed to acute diarrhea but according to this study there was no significant association between

areas of residence and acute diarrhea. Statistical tests did not prove significant association between areas of residence and occurrence of acute diarrhea. These study findings can be generalized to other urban areas in understanding acute diarrhea among children under 5 years since prevalence of acute diarrhea is consistent with findings conducted in Sub-Saharan countries. Also findings on bacterial causes of diarrhea and the prevalence rates of all bacteria agents is similar to other studies. However, risk factors for acute diarrhea among children cannot be generalized due to the fact that this study identified two factors of acute diarrhea. There is need to examine other social factors apart from area of residence and water sources.

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The study identified and characterized *Escherichia coli* as the most common bacterial pathogen causing acute diarrhea among children below 5 years of age. Ninety three percent (93.83%) of study population harbored *E.coli*. The strains enteroaggregative *E. coli* -EAGG, enteroinvasive *E.coli* EINV enterotoxigenic *E.coli*-ETEC shigatoxigenic *E.coli*-STEC were all isolated in the study population. All STEC strains isolated were non-0157 the fatal *E.coli* strain.. *Salmonella* and *Shigella* pathogens are rare among young children. All diarrhea specimens that were analyzed were negative for *Campylobacter* and *Vibrio Cholerae* bacteria. *Vibrio Cholerae* is known to occur in cholera outbreaks.

The prevalence of diarrhea caused by bacteria as determined by this study (n=246) are: *E.coli* 76; *Salmonella* 3; *Shigella* 2 total = 81 cases. 165 diarrheal cases were not caused by bacteria but possibly caused by viruses, parasites or other causes which were not examined in this study.

Diarrheal illnesses rank third among the most common diseases among children below 5 years. This age group is prone to unhygienic practices during weaning and also children in this age group are immunologically naïve and therefore easily exposed to diarrhea causing bacteria. There is no significant association between area of residence, water source and occurrence of the bacterial pathogens. Other social factors which are directly

related to hygiene practices in child care would be possible reasons for acute diarrhea among children under 5 years

5.2 RECOMMENDATIONS

It is thus recommended that:

1. An awareness on importance of personal and domestic hygiene is created among mothers with children under 5 years especially during weaning to prevent infection with *E.coli* and other diarrheal illnesses that are food and water-borne.
2. Continuous surveillance and reporting of diarrhea pathogens . This will serve to monitor changes of diarrhea pathogens within human populations hence promoting better prevention and therapeutic measures for diarrheal illnesses.
3. Need for further research on social factors that contribute to acute diarrhea among children under 5 years.

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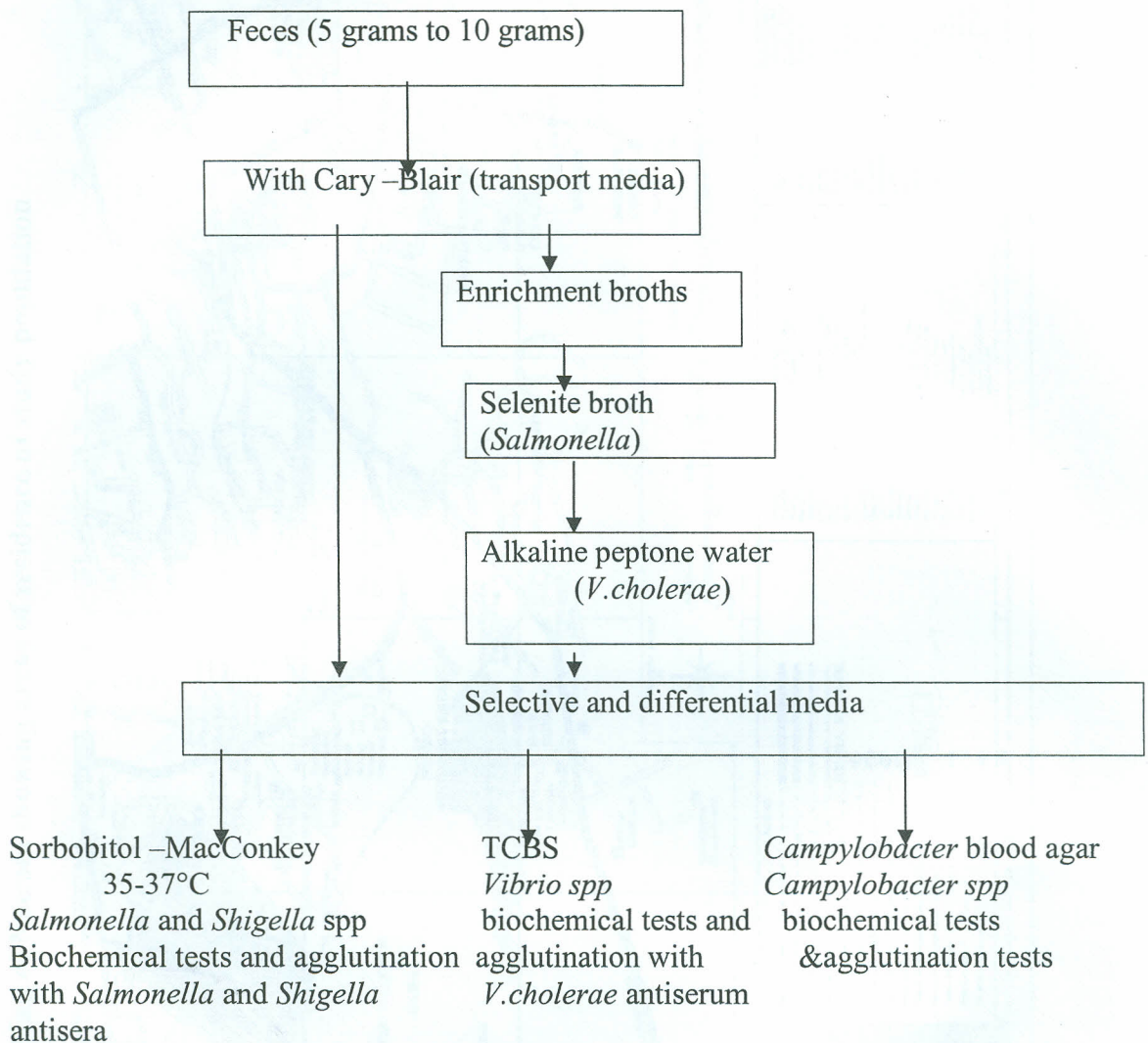
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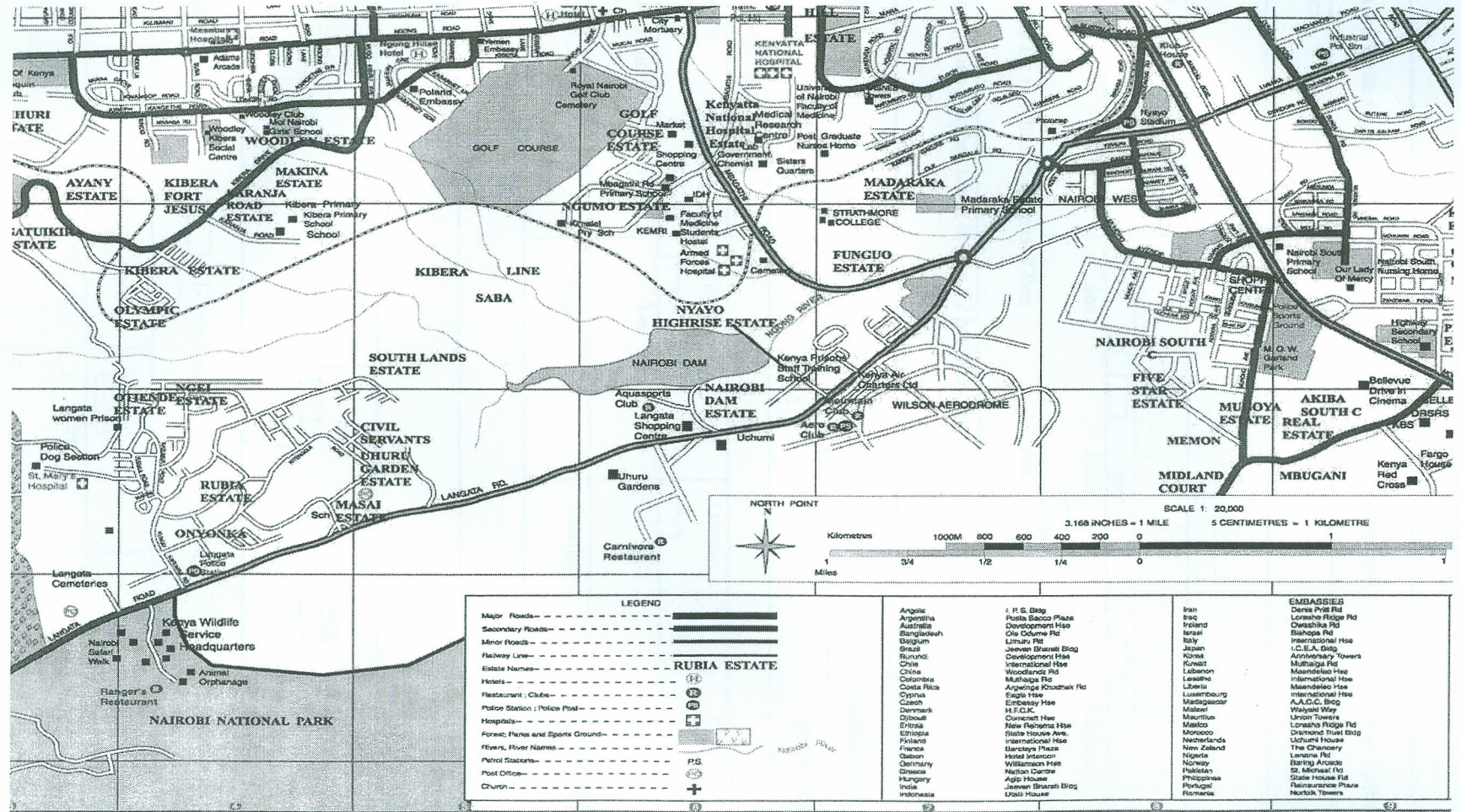
APPENDICES

Appendix 1: Study Conceptual Framework



Flow diagram of the methodology used for the investigation of potential pathogens in feces

Appendix 2: Map of Nairobi showing areas of residence of study population



Appendix 3: Questionnaire

QUESTIONNAIRE TO BE COMPLETED BY MEDICAL PROVIDERS AT SUBDISTRICT, DISTRICT, AND PROVINCIAL HOSPITALS			
Study Number			
Visit Date	_____ / _____ / _____ dd mm yyyy		
Date of onset of symptoms	_____ / _____ / _____ dd mm		
Age:	Sex	Male	Female
Any medications for diarrhea in the past 72hrs		Yes	No
List medication (s) for diarrhea taken in past 72 hrs if known:			
Where was medication for diarrhea obtained	<input type="checkbox"/> Shop/Chemist	<input type="checkbox"/> Health Institution	
	<input type="checkbox"/> Herbalist	<input type="checkbox"/> Home made	
PATIENT INFORMATION			
Place of resident	Village	District	Province
	<input type="checkbox"/> Yes	<input type="checkbox"/> No	
Have you visited another area in the last 2 weeks?			
Place visited last 2 weeks	Village	District	Province
Occupation:			
CLINICAL SYMPTOMS			
Diarrhea	Duration in days		
	<input type="checkbox"/> 1-2	<input type="checkbox"/> 3-4	<input type="checkbox"/> 5-6 <input type="checkbox"/> >7
When did the diarrhea begin? <input type="checkbox"/> 1-3 days ago <input type="checkbox"/> 4-6 days ago <input type="checkbox"/> >6days			
Mucus in stool	<input type="checkbox"/> Yes	<input type="checkbox"/> No	
Any bloody stool	<input type="checkbox"/> Yes	<input type="checkbox"/> No	

Vomiting		<input type="checkbox"/> Yes		<input type="checkbox"/> No	
Fever		<input type="checkbox"/> Yes		<input type="checkbox"/> No	
Abdominal cramp		<input type="checkbox"/> Yes		<input type="checkbox"/> No	
Other symptoms		<input type="checkbox"/> Headache		<input type="checkbox"/> Nausea	
				<input type="checkbox"/> Loss of appetite	
List any other symptoms:					
Type of water source		<input type="checkbox"/> Municipal		<input type="checkbox"/> Well	
				<input type="checkbox"/> Rain Water	
				<input type="checkbox"/> Borehole	
Do you boil your water		<input type="checkbox"/> Yes		<input type="checkbox"/> No	
PHYSICAL EXAMINATION					
General condition		Stable (Walking unsupported)		Sick looking (Require support)	
		<input type="checkbox"/>		<input type="checkbox"/>	
				<input type="checkbox"/> Very sick (Unconscious/confused)	
Temperature					
Eyeballs		<input type="checkbox"/> Normal		<input type="checkbox"/> Sunken	
Tongue		<input type="checkbox"/> Normal		<input type="checkbox"/> Dry	
Skin elasticity		<input type="checkbox"/> Normal		<input type="checkbox"/> Reduced	
Other evidence of dehydration					
Is the child malnourished		<input type="checkbox"/> Yes		<input type="checkbox"/> No	
Treatment(s)					
Name of person completing form: _____					
Signature _____			Date: _____		